

Fungal Biology

Ajar Nath Yadav *Editor*

Recent Trends in Mycological Research

Volume 2: Environmental and Industrial
Perspective



Springer

Fungal Biology

Series Editors

Dr. Vijai Kumar Gupta
Biorefining and Advanced Materials Research Center
Scotland's Rural College (SRUC), SRUC Barony Campus, Parkgate
Dumfries, Scotland
United Kingdom

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.

More information about this series at <http://www.springer.com/series/11224>

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Editor

Recent Trends in Mycological Research

Volume 2: Environmental and Industrial
Perspective

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Editor

Ajar Nath Yadav
Department of Biotechnology
Eternal University
Baru Sahib, Himachal Pradesh, India

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Foreword



Fungi are a diverse group of eukaryotic heterotrophic organisms. They are ubiquitous in the environment and can survive in most habitats. Some fungal classes exhibit an astonishing ability to tolerate extreme environmental conditions including low and high temperature, acidity and alkalinity, and the presence of heavy metals. They play a major role in the ecosystem and are even excellent degraders of pollutants. Agricultural practices, industrial processes, and the utilization of a range of chemicals in our day-to-day life result in the release of various hazardous contaminants into the environment.

These hazardous chemicals can be transported via the atmosphere and water, and, in many cases, find their way into sediments and soils after their release into the environment. Fungal communities produce a wide range of enzymes, exopolysaccharides, and organic acids for efficient degradation and transformation of metals, metalloids, organic chemicals, and radionuclides. White-rot fungi are gaining greater attention due to their versatility and robust nature, with enormous potential for oxidative bioremediation of pollutants due to high tolerance to toxic substances in the environment. Fungal species have been utilized for bread making and wine production, as well as drug production. The production of fungal food enzymes through fermentation processes has been growing continuously in many industrial sectors. The current volume of this book clearly describes that emerging environmental problems and expeditious industrial growth requires more exploration of fungal communities and a search for their novel enzymes and bioactive compounds. In this regard, the editor and authors have made an outstanding contribution in collecting and compiling past and current data on the role of fungal communities in each possible perspective with major emphasis on environment and industry.

I recommend this book to researchers and students working on the emerging and fascinating field of mycology. This book will advance the knowledge to a greater

extent in these areas with significant broader research on fungal communities. The editor of this book deserves credit for such a splendid and innovative contribution on mycological research.

Vice Chancellor
Eternal University
Baru Sahib, Himachal Pradesh, India



Dr. Davinder Singh

Foreword



Fungi are a unique group of organisms that consist of both microorganisms such as yeast, and moulds, which are grouped under the eukaryotes. The organisms of this kingdom have been the subject of debate for more than 200 years, and they remain the most discussed topic among mycologists. Earlier, fungi were often viewed as damaging and pathogenic organisms which infect and kill plants and animals, but as time passed, the dark ages of mycology faded away as humans began to realize the benefits of fungi. Nowadays, fungal communities play an essential role in addressing the major global challenges affecting agriculture, the

environment and industries like food, pharmaceutical, textiles, detergents and many more.

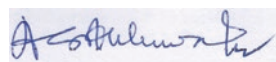
These new uses of fungi in a wide range of fields stand on the shoulders of mycologists over generations, who discovered them by studying the diversity, classification, genetics, evolution and physiology of this kingdom, without which applied mycology could not have progressed. In the environment, fungi can be applied for cleaning up the pollutants in the ecosystem spread by human activity, as they are good decomposers of pollutants and have hence been given the title “Nature’s Sweeper.” A huge amount of pollutants like heavy metals, oil, xenobiotics including DDT, pesticides, halogenated hydrocarbons and synthetic azo-dyes, which are degrading the earth’s diversity and structure, can be overcome by using organisms within the fungi kingdom. On the other hand, fungi can also be applied in industries as they have the capacity to produce several types of extracellular digestion enzymes and secondary metabolites, which can be used in the food, pharmaceutical, textile, detergent, paper and pulp industries.

The present volume, *Recent Trends in Mycological Research, Vol 2: Environmental and Industrial Perspective*, is a very timely publication, which provides state-of-the-art information in the area of mycology, broadly involving fungi and fungal-based products for sustainable developments in industry and the environment. The book

comprises 18 chapters. The first chapter by Agrawal et al. describes the bioprospecting and applications of fungi in the present scenario, while Hota et al. highlight diverse fungal communities from different sources and their roles in bioremediation of contaminated soil for sustainable environments in Chap. 2. Chapter 3 by Chandra et al. describes white-rot fungi for bioremediation of polychlorinated biphenyl contaminated soil. Chapter 4 by Singh et al. highlights the opportunities and challenges of fungal secondary metabolites for bioremediation of hazardous heavy metals. Kumari et al. describe enzymes from different fungal communities and their potential applications for degradation and detoxification of organic and inorganic pollutants in Chap. 5. In Chap. 6, Singh and Roy have presented details on fungal communities for the remediation of environmental pollutants. Vijayalakshmi highlights the diverse fungal consortia for effective degradation and decolourization of textile effluents in Chap. 7. In Chap. 8, Singh et al. describe the current status and future outlook of remediation of hazardous wastes by fungi. Joshi et al. highlight the potential applications of myco-nanoparticles in remediation in Chap. 9. Shahrajabian et al. explain metabolic engineering for secondary metabolites of marine fungal communities and their industrial applications in Chap. 10. The industrially important fungal enzymes and their productions and applications have been described by Dhevagi et al. in Chap. 11. Chapter 12 by Selvasekaran et al. describes fungal exopolysaccharides and their production and biotechnological industrial applications in food and allied sectors. Nanda et al. highlight the neoteric trends in medicinal plant-AMF association and elicit accumulation of phytochemicals in Chap. 13. Chua et al. discuss the fungal endophytes from orchidaceae and their diversity and applications for different sectors in Chap. 14. Fungal mycotoxins occurrence and detection is discussed in Chap. 15 by Kumari et al. Mohan et al. describe the preservative efficacy of essential oils against post-harvested fungi and insects of food commodities – a prospect to go green – in Chap. 16. Kapahi et al. explain the impact of fungal biorefineries on biofuel production for sustainable future energy systems in Chap. 17. Finally, the conclusion and future prospects of environmental and industrial perspectives of beneficial fungal communities have been described by the editor and co-authors in the last chapter.

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 and up-to-date source on recent trends in mycological research for students, researchers, scientists and academicians. I hope that the readers will find this book highly useful and interesting during their pursuit of mycology.

Pro-Vice Chancellor
Eternal University
Baru Sahib, Himachal Pradesh, India



Prof. Amrik Singh Ahluwalia

Preface

Fungi range from microscopic, single-celled yeasts to multicellular and heterotrophic in nature. Fungal communities have been found in vast ranges of environmental conditions. They are associated with plants in the forms of epiphytic, endophytic, and rhizospheric. Extreme environments represent unique ecosystems that harbor the novel biodiversity of fungal communities. Interest in the exploration of fungal diversity has been spurred by the fact that fungi are essential for life, as they perform numerous functions integral to the sustenance of the biosphere, including nutrient cycling and environmental detoxification, which involve processes such as augmentation, supplementation, and recycling of plant nutrients, which are vital to sustainable agriculture. Fungal communities are useful for sustainable environments as they are used for bioremediation, which is the use of microorganisms' metabolism to degrade waste contaminants (sewage, domestic, and industrial effluents) into non-toxic or less toxic materials by natural biological processes. Fungi could be used as mycoremediators for the future of environmental sustainability. Fungi and fungal products have the biochemical and ecological capability to degrade environmental organic chemicals and to decrease the risk associated with metals, semi-metals, and noble metals either by chemical modification or by manipulating chemical bioavailability.

The aim of this volume, *Recent Trends in Mycological Research, Vol 2: Environmental and Industrial Perspective*, is to provide an understanding of fungal communities from diverse environmental habitats and their potential applications in environment and industry. The book will be useful to scientists, researchers, and students in the fields of microbiology, biotechnology, agriculture, molecular biology, environmental biology, and related subjects.

Baru Sahib, Himachal Pradesh, India

Ajar Nath Yadav

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All authors are sincerely acknowledged for contributing up-to-date information on the beneficial fungi, their biodiversity, and biotechnological applications for sustainable agriculture and environments. I am thankful to all the authors for their valuable contributions.

My sincere thanks to the entire Springer team that was directly or indirectly involved in the production of the book. I am grateful to the many people who helped to bring this book to light. I wish to thank Eric Stannard, Senior Editor, Botany, Springer; Dr. Vijai Kumar Gupta; Prof. Maria G. Tuohy, Series Editor, Fungal Biology Springer; Mr. Rahul Sharma, Project Coordinator, Springer; and Sudha Kannan (Ms.), for generous assistance, constant support, and patience in initializing the volume.

I give special thanks to my exquisite wife Ms. Neelam Yadav for her constant support and motivation in putting everything together. I am grateful to my Ph.D. research scholars, Dr. Divjot Kour, Ms. Tanvir Kaur, and Ms. Rubee Devi, and colleagues for their support, love, and motivation in all my efforts during this project.

This book should be useful to scientists and scholars investigating fungal biology and biotechnology.

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Contributors

Editor



Ajar Nath Yadav is an assistant professor (senior scale) and assistant controller of examinations at Eternal University, Baru Sahib, Himachal Pradesh, India. He has 5 years of teaching experience in UG, PG, and Ph.D. level courses related to microbiology as well as microbial biotechnology in the Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University. He has 11 years of research experience in the fields of microbial biotechnology, microbial diversity, and plant–microbe interactions. Dr. Yadav obtained a doctorate in microbial biotechnology, jointly from ICAR–Indian Agricultural

research Institute (IARI), New Delhi, and Birla Institute of Technology, Mesra, Ranchi, India; an M.Sc. (biotechnology) from Bundelkhand University; and a B.Sc. (CBZ) from the University of Allahabad (a Central University), India.

Dr. Yadav has 238 publications, with h-index of 53, i10-index of 150, and 7600 citations (Google Scholar- on 03/03/2021). Dr. Yadav is editor of 18 Springer-Nature, 7 CRC Press Taylor & Francis, 2 Elsevier, and 1 Wiley book. He has to his credit one granted patent, “Insecticidal formulation of novel strain of *Bacillus thuringiensis* AK 47”. He has received 12 Best Paper Presentation Awards and 1 Young Scientist Award (NASI-Swarna Jayanti Purskar). Dr. Yadav received the “Outstanding Teacher Award” in the 6th Annual Convocation in 2018 from Eternal University, Baru Sahib, Himachal Pradesh. Dr. Yadav is currently handling two projects, one funded by the Department of Environment, Science & Technology (DEST), Shimla, and one by the HP Council for Science, Technology & Environment (HIMCOSTE). Dr. Yadav has guided one Ph.D. and one M.Sc. scholar, and presently he is guiding four Ph.D. scholars. To his credit, ~6700 microbes (archaea, bacteria, and fungi) have been isolated from diverse sources and ~550 potential and

efficient microbes deposited at the culture collection of ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh, India. He has deposited **2423** nucleotide sequences and **03** whole genome sequences (*Bacillus thuringiensis* AKS47, *Arthrobacter agilis* L77, and *Halolamina pelagica* CDK2) and **02** transcriptome to NCBI GenBank databases in the public domain. The niche-specific microbes from extreme environments were reported as specific bio-inoculants (Biofertilizers) for crops growing in normal and diverse abiotic stress conditions. Dr. Yadav and group have developed technology for screening of archaea for phosphorus solubilization for the first time. He has served as an editor/editorial board member and reviewer for different national and international peer-reviewed journals. Dr. Yadav has lifetime membership of the Association of Microbiologists of India and the Indian Science Congress Council. Please visit <https://sites.google.com/view/ajarnathyadav> for more details.

Authors

Ahmed M. Abdel-Azeem Botany and Microbiology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt

Komal Agrawal Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Bandarsindri, Kishangarh, Ajmer, Rajasthan, India

Partibha Ahlawat Centre of Food Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Amrik Singh Ahluwalia Department of Botany, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

Ansar Alam Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Bandarsindri, Kishangarh, Ajmer, Rajasthan, India

Sakthivel Ambreetha Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Lavanya Agnes Angalene School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Padmini Baruah Central Muga Eri Research & Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India

Chanda Parulekar Berde Marine Microbiology, School of Earth, Ocean and Atmospheric Sciences (SEOAS), Goa University, Taleigao Plateau, Goa, India

Vikrant B. Berde Department of Zoology, Arts, Commerce and Science College, Lanja, Maharashtra, India

Chandini School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

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

Qi Cheng Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Ramalingam Chidambaram Instrumental and Food Analysis Laboratory, School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Ru Wei Chua School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Selangor Darul Ehsan, Malaysia

Rubee Devi Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

Abhijit Dey Department of Life Sciences, Presidency University, Kolkata, India

Periyasamy Dhevagi Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Murat Dikilitas Department of Plant Protection, Faculty of Agriculture, Harran University, S. Urfa, Turkey

Enespa Department Plant Pathology, Sri Mahesh Prasad Degree College, University of Lucknow, Lucknow, Uttar Pradesh, India

Shiv Shanker Gautam Department of Microbiology, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Krishna Giri Rain Forest Research Institute, Jorhat, Assam, India

Sanjay Gupta Department of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, India

S. Zafar Haider Centre for Aromatic Plants (CAP), Selaqui, Dehradun, Uttarakhand, India

Surabhi Hota ICAR-National Bureau of Soil Survey and Land Use Planning, Regional Center, Jorhat, Assam, India

Suyog A. Joshi Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

Rehema Joshua Centre of Food Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Meena Kapahi Department of Chemistry, Manav Rachna University, Faridabad, Haryana, India

Tanvir Kaur Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

Kashish Kohli Department of Chemistry, Manav Rachna University, Faridabad, Haryana, India

Divjot Kour Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

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

Rakesh Kumar Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Anju Kumari Centre of Food Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Rekha Kumari Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

Anathi Magadlela School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

Mahalakshmi School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Manindra Mohan Uttarakhand Council for Biotechnology, Haldi, U.S. Nagar, Uttarakhand, India

Anuradha Mukherjee MMHS, South 24Pgs, West Bengal, India

Banadipa Nanda Department of Life Sciences, Presidency University, Kolkata, India

Samapika Nandy Department of Life Sciences, Presidency University, Kolkata, India

Ambikapathi Nivetha Department of Chemistry, Bharathiar University, Coimbatore, Tamil Nadu, India

Devendra Kumar Pandey Department of Biotechnology, Lovely Faculty of Technology and Sciences, Lovely Professional University, Phagwara, Punjab, India

Durbaka V. R. Prasad Department of Microbiology, Yogi Vemana University, Vemana Puram, Kadapa, Andhra Pradesh, India

Sengottiyar Priyatharshini Department of Crop Management, Vanavarayar Institute of Agriculture, Pollachi, Tamil Nadu, India

Pankaj Kumar Rai Department of Biotechnology, Invertis Institute of Engineering and Technology (IIET), Invertis University, Bareilly, Uttar Pradesh, India

Ambikapathi Ramya Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Roopa Rani Department of Chemistry, Manav Rachna University, Faridabad, Haryana, India

Madhu Kumar Reddy Department of Microbiology, Yogi Vemana University, Vemana Puram, Kadapa, Andhra Pradesh, India

Felicia Roshini School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Arpita Roy Department of Biotechnology, School of Engineering & Technology, Sharda University, Greater Noida, India

Sagar P. Salvi Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

Satyajit Saurabh DNA Fingerprinting Laboratory, Bihar State Seed and Organic Certification Agency, Patna, Bihar, India

Department of Bioengineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

Pavidharshini Selvasekaran Instrumental and Food Analysis Laboratory, School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Neha Sen Pal College of Nursing and Medical Sciences, Haldwani, Uttarakhand, India

Aftab A. Shabnam Central Muga Eri Research & Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India

Mohamad Hesam Shahrajabian Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Department of Agronomy and Plant Breeding, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

Gulshan Kumar Sharma ICAR-National Bureau of Soil Survey and Land Use Planning, Regional Center, Jorhat, Assam, India

Sangeeta C. Sindhu Department of Food and Nutrition, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Aditi Singh Department of Biotechnology, Bennett University, Greater Noida, Uttar Pradesh, India

Archana Singh Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

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

Dipti Singh Department of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

Manali Singh Department of Biotechnology, Invertis Institute of Engineering and Technology (IIET), Invertis University, Bareilly, Uttar Pradesh, India

Prashant Singh Department of Chemistry, DAV (PG) College, Dehradun, Uttarakhand, India

Ram B. Singh Halberg Hospital and Research Institute, Civil Lines, Moradabad, Uttar Pradesh, India

B. V. Sivaprasad Department of Microbiology, Yogi Vemana University, Vemana Puram, Kadapa, Andhra Pradesh, India

Ravindra Soni Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, Chhattisgarh, India

G. Subrahmanyam Central Muga Eri Research & Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India

Wenli Sun Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Deep Chandra Suyal Department of Microbiology, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

Kalyanasundaram Geetha Thanuja Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Adeline Su Yien Ting School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Selangor Darul Ehsan, Malaysia

P. Veera Brahmma Chari Department of Microbiology, Yogi Vemana University, Vemana Puram, Kadapa, Andhra Pradesh, India

Pradeep Verma Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Bandarsindri, Kishangarh, Ajmer, Rajasthan, India

D. Vijayalakshmi Department of Microbiology, Yogi Vemana University, Vemana Puram, Kadapa, Andhra Pradesh, India

Ajar Nath Yadav Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

Ashok Yadav Department of Botany, Institute of Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Praveen Kumar Yadav Department of Botany, Institute of Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Farhan Zameer Department of Biological Sciences (Biochemistry), School of Basic and Applied Sciences, Dayananda Sagar University, Shavige Malleshwara Hills, Kumaraswamy Layout, Bengaluru, Karnataka, India

Chapter 1

Bioprospecting and Applications of Fungi: A Game Changer in Present Scenario



Komal Agrawal, Ansar Alam, and Pradeep Verma

1.1 Introduction

The fungal kingdom is a very diverse group, characterized in seven different phyla. The knowledge of this group is limited to relatively few species. Their application is very versatile for economically significant biotechnological applications which were due to the invention of penicillin uprising in the field of medicine that promoted the discovery and screening of fungi for their valuable properties (Yadav et al. 2020a, b). Apart from Ascomycetes, Basidiomycetes are also explored and studied as they are a good producer of secondary biomolecules. The unexplored fungi like endophytic fungi are reservoir of unique biologically active molecules (Kusari et al. 2012; Imhoff 2016; Jones et al. 2019; Rana et al. 2019a, b).

Fungi and their associated biomolecules have a great influence on mankind as they produce various biomolecules like enzymes, antibiotics, antiviral, immunosuppressor, and antitumor as well as toxins that contaminate food and the environment and causes health issues in their respective host (Devi et al. 2020a). There is a need for both biomolecules and toxins of fungal origin for their beneficial use in medicine, pharmaceutical, agriculture, food industries, cosmetics, textiles, etc. Fungal biomolecules are very imperative for the evolution and ecology of living organisms and cannot be considered as waste products (Brakhage and Schroeckh 2011; Rateb and Ebel 2011; Agrawal and Verma 2020a), e.g., a secondary molecule like patulin, yeast killer toxin, and penicillic acid have also shown quorum sensing inhibitory activities (Schulz et al. 2002). The slight concentration of these molecules has stout lethal activity (Villa et al. 2013) or can stimulate a target effectively. Thus, considering its diverse potential the present chapter would discuss about various

K. Agrawal · A. Alam · P. Verma (✉)

Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, NH8 Bandarsindri, Kishangarh, Ajmer, Rajasthan, India

e-mail: pradeepverma@curaj.ac.in

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applications of fungi, and its biomolecules such as enzymes and mycotoxins. In addition the limitations and future prospect has also been discussed.

1.2 Fungi

Fungi are ubiquitous in nature and play a crucial role as a primary decomposer. They produce various low molecular weight biomolecules, and the main ecological properties of maximum biomolecules are still cagey; however they emerge as biotic and abiotic protectants (Devi et al. 2020b). Their noteworthy impression has both positive and negative impacts on mankind and has made them an attractive study material. Fungal secondary biomolecules possess a wide range of biological activity that has a good impact on mankind and environmental health. Fungal mycotoxins has caused loss of millions of tons of crop annually worldwide. Their contamination and the resulting mycotoxicosis have health and economic benefits and consequences. On the contrary, the pharmacodynamics of medically applicable secondary metabolites like ergot, ribotoxin, β -lactam antibiotics, penicillin, cholesterol-lowering agent lovastatin, and antitumor agent taxol are few examples of fungal secondary metabolites that had constructive impressions on mankind (Kour et al. 2019b) (Fig. 1.1).

1.2.1 Applications of Fungi

The harmful wastes generated from various chemical operations and processes are treated via various physicochemical and biological treatment methods in order to meet the prescribed standard as per the Environmental Protection Act, 1986 (Singh

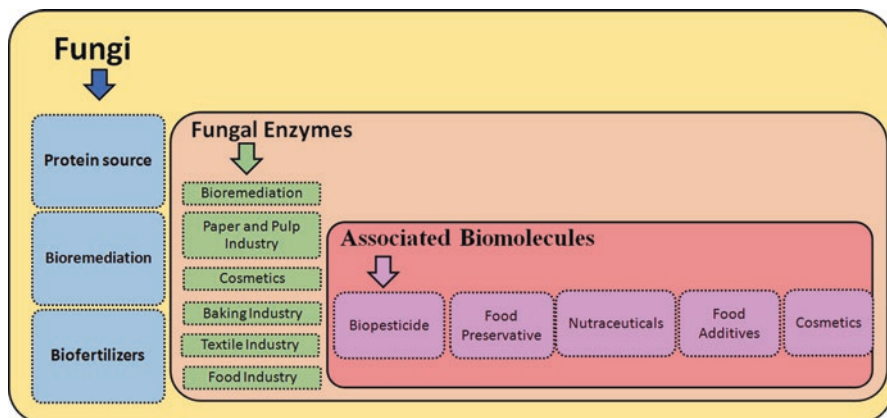


Fig. 1.1 Various applications of fungi, fungal enzyme, and their associated biomolecules

et al. 2014). Bioremediation is referred for transforming toxic contaminants into benign products using biological treatment methods and is the most potent tool to treat pollution in the environment to make it a sustainable ecosystem. Fungi due to their diverse morphology and metabolic capacity play a vital role in bioremediation (Agrawal et al. 2020; Kour et al. 2020a; Kumar et al. 2019).

Fungi are capable to degrade various toxic pollutants coming from various industries such as textile, pulp, leather, petroleum, etc. (Deshmukh et al. 2016; Kour et al. 2020b). Fungi can survive in a diverse range of habitats, e.g., soil matrix and fresh and marine water habitats (Yadav et al. 2020c). The diversity of fungi with respect to habitats and secretion of various biomolecules makes fungi a potential candidate for bioremediation of various recalcitrant molecules (Anastasi et al. 2013; Rastegari et al. 2020a, b). In addition, fungi can also degrade environmentally present organic chemicals in order to minimize the risk that is associated with metals, metalloids, and radionuclides (Harms et al. 2011).

1.2.1.1 Bioremediation of Contaminated Water

Water pollution is becoming a major problem since the last decade. In mill effluents, different dyes, starch, and other complex molecules from desizing process are major contaminants that need to be removed from the effluents. Textile dye effluents pose environmental hazards because of color effluent and its toxicity. Azo dyes and their degraded products both are toxic, harmful for the environment, and difficult to deplete. The removal of dye wastewater has been performed using various methods (ion exchange, adsorption, electrokinetic coagulation, etc.) which were developed but not executed at a large scale because of their narrow application assortment and high operation cost. The rise in the application area has been documented and explored due to increased pressure of legislative restriction on colored effluent and emerging environmental awareness. Fungal biomass and associated enzyme have shown an effective role in the decolorization and detoxification of textile effluents. Ascomycetes have been reported to remove color by adsorption, whereas laccase from basidiomycetes has been reported to play a major role in the decolorization of textile effluents (Agrawal and Verma 2020b, 2019a, 2019b, Verma et al. 2010, Verma and Madamwar 2002, 2005).

Asamudo et al. (2005) stated that large quantities of effluents are generated at various steps of the textile manufacturing process, and also result in the use of abundant amounts of chemicals and dyes. The effluents possess toxic reactive dye, chlorolignin residues, and dark coloration. The biological breakdown of such complex mixtures can be attained using enzymes produced by a white-rot fungus, *Phanerochaete chrysosporium*, by degrading the chromophoric groups. Malaviya and Rathore (2007) used two Basidiomycetes and Deuteromycetes fungi to bioremediate effluent pulp and paper mill using a continuously aerated bench-top bioreactor, and reduction in color, lignin, and chemical oxygen demand was attained on the fourth day of incubation. The physicochemical parameters of textile wastewater

such as COD and toxicity were also found to be reduced when a fungal biomass strain *Trametes pubescens* MUT2400 was used (Anastasi et al. 2012).

1.2.1.2 Bioremediation of Contaminated Soil

The bioaugmentation in composting of a flare pit soil was studied at lab-scale composters, and total petroleum hydrocarbon (TPH) content reduced by an average of 29% reduction in most jar after 98 days of incubation, and 70–99% reduction in the peak area of the selected hydrocarbons was observed after gas chromatograph analysis of the oil extract (Baheri 2002). A similar study of contaminated soil through biostimulation–bioaugmentation with filamentous fungi was done by Mancera-López et al. (Mancera-López et al. 2008). The three species of fungi such as *Rhizopus* sp., *P. funiculosus*, and *A. sydowii* removed 36%, 30%, and 17% polycyclic hydrocarbons (PAH). Additionally, the biodegradation in soil microcosms was studied using a mixture of toluene, ethylbenzene, benzene, xylene, and methyl-*tert*-butyl ether. The biodegradation profile was examined at two pH: at neutral conditions, the fungus had little effect, whereas at acidic pH, the activity was inhibited followed by the increase of strain *Cladophialophora* sp. and enhancement in the biodegradation of toluene and ethylbenzene (Prenafeta-Boldú et al. 2004). D’Annibale et al. (D’Annibale et al. 2006) investigated heavily contaminated soil, and nine fungal strains were identified and screened for degradation potential. After 30 days, fungal colonization was observed, and it removed dichloroaniline isomers, naphthalene, o-hydroxybiphenyl, and 1,1-binaphthalene. The *Stachybotrys* sp. strain DABAC 3 was the most effective among them. The results reveal that autochthonous fungi are capable of decreasing soil toxicity.

Similarly, five fungal isolates *Cladosporium*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Pleurotus* were found as a suitable candidate in the removal of PAHs from creosote-contaminated soil. The higher percentage of creosote removal was between 78 and 94%. It was also observed that the mixed population of fungi was more effective about 94.1% in the removal of creosote as compared to the single population with a maximum of 88% (Atagana et al. 2006). In another report, the degradation of PAHs in aged-creosote contaminated soil was observed using spent compost of mushroom and oyster mushroom. It was incubated for 7 weeks at ambient temperature, and results showed effective removal of the total 16 PAHs (86%), 3-ring (89%), 4-ring (87%) and, 5-ring (48%) PAHs, respectively (Eggen 1999).

1.2.1.3 Biodegradation of Xenobiotic

The white-rot fungi have the ability to degrade/mineralize a wide spectrum of toxic pollutants (Singh et al. 2020b). The extracellular peroxidases are capable to biodegrade xenobiotic compounds (Reddy 1995). Paszczynski and Crawford (1995) have reported that *Phanerochaete chrysosporium* can be used as potential bioremediation

agents for the degradation of xenobiotic. Magan et al. (2010) reviewed that *Trametes versicolor* and *Phanerochaete chrysosporium* also biodegraded xenobiotic compounds.

1.3 Next Generation Promoter of Cereal Plants

In the last century, according to Giannopolitis and Ries (1977) many environmental and world climate fluctuation issues happened. The fluctuation in climate constraints has a global impact and decreased the production of cereal crops (El-Ramady et al. 2013; Nelson et al. 2018). The Food and Agricultural Organization (FAO) of the UN reported that due to population inflammation cereal crops demand amplified. Thus, for boosting the agricultural productivity and sustainability, research is carried out globally to overcome the challenges. The use of plant–fungal symbionts has been an innovative program and is currently getting more consideration due to its benefits like its positive influence on plant growth and health, upgraded agricultural qualities, and improved soil quality and nutrient cycling (Ripa et al. 2019; Rana et al. 2019c; Verma et al. 2017). Fungi being a natural decomposer of natural and manufactured material (Burford et al. 2003; Gadd 1999, 2007) play an important role in upholding soil health. In addition, it enhances the coping mechanism against biotic and abiotic stresses, reduces the consumption of water, and increases the biomass. Endophytic fungi in plant produces plant growth regulator that enhances the growth and development of plants specifically in applicable cases of crops (Gadd 1999, 2007). Endophytic fungi are considered functionally dynamic associates of the plants, without causing visible damage in their host (U'Ren et al. 2012; Furtado et al. 2019; Rana et al. 2020a).

Endophytic fungi have been observed to assist their hosts to overcome environmental stress (Redman 2002; Gonthier et al. 2006). The plant growth-promoting fungi are used as bio-inoculants (Singh and Yadav 2020). They have multiple assistances in order to increase the quantity and quality of plants (Verma et al. 2015, 2016; Rana et al. 2020b). Mycorrhizal fungi show a better ability to tolerate acidic conditions and are excellent mobilizers of nutrients (Johansson et al. 2004; Subrahmanyam et al. 2020). They are examined to produce various types of phytohormones such as indole-2-acetic acid, gibberellins, and siderophores (Milagres et al. 1999). Khan et al. (2012) observed that fungal strain *Paecilomyces formosus* producing gibberellins and indoleacetic acid reduces the negative impact of salt stress on cucumber plants. The increased activity of the antioxidant enzyme was observed in the saline environment, and high tomato yield was achieved when the higher colonization of *Trichoderma harzianum* was found (Yasmin et al. 2018). Gaiind (2016) stated that *Aspergillus niger* and *Trichoderma harzianum* solubilizing phosphorus and producing indoleacetic acid jointly reduced salt stress of wheat seedlings.

1.3.1 Fungal Pigments

During the industrial uprising, food and processed foods industries developed swiftly, and the use of natural colorants was replaced by chemically synthesized colors in the nineteenth century. However, increasing safety concerns about the application of coloring agents and various regulations on the use of it throughout the world have led to the revival of natural-based food colorants. They are mostly derived from flowering plants and insects and are extracted using food-grade solvent and are also dried or are also available as powders that are oil soluble. However, the production of natural colorants strongly relies on the seasonal supply of the raw materials (Spears 1988). The natural colorants are, in most cases, the mixture of fluctuating composition with the difficulty in characterizing its purity and the contaminants. The various classes are already existing natural food colorants showing excessive variation in stability and functionality. Fungi particularly Ascomycetes and basidiomycetes are best known about the production of diverse classes of pigments naturally. Fungi are the major source of renewable and reliable pigment production (Poorniammal et al. 2013; Chen et al. 2015; Vendruscolo et al. 2016), and the pigments as secondary metabolites have either known/unknown functionality with diverse array of colors (Poorniammal et al. 2013). The potential of fungal biodiversity exploration, for safe and novel pigment via suitable techniques, is untouched (Mapari et al. 2005).

According to Wissgott et al. (1996), Ascomycetes are better suited than mushrooms and other fungal species as they give comparatively high yield under optimized cultivation conditions. Riboflavin produced by *E. ashbyii* and *A. gossypii* is a natural yellow food colorant produced by semi-fermentative technique (Stahmann et al. 2000). A very expensive carotenoid lycopene also produced by *B. trispora* is approved by the European Union food legislation which leads to the successful adaptation of fungal sources for natural colorant productions (Mantzouridou and Tsimidou 2008).

The fungal colorants are chemically classified as carotenoids and polyketides (Frisvad and Samson 2004). Kaur et al. (2019) reported that a yellow pigment produced by *E. nigrum* has antioxidant activity similar to curcumin and can be a potential food colorant. Arpink red, riboflavin, lycopene, and beta-carotene from *Penicillium oxalicum*, *Ashbya gossypii*, *Blakeslea trispora*, and *Monascus pigments* (Ogbonna 2016) are some fungal pigments approved by the European Union and are currently in use.

Aside from providing color to food, it has shown other excellent properties such as antimutagenic, antimicrobial (Sen et al. 2019; Sibero et al. 2017), antioxidants, anti-cancerous, and anti-obesity activities (Ogbonna 2016; Gessler et al. 2013). Such properties of pigment point out toward the use of nutraceuticals when added to some specific food products. Guo-Ping et al. (Guo-Ping et al. 2016) reported that pigment secreted by the fungus *M. purpureus* inhibits both fungi (*Aspergillus*, *Trichoderma*, *Mucor*, etc.) and bacteria (*Bacillus*, *Pseudomonas*, *Escherichia*, and *Streptomyces*). Pigments from *M. castaneae* SVJM139 (Visalakchi and Muthumary

2009), *Sporobolomyces* sp. (Manimala and Murugesan 2014), *Fusarium* sp. (Sharmila et al. 2012), *Aspergillus* sp., and *Penicillium* species (Teixeira et al. 2012) also have antimicrobial properties against many fungal and bacterial species.

1.4 Fungal Siderophores

Fungal siderophores are mostly hydroxamate and carboxylate type and are produced extracellularly or intracellularly under iron-limiting conditions. It has affinity for ferric iron and results in the formation of a complex ferric iron–siderophore that is transported to the cytosol. Currently, siderophores have much more applications, and various fungal strains reported for the production include *Aspergillus fumigatus* and *Aspergillus nidulans* (Schrettl et al. 2010; Kour et al. 2019a). The fungal strain *A. nidulans* has also been reported for the production of ferricrocin, ferrihordin coprogen, and triacetylfusarinine C (Oberegger et al. 2008). The other ester-containing siderophore linear fusigen has been produced by *L. laccata* and *L. bicolor* (Haselwandter et al. 2013; Harrington et al. 2015).

The fungal strain *Trichoderma* spp. and *Fusarium* sp. has been reported to produce coprogen and fusigen (Sharma et al. 2019). These are hydroxamate-type of siderophore (Diekmann and Zahner 1967; Sayer and Emery 1968). Further, *T. harzianum* has been reported to produce hydroxamate and carboxylate (Ghosh et al. 2015), and Hussein and Joo (2014) stated that 92.33% of siderophores were produced by *T. harzianum*. In addition ferricrocin has also been reported in various ericoid mycorrhizal fungi along with fusigen (Haselwandter et al. 1992). Ectendomycorrhizal fungus and ectomycorrhizal fungus *W. rehmi* and *C. geophilum* have the capacity to produce siderophore, and they have been identified as ferricrocin. Basidiochrome, a novel siderophore, has been isolated from *Ceratobasidium* and *Rhizoctonia* (Zou and Boyer 2005; Haselwandter et al. 2006).

In agriculture siderophores promote plant growth and increase yield by improving uptake of Fe (Rai et al. 2020; Singh et al. 2020a). In addition, it is also a potential biocontrol agent against harmful phytopathogens (Suman et al. 2016). Siderophores can also detoxify heavy-metal-contaminations and thus its application can be extended to bioremediation as well. The iron detection potential of siderophores suggests its potential role as a biosensor. In case of bacteria the poor penetration of bacterial envelope has contributed to the problem, and thus it plays a key role in the resistance especially for *P. aeruginosa*. The use of a “Trojan Horse” strategy has been proposed as a means to increase biological activity of *P. aeruginosa* (Mislin and Schalk 2014). The “Trojan Horse strategy” of siderophore has been used in medical field to form complexes with antibiotics and delivery to antibiotic-resistant bacteria. Similarly, it can also help treat certain diseases such as sickle cell anemia, malaria, cancer, and removal of transuranic elements from the body.

There has been very less work done on antifungal agent using siderophore-pharmacophore conjugates. Antifungal drugs conjugated to siderophores can improve the activity (Bernier et al. 2005). The overload of iron in humans due to

Table 1.1 Fungal siderophores and their applications

S.No.	Siderophores	Organisms	Application	Reference
1.	Coprogen	<i>Alternaria cassiae</i>	Virulence factors	Ohra et al. (1995)
	Ferricrocin	<i>Neurospora crassa</i> , <i>Trichoderma virens</i>	Iron storage, growth promoter	Horowitz et al. (1976), Mukherjee et al. (2018)
3.	Rhodotorulic acid	<i>Rhodotorula glutinis</i>	Bio-control of diseases	Calvente et al. (1999)
4.	Triornicin	<i>Epicoccum purpurascens</i>	Inhibitory effect on tumors	Frederick et al. (1981)
5.	Hydroxamate siderophores	<i>Gloeophyllum trabeum</i>	Bio-bleaching of pulps	Xu and Goodell, (2001), Arantes and Milagres (2007)
6.	Carboxylate type siderophore	<i>Glomus</i> , <i>Rhizopus</i> , <i>Mucor</i>	Plant growth promoter	Winkelmann (2017)
7.	Dimerum acid, Coprogens	<i>Histoplasma capsulatum</i>	Data not available	Crawford and Wilson (2015)
8.	Rhizoferrin	<i>Rhizopus oryzae</i>	Ferrioxamine B type xeno-siderophore	Ibrahim et al. (2008)

repeated transfusion therapy and no particular physiological mechanisms for iron elimination leads to disease development and can be treated using siderophore-based treatment, e.g., desferal a type drug can be used for the treatment of sickle cell anemia and thalassemia major (Nagoba and Vedpathak 2011; Nagoba and Vedpathak 2011; Szebesczyk et al. 2016; Braun et al. 2009) (Table 1.1).

1.5 Fungal Enzymes

Fungi survive as saprophyte, parasite, or mutualistic symbionts and are present on a wide range of ecological niche. The unique mode of life cycle and nutrition makes fungi a perfect candidate for mankind's welfare applications. Fungi are applied for the industrial production of enzymes, vitamins, pigments, pharmaceuticals, etc. (Subrahmanyam et al. 2020). It is also used in agriculture as a biofertilizer or biological control agent, and fungal enzymes are more suitable for recycling paper waste, the deinking process of printed papers over bacterial enzymes, and synthetic chemicals (Rana et al. 2019a). The fungal species *Trichoderma* produces cellulase capable of breaking down cellulosic substrate to glucose. It also has its application in various fields like textile, detergent, animal feed, baking, bioremediation, etc. According to Gopinath and co-workers (Gopinath et al. 2017), *Aspergillus* and *Penicillium* species are the chief producers of amylase. Amylase is chiefly used in food industries to produce glucose syrup, corn syrup, and juice and in alcohol fermentation process. There are several fungal species that are used for various enzymes like protease, amylase, pectinase, lipase xylanase, laccase, etc. production

(Yadav 2020; Yadav et al. 2020a, b). These fungal enzymes can be applied in numerous fields that need more exploration and development.

1.5.1 Various Prospects of Fungal Enzymes

The fungal enzymes have diverse range of applications (Fig. 1.2) which have their utility in various sectors which have been described as follows:

1.5.1.1 Dishwashing and Household Detergents

Detergent and dishwashing agent removes various types of complex stain and food-stuff. Due to environmental aspects and consumer safety, at present different enzymes are incorporated with detergent. These enzymes are mostly hydrolytic in nature chiefly obtained by fungi and include proteases, α -amylases, lipase, hemicellulase, cellulases, etc. Their incorporation is required for the development of detergent and dishwashing agent. The enzymes used in dishwashing agents are the same as laundry detergent, but for specificity in performance, some groups of proteases and amylases are favored (Vasconcelos et al. 2006). These all belong to hydrolases that degrade their substrate by hydrolysis and break down the dirt or complex molecules into smaller and soluble groups. The enzymes used in automatic dishwashing detergent are not very specific because of the cleaning environment which involves an enormous assortment of foodstuffs.

In household detergent, mostly stains are incompletely detached by the surfactant or bleach system which leads to unsatisfactory end result depending on the washing conditions. The suitable detergent enzyme removes the soil and stains where the detergents have purely physicochemical action. It is a joint action of the enzyme and surfactant to get rid of the stain completely from the fibers. The first detergent using cellulase was developed for efficiently cleaning and removing stains. After the introduction of enzyme in detergent, genetic, and protein engineering, techniques were publicized for various first- and second-generation enzymes

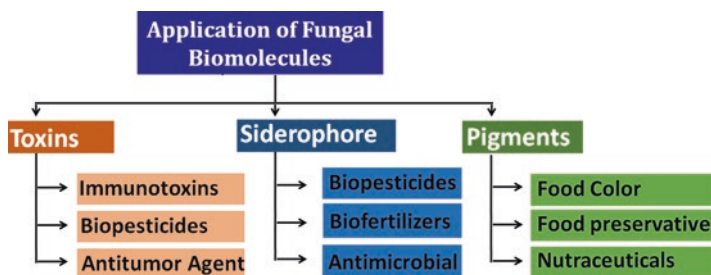


Fig. 1.2 Applications of fungal biomolecules in various sectors

that improved washing under harsh conditions and with single wash treatment. In the early stage of development, enzymes were used mostly for stain removal and cleaning of fabrics, but later on enzymes were developed for color brightening and fabric softening.

1.5.1.2 Animal Feeds

The chief component of animal feed contains antinutritional factors that restrict their use, and the endogenous enzymes produced by animal produces are not enough to digest all the feed. The addition of commercial enzymes with feed can resolve this issue and also increases animal performance and has been well accepted in industries. The use of commercial enzyme in animal feed would first result in the breakage of ANFs inherent in the feedstuff for better utilization and second would be to break down the chemical structure to release the nutrients, thereby increasing the feed value. In addition, the availability of protein, starch, and minerals to the animal can be enhanced that are generally not accessible to the animals. In the European Union countries, maximum poultry feed contains enzyme supplements, and the frequency of their use is also high.

1.5.1.3 Textile Industries

The fungal enzyme technology has been used in processing natural fibers as they are environmentally friendly and the reactions are specific in nature. In 1912 amylases were used in the desizing process which later resulted in increase of different hydrolytic enzymes, e.g., cellulase, protease, xylanase, laccase, etc.; as they were economically feasible, better quality products were generated and were eco-friendly in nature (Mojsov 2011). Water pollution has become a major problem since the past few decades, and the wet processing of textiles is the major spring of it. In mill effluents, different dyes, starch, and other complex molecules from desizing process are major contaminants that need to be removed from the effluents. Azo dyes and their degraded derivatives are toxic, harmful for the environment, and difficult to deplete. The removal of dyes from wastewater has been performed using various methods but not executed at a large scale because of its narrow application assortment and high operation cost. The rise in the application area has been documented and explored due to increased pressure of legislative restriction on colored effluent and emerging environmental awareness. Many researchers stated that complete or almost complete decolorization is achieved with the use of different fungal microorganisms. The fungal strains *Pleurotus ostreatus* and *Phanerochaete chrysosporium* has been reported for the complete degradation of azo dyes by Senthilkumar et al. (2014).

1.5.1.4 Cosmetics

There have been many investigations on fungal derivatives for their potential use in cosmetics and great impact on society. Enzyme-containing products in the market are still less due to the high capital cost as compared to conventional products (Gupta et al. 2019). The biological derived cosmetic product in India of various international and national brands has got momentum as the immense appeal of traditional herbal and ayurvedic ingredient among urban and rural consumers as well as the matured population increased (Gupta et al. 2019; Agrawal et al. 2018). Fungi produce a variety of products which may have potential application in cosmetics such as oligosaccharide, exopolysaccharide, biosurfactant, enzyme, pigments, etc. Buschmann and Schollmeyer (2002) reported that the cyclodextrin which is an oligo-polysaccharide is used in perfume and room freshener gel to reduce the volatility of esters and also used in detergent to provide a long-lasting effect. Cyclodextrin powder is used in the napkin, talcum, menstrual disc, etc. for odor control.

Biosurfactant has multi-functional properties like the skin-hydrating agent, emulsifier, detergent, foaming agent, etc. It is used in many cosmetic products because of its non-toxicity and bio-degradable properties (Gupta et al. 2019). Mannosylerythritol lipid (MEL) is mostly used as a biosurfactant in the production of cosmetic, e.g., lipsticks, lip makers, eyeshades, soap, etc. (Gupta et al. 2019; Ueno et al. 2007). Many oxidative enzymes have been used as the substitute of hydrogen peroxide which makes a milder hair coloring process, compatible with enzyme and neutral pH.

1.5.1.5 Dental Care

The enzymes produced by fungus have great value in various dental caries, such as the enzymes that are oxidative in nature inhibit pathogenic bacteria responsible for causing various oral-related issues. The starch- or protein-hydrolyzing enzymes can help eliminate food residues and avert staining of tooth. On the other hand, lysozyme hydrolyzes the cell walls of pathogenic bacteria which if present would cause oral-related issues.

The use of amyloglucosidase and glucose oxidase in toothpaste breaks down starch to glucose, and glucose oxidase further oxidizes glucose to gluconolactone and hydrogen peroxide. The hydrogen peroxide so produced is used by saliva peroxidase to produce hypothiocyanite that acts as an antibacterial agent. In various literature, the use of proteases, amylases, and cellulases to degrade food residues (Mazzucotelli et al. 2013) has been reported (Callewaert et al. 2011). Proteases, amylases, glucosidases, and oxidative enzymes are known for cleaning of artificial teeth or dentures. A study suggested that the enzymes dextranases, mutanases, and levanases can dissolve special sugar components of dental plaque (de Castro et al. 2011) and proteases, amylase, and endoglucanases break down food residues into smaller particles and support to dissolve them from the dentures. The cleaning of the denture usually takes place at a moderate temperature and takes several hours.

The safety measures are taken for the formulation of an enzyme-containing denture the same as the preparation of enzymatic automatic dishwashing detergents.

1.5.1.6 Membrane Cleaning

The fungal enzyme application is in fast track lane as the use of membrane technology increases in the different industries. Mostly food processing industries like the fruit juice industry use enzyme directly to improve the membrane separation performance. There are many studies on enzymes; it always demonstrates the benefit of enzymatic cleaning on organic ultrafiltration membranes. The use of enzymes such as subtilisin protease alcalase is more stable than another reported enzyme (Sumantha et al. 2006; Anwar and Saleemuddin 1998).

1.6 Mycotoxins

Every ecological niche of planet earth is populated with a diversity of organisms. These organisms have a variety of biological interactions, e.g., mutualism, parasitism, antagonism, etc. which enables their survival. On the other hand, collembolan, mites, and insects are fungivores as fungi are a rich source of phosphorus and nitrogen (Berenbaum and Eisner 2008) also necessary for their survival; however, there are certain exceptions, e.g., fungus-farming ants which established a mutualistic relationship with fungi, thereby enabling the survival of both the counterparts. The survival of fungi is enabled due to various mechanisms like predatory, defensive, and mutualistic. They also produce varieties of toxins, e.g., patulin, ergot, yeast toxin, ribotoxin, etc. which generally are secreted as secondary metabolites by the fungi. These toxins have both positive and negative impacts and have been used in various industrial applications as well. Thus, the present chapter would deal with various applications of toxins produced by fungi. The fungi are reported for the production of various types of toxins and are discussed as follows:

1.6.1 Applications of Mycotoxins

1.6.1.1 Ribotoxin

Ribotoxins are extracellular RNases produced by several filamentous fungi like *Aspergillus*, *Penicillium*, and *Hirsutella* species. These toxins are a small protein of about 150 amino acids, larger than non-toxic fungal RNases. They contain elongated and positively charged loops and have been purposed to be the structural basis of its toxicity. This feature makes ribotoxins an efficient natural killer and can be utilized as targeted armaments against pathogens. The best studied ribotoxins

included sarcin, restrictonin, Aspfl, and hirsutellin A (HtA). Hirsutellin A is produced by *Hirsutella*, and the rest are chiefly produced by *Aspergillus* sp. (Olombrada et al. 2017; Olson and Goerner 1965). It was found that sarcin was produced by mold *Aspergillus giganteus* and has the potential to inhibit sarcoma and carcinoma induced in mice (Olson and Goerner 1965). Soon after, restrictocin and mitogillin two other antitumor proteins having similar activity in *Aspergillus restrictus* were discovered and were commonly called as ribotoxins. Thus, a study was conducted, and it was reported that all species of *Aspergillus* contain ribotoxin genes (Varga and Samson 2008).

The extended study of ribotoxin has shown that they exhibit insecticidal features, supporting their participation in defense and parasitism (Brandhorst-Hubbard et al. 2001), and highlighted potential use in biotechnological applications (Tomé-Amat et al. 2015a, b). The world population is projected to reach 9.5 billion by 2050. The exponential growth of the population needs an equivalent growth of food resources. According to a study it was suggested that approximately 40% of the loss in agriculture production is due to pest diseases every year globally. For decades, chemical insecticides were used to control pests such as DDT (dichlorodiphenyltrichloroethane). Chemical insecticides were highly effective, cost-effective, and easy to deliver and fast-acting, but their toxicity and development of resistance among mites and insects forced many countries to ban chemical insecticides. Biological control methods have risen as an alternative due to the cost of discovering, developing, registering new synthetic pesticides. Bio-pesticide became a vital factor in eco-friendly green pest management. Still, its use has not yet reached its potential, participating only 3–4% of the insecticidal market (Glare et al. 2012). Thus, the ribotoxin can significantly contribute toward the shifting of biological control methods against the chemical-based insecticides.

Ribotoxins could be also used in the field of ribosome biogenesis. It can also be used as a specific tool for the study of human ribosome-related disease ribosomopathies (Narla and Ebert 2010; Nakhoul et al. 2014). Immunotoxins are small in size, thermostable, highly efficient to inactivate ribosomes and resistant to protease, composed of a very specific antibody fragment, and accountable to target specific cell surface antigen. Ribotoxins have been tested for therapeutic uses and have all features to make it an appropriate candidate for the construction of immunotoxins (Reiter 1998). The engineered variant of immunotoxins, using fungal ribotoxins or other fungal RNases as a toxic component, can be unable to cross membranes but retains the ribonucleolytic activity (Tomé-Amat et al. 2015a). This engineered variant reduced unwanted side effects during cancer therapy.

1.6.1.2 Patulin

Patulin, a mycotoxin, is produced by several filamentous fungal families like *Penicillium*, *Aspergillus*, and *Byssoschlamys* as secondary biomolecules, habitually found in fruit and vegetable, and its derivative products also reported persuading apoptosis in carcinoma cells (Saxena et al. 2009; Zhou et al. 2010). Patulin via two

mechanisms initiates noxiousness in humans, first by generating oxidative stress response in cells and second by binding to responsive sulfhydryl groups in the cellular proteins via covalent bonds (Alavizadeh et al. 2016; Liu et al. 2006). It is also reported to affect testis tissue in rats and increase levels of serum testosterone. However, the toxic/apoptotic effects on the female reproductive system of rats have to be studied as they have not been reported (Selmanoglu and Koçkaya 2004).

Patulin also has its application in medical sectors, and as per International Agency for Research on Cancer, it is “not carcinogenic to humans” (Nicolay et al. 2009). The second and third most frequently diagnosed cancers in women globally are colorectal and cervical cancers (Kohler et al. 2015), and a study was conducted where it was observed that patulin exhibited antitumor activity. The effect of patulin was determined on B16F10 cell proliferation, and during 4 days of analysis it was observed that patulin exhibited an anti-proliferative effect, thereby signifying the potential role of patulin against melanoma cells. Alam et al. (2020) stated that a combination of oxaliplatin with patulin formed synergism counter to human colorectal cancer models conditional on dose and order of drug administration.

1.6.1.3 Ergot Alkaloids

Ergot is a plant disease caused by fungi *Clavicipitaceae*, and the sclerotia of fungus contain a high amount of alkaloid ergotamine and several other peptide alkaloids of the ergotamine group including ergosine and ergocristine (Sharma et al. 2016). They have high structural diversity, have varied effects on animals and humans, and have also contaminated grains. It has also been reported to cause mass poisonings, with effects ranging from dry gangrene to convulsions and death. The chemical structure of ergot alkaloid possesses very similar homology to several neurotransmitters like noradrenaline, dopamine, or serotonin which make it useful sources of pharmaceuticals for a variety of medical purposes like migraine and Parkinson’s disease. Agonist or antagonist behavior of ergot alkaloid targeting alpha-adrenergic, dopaminergic, and serotonin (5-HT) receptors (Azizan et al. 2016) leads to various physiological changes like vasoconstriction, uterine contraction (mimicking oxytocin effect), and neurotrophic activities (Azizan et al. 2016; Berde and Stürmer 1978).

Ergometrine is an important therapeutic drug in obstetrics and is used primarily with the drug oxytocin in the management of the third stage of labor or the treatment of postpartum hemorrhage (Liabsuetrakul et al. 2018). The effect of ergot alkaloids on the uterus varies with hormonal status of the pregnant woman. Small doses of ergot alkaloid provisions can carry rhythmic contractions and relaxation of the uterus, while higher doses result in a strong and prolonged contraction (Sharma et al. 2016). Ergonovine directly stimulates uterine smooth muscle contraction consequential in amplified muscular tone and contraction which makes it a more suitable choice in obstetrics compared to other ergot alkaloids.

The prophylactic use of ergot alkaloids has also been studied and has been reported to reduce postpartum blood loss and moderate to severe postpartum hemorrhage (PPH) in the third stage of labor. PPH is defined as the blood loss of 500 mL

or more within 24 hours after birth. The use of ergot alkaloids in the third stage of labor means blood loss, i.e., at least 500 mL, increased maternal hemoglobin concentration (g/dL) at 24–48 hours postpartum eventually decreasing the use of therapeutic uterotonics. The risk of the retained placenta or manual removal of the placenta, or both, has been reported to be inconsistent with high heterogeneity. Results for intravenous (IV)/intramuscular (IM) ergot alkaloids versus no uterotonic agents were similar to those of ergot alkaloids administered by any route since most of the studies (seven of eight) used IV/IM route. Only one small study (289 women) compared oral ergometrine with placebo, and it showed no benefit of ergometrine over placebo; however, no maternal adverse effects were reported.

Individual agent and blood vessel type fluctuate the ergot alkaloid effect on vascular smooth muscles. The natural alkaloid ergotamine compresses all human blood vessels including both veins and arteries, whereas dihydroergotamine is more effective in capacitance rather than on resistance vessels. It also plays a vital role to act as α_1 -receptor antagonist and causes the reversal of pressor effects of adrenaline. Ergotamine, ergonovine, and methysergide all are nonspecific partial agonists of 5-HT₂ vascular receptors (Sharma et al. 2016). These drugs stimulate serotonin, decrease inflammation, and reverse blood vessel dilation around the brain, thereby relieving the migraine or cluster headache symptoms (Olesen et al. 2009).

1.6.1.4 Yeast Killer Toxins

The production of toxins is a common phenomenon in nature. This is due to antagonistic activities of yeast against another microorganism. Further studies showed that the antagonistic activities of yeast against other microorganisms can be attributed to some different properties, including competition for nutrients and space, acidification of the medium, production of ethanol, and secretion of antimicrobial compounds, such as volatile acids, hydrogen peroxide, secondary metabolites, and the so-called killer toxins. For yeast, the first reports regarding their killer phenotype date to over 50 years ago, with the initial isolation of a *Saccharomyces cerevisiae* strain that inhibited the growth of other *S. cerevisiae* strains (Mannazzu et al. 2019; Woods and Bevan 1968). According to these early investigations, killer (K) yeast secreted a toxin that was lethal to sensitive (S) strains of the same or related species but was harmless to neutral (N) strains, which were immune to their killer effects. Most killer yeast can kill other yeast of the same or different species and genera. Some of them are active against filamentous fungi (Izgu et al. 2011; İzgü et al. 2006), while others are also active against bacteria (Meneghin et al. 2010). The action spectrum of yeast killer toxins encompasses spoilage microorganisms relevant to the fermentation (Todd et al. 2000) of food and feed industries (Lowes et al. 2000). It also includes microbial pathogens of clinical interest and plant pathogens (Santos and Marquina 2004a, b). Thus, they can be used as natural antimicrobials in the agro-food industry, as well as antimicrobials against animal and human infections and for biological control of plant pathogens, both in the field and for postharvest applications (Rosa-Magri et al. 2011; Muccilli and Restuccia 2015) (Table 1.2).

Table 1.2 Various fungal secondary metabolite and their applications

S.No.	Secondary metabolites	Application	Fungi	Reference
1.	Astaxanthin	Skin protectant/ anti-oxidant	<i>Rhodotorula</i> , <i>Phaffia</i> , <i>Xanthophyllomyces</i>	Corinaldesi et al. (2017)
2.	Mycosporine	UV protectant	<i>Phaeothea triangularis</i> , <i>Trimmatostroma salinum</i>	Kogej et al. (2006)
3.	Sophorolipids	Treatment of acne, dandruff and body odors	<i>Candida bombicola</i>	Varvaresou and Iakovou (2015)
4.	Sarcin, restrictonin	Immunotoxin, to inhibit sarcoma and carcinoma	<i>Aspergillus giganteus</i> , <i>Aspergillus restrictus</i>	Olson and Goerner (1965)
5.	Ergotamine	Vasoconstriction, uterine contraction	<i>Claviceps purpurea</i>	Sharma et al. (2016), Azizan et al. (2016)
6.	Naphthoquinonic pigment	Antibacterial and antifungal activity	<i>Fusarium oxysporum</i>	Lebeau et al. (2019)

1.7 Limitation of Fungal Biomolecules and Its Future Prospect

Mycological claim for human welfare is an important economical and renewable factor. It has been estimated that more than 40% of the commercial drugs available in the market are natural or biologically modified natural metabolites of fungi. There is a potential to produce mycoprotein and fungal associated biomolecules effectively, and industries must grasp the chance. According to Kurt et al. 2018, the production of enzyme and secondary biomolecules highly depends upon the origin of strain and culture conditions. Secondary biomolecule is produced under stressed conditions, while enzyme production requires optimum media condition and specific substrate concentration, pH, and temperature. The optimum pH and temperature vary from enzyme to enzyme secreted by fungal species. The enzyme stability also is a limiting factor that affects its application and production. For some biomolecules and enzymes, high production cost is also a key limiting factor that restricts their application in various fields. Fungi and its biomolecules like secondary metabolites, enzymes, pigment, siderophores, etc. represent the future of various fields like agriculture, cosmetics, food, remediation, pharmaceuticals, etc. Biological properties of fungal biomolecules in various fields have gained attention, but still there is a lack of exploration for their application. For example, mycosporines and mycosporine-like amino acids are an effective natural UV-filters, with strong anti-oxidant activity produced by marine fungi. Even PUFA and carotenoids produced by fungi might have an important role in cosmetic and food applications. Similarly, antimicrobial compounds such as chitosan and derivatives extracted from marine fungi provide a valid alternative to other synthetic preservatives not being harmful for skin and environmental health. Fungal enzymes such as amylase, cellulase, and

laccase have various applications in different fields. The fungal biomolecules have a wide spectrum of applications. However, still there is need to explore fungi and their associated biomolecules for wide range of applications.

1.8 Conclusion

The fungal metabolites have tremendous scope, and its application can be explored for future industrialization. As much research is required, and it is still a work in progress, it can offer huge scope for its effective utilization for the benefit of mankind and environment as it is renewable in nature, though rigorous optimization and scale-up studies have to be performed for the effective utilization of the fungal strains producing desired metabolites. Thus, with advancement of technology various fungal strains can be effectively screened for a huge array of identified and unidentified metabolites followed by exploring their application in various biotechnological sectors.

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Chapter 2

Fungal Communities for Bioremediation of Contaminated Soil for Sustainable Environments



Surabhi Hota, Gulshan Kumar Sharma, Gangavarapu Subrahmanyam, Amit Kumar, Aftab A. Shabnam, Padmini Baruah, Tanvir Kaur, and Ajar Nath Yadav

2.1 Introduction

Accelerated human activities in the form of rapid industrialization and urbanization have led to contamination of the natural ecosystems. The major sources of environmental degradation are industrial effluents, sewage water, oil spills, fertilizers and pesticides that are persistent in soils due to longer half-life periods (Kumar et al. 2020a; Singh et al. 2013a; Malyan et al. 2019). These materials when released into a soil system contaminate it with heavy metals and complex organic and inorganic compounds, which have a great threat to soil organisms like microbes and plants (Kumar et al. 2020b; Bhatia et al. 2015). In the long run, such contaminants in natural environment may lead to the degradation or permanent destruction of soil and soil fertility (Singh et al. 2013b; Borowik et al. 2017). In addition to the detrimental effects on animal health, ecosystem functions and food security, soil contamination may pose direct hazards to human health (Subrahmanyam et al. 2020; Gupta et al. 2019). Contamination of soils may also gradually lead to contamination of the groundwater (Mishra et al. 2018) when these contaminants move in with irrigation or rain water. Soil contamination has become a global issue as a result of the last 200 years of industrialization and has affected at least one third of the world's ecosystem.

S. Hota · G. K. Sharma

ICAR-National Bureau of Soil Survey and Land Use Planning, Regional Center,
Jorhat, Assam, India

G. Subrahmanyam · A. Kumar · A. A. Shabnam · P. Baruah

Central Muga Eri Research & Training Institute, Central Silk Board,
Lahdoigarh, Jorhat, Assam, India

T. Kaur · A. N. Yadav (✉)

Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture,
Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

Several works and developments have been done towards remediation of these contaminants. These contaminations can be corrected by certain measures like chemical remediation, physical remediation and biological remediation. Biological remediation or bioremediation is a new dimension in this area, as physical remediation is a costly affair and chemical remediation has long-term side effects to the environment (Kumar et al. 2017a). Bioremediation is eco-friendly and cost-effective, and it includes the remediation using biological processes by microbes, in common (Rastegari et al. 2020a; Rastegari et al. 2020b; Singh et al. 2020). It can be in situ or ex situ. For the past two decades, many agents have been used towards bioremediation, but there is no thumb rule in the application of these agents of remediation, as they are target specific and the choice of the agents depends upon the environment in which they are being used (Azubuike et al. 2016). They may be algae (Khan et al. 2018), bacteria, fungi, or most recently, biochar which is also being successfully used in bioremediation (Sharma et al. 2020).

In this chapter emphasis will be given on bioremediation of contaminated soil by fungi. Fungi have been proved to be very much effective in implementing bioremediation. Fungi, because of their robust nature and capacity to withstand extreme environmental conditions, can be effectively used in remediation of a wide range of contaminants such as persistent organic pollutants, polyaromatic hydrocarbons, leather tanning industries, textile dyes, effluents from textile, petroleum, bleached kraft pulp and pesticides (Deshmukh et al. 2016; Yadav et al. 2020e). In this chapter, the emphasis will be given on the efficient role of fungi in bioremediation, its mechanism and factors affecting the bioremediation.

2.2 Fungal Communities Participating in Bioremediation of Contaminated Soils

There are a number of fungal communities which are effective in bioremediation of contaminated soils. These communities are target specific, i.e. all communities cannot be used for all kinds of pollutants. Some of the most commonly used fungal communities are *Penicillium* sp., *Ganoderma* sp., *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., *Candida* sp., *Agaricus* sp., *Pleurotus* sp., *Fusarium* sp. and *Volvariella* sp. The fungal species with their target contaminant have been given in Table 2.1.

2.3 Mechanisms of Bioremediation by Different Fungal Communities

2.3.1 Fungal Mechanisms of Bioremediation

Fungi have tremendous capacity to degrade a wide range of substances by secreting several extracellular enzymes which help in decomposing two essential components lignin and cellulose. Fungi are one of the most responsible terrestrial organisms that

Table 2.1 Categorical classification of fungal species targeting metal remediates for fungal phytoremediation

Fungal Species	Metal Remediation										References
	Mn	Zn	Cu	Ar	Ni	Cr	Pb	Fe	Cd	Hg	
<i>Agaricus bisporus</i>											Ita et al. (2008)
<i>Agaricus bisporus</i>											Nagy et al. (2014)
<i>Agaricus bitorquis</i>											Prasad and Sachin (2013)
<i>Alternaria alternata</i>											Seshikala and Charya (2012)
<i>Alternaria alternata</i>											Shoaib et al. (2011)
<i>Armillaria mellea</i>											Ita et al. (2008)
<i>Ascohyta betae</i>											Seshikala and Charya (2012)
<i>Aspergillus awamori</i>											Joshi et al. (2011)
<i>Aspergillus</i>											Fulekar et al. (2012)
<i>Aspergillus flavus</i>											Thippeswamy et al. (2012b)
<i>Aspergillus flavus</i>											Shoaib et al. (2011)
<i>Aspergillus flavus</i>											Joshi et al. (2011)
<i>Aspergillus flavus</i>											Iram et al. (2013)
<i>Aspergillus flavus</i>											Talukdar et al. (2020)
<i>Aspergillus foetidus</i>											Prasenjit and Sumathi (2005)
<i>Aspergillus fumigates</i>											Kumar Ramasamy et al. (2011)
<i>Aspergillus fumigatus</i>											Rao et al. (2005)
<i>Aspergillus fumigatus</i>											Shazia et al. (2013)
<i>Aspergillus fumigatus</i>											Talukdar et al. (2020)
<i>Aspergillus niger</i>											Pal et al. (2010)
<i>Aspergillus niger</i>											Kumar Ramasamy et al. (2011)
<i>Aspergillus niger</i>											Thippeswamy et al. (2012a)
<i>Aspergillus niger</i>											Iram et al. (2013)
<i>Aspergillus ochraceus</i>											Seshikala and Charya (2012)
<i>Aspergillus oryzae</i>											Nasseri et al. (2002)
<i>Aspergillus terreus</i>											Shoaib et al. (2011)
<i>Aspergillus terreus</i>											Seshikala and Charya (2012)
<i>Aspergillus versicolor</i>											Taştan et al. (2010)
<i>Aspergillus versicolor</i>											Çabuk et al. (2005)
<i>Candida tropicalis</i>											Akhtar et al. (2008)
<i>Circinella</i> sp.											Alpat et al. (2010)
<i>Cladosporium resinae</i>											Gadd and de Rome (1988)
<i>Cunninghamella echinulata</i>											Shoaib et al. (2011)
<i>Curvularia lunata</i>											Seshikala and Charya (2012)
<i>Drechslera rostrata</i>											Seshikala and Charya (2012)
<i>Fusarium oxysporum</i>											Amatussalam et al. (2011)
<i>Fusarium solani</i>											Sen and GHOSH (2011)
<i>Ganoderma lucidum</i>											Loukidou et al. (2003)
<i>Ganoderma lucidum</i>											Muraleedharan et al. (1995)
<i>Gliocladium</i> sp.											Tahir (2012)
<i>Lactarius piperatus</i>											Nagy et al. (2014)
<i>Metarhizium anisopliae</i>											Çabuk et al. (2005)
<i>Mucor</i>											Fulekar et al. (2012)
<i>Mucor hiemalis</i>											Srivastava and Hasan (2011)
<i>Mucor rouxii</i>											Majumdar et al. (2010)
<i>Mucor</i> sp.											Tahir (2012)
<i>Penicillium</i>											Fulekar et al. (2012)
<i>Penicillium canescens</i>											Say et al. (2003)
<i>Penicillium canescens</i>											Say et al. (2003)
<i>Penicillium chrysogenum</i>											Tan and Cheng (2003)
<i>Penicillium cyclopium</i>											Ianis et al. (2006)
<i>Penicillium decumbens</i>											Levinskaite (2001)
<i>Penicillium digitatum</i>											Galun et al. (1987)
<i>Penicillium notatum</i>											Seshikala and Charya (2012)
<i>Penicillium pupurogenum</i>											Say et al. (2003)
<i>Penicillium</i> sp.											Loukidou et al. (2003)
<i>Penicillium verrucosum</i>											Çabuk et al. (2005)
<i>Phanerochaete chrysosporium</i>											Joshi et al. (2011)
<i>Phanerochaete chrysosporium</i>											Mamun et al. (2011)

can decompose wood. The main component of the fungi is the mycelium (the vegetative part of the fungus which is often observed as fine, white threads), in most cases, that is responsible for decomposing lignin and cellulose (Rhodes 2014). White-rot fungi have attracted most of the attention in this field and contribute to 30% of the total reported fungi used in mycoremediation (Singh 2006) due to their capacity to degrade a wide range of toxic compounds which are very much persistent in the soil system (Barr and Aust 1994).

The mechanism of white-rot fungi in degrading the lignin and cellulose is non-specific. But the interesting thing is it does not use lignin as source of carbon (Devi et al. 2020a; Devi et al. 2020b). The possible mechanism is they secrete oxidase enzyme, which is extracellular. The enzyme utilizes the components of lignin, methylglyoxal, glucose and glyoxal and breaks it down to H_2O_2 or CO_2 . *Phanerochaete chrysosporium* has been reported to be an important decomposer (Barr and Aust 1994; Rhodes 2014). *Trametes versicolor*, *Bjerkandera adusta* and *Pleurotus* sp. are some other white fungi which are capable of producing various ligninolytic enzymes, i.e. peroxidases and laccases. Other enzymes are lignin peroxidase and manganese peroxidase, which also take part in degradation of the compounds. The mechanism of degradation of lignin and cellulose by white-rot fungi is given in Fig. 2.1.

Fungal inoculation may facilitate the bioremediation also by accelerating the bacterial degradation of organic soil pollutants. The two mechanisms involved in bioremediation are called as Highways and Pipelines (Banitz et al. 2013). Highway involves the mycelial network while pipelines include the hypha. Soil is a heterogeneous system and irregular in terms of air-filled porosity, moisture content and distribution of pollutants, which make it harder for the bacteria to thrive and act. Fungi, on the other hand, can thrive better in this condition. The highways or the hydrophilic mycelial network can provide a continuous film of moisture around their hyphae for the bacteria to have plenty of substrates moved to them to act on (Furuno et al. 2010; Kohlmeier et al. 2005; Yadav et al. 2020b, c). This mechanism

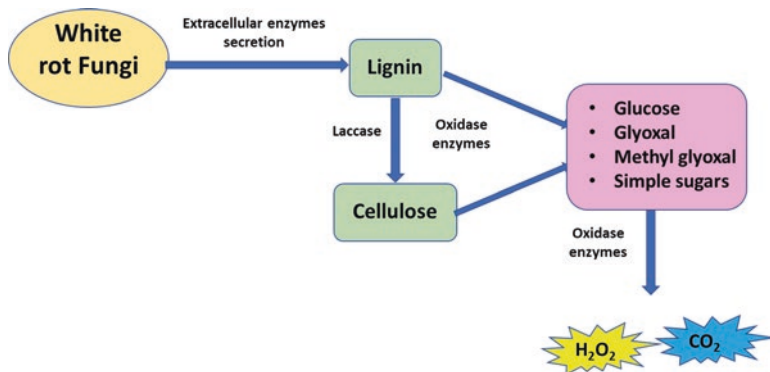


Fig. 2.1 Mechanism of degradation of polysaccharides, viz. lignin and cellulose, by white-rot fungi

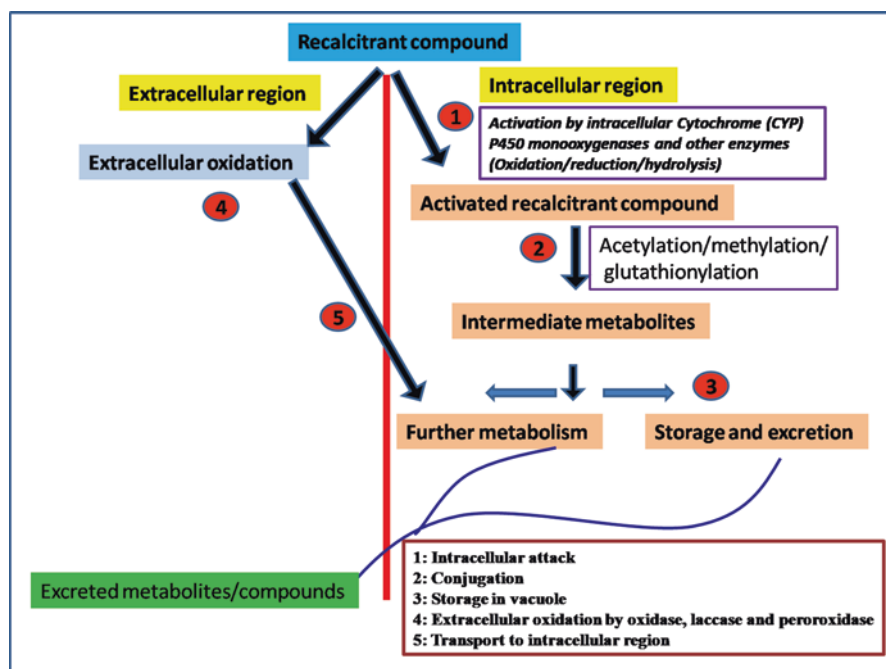


Fig. 2.2 Various extracellular and intracellular mechanisms of fungi for bioremediation of recalcitrant toxic compounds. The bioremediation mechanisms illustrated in this figure were adopted. (Modified and redrawn from Deshmukh et al. (2016))

has been reported to be successful in bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs) (Furuno et al. 2012). Pipelines mechanism acts where hyphal translocation occurs via cytoplasmic streaming. Highways and pipelines may operate separately in different conditions or may act simultaneously. Fungi act by mechanisms of detoxification by various enzymatic degradation, bio-adsorption and chelation towards remediation of heavy metals and PAHs (Liu et al. 2017) (Fig. 2.2).

2.3.2 Mechanisms of Bioremediation by Mycorrhizal Fungi

The mycorrhizal fungi are commonly found in symbiosis within and on the rhizosphere of the host plant in a mutualistic relationship. The host plant provides soluble carbon to fungi, and the fungi provides the host plant with water and nutrients by its enhanced absorption through its hyphal network. There are two types of mycorrhizal fungi ectomycorrhizal and endomycorrhizal. The arbuscular mycorrhizal fungi are endomycorrhizal fungi (Donnelly and Entry 1999). They were originally called as the endotrophic mycorrhizae and are the most common of the mycorrhizae types. AMF may be beneficial for PAH rhizodegradation by the way of enhancing root

exudation and root associated microbial populations because of their tremendous capacity of extending their fungal hyphae to reach out to the soil environment beyond the capacity of roots alone (Hesham et al. 2021, Singh and Yadav 2020). AMF can help remediate metal toxicity to plants by reducing metal translocation from root to shoot (Leyval et al. 2002). The mycorrhizal fungi mainly act by enhancing the phytoremediation, phytostabilization, phytoextraction and rhizofiltration in its host plant and also help in bioremediation by the mechanisms of biosorption, biostimulation and hyper-accumulation (Yadav et al. 2020c, d).

2.4 Factors Affecting the Efficacy of Bioremediation by Fungi

Although fungi are less sensitive to extreme conditions, the substrates they act upon are affected by various factors, and hence, the effectiveness of a bioremediation process is ultimately affected. They may be environmental factors or abiotic/environmental factors, chemical factors and biotic factors (Fig. 2.3).

2.4.1 Abiotic/Environmental Factors

Environmental factors such as the pH of soil or soil reaction, soil moisture condition, temperature and aeration affect the efficacy of bioremediation. Studies have established that pH is a major deciding factor in bioremediation efficiency of PAHs and heavy metals (Kumar et al., 2020c; Liu et al. 2017). Apart from this, microorganisms are also affected by pH as each species operate optimally at a particular pH. Therefore, the enzyme activities which are secreted by fungi are affected by PAHs and heavy metals as they can alter the pH of the soil environment as well as the oxygen condition and other environmental elements (Brito et al. 2015; Liu et al. 2017). pH also governs the redox and solubility of heavy metals (Liu et al. 2017). The variable valence states and forms of heavy metals sometimes have a reverse effect on microbes where they become toxic to the microbe, which influence their remediation efficiency to heavy metals ultimately. Highly acidic or alkaline conditions may sometimes inhibit the activities of the microbes where they cannot degrade the complex organic compounds and heavy metals, especially those microbes which grow in situ. Therefore, it is recommended to adjust the pH of the affected site first (Bamforth and Singleton 2005).

Availability and forms of contaminants directly influence available soluble materials, osmotic potential and pH, which control the rate of contaminants' metabolism through soil moisture. Soil moisture also governs the aeration, which affects the activity of the enzymes and also the redox state of the heavy metals, thereby affecting the bioremediation process (Yadav et al. 2020a; Yadav et al. 2020b). An

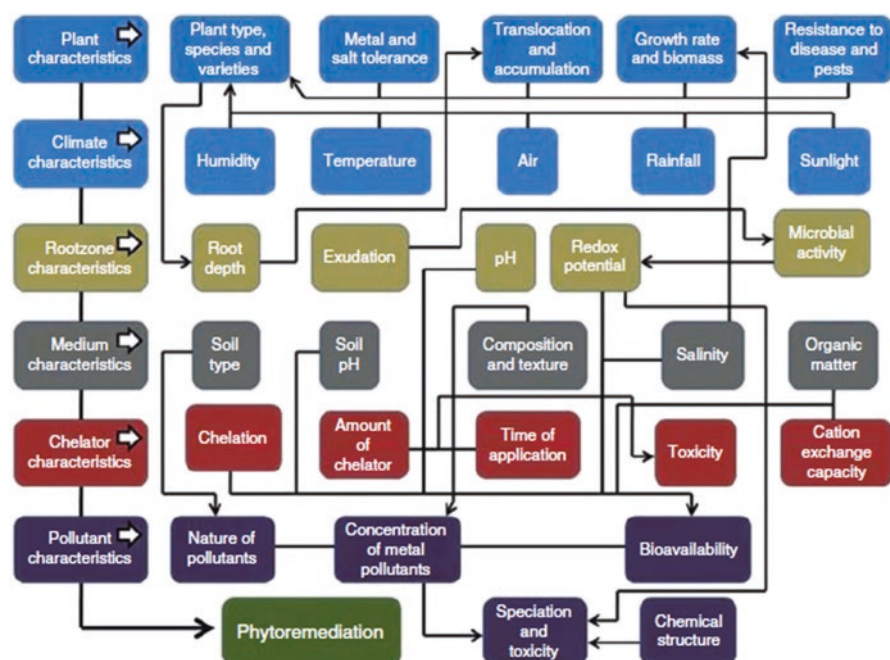


Fig. 2.3 Relationships among the factors affecting phytoremediation efficiency. (Adapted with permission from Saxena et al. (2019))

optimum moisture condition is always desirable for maximum result. Moisture also governs the temperature and vice versa.

Bioremediation system of heavy metals and complex organic contaminants are widely affected by temperature changes. With rise of temperature, the solubility of PAHs and heavy metals increases, thereby increasing the bioavailability of these contaminants (Bandowe et al. 2014). Also, the rise in temperature accelerates the activities of microbes up to a certain range because microbial metabolism and enzyme activity are enhanced with increasing temperature, accelerating the bioremediation process of contaminants. Temperature changes also govern the processes of adsorption and desorption of the toxic compounds/heavy metals on microorganisms or particle surface. The adsorption capacity and adsorption intensity increase with the rise in temperature (Chung et al. 2007). The temperature fluctuation may sometimes lead to the increased competition between the heavy metals and PAHs for the adsorption site, as at a particular temperature, the solubility of the organic contaminants and heavy metals is different and, hence, the affinity of one may be higher than the other for non-specific sites. It has been reported that the co-existing PAHs may promote heavy metals adsorption because of the pollutants' capacity to redistribute between weakly bound and strongly bound fractions (Chen et al. 2015; Liu et al. 2017).

2.4.2 Chemical Factors

Microorganisms need various nutrients (carbon, nitrogen and phosphorus) to survive and continue their microbial activities. Adequacy of nutrients increases the metabolic activity of microorganisms and ultimately the biodegradation rate. On the other hand, the higher concentration of some metal ions may retard the metabolism of the microbes. Soils have an abundance of low molecular weight organic acids and humic acids, and the amount varies according to soil type. These are released from the decomposition and degradation of organic matters. These compounds interact with the complex organic molecules and heavy metals by various mechanisms and govern the bioremediation fate of these contaminants. The migration, transformation and bioavailability of heavy metals are affected or hindered by ion exchange, surface adsorption and coordinate complexation (Qin et al. 2004; Wu et al. 2003). Major functional groups such as amino and sulfhydryl of humic acids, carboxylic, phenolic and quinine serve as ligands for heavy metals and form compounds (Wang et al. 2009).

The strength of bond depends upon the electrostatic interactions and other proton competition (Benedetti et al. 1995; Wang and Chen 2009). Low molecular weight organic acids and humic acids improve the mobility of PAHs and their bioavailability to microbes by decreasing the adsorption onto soil particles by directly competing with adsorption sites or binding with the minerals and indirectly facilitate the release of bound PAH residues in soil particles and enhance the degradation rate of PAHs by microbes. Addition of low molecular weight organic acid at the rate of 10–100 mmol/kg increases the concentration PAH in soils by 54–75% after 40 days of treatment compared to untreated groups (Gao et al. 2015; Liu et al. 2017).

Another chemical factor is the matrix effect (Liu et al. 2017) which refers to the impact of structure, concentration, solubility and adsorption of contaminants on their bioavailability. This matrix effect can be modified using surfactants. Surfactants have been widely used in PAHs and heavy metals restoration. Surfactants may act by the way of augmenting membrane fluidity and thereby promoting transmembrane transport of the contaminant into microbial cells. Li et al. (2015) have reported that the addition of sodium dodecyl benzenesulfonate (SDBS) could increase the D9 fatty acid desaturase level and in result increases unsaturated fatty acid content and enhances transmembrane transport. The surfactants may also act by enhancing microbial cell surface hydrophobicity and intracellular degradation for PAHs by regulating genes such as 1H2Nase and RHDase. Biosurfactants act as microbial surfactants (amphiphilic compounds) excreted by extracellularly hydrophobic and hydrophilic moieties. These moieties give ability to microbes to accumulate between fluid phases, thus reducing surface and interfacial tension. It has been reported that *Candida sphaerica* released surfactant could remove 95%, 90% and 79% of Fe, Zn and Pb, respectively (Luna et al. 2016). Surfactants can form complexes with metal ions and can reduce surface properties by decreasing interfacial tension and fluid forces, deflating the adhesion between metal and soils (Hu et al. 2011; Liu et al. 2017).

2.4.3 Biotic Factors

The biotic factors include the species of microbes, screening conditions and genes of organisms the microbes. The microbial communities vary in their capacity to reproduce and may compete for substrates in some environments. Sometimes excessive amount of contaminants lead to the formation of new microbial communities in order to adapt to the hazardous environment (Singh et al. 2020; Yadav 2020). The strains isolated from such environments are reported to show the high capacity to remediate the contaminants. These also have to compete with the indigenous strains of that environment which again affects the efficacy (Momose et al. 2008). The strains with genes that have high capacity to degenerate the contaminants are being studied, and the resistant strains are being developed by genetic modification of existing strains.

2.5 Process of Implementing Fungal Bioremediation

The preparation of the fungal agent to be used as bioremediation is a four-phase process (Lamar and White 2001). It includes bench-scale treatability, on-site pilot testing, production of inoculum and finally full-scale application.

The first process starts with treating a large amount of substrate which are mostly nutrient-rich, such as wood chips, peat, sawdust, corn cobs, bark, rice and wheat straw, alfalfa, spent mushroom compost, sugarcane bagasse, coffee and sugar beet pulp and cyclodextrins. The substrate may also be biofortified with nutrients. Biosurfactants produced from fungi may also be used as inoculum, both in situ and ex situ. Obtaining the perfect C:N ratio is most important for proper multiplication of the fungi. After treating the substrate, fungal inoculum is obtained. Encapsulation of fungal inoculum with alginate, gelatin, agarose, carrageenan, chitosan, etc. in the form of pellets offers a better efficacy than with inoculum produced using bulk substrates. The success of inoculum depends upon the accuracy of the first phase. In the second phase, it is confirmed in small experimental sites. If the result is successful, the inoculum is bulk produced in stage three to be released for final large scale application. Success in stages three and four are affected by the factors mentioned above in this chapter. Especially the native communities give a great competition to these inoculated communities.

2.6 Limitations of Using Fungi for Bioremediation

Many fungal strains have been identified and tested for biodegradation and bioremediation of different chemical contaminants may it be complex organic and inorganic compounds or heavy metals. But there are several limitations that hinder the

wide application of fungi in this field. It has been reported that the bioremediation by fungi is a slow process and sometimes it leaves the process incomplete (Sasek and Cajthaml 2005). Fungi require more time to adapt into a newer environment, hence, the process of initiation of bioremediation. Soil is a very much heterogeneous system, and sometimes this fact results in a limitation for proliferation of the inoculum in field conditions. Similarly, accessibility and bioavailability of the pollutants also serve as a limitation in bioremediation including fungal mediated bioremediation of pesticides (Kumar et al. 2017b). Sometimes the partial degradation of some organic or inorganic compound may leave secondary metabolites behind, which may be toxic in nature. It has been reported that, sometimes, these secondary metabolites have been found to be even more harmful as compared to their parent compounds (Boopathy 2000; Kumar et al. 2017b). There are very few fungi that can mineralize pesticides. Most importantly, there are very less information available about the fate of the metabolites resulted from fungal degradation (Sharma et al. 2021; Yadav 2021).

Bioremediation is not useful for treatment of all kinds of organic compound; in fact, it is limited to a very few groups of aromatic compounds. There are also some limitations that are realized while testing the inoculum and implementing it at field level. Highly permeable soils are important for the in situ application (Kumar et al. 2018). The demand of a clean site makes the performance evaluations difficult because there is not a defined level and therefore performance criteria regulations are uncertain. The metabolites remaining after treatment may be mobilized to groundwater if not controlled. In ex situ process of application, controlling of volatile organic compounds may be tough.

2.7 Conclusion and Future Perspectives

Bioremediation is an adaptable, eco-friendly treatment approach and a fast-growing field of environmental restoration so that it disintegrates or detoxifies environmental pollutants into less toxic forms. It is based on the idea that different organisms will work together to remove the waste substances or pollutants from the environment. Fungi have greater potential by virtue of their growth, biomass production, and extensive hyphal reach in soil. The unique biochemical properties of fungi elucidate its importance to transfer hazardous substances to non-hazardous and are highly in demand to translate powerful ecosystem services. Overall, this chapter discusses species of the fungi and pollutants, briefs the factors affecting the bioremediation efficiency and depicts the remediation mechanisms of the potent contaminants in soil environment by fungi. In order to promote bioremediation efficiency fungi, future studies should concentrate on enhancing competitiveness of dominant strains improving bioavailability of pollutants, and exploring novel technologies to increase detoxification of microbes.

The future researches should focus on alleviating the existing limitations. The fact that all the sites are different from each other and have wide variations

possesses limitation on application of fungi. Hence, the remediation must be curtailed to a site-specific approach by understanding the mechanisms involved in regulating the behaviour of fungi under a certain environmental condition. The problem of competition by indigenous strains may be overcome by the use of microbial combination including both fungal and bacterial strains and also by genetic modifications. In genetic modification, there should be added a specific gene that can render specific degradation capability to indigenous microorganism.

Bioremediation is very effective in small quantities as small quantities of substrates often decompose quickly. But for large scale implementation, the rapid multiplication ability of bacteria should be explored. More researches should be done towards co-culturing the two microbes for a much effective strain. Also, rigorous searches are to be done to identify the genus that can mineralize pesticides. Simulation models can be developed including the nature of chemical, fungi, environment and the products of degradation which can help understand the fate of a biodegradation process. The understanding of microbial diversity of the contaminated site is important to develop broad insight about potential agents of degradation as well as comprehending their genetics and biochemistry which will fasten the process of evolving suitable bioremediation approaches.

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Chapter 3

White-Rot Fungi for Bioremediation of Polychlorinated Biphenyl Contaminated Soil



Prem Chandra, Enespa, and Devendra Pratap Singh

3.1 Introduction

Polychlorinated biphenyls (PCBs) have been used for several industrial applications due to their thermal and chemical stability, flame resistance, and dielectric properties and are known as xenobiotic compounds (Dhakal et al. 2018; Brown 1987; Ulbrich and Stahlmann 2004). PCB contamination is still widespread in all types of ecosystems and affects both natural environments and wildlife due to their inertness (Markowitz and Rosner 2018; James et al. 1993). Subsequently, due to the teratogenicity and carcinogenic and endocrine-disrupting features of these xenobiotics, the cleanup of PCB-contaminated sites has become a priority of great relevance (Reddy et al. 2019; Stella et al. 2017). Extensively in numerous industrialized applications until the mid-1970s, polychlorinated biphenyls (PCBs) were used (Schantz et al. 2001). Due to their inertness and insulating properties (Gakuba et al. 2015), polychlorinated biphenyls (PCBs) include 209 organic chemicals widely used since the 1920s in industrial application. Their persistency, bioaccumulation properties, toxicity, and increasing concern about their environmental risks led to their prohibition in the early 1980s by several countries' governments (Matthies et al. 2016). PCBs can be reemitted by reservoir compartments such as contaminated soils, whose reclamation represents therefore a primary issue for our society (Nieder et al. 2018; Kumar et al. 2019a).

Polychlorinated biphenyls (PCBs) are more toxic and carcinogenic effects categorized a synthetic semi-volatile organic compound so declare as one of the legacies persistent organic pollutants (POPs) at the Stockholm Convention in 2001 (UNEP

P. Chandra (✉) · D. P. Singh

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

Enespa

Department Plant Pathology, Sri Mahesh Prasad Degree College, University of Lucknow,
Lucknow, Uttar Pradesh, India

2001; Wania and Mackay 1996; Jones and De Voogt 1999). The physical-chemical properties of PCBs such as the long half-life of 60 days to 27 years in water and 3–38 years in sediment, more affinity to organic carbon and black carbon (BC), and resistance to transformation make them a representative POP, forming an ideal marker for re-distribution globally (Wiberg and Josefsson 2009). In several developed countries concerns about their potential carcinogenic role and their magnification in the food chain resulted in a ban in the usage of PCBs (Walters et al. 2010; Chandra and Enespa 2019a). However, for several years due to lack of chemical reactivity and their low water solubility, PCBs have persisted in the environment, widespread in several former industrial sites and related terrestrial and aquatic habitats (Wagner et al. 2017). Due to the accumulation in food chain and their removal from soil and residue is a primacy of great relevance in numerous industrialized states (Thornton et al. 1996). Inevitably, they have been linked to chronic effects in humans including immune system damage, decreased pulmonary function, bronchitis, and interferences with hormones leading to cancer (Chang 2010).

Additionally, studies indicated that children will show serious developmental problems such as low birth-weight, behavioral disorders, and hearing loss at a relatively high exposure to PCBs (more than 10 pg/kg body weight per day) (Schardein et al. 1989). Literature also provides evidence of the effects of PCB exposure to animals (e.g., rats) such as liver damage, immune system suppression, abnormalities in fetal development, enzyme induction, sarcomas, non-Hodgkin lymphomas, and serum lipids (Mitrou et al. 2001; Yadav et al. 2020a, b). For remediating contaminated soil, bioremediation may be cost-competitive, eco-friendly, and an effective approach using low concentrations of mixtures of low chlorinated PCBs. On the isolation of microorganisms, extensive efforts have been focused to degrade a broad range of PCB congeners (Kuppusamy et al. 2016). Most naturally occurring microorganisms can only degrade low to moderate concentrations of lightly chlorinated PCBs. The degradation of PCBs by fungi has received much less attention in comparison to the extensive information on bacteria (Pieper 2005; Rastegari et al. 2020a, b). For the degradation of environmental pollutants, including PCBs, the white-rot fungi are the most favorable eukaryotic microorganisms. *Phanerochaete chrysosporium* are the major degraders of lignin in nature and are the best organism (Harms et al. 2011).

Lignin is a polymer of phenylpropane substructures, a derivative from phenolic precursors, and the main structural component of wood. White-rot fungi have been renowned as proficient biodegraders of several recalcitrant organo-pollutants (Janusz et al. 2017). Several ligninolytic enzyme combinations are known to confirm efficient biodegradation of recalcitrant lignin and several xenobiotic molecules (Ruiz-Deñás and Martínez 2009; Singh et al. 2020). Removal of individual PCB congeners from Aroclors 1242, 1254, and 1260 was revealed using the capability of *Phanerochaete chrysosporium* (Billingsley et al. 1997). For designing effective remediation technology, filamentous fungi display features that make them excellent candidates (D'Annibale et al. 2006). The hyphal arrangements of fungi, demarcated as “fungal highways,” allow them to simply penetrate through environmental matrices and to act as dispersion vectors of both pollutant-degrading bacteria and

pollutants (Ritz and Young 2004). An ecological subgroup of filamentous fungi denoted as “white-rot fungi” can degrade a wide range of aromatic organo-pollutants using the secretion of oxidative enzymes (Stella et al. 2017; Yadav 2020; Yadav et al. 2020c, d).

In the extracellular environment, the nonspecific radical-based system is active, and fungi can easily enter a polluted matrix and reach even poorly bioaccessible waste products. To decompose PCBs under model laboratory-scale conditions in liquid systems, several white-rot fungi were tested for their competency (Chun et al. 2019). *Phanerochaete chrysosporium*, *Trametes versicolor*, *Lentinus edodes*, *Phlebia brevispora*, *Irpex lacteus*, *Bjerkandera adusta*, *Pycnoporus cinnabarinus*, *Phanerochaete magnoliae*, and *Pleurotus ostreatus* are the most well-known degrading strains (Cajthaml et al. 2009; Green and Clausen 1999; Chandra and Enespa 2019b; Devi et al. 2020). One or more of three extracellular enzymes are essential for lignin degradation secreted by white-rot fungi and combine with other methods to effect lignin mineralization (Leung and Stephen 2002). Two glycosylated heme-containing peroxidases, lignin peroxidase (LiP, E.C. 1.11.1.14) and Mn-dependent peroxidase (MnP, E.C. 1.11.1.13), and a copper-containing phenoloxidase, laccase (Lac, E.C. 1.10.3.2), are the three enzymes involved also known as lignin-modifying enzymes or LMEs (Vrsanska et al. 2016; Janusz et al. 2017). This chapter describes the biomineralization and bioremediation of polychlorinated biphenyl using white-rot fungi.

3.2 Site Characterization

At electric power substation sites, PCB-contaminated soils have unique characteristics, which set them at a distance from other well-known PCB-contaminated sites like lake and river sediments (Weber et al. 2018). These contaminated soils are frequently, protected with gravel, issue to harsh physical and chemical treatments like weed control, and poor nutrient (Braddock et al. 1997; Chan). For our field demonstration, the site chosen has nearly 100–300 parts per million (ppm) of weathered PCBs and was found to have insignificant regular microbial inhabitants which lacked PCB-degrading movement (Leigh et al. 2006; Sayler et al. 1978). PCBs can transform using soil microflora by either growth on specific chlorinated biphenyls, or congeners, as carbon source (Field and Sierra-Alvarez 2008) or by cometabolism (Borja et al. 2005; Chandra and Enespa 2019c). The natural growing inhabitants could not to degrade the PCBs by adding of ingredients alone or nutrients adding, so it is confirmed that these sites are not likely to go through natural re-establishment (Singer et al. 2000; Passatore et al. 2014; Enespa and Chandra 2017).

Bio-stimulation is an effective approach for PCB degradation (Kumar et al. 2019b). Although amended PCB degradation has been revealed to result from nutrient stimulation alone (Field and Sierra-Alvarez 2008), for PCB degradation the adding of biphenyl is a more important factor (Liu and Chen 2006). Further in PCB degradation, the improvement may be succeeded through bioaugmentation, the

addition of known degrading bacterial strains, in comparison to degradation resulting from stimulation of native populations (Mrozik and Piotrowska-Seget 2010). *Alcaligenes eutrophus* H850, *Pseudomonas* sp. LB400, and *Corynebacterium* sp. MBI bacterial strains determine a broad range of PCB congener attacks, including several extremely chlorinated biphenyls (Bedard and Haberl 1990). White-rot fungi which have the potential for the degradation of many PCB congeners include *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Irpex lacteus*, *Trametes versicolor*, *Lentinus edodes*, *Coriolus versicolor*, *Cyathus stercoreus*, *Heterobasidion annosum*, and *Ceriporiopsis subvermispora* (Sadañoski et al. 2018; Gao et al. 2010; Enespa and Chandra 2019).

3.3 White-Rot Fungi

Basidiomycetes are white-rot fungi that have the capability of the lignin component degrading of lignocellulose substrates. But the brown- ligninolytic enzymes Basidiomycetes are white-rot fungi that have the capability to lignin component degrading of lignocellulose substrates, and the brown-rot fungi do not produce the same ligninolytic enzymes but have the ability to degrade the same ligninolytic enzymes (Ohkuma et al. 2001; Andlar et al. 2018). White-rot fungi have a high tolerance to toxic environments and are known as robust organisms making them ideal to use for remedial purposes (Yadav et al. 2020e). It further enhances their hardy capabilities, and they can also withstand high temperatures and a wide range of pH (Ellouze and Sayadi 2016; Deshmukh et al. 2016).

Oxidative lignin breakdown depends on a panel of enzymes including:

- A heme (Fe)-containing protein, which catalyzes H_2O_2 -dependent oxidation of lignin: *lignin peroxidase*
- A heme protein which also catalyzes H_2O_2 -dependent oxidation of lignin: *manganese peroxidase*
- A copper-containing protein which catalyzes the demethylation of lignin components: *laccase*

Glyoxal oxidase is extracellular peroxide-generating enzymes are the key enzymes, have important function in the breakdown of lignin and produce veratryl alcohol (Ruiz-Dueñas and Martínez 2009; Pollegioni et al. 2015).

In the catabolic lignin degradation processes, the following are involved:

- (a) Between monomers cleavage of ether bonds
- (b) Propane side-chain oxidative cleavage
- (c) Demethylation
- (d) Benzene ring cleavage to ketoadipic acid which is fed into the tricarboxylic acid cycle as a fatty acid

On white-rot basidiomycete fungi, such as *Phanerochaete chrysosporium* (*Sporotrichum pulverulentum*), mostly research has been determined (Raeder and

Broda 1984; Wymelenberg et al. 2010). This has the capabilities to mineralize lignin to CO_2 and water completely. After cessation of primary growth, the lignin-degradative system of *Phanerochaete chrysosporium* appears prompted by nitrogen starvation (Leisola et al. 1982). Genes encoding laccases, lignin peroxidases, and manganese peroxidases are the genome of white-rot fungi structure families. In fungal physiology (e.g., laccases contribute to plant pathogenesis, sporulation, and pigment formation), the diversity of these extracellular enzymes may be a result of their having multiple roles but could also be a response to the diversity of the lignin substrate (Janusz et al. 2017; Hakala et al. 2005).

3.3.1 Advantages of White-Rot Fungi

The technology of white-rot fungi is very different approaches of bioremediation. For the pollutant degradation with several benefits the apparatuses used by the fungi afford them (Gao et al. 2010). The lignin-degrading system has progressed to degrade insoluble chemicals like lignin and several hazardous ecological pollutants at significantly more concentrations (Yang et al. 2013; Chandra et al. 2020a, b). The bacterial intracellular apparatus is poorly accessible to the pollutants, and their uptake may prevent growth. The lignin-degrading system is free radicals-based in nature and nonspecific, is non-stereoselective, and agrees with WRF to degrade a wide variability as well as compound mixtures of contaminants (Bilal et al. 2019; Kamei et al. 2006). Non-detectable level or very low concentrations of pollutants can efficiently be degraded by WRF. WRF can be cultured such as wheat straw, corncobs, wood chips, or another crop residue on economic growth substrates that stimulate the usage of WRF for bioremediation (Lechner and Papinutti 2006; De Wilde et al. 2007). The fungi also produce hydroxyl radicals such as OH, in addition to being capable to cultivate under nutrient control, which can oxidizing biomolecules, like proteins and DNA that could result in the death of other microorganisms (Phaniendra et al. 2015; Chandra et al. 2020c). The fungus is capable to modify the pH of its adjacent environment using the plasma membrane-dependent redox system (Morth et al. 2011; Husson 2013).

3.4 Lignin Peroxidase (Ligninase) Mechanism of White-Rot Fungi

3.4.1 Direct Oxidation

Peroxidase catalytic mechanism are found in lignin peroxidase (LiP) classical peroxidase catalytic mechanisms are found, HCO oxidized native enzyme and two electron-deficient compound generated (Houtman et al. 2018). Compound-I

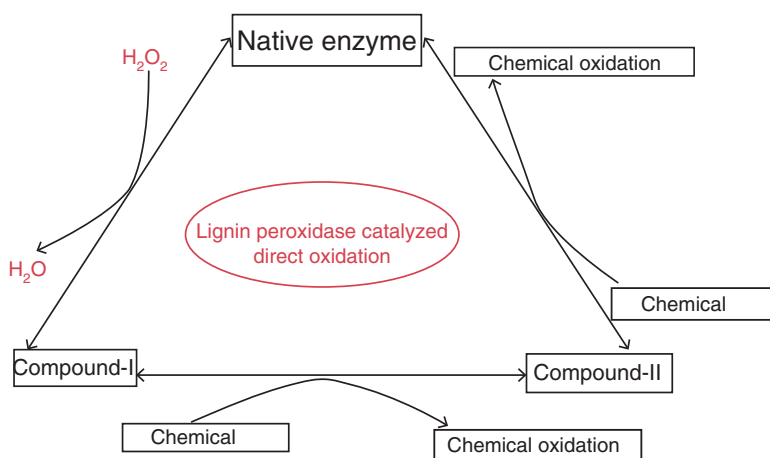


Fig. 3.1 Mechanism of direct oxidation by lignin peroxidase

chemically oxidized and reduce to one electron-deficient compound-II, A subsequent oxidation of another molecule by compound II proceeds the peroxidase to its native latent stage (Fig. 3.1). LiP has relatively high redox potential, so the chemicals with high redox potentials that are not oxidized by other enzymes are oxidized by LiP (Wong 2009; Hammel and Cullen 2008).

Both phenolic and non-phenolic compounds can be oxidized by LiP subsequent to carbon-carbon bond cleavage, demethoxylation, methylation, hydroxylation, phenolic oxidation, aromatic ring fission, and dimerization reactions same as with lignin (de Gonzalo et al. 2016). Lignin peroxidase are strongly oxidative and involves extracellular glycosylated heme proteins of the *Phanerochaete chrysosporium* (Dashtban et al. 2010; Vasina et al. 2017). H_2O_2 production increased in low-nitrogen medium and secretes lignin peroxidases which compares with the arrival of ligninolytic activity; by adding the enzyme catalase powerfully inhibits the breakdown of lignin in experimental destruction of H_2O_2 (Forney et al. 1982). The enzyme is stimulated by itself being oxidized by H_2O_2 , the preliminary step connecting oxidation by one electron to create an unstable intermediate that can catalyze the oxidation of phenols, aromatic amines, aromatic ethers, and polycyclic aromatic hydrocarbons (Guengerich and Yoshimoto 2018; Chan et al. 2020). Motivated oxygen derived from H_2O_2 is complicated in degrading lignin but is held in the active site of a specific extracellular enzyme, the lignin peroxidase (Falade et al. 2017). The lignin degradation enzyme secreted from *P. chrysosporium* and produced veratryl alcohol (Fig. 3.2) (Schlosser and Höfer 2002). Veratryl alcohol oxidized to a cation radical is an excellent substrate for LiP which can oxidize other chemicals that are not oxidized directly by LiP (Romero et al. 2019).

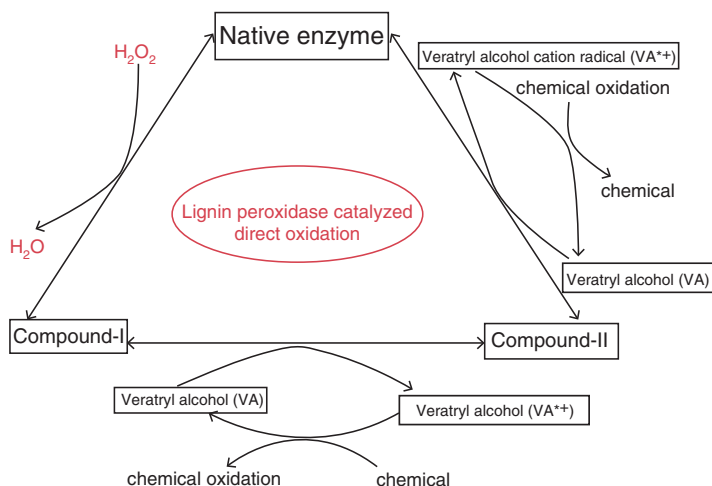


Fig. 3.2 Lignin peroxidase catalyzed indirect oxidation

Most of the white-rot fungi also produced manganese-dependent peroxidases that are another family of extracellular glycosylated heme proteins. The mechanism is very different in lignin peroxidase and manganese peroxidases, but H_2O_2 requires functioning (Dashtban et al. 2010; Bonnarne and Jeffries 1990). Low-molecular-weight oxidizing agents generated by the manganese peroxidase system diffuse into the lignin substrate and are capable to oxidize phenolic filtrates in the lignin (Xu et al. 2017).

The H_2O_2 uses enzyme to oxidize extracellular Mn (II) to Mn (III), and this becomes the diffusible oxidant that can reduce lignin at a distance. The metal ion manganese is the chief one from which the enzyme gets its name (Glenn et al. 1986). Glyoxal oxidase an extracellular enzyme generated H_2O_2 which is required by peroxidases that transfer electrons from aldehydes of low-molecular-weight (e.g., glyoxal and glycolaldehyde) to O_2 and so form H_2O_2 (Daou and Faulds 2017). An oxalate oxidase secreted by *Ceriporiopsis subvermispore* and the degradation of oxalate to carbon dioxide and H_2O_2 catalyzed by that and in this organism for manganese peroxidase could be the main provider of H_2O_2 (Urzúa et al. 1998; Grąz et al. 2016). Ligninolytic extracellular enzyme laccase is produced by few fungi in a wider range and also produced by non-ligninolytic members of Ascomycota, such as *Aspergillus* and *Neurospora*, as well as wood-rotting Basidiomycota (Liers et al. 2007; Kersten and Cullen 2007). Laccases are one of the most primitive revealed enzymes and have a large number of biotechnological applications ranging from the degradation of xenobiotics to biosensors and food preservation, also known as polyphenol oxidases (classified as EC 1.10.3.2) (Kunamneni et al. 2007; Rodríguez-Couto 2019; Gupta et al. 2010; Kour et al. 2019).

3.5 Oxidized Pollutant Degradation

White-rot fungi degraded several pollutants to CO₂, e.g., polychlorinated bi-phenyls, nitroaromatic munitions, and chlorinated phenols. The highly oxidized state of these chemicals makes them very resistant to secondary forms of microbial degradation (Furukawa 2000). The rates and amounts of degradation were substantially slow when the fungus was cultured with adequate nutrients (Boonchan et al. 2000). It was established that the LiP was involved in the degradation of these pollutants (Barr and Aust 1994). *P. chrysosporium* reduced 1, 1- bis (4-chlorophenyl) -2, 2, 2-trichloroethane (DDT) in to 1,1-bis (4-chlorophenyl) - 2, 2-dichloroethane (DDD) was (Purnomo et al. 2019; Miskus et al. 1965). In cultures of *P. chrysosporium*, the explosive 2, 4, 6-tri-nitrotoluene (TNT) of amino congeners were also determined. Another example of a highly oxidized pollutant that is degraded to CO₂ by white-rot fungi is Pentachlorophenol (PCP). So, the fungus performs several specific mechanisms for reducing these chemicals (Aust 1990; Tuomela et al. 1998).

3.6 Polychlorinated Biphenyl (PCB) Compounds and Their Mode of Actions

A large group of chlorinated biphenyls with 209 possible congeners denotes the term polychlorinated biphenyls (PCBs). Aroclor, Fenclor, Kanechlor, and Phenclor, among others, are the commercial names composed of complex mixtures containing 60–90 congeners (Jacobson and Jacobson 1996; Ross 2004). Commercial preparations of Aroclor are specified with a four-digit code that was produced in the United States. The first two numbers in the code refer to the parent structure (12 indicating biphenyl), and the second two digits refer to the weight percentage of chlorine (Tebo et al. 2010). For example, Aroclor 1242, 1248, 1254, and 1260 refer to PCB mixtures with an average weight percentage of chlorine of 42, 48, 54, and 60%, correspondingly (Mayes et al. 1998; Frame et al. 2001). Several observations have employed single congeners, which will be abbreviated as CBp, DCBp, TCBp, TeCBp, PeCBp, HCBp, HeCBp, OCBp, and NCBp for mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, and nonachlorobiphenyl, respectively (Field and Sierra-Alvarez 2007). PCBs released into the environment were several hundred million kilograms. The manufacturing and use from 1929 to 1978 as transformer fluids, hydraulic fluids, and other industrial products are the main source of PCB into the environment (Markowitz and Rosner 2018). PCBs tend to become absorbed by natural organic matter in soil, sediments, and sludges due to their hydrophobic properties. PCBs released into the aquatic environment majority, so partitioned into aquatic sediments (Tehrani and Van Aken 2014; Marvin et al. 2011).

3.6.1 *Mode of Actions*

PCBs exhibit a wide range of mechanisms of action that depend on the chlorine substitution pattern in the molecule. Due to the presence or absence of chlorine molecules on the ortho (2, 2', 6, 6') positions, the most conspicuous difference in the mode of action is found (Dudasova et al. 2017; Ohta et al. 2015). Those PCBs that do not hold ortho chlorines and have two pairs of neighboring chlorines on the meta- and para-positions can have high-affinity binding to the Ah receptor (e.g., PCBs 77, 126, and 169) (Pessah et al. 2019; Grimm et al. 2015). A remarkable similarity was found in both the biochemical and the toxicological modes of action to those of the 2, 3, 7, 8-substituted chlorinated dioxins (PCDDs) and dibenzofurans (PCDFs) (Schrenk and Chopra 2017). The possible planar configuration of the PCB molecule becomes increasingly more difficult with an increasing number of ortho chlorines (Alegbeleye et al. 2017). Consequently, the binding attraction of these (multiple) ortho PCBs to the Ah receptor declines radically, and so do the associated mechanisms of action. In this group of ortho-substituted congeners, only some mono-ortho-substituted PCBs (e.g., PCBs 105 and 118) display several binding to the Ah receptor, which effects in dioxin-like toxicity and biochemical effects (Balouiri et al. 2016; Petrosino et al. 2018; Herzig et al. 2019). Due to the absence of appropriate binding to the Ah receptor, PCB congeners that hold two or more ortho chlorines (e.g., PCB 153) do not display any substantial dioxin-like toxicity (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al. 2018). However, multiple-ortho-substituted PCBs do have other prominent mechanisms of action, counting effects on neurological progress, dopamine levels, and tumor promotion (Khezri et al. 2017; Gupta et al. 2018). The more meticulous properties of multiple-ortho PCBs are seen at substantially higher dose levels than the distinct dioxin-like effects that are accompanying with some non- and mono-ortho PCBs that are potent Ah receptor agonists. Feasibly the greatest distinctive alteration among several groups of PCB congeners is found in the way they induce cytochrome P450 enzymes (Qian et al. 2015; Gadupudi et al. 2016).

Moreover, the Ah receptor activation leads to changes in gene expression and signal transduction, prompting fluctuations in cell proliferation and differentiation, bodyweight gain inhibition, and thymic atrophy (Yan et al. 2017). But, to the Ah receptor mechanism of toxicity, a substitute includes an augmented endotoxin sensitivity and boosted liability treated with Aroclor 1242 to a malarial parasite in mice, signifying that the immunosuppressive effect may have been initiated by a PCB obstruction of the hepatic, splenic, and thymic constituents of the reticuloendothelial system (Gutiérrez-Vázquez and Quintana 2018). Endocrine disruptors and estrogen-like PCB congeners bind to estrogenic receptors. Subtle endocrine disturbances are caused by these PCBs and unfavorably affect the performance of the reproductive system (Pinson et al. 2016). Gonadotropin-releasing hormone increases by certain PCB congeners or harvest effects outside the receptor for gonadotropin-liberating hormone. The production and release of luteinizing hormone from the pituitary may also affect the PCB mechanisms unconnected to estrogenic action

(Nadal et al. 2017; Gore et al. 2015). The promising endocrine-disrupting role of assured hydroxylated and methyl sulfone PCB metabolites is of special toxicological interest besides the biological and toxicological effects of the parent compounds (Awad et al. 2016; Grimm et al. 2015). The retinol-binding protein as transthyretin also forms a complex, and the impact of PCBs on vitamin A can also be described (Berntssen et al. 2016).

3.7 Biodegradation of Polychlorinated Biphenyls

3.7.1 Biodegradation in the Environment

For anaerobic biotransformation of PCB in aquatic sediments, there are multiple lines of evidence. Evidence from Canada, Japan, the Netherlands, and the United States at 16 sites for the anaerobic natural attenuation of PCB were reviewed (Atashgahi et al. 2016). In dated sediment layers PCB concentrations were revealed to be considerably diminished when compared with archived sediments (Pavlova et al. 2016). From various historically polluted sites, sediment samples exhibit congener patterns with a smaller amount of chlorine substitution than the unusual polluting profitable PCB mixture (Bertrand et al. 2015). For the in situ anaerobic biotransformations of PCBs, the most fascinating substantiation was obtained from sediment core samples in Lake Hartwell (South Carolina, USA) in which the focus of para- and meta-chlorines was exposed to decline with age of the sediment layer, whereas resistant ortho-chlorines were exposed to be constant with the depth of core (Chen et al. 2017). Consequently, in aquatic sediments, the reductive dechlorination completed strong evidence provides in these results. Consequently, the reductive dechlorination completed strong evidence in aquatic sediments, provides in these results. On the detection of characteristic metabolites in sediment cores, the aerobic degradation of PCB has been also detected in the field example like chlorinated benzoates and 2,3-dihydro-2,3-dihydroxy-20-chlorobiphenyl and 2,3-dihydroxy-20-chlorobiphenyl (Long et al. 2015).

3.7.2 Biodegradation of PCB in Engineered Systems

To promote the biodegradation of PCB in engineered systems, the anaerobic and aerobic methods have been utilized. To supply electron-donating substrates to facilitate reductive dechlorination is the main method toward the anaerobic bioremediation of PCB (Maier and Gentry 2015; Rana et al. 2019). Sediments of Hudson River spiked with Aroclor 1242 were cured through or without the electron-donating

substrates (Onuska 2017). It is also found that no dechlorination arose if the electron-donating substrate was left out. In a similar study, a mixture of volatile fatty acids (VFA) was tested as an electron-donating substrate to stimulate the dechlorination of PCB in the Hudson River sediments spiked with Aroclor 1242 (Xu et al. 2018).

VFA stimulated the initial rate of dechlorination which assists 65% elimination of para- and meta-chlorines within 8 weeks, whereas without added VFA 11 months were required as the final extent of dechlorination to reach the same (Yoshikawa et al. 2017; Marco-Urrea et al. 2015). In an anaerobic batch reactor using landfill leachate as the electron donor, the reductive dechlorination of Aroclor 1248-spiked sediments was promoted (Matturro et al. 2016). Up to 23% of the chlorine/biphenyl ratio was decreased in 7 weeks. Reductive dechlorination of anaerobic sediments spiked with Aroclor 1248 enhanced biosurfactant additions (Vergani et al. 2017). The development was ascribed to a better bioavailability of the PCB. In a constantly fed anaerobic reactor, the anaerobic dechlorination of Aroclor 1242 has also been reconnoitered (Kaya et al. 2018).

In the anaerobic bioreactor, the microbial consortium had higher activity toward the dechlorination of 2, 3, 4-TCBp to 2, 4-DCBp with ethanol and formate as electron donors associated to pyruvate (Lu et al. 2019; Dąbrowska and Rosińska 2016). The key approach in the direction of promoting the PCB aerobic degradation has been through the addition of oxygen, co-substrates, surfactants, inducers, and bio-augmentation of PCB-degrading bacteria in selected cases (Long et al. 2015; Song et al. 2015). To arouse the aerobic degradation of PCB-contaminated soil and sediments, biphenyl which is an important primary substrate supporting PCB co-metabolism has successfully been utilized (Zhang et al. 2015; Singh and Yadav 2020). A PCB-degrading *Acinetobacter* strain and the impact of adding biphenyl to soil on the mineralization of [14C] Aroclor 1242 have been analyzed (Hu et al. 2015). Biphenyl also enhanced mineralization of the without-bioaugmented treatments, enabling up to 20% mineralization by the natural soil microflora (Hanney and Semple 2015; Pino et al. 2016).

3.8 Microbiology and Biochemistry of PCB Biodegradation

In aerobic and anaerobic metabolism, PCBs are a theme to both. Lower chlorinated PCB congeners can be co-metabolized as well as serve as growth supporting substrates under aerobic conditions (Matturro et al. 2016; Sun et al. 2016). The greater PCB congeners are subject to reductive dechlorination provided that electron-donating substrates are available in under anaerobic conditions (Zanaroli et al. 2015; Sohn and Häggblom 2016).

3.8.1 Aerobic Fungal Co-Metabolism

In various white-rot fungi, PCBs are metabolized. White-rot fungi decrease with increasing chlorine number with the extent of PCB mineralization as a general trend. With values ranging from 11 to 16%, radiolabeled tri-, di-, and mono-chlorinated biphenyls are mineralized to 14CO_2 (Huang et al. 2018; Stella et al. 2017). The substantiation for their mineralization is not very conclusive; hexa- and tetrachlorobiphenyls were less extensively mineralized for 0.4–1.4% demonstrating subsequently radiolabeled contaminations could also have accounted for the mineralization (Uekusa et al. 2017; Enespa et al. 2020b). For instance, PCB concentrations of Aroclor 1242, 1254, and 1260 decreased using *Phanerochaete chrysosporium* (42, 54, and 60% chlorinated) by 60.9, 30.5, and 17.6%, respectively (Siebielska and Sidelko 2015; Astoviza et al. 2016). *Pleurotus ostreatus* remediate Delor 103 to lower chlorine, number congeners of were degraded more expansively compared to those of higher chlorine number (Gąsecka et al. 2015). Two types of metabolites, 4-chlorobenzoic acid and 4-chlorobenzyl alcohol, have been determined during the degradation of 4, 40-dichlorobiphenyl using *P. chrysosporium* fungi (León-Santisteban and Rodríguez-Vázquez 2017). *Phlebia brevispora* white-rot fungus tarnished TeCBp, PeCBp, and HCBp congeners to methoxylated intermediates and para-dechlorinated methoxylated intermediates (Covino et al. 2016; Li et al. 2018). The filamentous fungus *Aspergillus niger* degraded a PCB technical mixture (Chlophen A) and is also reported (Al-Hawash et al. 2019; Enespa et al. 2020a). Only the mixture with the lowest total chlorine content was biodegradably tested from the three Chlophen mixtures (A30, A50, and A60 of 42, 54, and 60% chlorine content, respectively) (Marco-Urrea et al. 2015; Jesus et al. 2016; Smulek et al. 2019).

3.9 Conclusion

The continually growing worldwide hazardous waste problem must be dealt with by the present as well as future generations. Past production and improper disposal of large capacities of environmentally persistent and toxic chemicals by both the government and the private sector has generated very legitimate public health concerns. Widespread contamination of soils, as well as groundwater and surface water, has brought this problem to the forefront. The cleanup of environmental contamination presents a serious economic burden on society. For the bioremediation of contaminated soils, the potential use and application of WRF is an eco-friendly biotechnology which has been industrialized over several centuries. Extracellular enzymes of WRF can degrade ecological contaminants like pesticides, dyes, explosives, and antibiotics, among others. White-rot fungi are the most significant feature of pollutant degradation that occurs extracellularly. The species of WRF generate very potent oxidizing species (i.e., the veratryl alcohol cation radical and the $\cdot\text{OH}$) in the

lignin-degrading system which would be fairly toxic if produced inside the cell. Also, toxic pollutants need not be internalized for metabolism, providing another advantage.

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Chapter 4

Fungal Secondary Metabolites for Bioremediation of Hazardous Heavy Metals



Archana Singh, Rekha Kumari, and Ajar Nath Yadav

4.1 Introduction

Widespread population explosion as well as increasing urbanization in the developing countries have imposed a serious threat to the environment which in turn has resulted in its large-scale degradation (Singh et al. 2020a). Similarly, industrialization and technologically advanced agricultural practices and day-to-day activities are deliberately or accidentally releasing potentially toxic chemicals into the environment (Kour et al. 2020). These released chemicals can easily be transported via atmosphere and water and in several cases deposited into sediments and soils. Various chemicals were categorized on the basis of their level of toxicity to the environment such as heavy metals, metalloids, and radionuclides, agricultural chemicals, petroleum hydrocarbons, industrial sources-based halogenated solvents, endocrine-disrupting agents and drugs, and explosives (Harms et al. 2011; Kumar et al. 2019b). Applications of various microbial catalysts are required for the biological degradation and detoxification of the toxic chemicals released into the environment. However, in heterogeneous environments due to the escape of many organic and inorganic chemicals from the aqueous microhabitats of degrader organisms, this microbial degrading tendency is impeded by the tendency to escape. Also, the process of chemical precipitation, its surface adsorption, as well as its accumulation in organic matter and in tiny pores of solid matrices cause a decline in their bioavailability (Semple et al. 2004; Yadav et al. 2020c, d). Such accumulation often predominates in hostile, toxic environments that are nutrient-free, and the presence of

A. Singh (✉) · R. Kumari
Department of Bio-Engineering, Birla Institute of Technology, Mesra,
Ranchi, Jharkhand, India

A. N. Yadav
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture,
Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

water or appropriate electron acceptors would be required to support microbial growth with the ability of remediation (Harms et al. 2011).

To combat the accumulation of the abovementioned contaminating chemicals, various decontaminating approaches have been explored and termed as the ideal decontamination machinery due to their ability to explore the contaminated environment thoroughly and could easily track chemicals present within the pores and organic matter. Also, the availability of the pollutants as substrates is not essential to induce the catabolic potential of this machinery but can be easily maintained by the presence of other similar compounds or their trophic interactions with plants instead. The active presence of such machinery is essential in a wide array of extreme (dry, toxic, or acidic) environments with the inherent capacity to transfer catabolically active organisms to contaminated spots followed by the transfer of water, electron acceptors, as well as essential nutrients.

Nowadays, severity of the environment-associated threats has also been increasing due to the huge quantum of hazardous heavy metal contaminants as well as recalcitrant compounds like polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). Hence, another major concern has been associated with chemical and solid waste management. Among all, PAHs are considered as the ubiquitous contaminants due to their abundance in soils and surface or groundwaters (Aranda 2016; Kumar et al. 2019a; Rana et al. 2019b).

Textile industries are the major cause of releasing potentially hazardous compounds into the ecosystem during textile production at various stages of the operation. However, the release of these compounds is the result of the production of goods to fulfill the market demand and in an attempt to improve the human standard of living and fashion, but incongruously these goods base has also raised the production of pollutants globally. As a result, the effort to maintain the standard of living has imposed reverse negative effects on the environment due to the unplanned intrusion of hazardous pollutants into the environment. Improper treatment of the textile effluent before discharge results in its seepage into the aquifer and the underground water which in turn leads to pollution. These pollutants are universal and can affect life forms in an enormous way, since no specific boundaries could be assigned to confine them.

Sources of pollutions in the water bodies are majorly due to metallic effluents having ecological impacts. Also, nutrient load in the water bodies can be correlated with the increased amount of essential metals. Effluent enriched with these metals has a positive effect on sediment fertility and water column, whereas its presence in open waters consequently leads to eutrophication, a major cause of oxygen deficiency, algal bloom, as well as the death of aquatic life (Asamudo et al. 2005; Singh et al. 2020b; Singh and Yadav 2020).

According to the previous reports based on water pollutions, it was found that metallic effluent-based water pollution is causing several health-related threats. For instance, the presence of minute concentration of lead can severely affect nerves and brain. It often interferes with the formation of red blood cells and responsible for impaired enzyme activities. Similarly, bioaccumulation of other heavy metals

such as mercury, cadmium, and chromium and its passage through the food chain can affect the health of human beings. Apart from lead, certain locomotor disorders, numbness, convulsion, brain damage, as well as nervous problems are associated with mercury. Cadmium is the causative agent for osteomalacia and kidney tubular impairment. Earlier reports were also based on the effects of cadmium, zinc, and manganese on the regulation of ions if present in adequate concentrations (Howells 1990). Long-term effects of cadmium and manganese on recruitment of fish, spawning, and other aquatic lives are also known. These two heavy metals are reportedly affecting calcium metabolism, development, and skeletal calcification.

Along with the serious effects on living organisms, changes in the chemistry of water due to pH fluctuations is a result of the influx of effluent, due to which resources, especially around the coastal areas, can be severely affected. Hence we can say that all the abovementioned effects are significant on water bodies. Effluents polluted with dyes not only affect photosynthetic organisms in water bodies but also have subsequent negative impact on the food chain. Apart from the improper effluent treatment, weather systems and the biogeochemical cycling of elements are the reason for this global effect which acts as an aid in the rapid dispersal of the pollutants.

Physical treatment techniques such as sedimentation and filtration as well as subsequent chemical treatments like flocculation, neutralization, and electro-dialysis were part of traditional methods to clear out the pollutants and unwanted materials before disposal. These processes are popular but may not guarantee complete effluent treatment. Moreover, these methods are often laborious and expensive for the treatment of voluminous wastes discharged during the industrial production process. Though all of the abovementioned conventional physico-chemical methods for adequate treatment/removal of harmful pollutants are effective, they cannot be feasible for large-scale applications (Deshmukh et al. 2016).

To combat the associated drawbacks of convention treatment methods, environment-friendly and economical technique of bioremediation has been explored. It is recognized as an efficient and environmentally friendly technique to treat the contaminated land and water, based on the application of natural biological processes (use of living organisms or their enzymes) for the conversion of toxic, recalcitrant compounds into their non-toxic forms. Successful implementation of bioremediation is dependent upon two mechanisms: (1) biodegradation using the proper exploitation of the microorganisms' enzymatic machinery for the degradation of organic contaminants into its less toxic form, leading to their mineralization, and (2) another mechanism is based on the phenomenon of biosorption which involves the process of binding of solutes to the biomass and carried out without the involvement of metabolic energy or transport (Varese et al. 2011).

Application of suitable microbial tools is explored in this technique for the treatment of polluted system, based on their metabolic physical and chemical reactions resulting into the adequate removal and degradation of pollutants (Gillespie and Philip 2013; Mishra and Malik 2014; Rastegari et al. 2020a, b). Application of individual processes such as natural attenuation, biostimulation, and bioaugmentation or their combinations is most commonly employed for bioremediation of pollutants

or a combination. Earlier studies on soil microcosms-based bioremediation of atrazine (Sagarkar et al. 2013), petroleum hydrocarbons (Lien et al. 2015; Qin et al. 2013), and trinitrotoluene (TNT) (Claus 2014; Nolvak et al. 2013) have been the pertinent demonstration of the above-illustrated techniques. Also, there are well-established bioremediation technologies for industrial chemicals based on activated sludge microorganisms (Ni et al. 2015; Sakaki et al. 2013), however considered as relatively less efficient for the removal of persistent trace organic contaminants (TrOCs) (Wang et al. 2015; Yang et al. 2013b).

4.1.1 Bioremediation Mediated Using Fungi

Due to the robust morphology and diverse metabolic potential, fungi are the most common decomposers and symbionts in soil and aquatic habitat-based ecosystems and hence can be especially suited for bioremediation (Devi et al. 2020b). Also, certain advantages of fungal mycelium were documented earlier in the solubilization of insoluble substrata by the action of extracellular enzymes during biodegradation as compared to single-cell organisms. These advantages are attributed to their increased cell-to-surface ratio and their ability to contact with the environment both physically and enzymatically. Due to the presence of various extracellular enzymes, fungi have an added advantage of tolerating high toxicant concentrations (Kaushik and Malik 2009; Devi et al. 2020a; Kour et al. 2019c).

Bioremediation in which fungal species are used to decontaminate contaminated areas is termed as mycoremediation. Exploration of fungal tools for the degradation of pollutants has been gaining interest because of their immense potential for detoxification and biodegradation due to the presence of certain extracellular and intracellular enzyme systems including laccase, oxidase, peroxidases, epoxide hydrolase, and cytochrome P450, respectively (Jebapriya and Gnanadoss 2013; Morel et al. 2013). Figure 4.1 depicts the basic bioremediation mechanisms adopted by fungi for the removal/degradation of toxic and recalcitrant compounds. In the current chapter, the multifaceted role of fungi has been highlighted in the field of bioremediation of xenobiotic compounds along with the emphasis on fungal properties directed toward complete detoxification and consequent bioremediation of toxic waste. Despite efforts done in the area of mycoremediation, various degradation pathways need to be elucidated. The advent of various molecular biology tools helps in the better understanding of the mechanism involved in bioremediation as well as helps in the designing of better expression systems for bioremediation. This chapter aims at highlighting various aspects of mycoremediation and provides insight into the broad-spectrum bioremediation potential of fungi.

Widespread survival and colonization of fungi have been reported in the habitats like soil matrix, freshwater, as well as in marine habitats. Fungi also play as a major contributor toward the maintenance of ecosystem, as they have an inherent ability to flourish in the soil habitat of different and adverse climatic conditions and can easily propagate in the surrounding air through the dispersal of spores (Anastasi et al.

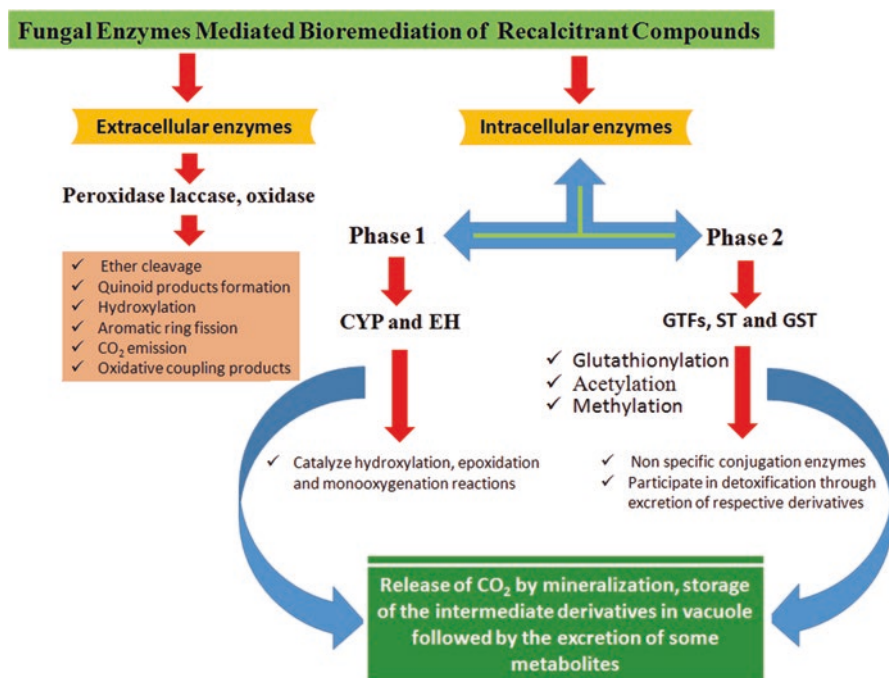


Fig. 4.1 Mechanism involved in bioremediation of toxic, recalcitrant compounds

2013; Rana et al. 2019a). Further, stable survival of fungi has been also reported in effluent treatment plants (ETPs) used for treating various wastewaters (Badia-Fabregat et al. 2015; Zhang et al. 2013). Hence, the ability of fungi to thrive the diversified habitats and secrete a multitude of enzymes makes them potential candidates for bioremediation at various sites (Deshmukh et al. 2016). It is considered as an important biotechnological tool to remediate polluted soil, water, and air and can act individually or in collaboration with certain bacterial and plant species.

Despite having various ecological benefits and extensive metabolic capabilities, fungi have not received much attention as a potential tool for bioremediation and waste treatment. Fungal activities are the result of heavy exposure of their aquatic and terrestrial fungal habitats with anthropogenic chemicals, due to which the metals serve as the natural micronutrients as well as organics are inherently utilized as the heterotrophic substrates (Harms et al. 2011).

In the earlier studies, wood-rot fungi were reported for biodegradation of wastewater pollutants due to their ability to produce lignin-degrading enzymes (i.e., laccases and peroxidases). They were reported for their high redox potential as well as their effectiveness to detoxify/degrade a variety of recalcitrant pollutants such as a wide range of industrial dyes (Anastasi et al. 2010). Though various researchers have demonstrated the excellent capacity of fungi to degrade wastewater-based pollutants, its real application has not been explored effectively due to difficulty in the selection of organisms showing growth and degradation potential under variable

and restrictive conditions of wastewaters generated through textile industries (Vanhulle et al. 2008). In addition, various screening methodologies have been adopted to measure actual degradation efficiency in real effluents of the areas where its measurement is difficult. To date, only a few reports are available on the continuous treatment of wastewaters in bioreactors (Blanquez et al. 2008).

Very recently, a bioreactor was developed by a group of researchers for the treatment of real wastewaters operated under the industrial conditions (Anastasi et al. 2010). Screening of 12 basidiomycetes was performed on the basis of their ability to decolorize and detoxify three simulated wastewaters followed by the selection of a wild strain of *Bjerkandera adusta* (Willd.). This selected fungal strain was extremely useful for application purposes and showed significant physiological versatility which is very useful. A fixed-bed bioreactor was used to evaluate true bioremediation potential for the treatment of large volumes of a real wastewater of this strain followed by its appropriate packing into the reactor bed. The selected fungus was found effective for over 10 cycles of decolorization and can even remain active in non-sterile conditions for a longer time period.

Apart from the other mentioned advantages of fungi over other organisms, it also displays several advantages in biosorption too. Numerous physical and chemical properties are associated with the fungal cell wall and act as an aid in pollutants binding during biosorption. Moreover, as compared to the separation of single cell organisms, separation of mycelial biomass from the liquid is simpler. Also, fungal biomass can be supplied as a biosorbent due to its easy and productive growth on different media and having low nutritional requirements. Most of the fungal species have been extensively used in large-scale industrial fermentation processes and also represent a potential source of cheap adsorbent materials (Gadd 2009).

Both living and dead forms of fungi can be employed for pollutant biosorption, and even its dead form can be utilized effectively as an efficient and environmentally safe tool for pollutant removal (Kaushik and Malik 2009). Most of the biosorption studies have been conducted on anamorphic fungi and Zygomycetes. In particular, Zygomycetes seem to be most effective for pollutants' biosorption because of the abundant concentration of acid polysaccharides such as chitin and chitosan in their cell walls; these components constitute about 50% of the cell wall components (Tigini et al. 2011). These macromolecules are highly responsible for biosorption of phenolic compounds, dyes, and heavy metals and characterized by amino and hydroxyl groups (Prigione et al. 2009).

To date, various literatures have focused attention on the configuration of bioreactors and physico-chemical conditions to enhance biosorption and biomass modification by a selection of pre-culture medium and biomass pretreatments (Gadd 2009; Tigini et al. 2010). Earlier, biosorption was also reported using *Cunninghamella elegans* Lendn in scale-up pilot trials to treat pollutants. This fungus has been effectively reported in simultaneous removal of heavy metals, dyes, surfactants, salts and toxicity from wastewaters (Tigini et al. 2010).

4.2 Pollutant Catabolism Using Fungal Diversity

A diverse range of fungi has been reported for the transformation of a variety of organic pollutants (Hawari et al. 2000; Pointing 2001; Prenafeta-Boldú et al. 2006; Chang 2008; Pinedo-Rilla et al. 2009). Among the kingdom Fungi (an estimated count of around 80,000–100,000 described species), phyla Ascomycota and Basidiomycota are considered to be the potential pollutant degraders followed by the subphylum Mucoromycotina. However, there are only a few documented examples dealing with the other fungi (Harms et al. 2011).

4.2.1 White-Rot Fungi

White-rot fungi are the common contributor to the global carbon recycling and activity involved in biodegradation of nature abundant lignininous material. Chemicals such as endocrine-disrupting chemicals (EDCs) and TrOCs such as pharmaceuticals and personal care products (PPCPs) are the prime target of white-rot fungi. These chemicals caught much attention concerning their degradation due to bioaccumulation, acute and chronic toxicity to aquatic organisms, as well as its possible adverse effects on human health. The bioremediation potential of white-rot fungi like *Phanerochaete chrysosporium*, *Trametes versicolor*, *Bjerkandera adusta*, and *Pleurotus* sp. has been demonstrated; also their potential was correlated to the production of different ligninolytic enzymes such as laccases and peroxidases (Dos Santos Bazanella et al. 2013). Due to the presence of ligninolytic enzymes, white-rot fungi promote microbial activity using a biopurification system (BPS), thereby involved in the transformation of a variety of organic pollutants such as pesticides from contaminated wastewaters (Rodriguez-Rodriguez et al. 2013).

Due to the limited or restricted access of ligninolytic enzymes to lignin granules (found deposited on the surface of lignocellulosic fibers), a technique of pressure refining was applied for separation of fibers of lignocellulosic materials. Due to the adopted strategy of pressure refining, accessibility of ligninolytic enzymes from white-rot fungus *Ceriporiopsis subvermispora* was enhanced which in turn showed higher delignification from pressure refined *Miscanthus* than milled *Miscanthus* (Baker et al. 2015). These ligninolytic enzymes are extracellular and provided with the capacity to adsorb dyes. Their presence in white-rot fungi makes them a potential and dominant candidate in the area of dye degradation or decolorization. Decolorization of Direct Blue 14 and Remazol Brilliant Blue-R by various species of *Pleurotus* and Agaricomycete (white-rot fungus from Amazon forest), respectively, was the example of the abovementioned properties of white-rot fungi (Singh et al. 2013a; Dos Santos et al. 2015).

Decolorization of dye effluent was also demonstrated in the earlier studies using the diverse fungal groups such as *Coriolus versicolor*, *Hirschioporus larincinus*, *Inonotus hispidus*, *Phanerochaete chrysosporium*, and *Phlebia tremellosa* (Jebapriya and Gnanadoss 2013), while 38 species of white-rot fungi were reportedly involved in the reduction of total phenolic content (>60%) and color ($\leq$ 70%) of olive-mill wastewater (Ntougias et al. 2015). Similarly, remediation of the cresolate-contaminated soil using the bioaugmentation of two strains of white-rot fungi such as *T. versicolor* and *Lentinus tigrinus* has also been demonstrated in the previous studies (Llado et al. 2013).

Fractions of high molecular weight PAH remain after a biopiling treatment, as well as residual recalcitrant petroleum hydrocarbons are the common contaminants of the cresolate-polluted soil. Biostimulation using lignocellulosic substrate along with fungal-based bioaugmentation is the common strategies for the significant degradation of the residues. However, validation of these two degradation strategies has been suggested at a small scale before field applications to rule out the possibility of growth of local microbes at the site of treatment which might consequently dominate the augmented organism.

In addition to the above, ligninolytic enzymes-based applications of white-rot fungi in bioremediation of variety of compounds and enhanced removal efficiencies of white-rot fungi have also been reported for the degradation substituted organic compounds due to the presence of laccase enzymes (Cutright and Erdem 2012; Fan et al. 2013; Purnomo et al. 2013). Based on the significant features of white-rot fungi on bioremediation, two strains of white-rot fungi such as *T. versicolor* and *P. ostreatus* were targeted for enhanced laccase production using solid-state fermentation on orange peels followed by the screening to test their ability to degrade PAHs such as phenanthrene and pyrene (Rosales et al. 2013). Laccase production from the cultures of *T. versicolor* and *P. ostreatus* was reported up to 3000 U/L and 2700 U/L, respectively. In spite of low laccase activity, *P. ostreatus* was found to remove both phenanthrene and pyrene much effectively. However, these reported fungi have to be studied at genomic level for the better understanding of their enzymatic route of degradation and exploitation of their bioremediation potential of fungi to the fullest.

4.2.2 Marine Fungi

Applications of marine fungi were also demonstrated in bioremediation of hydrocarbons and heavy metals as well as their immense contribution toward the production of novel enzymes, secondary metabolites, biosurfactants, polysaccharides, and polyunsaturated fatty acids (Damare et al. 2012). These fungi offer several biological advantages over terrestrial fungi such as their potential to survive/adapt at extreme conditions of pH and salinity. Hence, these marine microorganisms are categorized as the extremophilic organisms with the efficiency of metal ion as well as their huge applications in the field of bioremediation and nanotechnology (Rana

et al. 2020). In the earlier studies, the role of different marine fungi from mangrove areas was reviewed for their different potential applications which emphasized on their biotechnological potential for bioremediation, their diversity and immense ecological role, and ability to serve as the excellent source of novel drugs, enzymes, biodiesel, and biopesticides (Thatoi et al. 2013).

Recently, Bonugli-Santos et al. (2015) have demonstrated the biotechnological relevance and the significant role of enzymes derived from marine fungi. Apart from the abovementioned advantages, marine fungi are provided with the ability to tolerate heavy metals such as lead and copper at high concentration (Gazem and Nazareth 2013). These fungi were also documented for their applications in the synthesis of metal nanoparticles of desired properties based on their interaction with metal ions in marine ecosystems (Baker et al. 2013; Rastegari et al. 2019b). These fungal-based nanoparticles can be synthesized both extra- and intracellularly and can be employed for diverse applications in the field of textile industries, medical and clinical microbiology, as well as in food preservations (Kathiresan et al. 2010; Saxena et al. 2014; Singh et al. 2015).

Bioremediation of toxic and persistent organic pollutants using fungi applications is dependent upon several factors to further enhance its rate. Divya et al. (2013) reported the isolation of *Trichoderma viride* Pers. NFCCI-2745 from an estuary polluted with phenolics and aptly demonstrated the ability of this isolated marine fungi to adapt at high salinity and phenolics due to the presence of laccase. Similarly, isolation of three basidiomycetes was performed from marine sponges, and their respective applications were demonstrated in the bioremediation or decolorization of Remazol Brilliant Blue-R dye (Bonugli-Santos et al. 2012). A marine white-rot basidiomycete *C. unicolor* was reported for the decolorization of anthraquinone dye Reactive Blue 4. Gao et al. (2013) proposed the effectiveness of biostimulation and bioaugmentation in the biotransformation of persistent organic pollutants (POPs). One such application was reported in the biotransformation of PCB 118 in presence of maifanite by the two marine fungi belonging to genus *Penicillium* (Verma et al. 2012). In the other study, a fungus derived from marine water, i.e., *Trichoderma harzianum*, was reported for biotransformation of pentachlorophenol (POP) at high concentration (Vacondio et al. 2015). Similar bioremediation potential was demonstrated by other marine-derived fungi including *Mucor*, *Aspergillus*, *Penicillium*, and slime mold. These fungi could easily be applied in water-soluble crude oil fractions ranging in the concentration from 0.01 to 0.25 mg/mL. However, a higher concentration of oil fractions resulted in toxicity to the organisms (Hickey 2013).

4.2.3 Extremophilic Fungi

Industrial applicability of fungi from extreme environments is increasing due to their extremophilic enzymes with the unique ability to provide pH tolerance, thermotolerance, and tolerance to other harsh conditions (Neifar et al. 2015; Kour et al. 2019a; Yadav et al. 2020e). Due to the fungal ability to tolerate high levels of

pollutants from industrial effluents, it can be applied for diverse bioremediation applications (Rastegari et al. 2019a). The effluent treatment plants can be effectively used as a potential niche among the extreme environments, to explore fungal bioremediation capacity. Based on the special characteristics, fungi represent the ideal and potential candidate to carry out the raw materials based on bio-conversions and bioremediation in an economical and environment-friendly manner. These can effectively process raw materials generated from leather processing, textile manufacture, food industries, and animal feed preparation (Nigam 2013). Recently, potential applications of metallophilic microbes were demonstrated in the bioremediation of environmental-based problematic heavy metals followed by their application in the synthesis of nanoparticle which can further be helpful for bioremediation (Sinha et al. 2014).

A psychrophilic fungal (*Cryptococcus* sp.) isolate of deep-sea sediments was reported for their ability to tolerate and grow in the presence of heavy metals such as ZnSO_4 , CuSO_4 , $\text{Pb}(\text{CH}_3\text{COO})_2$, and CdCl_2 at the highest concentration of 100 mg/L indicating their mode of adaptation beyond extremities (Singh et al. 2013b). Many bioremediation-specific hydrolytic enzymes are known for their activity under extremophilic conditions and documented for their efficient remediation processes of oil belt-based drilling waste contaminated with extra-heavy crude oil (ECHO) as well as under extreme conditions of high salinity. Bioremediation activity in *Pestalotiopsis palmarum* was attributed to the presence of extreme acting laccases and lignin peroxidases induced in the presence of wheat bran and extra heavy crude oil, respectively, as the only carbon and energy source (Naranjo-Briceno et al. 2013; Betancor et al. 2013).

A psychrophilic fungus, i.e., *Lecanicillium muscarium*, was demonstrated earlier with the ability to actively enhance the activity of insecticides (acts on insect chitin exoskeleton) due to the presence of chitinase enzymes (Li et al. 2013; Narayanan et al. 2013). Apart from the applications of the abovementioned extremophilic fungi in bioremediation, other extreme environments-based fungal isolates such as those obtained from deep biosphere habitat (represented by fumarolic ice caves on Antarctica's Mt. Erebus) can also be used as model organism to identify their capability to utilize energy sources other than photosynthesis, which indirectly can be informative to know the possible sources of human contamination of such extreme regions (Narayanan et al. 2013; Connel and Staudigel 2013).

4.3 Symbiotic Association Between Fungi, Plants, and Bacteria

Fungi maintain a symbiotic association with plants and bacteria to combat barrier related to restricted growth under different environmental conditions. One such fungal association with plant roots is represented by arbuscular mycorrhizal fungi (AMF), wherein the high surface area of fungal hyphae and spores adsorb the pollutants, thereby causing its mobilization and removal. Groundwaters contaminated

with the pollutants of constructed wetland are often treated using phytoremediation in which root samples of the plant colonized with AMF are employed for the treatment (Fester 2013). Various other plant-associated fungi (*A. nidulans*, *Bjerkandera adusta*, *Trametes hirsuta*, *T. viride*, *Funalia trogii*, *Irpex lacteus*, *P. ostreatus*) were also reported on the basis of their survival in the presence of textile industry effluents, and their ability of dye decolorization (Tegli et al. 2013). Other examples of AM fungus include *Rhizophagus custos* which colonizes under root-organ cultures and can tolerate PAHs especially anthracene at higher concentration and reportedly produces a lesser amount of toxic by-product anthraquinone (Aranda et al. 2013; Verma et al. 2017).

Several other advantages are also associated with the mycorrhizal effect of AM product due to its root colonization property. One such example was based on the enhanced ^{137}Cs uptake by quinoa plants on loamy soil as a result of inoculation with a commercial AM product (Vinichuk et al. 2013). Recently, ectomycorrhizal fungi, *Suillus bovinus* and *Rhizopogon roseolus*, were reported for the removal of cadmium in association with *Pinus*. However, certain environmental factors like the type of nutrients and pH also influence the cadmium removal (Sousa et al. 2014). Apart from the fungal applications related to bioremediation, there are other reported applications such as complete removal of single algal cells from fermentation mediums is a result of microalgae and fungi cocultivation which in turn acts as an aid to overcome technical barriers of algal biofuels and photosynthetic biorefineries. Also, due to this strategy of cocultivation, it is easier to enhance the biomass, lipid, and bio-product yields followed by their extraction and harvest using simple filtration (Xie et al. 2013). Though various benefits are associated with the technique of cocultivation, it is also tedious to unravel the basic mechanism behind the interaction between multitudes of metabolic pathways from different organisms.

4.4 Fungi Potential of Bioremediation

Fungi play an indispensable role in bioremediation of a variety of pollutants such as textile dyes, pulp and paper industry effluents, leather tanning effluents, petroleum hydrocarbons, pesticides, POPs, PAHs, and PPCPs (Yadav et al. 2020a, b). Metal tolerance ability of other filamentous fungi (*Aspergillus*, *Curvularia*, *Acrimonium*, and *Pythium*) has also been studied (Akhtar et al. 2013; Yadav 2020). Degradation of model PAHs by *T. versicolor* and white-rot fungi *Pleurotus ostreatus* (members of the Basidiomycota) has been reported earlier in solid-state fermentation (SSF). This degradation was growth-dependent in which the fungal growth was based on the availability of agro-industrial wastes such as orange peels (Rosales et al. 2013). Diverse groups of fungi such as *Aspergillus*, *Penicillium*, and alkaliphilic white-rot fungi have been reported in the bioremediation/decolorization of colored effluents from textile and sugar industry, leather tanning effluents, and bleached kraft pulp mill. These reported fungi differ in their degradation potential as well as in their substrate preferences for growth-dependent degradation (Jebapriya and Gnanadoss

2013; Huang et al. 2014; Buvaneswari et al. 2013; Bennett et al. 2013; Duarte et al. 2013; Reya et al. 2013).

Under the controlled environmental conditions and in the presence of extra nutrients, fungi (*Aspergillus restrictus*, *Chrysosporium keratinophilum*, *Fusarium solani*, *Gliocladium roseum*, *Penicillium*, and *Stemphylium*) could be applied for the decaffeination of coffee pulp, thereby facilitating animal feed preparation or bio-ethanol production (Nayak et al. 2013). Bioremediation potential of fungi *A. niger* and *P. chrysosporium* was explored in the contaminated soil-based petroleum hydrocarbons along with petrol and diesel followed by the estimation of enhanced total organic carbon (TOC), thereby indicating substantial removal of these contaminants at short incubation periods (Maruthi et al. 2013). According to the reports of Silambarasan and Abraham (2013), a fungal strain of *A. niger* JAS1 had the potential to remove chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol (TCP) from contaminated soils completely without requiring any additional nutrients. TCP exhibits an antimicrobial and catabolite repression property; hence its significant degradation was reported by chlorpyrifos-degrading strain.

4.5 Toxic Recalcitrant Compounds and Their Bioremediation

Taking into consideration the carcinogenic and mutagenic properties of many toxic, organic compounds present in the industrial effluents, bioremediation becomes an essential pre-requisite for their release into the environment. Also, their persistence in soil, water, and the air is associated with their biomagnification potential. PAHs are categorized as the most complex organic compounds and fungi are considered as an efficient tool for their bioremediation. PAHs are fused, highly stable, polycondensed aromatic rings that can be easily degraded by the action of fungal lipase enzyme. Higher lipase production was observed in the case of 21 fungal strains (*Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma*) isolated from PAH-contaminated soil and reported for its efficient degradation (Llado et al. 2013; Chang et al. 2015; Balaji et al. 2014). Certain nonspecific fungal extracellular enzymes are reported for the fungal degradation ability for explosives such as TNT in the presence of cellulose and lignin like co-substrates (Nolvak et al. 2013). Conventionally, toxic effluents were the result of continuous and successive use of many toxic chemicals in agro-industrial operations such as paper mills-based bleaching of agro-residual pulp.

Green technology was developed for pulp and paper industry by Dhiman et al. (2014) based on bacterial xylanase and fungal laccase-mediated enzymatic pretreatment system which in turn results in significant toxicity reduction of the paper mill effluent. Dyeing industries have manifested following criteria to monitor the chemical toxicity level such as crop plant growth retardation, reduction in the rate of seed germination, decreased protein, chlorophyll and carbohydrate content, as well as increased proline content in exposed plants (Marjadi 2013; Ferraz et al. 2013).

According to the reports, aquatic organisms such as including algae and fishes are mainly affected by the toxicity of basic and acid dyes. These dyes are the probable cause of various physiological disorders in human beings due to their tendency to pass through the food chain and ultimately reach the human body (Bayramoglu and Arica 2013; Watharkar et al. 2015). Due to well-defined variable degradative capacities, the potential of white-rot fungi has been extensively exploited for optimum dye degradation in co-culture or sequential degradation studies studied (Jebapriya and Gnanadoss 2013).

Based on the data, reactive Remazol Blue was decolorized up to 89.4% at pH 6 and 69.23% at pH 3 by co-culture of *Aspergillus versicolor* and *Rhizopus arrhizus*. This decolorization percentage was achieved in 6 days of incubation using dye at the initial concentration of 100 mg/L and facilitated by dodecyltrimethylammonium bromide (DTAB) (Gulu and Donmez 2013). Two other direct dyes dye Solar Brilliant Red 80A and an azo dye, Acid Orange 7, were shown to be decolorized completely by the stain of white-rot fungus *Schizophyllum commune* IBL-06 and *C. versicolor*, respectively (Asgher et al. 2013; Hai et al. 2013).

Among the other toxic compounds, degradation of pesticide chloropyrifos and its major metabolites was elucidated in the mineral medium by *Aspergillus terreus* completely within 24 h of incubation (Silambarasan and Abraham 2013). Degradation studies of similar pesticide dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) by *Trichoderma atroviride* and its related gene expression studies revealed that the functioning of ABC transporters and alteration in expression of 5382 genes are associated to its dye tolerating ability (Zhang et al. 2015b). A strain of *Mucor racemosus*, DDF, was reported for the degradation of dieldrin within 10 days along with the generation of 9% aldrin trans-diol. Due to its specificity for a diverse range of substrates, this strain could degrade other pesticides as well such as endosulfan sulfate (95%), heptachlor (94%), endosulfan (80%), and heptachlor epoxide (67.5%) (Kataoka et al. 2010).

4.5.1 Heavy Metals Bioremediation

Applications of heavy metals in multiple areas lead to its wide-scale distribution into the environment which in turn causes systemic toxicity to human health even at low concentrations (Yadav et al. 2020f). Due to the high degree of toxicity, heavy metals such as arsenic, cadmium, chromium, lead, and mercury are major causes of health hazards related to multiple organ failure and carcinogenic effects. These metals are also categorized as priority metals and need to be removed from the environment to prevent its impact on public health and the environment. Heavy metals production, their environmental occurrence, and their role in posing serious human threats upon human exposure have been analyzed earlier followed by the revelation of molecular mechanisms behind their toxic effects (Tchounwou et al. 2012). However, the existence of different microbes and their ability to tolerate these heavy metals using diverse mechanisms have been proved as a boon toward their removal

from the environment. Potential of filamentous fungi was explored toward their ability to tolerate heavy metals like Cd, Cu, and Ni (up to 1500 mg/L) and reported for their significance in heavy metals bioremediation from contaminated soil and wastewater (Akhtar et al. 2013).

A diverse range of toxic compounds such as heavy metals, textile dyes, aromatic compounds, pesticides, etc. are reported to be degraded by the members of genus *A. flavus* and *A. niger*. Also, fungi has an immense ability to reduce heavy metals such as Cr^{6+} to Cr^{3+} (Bennett et al. 2013). Another species of *Aspergillus* was isolated from wastewater treatment plant, i.e., *A. foetidus*, and known for its ability to tolerate lead (Pb) up to the concentration of 200 mg/L and can effectively remove Pb through biosorption (Chakraborty et al. 2013). Similarly, a strain of *A. flavus* was documented in the bioremediation of aqueous substrates containing mercury (II); 98% removal of mercury was reported by using mercury at the concentration of 10 mg/L in the medium (Kurniati et al. 2014).

Bioremediation potential of fungi like *Aspergillus*, *Cryptococcus*, *Penicillium*, and *Curvularia* was also explored in the treatment of uranium-contaminated soils due to their uranium binding ability (Mumtaz et al. 2013). Plant growth in metal-contaminated soils can be attributed to the fact that symbiotic association of AM fungi with the plant roots promotes heavy metal immobilization, thereby providing tolerance in plants toward metals. Similar tolerance was observed in plants toward the enhanced level of Cd in the soil (Yang et al. 2015; Garg and Bhandari 2014). Further, the fungal potential for bioremediation of toxic compounds could be achieved by certain pretreatments. Das et al. (2013) revealed the role of gamma rays irradiation to enhance the Cd bioremediation efficiency in *Aspergillus* sp. In this study, better growth of *Aspergillus* sp. was observed when irradiated with gamma rays (20–100 Gy) in Cd supplemented media followed by its enhanced Cd removal efficiency as compared to unirradiated controls (Das et al. 2013).

4.5.2 Municipal Solid Waste (MSW) and Their Bioremediation

Urbanization and lifestyle of the developing countries are raising the major threat to public health and environmental problems by the generation of tons of municipal solid waste (MSW) (Habib et al. 2013; Soobhany et al. 2015). Various disposable practices are available for the treatment of MSW such as incineration, land-filling, composting, etc. Apart from their major role in MSW treatment, certain bottlenecks are associated with their applications. Among all, incineration and land-filling are widely employed practices for waste disposal but considered to be an expensive process, whereas land-filling sites are the ones leading to secondary environmental pollution including air fouling, bad odor, and enhanced soil-based pathogen content. On the other hand, methods of composting and land-filling suffer a problem of limited land availability in certain countries due to its requirement of vast land areas, hence not suitable in all countries (Khardenavis et al. 2013).

Earlier, various technological advances were highlighted for the treatment of misplaced resources. Composting and biomethanation by anaerobic digestion have been the most preferred and desirable solution for the management of MSW. Two benefits are associated with an aforementioned method such as easy management of MSW and their use in the generation of value-added products such as biogas, volatile fatty acids (VFAs), biogas, and organic residue/compost which in turn could be employed as a soil conditioner or fertilizer. Further, the application of fungi and their hydrolytic enzymes such as cellulases, proteases, amylases, and lipases could be employed to enhance the efficiency and rate of these processes (Pandit et al. 2015). Fungi-based treatment of MSW involves the conversion of complex polymeric substances to simple compounds (precursors for VFA and biogas production).

This mechanism was further demonstrated by the evaluation of steam, acid, base, and pretreated kitchen waste residues with the potential to serve as substrate for solid-state fermentation using a locally isolated strain of *Aspergillus niger* which acts as the excellent source of amylolytic, pectinolytic, cellulolytic, and hemicellulolytic enzymes (Janveja et al. 2013). These are the beneficial enzymes for pretreatment with the ability to enhance the efficiency of hydrolysis and saccharification of selected biomasses. Preparation of fungal consortium using two fungi *Armillaria gemina* and *Pholiota adiposa* and their application has been demonstrated in willow and rice straw (Dhiman et al. 2015). However, strains of white-rot fungi could also be applied to enhance the composting of other residual biomass. Further, spent biomass could also be utilized for soil application (Marco et al. 2013). Research based on bioremediation of copper deposited wood indicated the tolerance of wood-rotting fungi *Antrodia xanthan* and *Fomitopsis palustris* to copper (Hattori et al. 2015).

4.6 Fungal Features for Detoxification and Bioremediation of Toxic Waste

4.6.1 Bioremediation Using Fungal Enzymes

Enzymes of fungal origin (amylases, proteases, cellulases, xylanases, lipases, lacases, peroxidases, catalases) are industrially important for the management and treatment of organic waste such as the organic fraction of MSW (OFMSW) (Marco et al. 2013; Yadav et al. 2019b). These enzymes are involved in the hydrolysis of polymeric substances (cellulose, xylan, starch, protein, and lipid) present in vegetable market, leaf litter, and other kitchen-based food and vegetable wastes which in turn act as the precursor for the generation of value-added products such as VFAs and biogas or could be further subjected to composting (Khardenavis et al. 2013; Hattori et al. 2015). Various white-rot fungi were reported in the production of one or more types of ligninolytic enzymes based on the species and the prevalent environmental conditions. These fungi are not only known for their role in lignin

degradation of natural lignocellulosic-based substrates but also have various applications in bioremediation studies. One such application involves the degradation of various xenobiotic compounds including dyes (Kour et al. 2019b).

The crucial role of ligninolytic enzymes in the modification of azo dye structure is attributed to the destruction of chromophoric assemblies, thereby causing a reaction leading to the formation of phenoxyl radicals (Gulu and Donmez 2013). White-rot fungi have been reported to secrete ligninolytic enzymes extracellularly to promote lipid oxidation. These enzymes have been categorized into two groups involving peroxidases-manganese, lignin peroxidases (MnP and LiP) and laccases (Jebapriya and Gnanadoss 2013; He et al. 2015). White-rot basidiomycetes were also established for the production of enzymes like laccases and some fungal class II peroxidases involved in the degradation of persistent organic pollutants (Ikehata 2015). Similar enzymes from extremophilic fungi were also reported for their remediation applications under extreme conditions such as remediation of extra-heavy crude oil, high salinity contamination, and remediation of oil belts-based drilling waste. Taking into consideration the widespread role of enzymes in remediation, there has been gaining interest in the application of protein engineering and recombinant expression in the development of tailor-made enzymes. Recombinant gene expression from white-rot fungi has been reported as an effective tool for the treatment of toxic wastes in an ecofriendly manner (Sakaki et al. 2013; Fonseca et al. 2013; Syed et al. 2013; Wong et al. 2013).

4.6.1.1 Laccase

Laccases comprise of copper-containing extracellular enzymes belonging to a group of blue oxidases requiring copper as a cofactor and molecular oxygen as co-substrate. Laccases have the most protuberant role in the oxidation of phenolic and non-phenolic compounds. Higher laccase activity (more than 20 times) is observed in fungi such as *T. versicolor* as compared to other organisms (Margot et al. 2013). These enzymes have diverse industrial applications based on their non-specific activity on the substrates and considered as an ideal catalyst due to their efficient bioremediation potential (Vishwanath et al. 2014). Deinking of offset printed paper in the recycled paper industry reflects one such application associated with laccase. Laccases isolated from three basidiomycetes (*Trametes villosa*, *Coriolopsis rigida*, *Pycnoporus coccineus*) and one ascomycete (*Myceliophthora thermophila*) were found to decolorize flexographic inks in presence of synthetic and artificial mediators (Fillat et al. 2012).

Textile dyes are synthetic in origin, and its toxicity is a major cause for concern which is attributed to its resistance to fading on exposure to water and sunlight followed by its persistence in the environment. The role of marine fungal laccase was initially suggested in decolorization, detoxification, and mineralization of Reactive Blue 4 (Verma et al. 2012; Vishwanath et al. 2014) at moderately high concentrations of 1000 mg/L. Bisphenol A is an endocrine-disrupting chemical, and its degradation was reported in the earlier studies using purified *Fusarium incarnatum*-based

laccase. Incubation of laccase with Bisphenol A at the concentration of 200 mg/L led to its degradation by 91.43% (Chhaya and Gupte 2013).

Himalayan region-based isolates of extremophilic fungi like *Penicillium pinophilum* also demonstrated the ability to produce laccase at low temperatures (Dhakar et al. 2014). However, less information is available regarding the laccase mechanism of action under extreme conditions. Till date crystal structure of only a few laccases has been explored including those from ascomycetes *Melanocarpus albo-mycus* (MaL) and *Thielavia arenaria* (TaLcc1). This study revealed the difference between these two laccases and other laccases is due to the presence of a conserved “C-terminal plug” probably in proton transfer processes (Kallio et al. 2011). Despite having multiple applications and incredible potential in bioremediation, the effectiveness of laccases suffers a drawback of low shelf life. However, these above drawbacks can be overcome through a technique of enzyme immobilization which provides high residual activities over a broad range of pH and temperature (Patel et al. 2014). Similarly, through the advent of innovative technologies and the application of tools, it is possible to tailor the enzymes of interest and create mutants that harbor enzymes with higher substrate affinity and could tolerate broader substrate ranges and environmental factors (Mate et al. 2011; Torres-Salas et al. 2013).

Also, the availability of high-throughput assays is required for the screening of tailored enzymes prepared using the above tools. A new colorimetric assay was developed earlier to screen engineered laccases based on their activity toward the oxidation of syringyl compounds (Pardo et al. 2013). Fungal laccases are not only known for their catabolic potential but also involved in dimerization, oligomerization, and polymerization reactions of numerous aromatic compounds. Based on the catabolic potential and improved selectivity, fungal laccase could be explored as an ideal biocatalyst for dye synthesis as well as the synthesis of colorants including azo dyes as well as those with phenolic, non-phenolic, phenoxazinone (Polak and Jarosz-Wilkolazka 2012).

4.6.1.2 Catalase

Reactive oxygen species in biological systems and its excess accumulation are the major cause of damage to cellular macromolecules which in turn affects cellular integrity. Enzymes like monofunctional catalases and bifunctional peroxidase/catalase are involved in the primary defense mechanism of fungi to ROS generation. The role of catalase has been discussed in the growth of *Saccharomyces cerevisiae* in the presence of pesticide lindane; it was found that catalase was inhibited in the presence of pesticide which led to augmented ROS generation and ROS-mediated damage, thereby inhibiting the growth of *S. cerevisiae* (Pita et al. 2013). ROS is also induced in microbial cells due to the presence of heavy metals such as lead (Pb), copper (Cu), cadmium (Cd), and zinc (Zn). Studies which dealt with the correlation of heavy metals with ROS generation also indicated a concomitant rise in the level of anti-oxidative enzymes.

This finding was confirmed by the reports of Chakraborty et al. (2013) showing the effect of Pb(II) in growth and tolerance of *Aspergillus foetidus* at the concentration of 200 mg/L which was directly correlated with the enhanced level of anti-oxidative enzymes including catalase to detoxify malondialdehyde and H_2O^2 . Similar tolerance was observed in the case of *Aspergillus* spp. for the oxidative stress condition which was a result of heavy metals induction (induced by 100 mg/L Cu(II) and 750 mg/L Zn(II) (Mitra et al. 2014). The authors also demonstrated that better expression of copper-amine oxidase enzymes augmented ROS generation while fungi ability to tolerate heavy-metal induced oxidative stress enhanced the catalase activities among other enzymes.

Though very few reports are available to demonstrate the effect of heavy metals on the fungal physiology, inhibition of catalase and peroxidase was reported in *P. chrysosporium* followed by increase in cytochrome P450 (CYP450) activity due to the exposure of cadmium or lead (50–100 μ M) (Zhang et al. 2015a).

In contrast to this study, higher catalase activity was reported due to the addition of Pb^{2+} and Cu^{2+} either individually or in combination with the fungal consortia prepared using *A. niger*, *Penicillium* sp., and *Rhizopus* sp. at the concentration of about 50 mg/L (Thippeswamy et al. 2014). An earlier study also demonstrated the role of catalase as the monitoring tool and bioremediation efficiency could easily be monitored based on its activity. In a similar study, the effect of increasing oil concentration was elucidated on catalase activity and revealed that a significant decrease in catalase activity was observed when the oil concentration was increased during bioremediation of oil-contaminated soil (Lin et al. 2009). So taking into consideration the significant effect of catalase in alleviating heavy metal tolerance in fungi, catalase-producing fungi have been proposed as a promising candidate for bioremediation of metal-contaminated sites.

4.6.1.3 Peroxidase

Based on the source and activity, peroxidases are classified into lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP). Out of all, LiP and MnP are mostly reported in the degradation of toxic compounds by white-rot and basidiomycete fungi. These two are categorized as heme peroxidases and require hydrogen peroxidase and manganese for activity, whereas, due to broad substrate specificity, VP enzymes can oxidize both phenolic and non-phenolic compounds and are widely accepted tools for biotechnological processes such as bioremediation (Karigar and Rao 2011). Additionally, along with the other organic compounds, catalysis of various non-phenolic lignin model compounds has been reported by another heme peroxidase such as dye-decolorizing peroxidases (DyPs) and unspecific peroxygenases (UPO) that require the presence of hydrogen peroxide. But these peroxidases do not fit in the above classification system (Hofrichter and Ullrich 2014; Liers et al. 2013; Strittmatter et al. 2013). Similar peroxidase was reported in *B. adusta* showing decolorization of azo and phthalocyanine dye by the disruption of phthalocyaninic ring in phthalocyanine dyes and cleavage of the azo

bond. Dye mineralization capacity mediated by the fungal enzyme was validated by identification of the transformation products using EPR spectroscopy and mass spectrometry (Baratto et al. 2015).

Recently, the stability of *C. subvermispora* at acidic pH was enhanced using the engineering of a MnP enzyme which provides the acidic stability to *C. subvermispora* even at pH 2. Expression of acid stability as well as high Mn²⁺ oxidizing activity into the strain was based on the study derived using the enzymatic crystal structure as a scaffold followed by the strain engineering to incorporate ability to oxidize Reactive Black 5 as well as veratryl alcohol (Fernandez-Fueyo et al. 2014). Five fungal DyPs were discussed earlier possessed with catalytic properties of high-redox peroxidases (both LiP and VP) and low redox potential peroxidases. The presence of high-redox peroxidases was monitored from the fungal ability to promote oxidation of non-phenolic aromatic compounds along with Reactive Black B, whereas low redox potential peroxidases were indicated by their ability to oxidize phenolic substrates (Liers et al. 2013). In the same study, the need to classify peroxidase activities carefully in fungi-derived crude enzyme mixtures based on their catalytic specificity has been highlighted due to difficulty in distinguishing DyPs from LiP and VP. Hence, purification of the different enzymes was suggested for their clear classification.

4.6.1.4 Fungal Cytochromes in Bioremediation

Various complex oxidative and hydrolytic enzymatic systems are characterized in fungi for detoxification of environmental-based toxic compounds. Apart from the above-discussed system, certain fungi constitute the xenome consisting of intracellular networks. This fungal xenome comprises of an enzyme such as cytochrome (CYP) P450 monooxygenases and the glutathione transferases with the ability to tolerate a diverse range of pollutants. Most of the members of the detoxification pathways commonly belong to multigenic families such as cytochrome P450 monooxygenases and glutathione transferases (Morel et al. 2013). Region- and stereospecific oxidation of non-activated hydrocarbons is initiated by the fungal cytochrome P450 system which not only acts as the versatile catalyst but also serves as ideal substitutes for chemical catalysts (Urlacher and Girhard 2012). The significant role of cytochrome P450 systems has also been highlighted in the metabolism of a series of endogenous and exogenous compounds (Ichinose 2013).

A strain of *Fusarium oxysporum* was reported with separate cytosolic and mitochondrial isoforms of P450 reported in the degradation of dioxins (Sakaki et al. 2013; Guengerich and Munro 2013). Similarly, CYP63A2 P450 monooxygenase was characterized in a strain of white-rot fungus *P. chrysosporium*, due to which the fungus has developed with the ability to oxidize endocrine-disrupting long-chain alkylphenols (APs), crude oil aliphatic hydrocarbon, n-alkanes, as well as mutagenic/carcinogenic fused-ring high molecular weight PAHs (HMW-PAHs) (Syed et al. 2013).

Significant production of ω -hydroxy fatty acids was reported in the case of *F. oxysporum*-derived CYP monooxygenases which act as promising catalysts (Durairaj et al. 2015). Treatment of PAH-contaminated site and its efficient removal require the application of pre-induction P450 monooxygenase. It was reported that pre-induction of P450 monooxygenase followed by its application could result in the enhanced removal of PAH (Bhattacharya et al. 2013). Application of molecular tools aimed at the enhanced and rapid production of cytochrome P450 monooxygenase could also be employed for the effective removal of pollutants. One such example has been reported such as the combined application of a broad-range yeast expression system with a viral vector (*Arxula adenivorans*) (Guengerich and Munro 2013).

4.7 Fungal Bioremediation and Their Technological Advances

Bioremediation is the most effective, most sustainable, and green route for the cleanup of toxic organics and other contaminated sites, and fungi play an important role in the ecosystem being its core component due to its inherent and tremendous potential to tackle the contamination-based problem owing to the presence of their multiple modes (Yadav et al. 2019a). However, various environmental factors are involved in their applications such as long lag phase and high sludge generation followed by the role of difficult process control in the direct application of fungal biomass in bioremediation. Apart from the associated advantages, fungal bioremediation also suffers certain shortcomings that can be overcome by the technological advances of fungal bioremediation. Under these advances, fungal-derived enzymes have gained priority over fungal biomass due to added advantages like minimal sludge generation and reduced bioremediation time with no lag phase followed by the easy process control.

Though enzyme applications themselves are not cost-effective and suffer the problem of low shelf life due to lower stability, development of techniques like immobilization of the whole cell and their enzyme has contributed toward enhancing the stability, thereby increasing shelf life and leading to the reuse of enzyme economically. Recent developments in the bioreactor-based applications of immobilized fungi have been successfully applied for bioremediation. Various bioreactors like fluidized beds and rotating biological contactors have been reported for bioremediation using immobilized fungi (Jebapriya and Gnanadoss 2013). Development of novel bioreactor systems for their application in the efficient removal of Reactive Green 19 dyes by white-rot fungi is continuously gaining importance (Sari et al. 2015).

Two white-rot fungi *P. ostreatus* IBL-02 and *P. chrysosporium* IBL-03 were successfully applied for aerobic treatment in combination with Photo-Fenton for the degradation of azo dye Reactive Blue 222 in a two-stage reactor (Kiran et al. 2013).

Continuous flow fungal membrane bioreactor was employed for the removal of two TrOCs (80–90% Bisphenol A removal and 55% diclofenac removal) using a white-rot fungus *T. versicolor*. In this study, significant removal of two TrOCs was reported in a non-sterile environment at 2 days hydraulic retention time (HRT) (Yang et al. 2013a). Bhattacharya et al. (2013) reported a strain of white-rot fungus *P. chrysosporium* for the degradation of HMW-PAHs using a novel strategy consisting of biphasic approach.

Under the culture conditions of ample nutrient, i.e., under ligninolytic culture condition, bioremediation of benzo[a]pyrene resulted in the concomitant formation of P450-hydroxylated metabolite due to up-regulation of PAH oxidizing monooxygenases. However, this metabolite was further removed during the subsequent non-ligninolytic phase.

Biopurification systems-based novel strategy has also been promoted in the bioremediation of pesticide-containing wastewaters, and its application was based on the use of highly active biological mixture, in particular, white-rot fungi which were highlighted earlier (Rodriguez-Rodriguez et al. 2013). As discussed earlier, bioremediation reflects a sustainable and environmentally friendly nature of treatment which was demonstrated using the application of mixed filamentous inoculum in a continuous large-scale bioreactor employed for bioremediation of sewage sludge derived from sewage treatment plant (Rahman et al. 2014). Synergistic application of fungi and their bacterial co-cultures has also been demonstrated to promote efficient degradation.

One such application of co-cultures consisting of *Fusarium* sp. PY3, *Bacillus* sp. PY1, and *Sphingomonas* sp. PY2 has been reported in synergistic degradation as well as the removal of pyrene and volatilized arsenic up to 96.0% and 84.1%, respectively. However, the bioremediation ability of the abovementioned co-culture was 87.2% in contaminated soil with 100 mg/kg pyrene (Liu et al. 2013). Another unique and innovative technique was developed using permeable novel reactive bio-barriers of *Trichoderma longibrachiatum* on nylon sponge. This approach was earlier adopted and demonstrated for PAH removal; phenanthrene was removed up to 90% in 14 days (Cobas et al. 2013). Textile wastewater treatment was also reported using whole-cell systems-based fungal biocatalysis (Spina et al. 2015).

4.8 Application of Fungal Proteomics and Genomics in Bioremediation

Numerous fungi can be exploited in the best possible way to biotechnological applications including bioremediation for the degradation of a diverse range of recalcitrant organic pollutants. A variety of fungi can be screened from their diverse habitats and based on their substrate selectivity. For instance, a fungal strain of *Byssoschlamys nivea* was reported for its well-defined growth in the presence of pentachlorophenol-contaminated soil samples; however, this reported fungal strain

could not be explored well for the benefit of humankind due to limited availability of their reference genomic data which in turn provides access on information related to biochemical processes. The advent of techniques like environmental genomics can also provide help in waste site treatment based on the clear insight into the ecology and microbial physiology that are being applied to the field of bioremediation (Srivastava 2015; Sharma 2015). Due to the developing interest in fungal genomics, it is possible to gather information related to complete sequences of fungal genomes which can further be applied in the wide comparison of genomes for their ability to execute bioremediation (Mougin et al. 2013).

Development of next-generation sequencing approach in deep sequencing has helped in the creation of 3'-cDNA libraries which in turn bridge the gap of sequence data and are helpful toward understanding the genetic basis of diversity. Due to the need of understanding the role of diverse catabolic processes contributing to the degradation of recalcitrant organic pollutants, the application of this sequencing approach could enable structural and functional investigations to gain access to the respective catabolic processes (Testa et al. 2012). Further application of whole-genome sequence analysis can be exploited to unravel the fungal capability for multiple metabolic adaptations due to their characteristic diversified enzyme functions such as cytochrome P450 monooxygenase (Ichinose 2013). However, there are some other genomic tools such as multiplex terminal restriction fragment length polymorphism (M-TRFLP) which helps in simultaneous profiling of multiple microbial taxonomic groups, thereby enabling deep understanding of different ecosystem-based taxa.

Apart from the studies related to genomic sequences and taxonomic groups, these tools can also be useful in the identification of pollution bio-indicators and environmental health as well as contribute toward studies related to environmental stress-based microbial response (Desai et al. 2010). Another advance in sequencing techniques involves the development of nuclease-mediated genome editing which is based on the application of a new engineered nuclease tool, i.e., TAL effector nuclease (TALEN), which is a specific nuclease tool for yeast and can be applied to other fungal species as well (Li et al. 2015).

4.9 Degradation Pathways in Fungi

Various downstream pathways are involved in fungal bioremediation, and their understanding is of extreme importance to get insight into mechanisms and reactions involved in bioremediation of pollutants. To date, various such degradative pathways like the MetaCyc database (MetaCyc.org) have been highlighted. Various databases have been investigated to describe different metabolic pathways and respective enzymes involved in reactions of bioremediation (Caspi et al. 2014). Apart from the action of enzymes such as laccases in pollutant removal, pollutant

removal using absorption onto the fungal biomass has been suggested as the removal mechanism. Applications of laccase derived from two strains of white-rot fungi, *T. versicolor* and *P. ostreatus*, were involved in the transformation of endosulfan to endosulfan sulfate along with the minute amount of endosulfan ether (Ulcink et al. 2013).

The key mechanism lying behind fungal decolorization has been indicated by a technique of electrospray ionization (ESI) analysis and revealed the mechanism of N-demethylation involved in synthetic dyes decolorization (VazAraLijo et al. 2013). On the other hand, two alternative routes were involved in the laccase-mediated ring cleavage reactions of anthracene during transformation by *Armillaria* sp. F022. In the first reaction, anthracene was oxidized to anthraquinone and benzoic acid followed by the secondary conversion of anthracene to other products such as 2-hydroxy-3-naphthoic acid and coumarin (Hadibarata et al. 2013). Though the research reports are flooded with the information regarding the application of fungal cultures and their purified enzymes in bioremediation studies, very few have reported the effect of bacterial-fungal ecological interactions and their mechanism for the removal of PAHs from soils.

Recently, metabolomics has been applied to reveal the degradation pathways involved in *Aspergillus nidulans* for the removal of monochlorophenols. Degradation of monochlorophenols involved intermediates like 3-chloro-cis,cis-muconate, whereas degradation intermediates of 4-chlorocatechol and 3-chlorocatechol degradation pathways were uncommon compounds like 3-chlorodienelactone and catechol, respectively (Martins et al. 2014). In vitro dissipation of PAHs was reported up to 46% after 21 days using *F. solani* and *Arthrobacter oxydans* (Thion et al. 2013). Degradation of polychlorinated PCDDs and PCBs was also reported using white-rot fungi. Dieldrin degradation using white-rot fungus, *Phlebia*, was studied, and its degradation was reported over 50% in 42 days.

The degradation pathway of this fungi involves hydroxylation reactions leading to three hydroxylated metabolite products. A similar strain of white-rot fungi has been reported for aldrin degradation over 90% in just 28 days. In this degradation mechanism, methylene moiety of aldrin was targeted by the fungi leading to new metabolite formation like 9-hydroxyaldrin followed by two carboxylic acid products (Xiao et al. 2011). Aromatic compound degradation involves a well-known 3-oxaloadipate pathway. A however better understanding of most of the steps in this pathway required the application of gene-replacement mutants, gene expression studies, as well as proteomics. Based on this study, catechol formation was detected using salicylate either directly or through 2,3-dihydroxybenzoate. Additionally, successive muconate isomerization reactions of the catechol branch have also been indicated (Martins et al. 2015).

4.10 Conclusion and Future Prospects

Though various isolated reports are available to demonstrate the bioremediation potential of fungi; however, due to the multifaceted role of fungi in bioremediation of xenobiotic compounds, only a few have reported the in-depth assessment of altogether all fungal features employed for performing this task. In this chapter, different fungal features have been highlighted, and efforts have been made to compile different fungal features on a common platform such as aspects describing diverse and novel metabolic capacities of fungi reflecting their bioremediation potential. Diverse fungal groups from extreme environments and their bioremediation potential have been elaborated followed by the in-depth discussion of the fungal role in the effective removal of heavy metals. Apart from effective pollutant removal, fungal-mediated remediation led to nanoparticle synthesis which has been highlighted as a potential area of research. In this chapter the major role of fungal-derived enzymes like peroxidases and laccases has been discussed.

However, in addition to well-studied enzymes that play an important role in providing tolerance to fungi toward pollutants, there is a presence of some stress response proteins like ABC transporters in fungi which help fungi to tolerate and grow in the presence of many toxic pollutants, thereby emphasizing the need for exploring these genes further. In this chapter the influential role of mycoremediation has been discussed, but this technique is facing a great hindrance, since information regarding the fungal activity, enzyme production in contaminated environments, as well as methodologies and ecological approaches required to sustain sufficient fungal biomass is lacking. In addition, knowledge related to bacteria-fungi associations and their spatiotemporal stability needs to be strengthened which not only improves our understanding of these interactions but also will be helpful in the development of novel and ecologically sound bioremediation approaches.

Further, advanced research is being carried to achieve a clear understanding of the bioremediation pathways. Also, whole-genome studies which are a result of advances in genomic research help to understand and explore the biodegradation pathways further. Identification and screening of the genes of interest thus obtained can not only be used to amplify the gene expression or engineer the respective organisms but can also be used to enhance bioremediation processes in various expression systems. In addition, fungal gene expression studies are significantly influenced by efficient biomarkers for bioremediation and can emerge out as an aid in bioremediation studies employing fungal systems.

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Chapter 5

Fungal Enzymes: Degradation and Detoxification of Organic and Inorganic Pollutants



Rekha Kumari, Archana Singh, and Ajar Nath Yadav

5.1 Introduction

The excessive use of chemicals in several industrial processes, agricultural practices, as well as in our day-to-day activities has resulted in the accumulation of various organic and inorganic pollutants in our environment. The hazardous pollutants of particular concern include industrial dyes, pesticides, fertilizers, halogenated solvents, petroleum hydrocarbons, endocrine disrupting chemicals and drugs, plastics, and heavy metals (Harms et al. 2011; Sharma et al. 2018; Devi et al. 2020b). Structurally, a major fraction of these pollutants have aromatic rings with phenyl groups attached to them. These pollutants are persistent in nature and pose a serious threat to our ecosystem. Also, evidences state that these pollutants are potent carcinogens and teratogens, capable of disrupting hormonal balance and reproductive capabilities in humans, birds, and several other mammals (Baker et al. 2019). The only concern is to protect the environment from degrading and avoiding the use of these chemicals to further reduce their pollution. Thus, to combat this growing environmental toxicity, cost-effective and efficient approaches are important (Yadav 2021).

A large number of conventional physio-chemical methods have been applied to treat or remove these toxic chemicals from environment. These traditional methods are effective but are also responsible for generating a huge amount of toxic by-products. Biological degradation or detoxification of these pollutants serves as an eco-friendly and economical tool in environmental cleanup. In order to be detoxified or degraded, these environmental pollutants need to be exposed to

R. Kumari (✉) · A. Singh
Department of Bio-Engineering, Birla Institute of Technology, Mesra,
Ranchi, Jharkhand, India

A. N. Yadav
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal
University, Baru Sahib, Sirmaur, Himachal Pradesh, India

suitable microorganisms (Singh et al. 2020a, b). A majority of these chemicals can easily be decomposed or transformed by microbes (Cameron et al. 2000). The metabolic potential of microorganisms is exploited to convert these toxic compounds into less toxic or non-toxic components (Watanabe 2001; Baker et al. 2019; Kour et al. 2020). Biological degradation or detoxification using fungal strains is attracting a large number of scientists due to diverse metabolic capacities of fungal strains (Rana et al. 2019a, b). Several bacterial strains have been reported as efficient decomposers of toxic pollutants; certain fungal strains have the capacity to tolerate higher concentrations of environmental pollutants. In case of high molecular weight pollutants such as polyaromatic hydrocarbons (PAHs), fungal strains can completely mineralize these compounds in comparison to bacterial strains that utilize these compounds as a source of carbon and energy for their survival (Mougin et al. 2009). Based on its ability to secrete a multitude of enzymes and also its capacity to thrive in diverse habitats, fungal strains serve as potential candidates for biological detoxification and degradation of different environmental pollutants (Deshmukh et al. 2016).

Most of the pollutant degraders from kingdom Fungi belong to the phyla Basidiomycota and Ascomycota which are followed by the subphylum Mucoromycotina (Harms et al. 2011). Several white-rot fungi belonging to division Basidiomycota are considered chief agents for degrading lignininous materials and are also capable of detoxifying endocrine disrupting chemicals and pharmaceuticals which are capable of adversely affecting human health and aquatic life (Deshmukh et al. 2016). The biodegradation potential of white-rot fungi is by the virtue of different ligninolytic enzymes produced by them such as laccases and peroxidases. These enzymes help in degrading several organic pollutants including pesticides and waste generated from textile industries (dos Santos Bazanella et al. 2013). Filamentous fungi, such as *Acrimonium*, *Curvularia*, *Pythium*, and *Aspergillus*, have been investigated for their potential to effectively degrade heavy metals (Akhtar et al. 2013).

Several marine fungi have been exploited for their potential to degrade and detoxify toxic and persistent organic pollutants, textile wastes, and heavy metals (Bonugli-Santos et al. 2012; Verma et al. 2012; Gao et al. 2013). This could be attributed to the fact that the marine fungi are capable of producing laccase enzyme tolerant to higher salt concentrations and also resistant to phenolics present in the waste (Divya et al. 2013). Fungal species isolated from extreme environments are significant from industrial point of view based on the fact that the enzymes extracted from these fungi are capable of tolerating harsh conditions such as extreme temperature and pH (Neifar et al. 2015). Effluent treatment plants serve as potential habitat that could be exploited for several fungi with capability for diverse biodegradation and detoxification applications, keeping in mind that they are exposed to higher levels of pollutants released with industrial effluents (Deshmukh et al. 2016; Yadav et al. 2020b, c). However, it might be a possibility that fungi capable of decomposing a particular pollutant may be sensitive to other toxic components present in the environment (Mougin et al. 2009). One way to deal with this problem is to isolate fungal enzymes or a mixture of enzymes (Baker et al. 2019).

Laccases, peroxidases, oxygenases, and oxidoreductases are a major class of fungal enzymes involved in catabolism of environmental pollutants (Harms et al. 2011; Sharma et al. 2018; Kour et al. 2019). Laccases are copper-containing enzymes that are extracellular in nature, reported to oxidize phenolic and non-phenolic compounds present in the environmental pollutants and also detoxify textile dyes and effluents (Fillat et al. 2012; Verma et al. 2012; Margot et al. 2013; Viswanath et al. 2014). Peroxidases are iron-containing group of enzymes that are involved in detoxification and degradation processes. They have been categorized into manganese peroxidase, lignin peroxidase, and versatile peroxidases (Doddapaneni et al. 2005). Oxidoreductases are extracellular enzymes capable of detoxifying pollutants using oxidative coupling reactions using different oxidizing agents (Baker et al. 2019). Certain fungal species possess intracellular network of enzymes, containing glutathione transferase and cytochrome monooxygenase to deal with several organic and inorganic pollutants. Biodegradation and detoxification of recalcitrant compounds serve as the most economical and green route for environmental cleanup, and fungal species happen to be the most important component in the ecosystem owing to the multiple ways that these fungi offer to deal with contamination (Deshmukh et al. 2016). In this chapter, we will review the mechanism involved in detoxification and degradation processes using fungal enzymes. Also, we will discuss about different classes of fungal enzymes involved in the biodegradation process.

5.2 Sources of Organic and Inorganic Environmental Pollutants

Several organic and inorganic pollutants accumulating in the environment are the result of various industrial, agricultural, and domestic practices carried out by humans on a daily basis. The textile industries are the major contributors of synthetic dyes released in the environment which are considered carcinogenic and toxic to the aquatic organisms. Other industries where synthetic dyes are being used and washed down the drains are paper and pulp industries, food industries, cosmetic, plastic, and drug industries (Levin et al. 2005; Asgher et al. 2008). Various pharmaceutically active compounds such as nonylphenols, Bisphenol A, estrogens, stilbene, triclosan, etc. are common endocrine disrupting agents which are of grave concern to human and animal health (Asgher et al. 2008; Kumari and Ghosh Sachan 2019). Polycyclic aromatic hydrocarbons such as naphthalene, benzopyrene, anthracene, and pyrene are widespread organic pollutants produced from incomplete combustion of fossil fuels, gas plants, and accidental spillage of fuels (Necibi and Mzoughi 2017).

The use of synthetic pesticides and fertilizers in agricultural sector also contributes to the presence of organic pollutants (halogenated aromatic compounds such dichlorophenol, trichlorophenol, and their derivatives such as chlordane, DDT, and lindane) in the environment. Other pollutants of concern include bleach effluents

generated from the paper and pulp industry, which contain chlorinated phenols and other compounds such as azo dyes and bleaching chemicals (D'Souza et al. 2006). The heavy metal pollution in the environment can be attributed to both natural and anthropogenic activities. The industrial effluents containing heavy metals constitute the major source of metallic pollution in the aquatic environment (Necibi and Mzoughi 2017).

5.3 Fungal Mechanism Involved in Degradation and Detoxification of Pollutants

Many researchers have categorized the fungal metabolism of pollutants into three major strategies (Fig. 5.1). In the first category, the target compound is used as a source of carbon and energy by fungal species. The degradation and detoxification of the recalcitrant compound is carried out directly, where it is completely mineralized into carbon dioxide and other simple compounds. This is the most preferred degradation mode as the pollutant is completely or nearly removed from the environment (Mougin et al. 2009). The major limiting factor in this method is the availability of nutrients as the substrate is used by fungal species for its growth and survival (Baker et al. 2019). Co-metabolism is another strategy, which the fungal species employ in detoxifying various pollutants. In this method, the target compound is attacked enzymatically but is not utilized as a source of carbon and energy by the fungi. This results in minor structural changes in the compound,

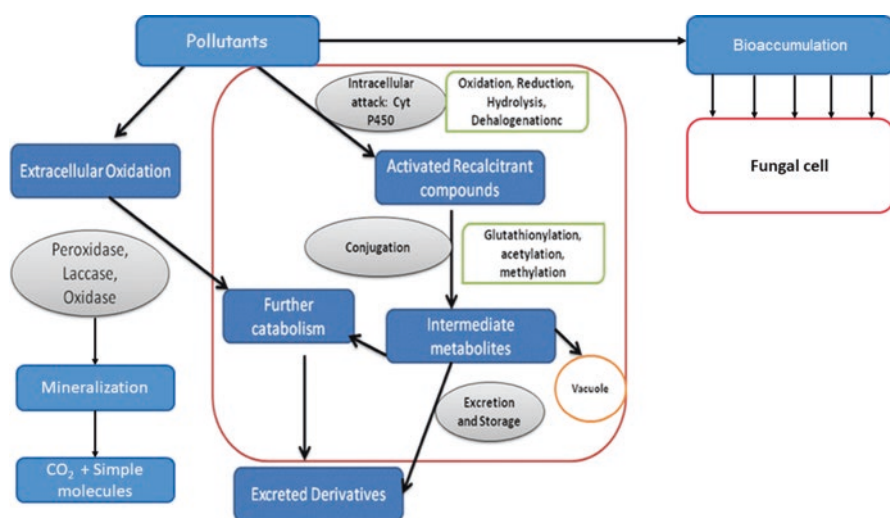


Fig. 5.1 Mechanisms involved in fungal degradation and detoxification of various environmental pollutants. (Adapted with permission from Deshmukh et al. (2016))

thereby reducing the bioavailability and toxicity of the target compound (Baker et al. 2019).

These transformed compounds can further be utilized by other microorganisms leading to complete mineralization of the target compound. Several fungi are capable of transforming toxic pollutants into high molecular weight components owing to the oligomerization and conjugation of the parent compounds (Singh and Yadav 2020; Yadav et al. 2020a). These structural modifications help in reducing the toxicity of the parent compounds, thereby removing it from the list of hazardous chemicals. Bioaccumulation is the third pathway involved in detoxification of pollutants by fungi. The toxic pollutant is taken up by the species and concentrated within the organism instead of being metabolized by fungal species. Pollutants such as synthetic dyes and heavy metals are detoxified using the bioaccumulation potential of several fungal species (Joutey et al. 2013).

5.4 Fungal Enzymes Involved in Degradation and Detoxification Processes

Fungi are capable of producing various industrially important enzymes such as lac-cases, lipases, catalases, peroxidases, proteases, cellulases, xylanases, amylases, etc. that can be potentially applied in removing organic pollutants from the environment (Marco et al. 2013; Rastegari et al. 2020a, b). These enzymes can be helpful in decomposing agricultural wastes and other effluents generated from food and beverage industries that contain polymeric substances such as xylan, cellulose, starch, protein, lipids, etc. The hydrolyzed end products generated can further be decomposed to produce certain value added compounds such as fatty acids and biogas (Khardenavis et al. 2013; Hattori et al. 2015).

The ligninolytic enzyme released by white-rot fungi is not only limited to degrad-ing natural lignin found in the environment but can also be helpful in degrading pollutants such as azo dyes. The structure of azo dyes is modified destructing the chromophore group in it, thereby resulting in the formation of phenoxyl radicals (Gul and Donmez 2013). These extracellular ligninolytic enzymes responsible for carrying out the oxidation of lignin are divided into two categories: peroxidases and laccases. Laccases and monooxygenases use molecular oxygen as terminal electron acceptors, whereas peroxidases use hydrogen peroxide as terminal electron accep-tors in oxido-reductive catalyzing reactions (Baker et al. 2019). There are several evidences that validate the involvement of these enzymes in detoxification and deg-radation of toxic environmental pollutants owing to their relatively lower substrate specificity (Harms et al. 2011; Ikehata 2015). The focus is to genetically engineer these enzymes using recombinant technology to further enhance the degradation rates of toxic recalcitrant compounds.

5.4.1 Laccase

Laccases are extracellular enzymes belonging to blue oxidase group that uses molecular oxygen as co-substrate and copper ions as co-factor (Deshmukh et al. 2016). These copper-containing enzymes are secreted by fungi belonging to class basidiomycetes, deuteromycetes, and ascomycetes (Harms et al. 2011). The potential application of laccase enzyme in the field of biodegradation and wastewater treatment includes detoxification and decolorization of dyes released from textile industries, decomposing effluents released from paper and pulp industries, and detoxification of recalcitrant compounds from agricultural sector and wastewater treatment plants (polycyclic aromatic hydrocarbons, chlorophenols, endocrine disrupting agents, and pesticides) and also helps in degrading metallic pollution and other hazardous compounds released from different anthropogenic activities (Harms et al. 2011; Yadav 2020).

Laccases are capable of catalyzing phenolic and non-phenolic components present in the effluents resulting in the formation of free radicals and release of molecular oxygen. The free radicals in turn undergo coupling reaction either with the same molecule or a different one, thereby reducing the toxicity of the pollutant. This coupled intermediate can further be degraded by demethoxylation, dechlorination, and decarboxylation (Deshmukh et al. 2016; Baker et al. 2019). The laccase-dependent oxidative coupling reaction results in the formation of high molecular weight compounds that potentially negates the toxic effects of parent compounds on human health (Tsutsumi et al. 2001; Junghanns et al. 2005). Laccase is considered to be a prominent enzyme involved in environmental cleanup of toxic pollutants owing to the fact that it uses oxygen as an oxidizing agent resulting in the formation of water as an end product (Baker et al. 2019). As compared to peroxidase enzyme, laccases were found to tolerate a wide range of pH (2–10) and also has broad substrate specificity (Xu 1996).

The activity of laccase on a variety of substrate is non-specific in nature, making it an ideal catalyst for different industrial applications of which this enzyme has been extensively exploited for its biodegradation potential (Viswanath et al. 2014). Laccase enzyme extracted from three basidiomycete species (*Corioloopsis rigida*, *Pycnoporus coccineus*, and *Trametes villosa*) and *Myceliophthora thermophila*, an ascomycete, was investigated for its potential to decolorize the flexographic ink used in paper and pulp industries (Fillat et al. 2012). Moreover, laccase enzymes secreted by marine fungal species were found capable of mineralizing, detoxifying, and decolorizing a synthetic dye, Reactive Blue 4, at a concentration of 1 gm/L (Verma et al. 2012; Viswanath et al. 2014). Laccase enzyme purified from basidiomycete species *Trametes versicolor* and *Pleurotus ostreatus* was helpful in detoxifying polychlorinated biphenyls (PCBs) (Keum and Li 2004). The mechanism involved in detoxifying PCBs revolves around dechlorination and substrate oligomerization. Laccases carry out the extracellular digestion of polyaromatic hydrocarbons by catalyzing the initial oxidation of these compounds (Pozdnyakova et al. 2018).

The purified laccase enzyme from *Coriopsis gallica* catalyzed the oxidation of different polyaromatic hydrocarbons such as dibenzothiophene, carbazole, and N-ethylcarbazole with the formation of certain mediators like 2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid and 1-hydroxybenzotriazole (Viswanath et al. 2014). An ascomycete, *Fusarium incarnatum*, secreted laccase enzyme that was applied to degrade an endocrine disrupting agent, Bisphenol A, at a concentration of 200 mg/L (Chhaya and Gupte 2013). In another study, it was observed that the laccase enzyme obtained from *Trametes versicolor* was capable of degrading different pharmaceutical drugs such as mefenamic acid and diclofenac present in municipal wastewater (Margot et al. 2013). The extremophilic fungi isolated from the Himalayan region, *Penicillium pinophilum*, has also shown the capacity to produce laccase enzyme at low temperatures (Dhakar et al. 2014).

Though the mode of action of laccase enzyme secreted under extreme conditions is less known, only the laccase enzyme extracted from extremophilic ascomycetes, *Thielavia arenaria* and *Melanocarpus albomyces*, has been structurally explored showing difference in proton transfer process than other laccases by having a "C-terminal plug" in its structure (Kallio et al. 2011). Despite having tremendous potential in the field of biodegradation, the use of laccase enzyme is limited owing to its low shelf-life. One way to improve the shelf-life of laccase enzyme is to immobilize the enzyme to different carriers that will help in enhancing the laccase activity over a broad range of temperature and pressure (Patel et al. 2014). The application of covalently immobilized laccase enzyme was observed in case of *Trametes versicolor*, and it was reported that this immobilized laccase enzyme efficiently degraded several polyaromatic hydrocarbons such as naphthalene, phenanthrene, and anthracene from the contaminated site (Bautista et al. 2015). Another strategy that could be employed in enhancing the catalytic activity of laccase enzyme is to genetically engineer this enzyme to improve its activity over a broader range of substrate and different environmental factors (Mate et al. 2011; Torres-Salas et al. 2013).

5.4.2 Peroxidase

Peroxidases are heme proteins containing iron (III) protoporphyrin IX as a prosthetic group with hematin compound as a co-factor. Peroxidases are responsible for carrying out the oxidation of several phenolic and non-phenolic compounds of which degradation of lignin is of prime importance that occurs at the expense of hydrogen peroxide (Piontek et al. 2001). Peroxidases are ubiquitous in nature and can be commonly found in all living organisms such as microbes, plants, and animals. Among the microbes, bacterial and fungal species are exploited on a regular basis to extract peroxidase enzyme for several industrial applications. Fungal peroxidases have shown significant potential in removing different organic and inorganic pollutants from the environment. Chlorinated phenols, cresols, synthetic azo dyes, and phenols can easily be oxidized by peroxidase enzyme,

thereby reducing the toxic effect of these compounds on the ecosystem (Karigar and Rao 2011).

Based on the presence of heme group, peroxidases are divided into two groups: (1) heme peroxidases and (2) non-heme peroxidases (Passardi et al. 2007). More than 80% of the known peroxidases are heme-containing peroxidases. The focus is on heme peroxidases which is further divided into two superfamilies: (1) peroxidase-cyclooxygenase superfamily (PCOXS) and (2) peroxidase-catalase superfamily (PCATS) (Zamocky and Obinger 2010). Animal peroxidases belong to PCOXS family which is basically involved in the innate immunity and hormonal regulation (Dick et al. 2008; Karigar and Rao 2011). The PCATS family contains peroxidases of non-animal origin secreted from plants, bacteria, fungi, and algae. The PCATS family is further classified into three classes, of which Class II peroxidase enzyme is of utmost importance. Class II peroxidases contain manganese peroxidase (MnP), lignin peroxidase (LiP), and versatile peroxidase that have been extensively investigated for their potential to degrade toxic pollutants (Karigar and Rao 2011).

5.4.2.1 Lignin Peroxidase

Lignin peroxidases are heme-containing enzymes that are secreted mainly by white-rot fungi during secondary metabolism and are involved in metabolizing various aromatic compounds in the optimum pH range of 2–5 (Shrivastava et al. 2005). These lignin peroxidases isolated from basidiomycete fungi are extracellular in nature and are generally 38 kDa to 43 kDa in weight (Falade et al. 2017). A major fraction of phenolic compounds is oxidized by lignin peroxidase enzyme in the presence of hydrogen peroxide as a co-substrate which is converted into water in the oxidation process. In this degradation process, veratryl alcohol is the mediator that donates an electron to lignin peroxidase to return to its native state resulting in the formation of veratryl aldehyde. Veratryl aldehyde is converted to veratryl alcohol in the end by gaining an electron from the substrate (Karigar and Rao 2011; Baker et al. 2019). LiPs play a pivotal role in mineralizing lignin present in the cell wall of plants and have also been found capable of completely mineralizing polyaromatic hydrocarbons (Karigar and Rao 2011; Kadri et al. 2017).

Phanerochaete and *Trametes* sp. are known producers of LiPs. LiP having a broad substrate specificity was isolated from *P. chrysosporium* which was found capable of transforming several polyaromatic hydrocarbons such as anthracene, 1-methylantracene, benzo[a]pyrene, 2-methylantracene, fluoranthene, dibenzothiophene, acenaphthene, 9-methylantracene, and 2-methylantracene (Pozdnyakova 2012). This expanded substrate range of LiPs can be attributed to the presence of veratryl alcohol as a co-substrate in the reaction that increases the rate of oxidation in terminal or weak substrates. LiP converts or oxidizes different aromatic compounds with 8 eV ionization potential and redox potentials higher than 1.4 V (Piontek et al. 2001; Baker et al. 2019). In non-phenolic compounds, LiPs are capable of cleaving the ester bonds, thereby signifying its biodegradation potential toward several aromatic toxic pollutants (Pozdnyakova 2012).

5.4.2.2 Manganese Peroxidase

MnPs are also heme-containing extracellular enzymes that function under a less acidic pH range of 4–7 unlike that of LiP (Asgher et al. 2008). It has been reported that MnP shares most of its characteristics and mechanisms to that of LiP (Deshmukh et al. 2016). MnPs are not found in the large gene family which is the key feature of many other ligninolytic enzymes such as laccase (Torres-Farrada et al. 2017). MnP enzyme is commonly found in white-rot fungi belonging to Basidiomycota group (Harms et al. 2011). Chlorophenol and monoamino-dinitrotoluene derivatives are formed when the MnPs cleave the aromatic rings present in the environmental pollutants (Harms et al. 2011; Baker et al. 2019). In a multistep reaction, Mn^{2+} is oxidized to Mn^{3+} by MnP. Mn^{2+} acts as a substrate for MnP, thereby enhancing the production of MnP. The Mn^{3+} ion produced by MnP acts as a mediator in the oxidative reactions of different phenolic compounds in the presence of MnP (Karigar and Rao 2011).

The substrate range is broad in the case of MnP, and also the transformation process of different metabolites is near complete owing to the fact that MnP degrades the compounds indirectly. Fungal MnP has shown tremendous potential in degrading phenolics from industrial wastes, decolorizing synthetic dyes from textile effluents, removes endocrine disrupting chemicals and chlorinated toxins released in the environment, degrades various polyaromatic hydrocarbons, and detoxifies insecticides, pesticides, and other agricultural wastes (Bansal and Kanwar 2013). Manganese peroxidase enzyme purified from a saprophytic fungus, *Phanerochaete chrysosporium* RP 78, was found capable of decolorizing different azo dyes under optimal conditions (Ghasemi et al. 2010). *Pleurotus ostreatus*, an edible macroscopic fungi, produced extracellular MnP that decolorized Remazol Brilliant Blue R dye and other structurally important azo dyes (Faraco et al. 2007). MnP isolated from fungus, *P. sordida*, was reported to degrade dioxins such as polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzodioxins (PCDD) (Kasai et al. 2010).

MnPs secreted from *Anthracyllum discolor* were investigated for its potential to degrade polyaromatic hydrocarbons such as phenanthrene, fluoranthene, anthracene, and pyrene along with their derivatives (Pozdnyakova 2012). Zhang et al. (2016) reported another MnP produced by *Trametes* sp. capable of oxidizing azo and indigo dyes along with other polyaromatic hydrocarbons. MnP produced by *Peniophora incarnata* demonstrated exceptional ability to degrade anthracene which is transferrable by expression in heterologous yeast, promising it to be a potential biodegradation tool (Bilal et al. 2017). 40 mM of bisphenol was degraded in 1 h using 10 U/mL of manganese peroxidase purified from *Pleurotus ostreatus* (Huang and Weber Jr 2005).

Cross-linked enzyme aggregates of MnP isolates from *Ganoderma lucidum* efficiently degraded two toxic endocrine disruptors, triclosan and nonylphenol (Bilal et al. 2017). The limiting factor in case of MnP being used as a detoxifying agent is the presence of suitable chelators (Bogan et al. 1996). Organic acids such as malonic and oxalic acids are used as common chelators in case of MnP (Kadri et al.

2017). Redox mediators such as Tween 80 have significantly increased the activity of MnP enzyme. In the presence of Tween 80, MnP has shown the ability to transform and detoxify compounds with an ionization potential of 8.2 eV (Baker et al. 2019).

5.4.2.3 Versatile Peroxidase

Versatile peroxidases (VPs) are heme-containing extracellular hybrid enzymes that lie between LiP and MnP and are capable of directly oxidizing Mn^{2+} to Mn^{3+} and also oxidize phenols and aromatic compounds with high redox potential (Harms et al. 2011). VPs combine the mechanisms and substrates of LiPs and MnPs, thereby showing a much broader range of substrate specificity (Kues 2015). So far, VPs have been isolated from basidiomycetes species (Harms et al. 2011). Camarero et al. (1999) characterized the initial VPs from *Pleurotus eryngii* that contained combined features from MnPs and LiPs – aromatic substrate oxidation center from LiPs and Mn oxidation domains from MnPs. VP has shown unusual substrate specificity and also the capacity to oxidize these substrates in the absence of manganese as compared to other peroxidases. VP oxidizes both phenolic and non-phenolic lignin model dimers (Ruiz-Dueñas et al. 2009). The role of VPs in the mediation of xenobiotic compounds is not clearly understood, but it has been evident the presence of xenobiotic compounds; specifically polyaromatic hydrocarbons stimulate the production of VPs (Pozdnyakova et al. 2018).

Versatile peroxidase purified from fungus, *Thanatephorus cucumeris*, was found capable of decolorizing anthraquinone dye Reactive blue 5 (Sugano et al. 2006). Another versatile peroxidase from *Bjerkandera adusta* transformed pentachlorophenol totetrachloro-1,4-benzoquinone in the presence of hydrogen peroxide by oxidative dehalogenation mechanism (Longoria et al. 2008). VP secreted by *P. eryngii* and *P. ostreatus* oxidized Mn^{2+} to Mn^{3+} as that of MnPs and also oxidized non-phenolic compounds similar to that of LiPs (Ruiz-Dueñas et al. 2009). Due to its broad substrate specificity and different detoxification mechanism, VP demands further exploration and investigation for its potential to be applied in the field of biodegradation and bioremediation.

5.4.3 Cytochrome Monooxygenases

Certain fungi possess intracellular network of enzymes involved in the transformation and detoxification of diverse range of pollutants. These are cell-bound enzymes commonly found in the organisms belonging to fungal classes Ascomycota, Basidiomycota, Mucoromycotina, and Chytridiomycota (Harms et al. 2011). Cytochrome monooxygenases belong to a larger group of enzymatic family, i.e., oxygenase, chiefly known to catalyze the aerobic detoxification and degradation of various aromatic pollutants using oxygen (Sharma et al. 2018). Monooxygenase

(addition of one oxygen atom) and dioxygenase (addition of two oxygen atoms) are the two different categories of oxygenase enzymes based on the number of oxygen atoms used in the oxygenation process. Many environmental pollutants such as alkanes, alkyl-substituted aromatics, polyaromatic hydrocarbons, and dioxins are detoxified by oxygenase enzyme, most extensively investigated being cytochrome P450 enzyme that utilizes NADPH as a co-factor in redox reactions (Doddapaneni et al. 2005).

Fungal cytochrome P450 can act as a suitable alternative to chemical catalysts as it is a versatile catalyst for stereo-specific and region-specific oxidation of different non-activated hydrocarbons (Deshmukh et al. 2016). The metabolism of various endogenous and exogenous compounds involving cytochrome P450 systems has been very well explained by Ichinose (Ichinose 2013). Mitochondrial and cytosolic isoforms of cytochrome P450 enzyme extracted from *Fusarium oxysporum* and other fungal species were involved in the detoxification of dioxins (Guengerich and Munro 2013; Sakaki et al. 2013). Moreover, these monooxygenase enzymes isolated from *F. oxysporum* have also been involved in the production of ω -hydroxy fatty acids (Durairaj et al. 2015). The genome of *Phanerochaete chrysosporium* contains 149 isozymes of cytochrome P450 enzyme making it a massive enzymatic gene family also found in some other fungi (Syed et al. 2011; Olicon-Hernandez et al. 2017; Baker et al. 2019).

The cytochrome P450 monooxygenase isolated from white-rot fungus, *P. chrysosporium*, was investigated for its potential to degrade aliphatic hydrocarbons (n-alkanes), endocrine disrupting agents, and different high-molecular weight polyaromatic hydrocarbons that are mutagenic and carcinogenic (Syed et al. 2013). It was observed that prior induction of P450 monooxygenase enzyme resulted in enhanced degradation of polyaromatic hydrocarbons (Bhattacharya et al. 2013). The cytochrome P51, a sub-family of cytochrome P450 enzyme isolated from Ascomycota and Basidiomycota phyla, was found capable of degrading and detoxifying benzoate derivatives. Moreover, cytochrome P405, another sub-family of cytochrome P450 monooxygenase degraded phenylacetate derivatives (Baker et al. 2019).

A non-ligninolytic fungi, *Scopulariopsis brevicaulis*, showed complete mineralization of anthracene with the production of 9,10-anthraquinone as a major metabolite in the presence of cytochrome P450 monooxygenase enzymatic system (Godoy et al. 2016). Cytochrome P450 monooxygenase has also been explored for its potential to detoxify hazardous pharmaceutical agents such as antibiotics, β -blockers, and anti-inflammatory drugs (Olicon-Hernandez et al. 2017). These cytochrome monooxygenases detoxified naproxen, an environmentally toxic pharmaceutical agent that found its way into drinking system due to its overuse in treating certain mammalian diseases. The degradation took place via hydroxylation and demethylation (Aracagok et al. 2017). Various molecular tools such as vector expression systems can be used to tailor monooxygenase enzyme to further enhance their capability to degrade environmentally toxic pollutants.

5.4.4 Catalase

One of the most common limitations with biological systems is the accumulation of reactive oxygen species (ROS), resulting in damage to cellular macromolecules which is hazardous for the cell. In case of fungal species, the primary defense mechanism adopted to cope with ROS generation involves the usage of monofunctional catalase enzyme and a combination of bifunctional catalase/peroxidase enzyme. One of the major reasons for the production of ROS in microbial cells could be attributed to the presence of heavy metals such as cadmium, lead, mercury, and zinc. It was observed that *Aspergillus foetidus* showed tolerance toward 200 mg/L of lead, resulting in the elevated levels of anti-oxidative enzymes such as catalase to detoxify malondialdehyde along with hydrogen peroxide (Chakraborty et al. 2013). Similarly, *Aspergillus* spp. showed enhanced levels of tolerance toward oxidative stress caused by heavy metals such as 750 mg/L of zinc and 100 mg/L of copper (Mitra et al. 2014).

The effect of heavy metals on fungal physiology is little known. Inhibition of catalase and peroxidase activity with an increase in cytochrome P450 monooxygenase enzyme was observed when *P. chrysosporium* was exposed to 50–100 μ M of lead or cadmium (Zhang et al. 2015). When the similar amount of lead and cadmium, individually or in combination, was used for a fungal consortia containing *Penicillium* sp., *Rhizopus* sp., and *Aspergillus niger*, a higher amount of catalase activity was recorded (Thippeswamy et al. 2014). Owing to the fact that several fungal species are tolerant toward toxic heavy metals due to the presence of catalase activity in them, this enzyme can be used in detoxification of metal-contaminated sites (Kumar et al. 2019).

5.4.5 Unspecific Peroxygenases

Functionally, unspecific peroxygenases (UPOs) are considered as a hybrid of peroxidase and monooxygenase enzyme. Talking phylogenetically, unspecific peroxygenase enzyme belongs to the heme-thiolate peroxidases family. Two structural motifs have been observed in case of UPOs: short UPOs and long UPOs of approximately 29 kDa and 44 kDa, respectively. Almost all the fungal classes are capable of secreting short UPOs, but only Ascomycota and Basidiomycota are capable of secreting long UPOs. Both these UPOs use hydrogen peroxide as a cofactor with a diverse range of substrates to act upon. Out of the priority pollutants listed by EPA, UPOs were capable of transforming 41 toxic pollutants. Despite their role in utilizing toxic pollutants as substrates, the physiological role of UPOs is yet to be explored (Olicon-Hernandez et al. 2017). It has been hypothesized by Karich et al. (2017) that these UPOs might be working in coordination with cytochrome P450 monooxygenase enzyme, where UPOs convert these toxic pollutants into some other metabolites which are further used as a substrate by monooxygenase enzyme,

which fine-tunes the metabolites to be further used by cells as a source of carbon (Karich et al. 2017).

Agrocybe aegerita produced UPO that was found capable of transforming a large number of toxic pollutants phenol, anisole, naphthalene, phenanthrene, benzidine, nitroarenes, anthracene, toluene, fluorine, 2,4-dimethylphenol, ethylbenzene, benzo[a]anthracene, acenaphthene, 1,4-diphenylhydrazine, and polychlorinated benzene (Karich et al. 2017). Another UPO extracted from *Coprinellus radians* was found capable of degrading brominated compounds, aryl alcohols, and naphthalene (Hofrichter et al. 2015). *Marasmius rotula*, a subtropical mushroom, secreted UPO that could not oxidize halides but instead degraded bulkier substrates (Poraj-Kobielska 2013). Even other mushrooms were capable of secreting UPOs, but these have not been purified and characterized yet. These UPOs are of great importance in treating the environment polluted with several toxic compounds.

5.5 Metabolism of Xenobiotic Degradation by Fungal Enzymes

There are basically two steps involved in the xenobiotic detoxification by extracellular enzymes secreted by fungal species (Rao et al. 2010). In the first step, the hydrolase activity is initiated upon the macromolecules by the hydrolytic system of the fungal species under investigation (Devi et al. 2020a). Secondly, the ligninolytic system covering specific oxidative enzymes is activated to further act upon the hydrolyzed substrate and transform it accordingly. In this section, we will focus on the enzymes belonging to oxidoreductase family whose mechanisms have been very well explained.

5.5.1 Laccase

These are multicopper oxidases belonging to a broad group of enzymes called polyphenol oxidases. They have copper atoms in the catalytic center of the enzyme. The blue color of the laccase enzyme is due to the presence of copper in it. Those who do not have blue color are called white or yellow laccases. Laccase enzymes in their resting state contain four copper atoms in +2 oxidation state. The four copper atoms present in the laccase enzyme are characterized as T1, T2, and T3, of which T3 is binuclear. The copper atom characterized as T1 helps in substrate oxidation, where T2 and T3 facilitate the storage of resulting electrons which later aids in converting molecular oxygen into water. Laccases mediate the coupled redox reactions involving a substrate and diatomic oxygen, thereby forming water and releasing a radical cation. Laccase enzyme is capable of binding to different substrates with the help of type I copper that is present on the enzyme surface in a wide cavity (Su et al. 2018).

The importance of laccase enzyme in biodegradation and detoxification of recalcitrant compounds can be enhanced by laccase-mediated systems found in several fungal species that act as an electron transfer chain and increase the kinetic favorability of detoxification by the enzyme along with broadening the substrate specificity of the enzyme (Wong et al. 2013; Rastegari et al. 2019b). The breakdown of different polyaromatic hydrocarbons by laccase enzyme occurs with the oxidation of one electron and reduction of one oxygen molecule resulting in the formation of water (Brijwani et al. 2010). Oxygen-centered free radical formation takes place following the oxidation reaction which in a second-enzyme catalyzed reaction is converted to quinone. Laccase-mediated catalysis of xenobiotic compounds takes place in three steps: (1) reduction of type I copper by the substrate; (2) type II copper and type III copper forming trinuclear cluster gain electron from type I copper in the electron transfer process; and (3) water molecule is formed at the trinuclear cluster due to reduction of oxygen (Gianfreda et al. 1999; Jones and Solomon 2015).

The catalysis of non-phenolic substrates by laccase enzyme occurs with the help of mediators in this process. In case of non-phenolic compounds as substrates, the laccase-mediated catalysis takes place in two steps: (1) the low molecular weight organic compounds called mediators are oxidized by laccase enzyme to form high molecular weight cations and (2) oxidation of non-phenolic substrates by these cations along with the laccase enzyme (Fig. 5.2). Some of the commonly involved synthetic mediators are 2,2'-azinobis-3-ethyl-benzthiazoline-6-sulfonate (ABTS),

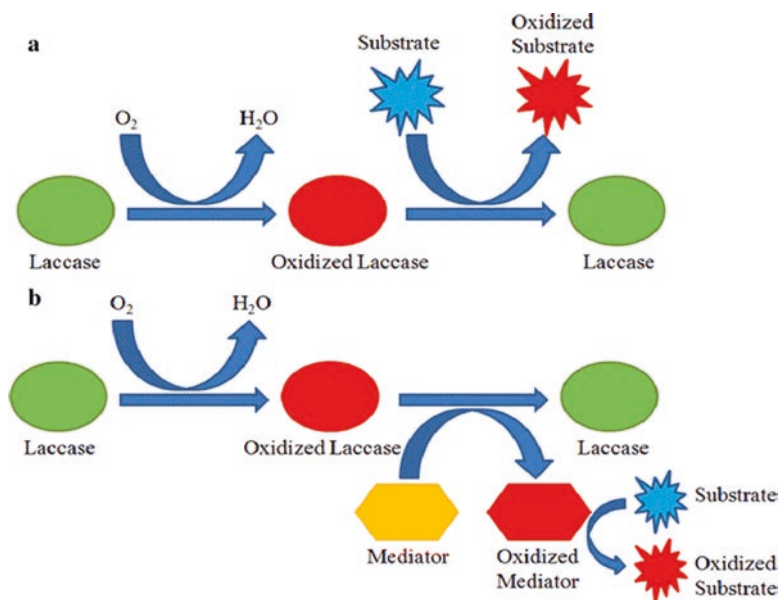


Fig. 5.2 Laccase-mediated catalysis of phenolic and non-phenolic compounds. (Adapted with permission from Agarwal et al. (2018))

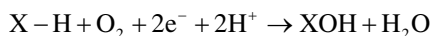
1-hydroxybenzotriazole (HOBT), and N-hydroxyphthalimide (NHPI) (Gochev and Krastanov 2007).

Laccases tend to directly catalyze compounds with a relatively low redox potential ranging between 0.4 and 0.8 V. Compounds having high redox potential or showing steric hindrance cannot be directly oxidized by laccase-mediated systems. Laccase enzymes are mostly active in the acidic pH range and show less or rare activity in the alkaline or neutral pH range (Baldrian 2006; Majeau et al. 2010).

5.5.2 Cytochrome P450 Monooxygenase

Cytochrome P450 (CYP450) belongs to heme-containing enzymatic superfamily that shows remarkable variation and plays a pivotal role in metabolism of various xenobiotic and endogenous compounds (Moktali et al. 2012; Baker et al. 2019). Depending upon different proteins and reactionary motifs involved in this enzyme, CYP450 enzymes are divided into ten different classes. Three classes of these enzymes have been identified in fungal species: Class II, Class VIII, and Class IX (Cresnar and Petric 2011). CYP and CPR are the two integral membrane proteins found in the Class II CYP450 enzymes, which contain FAD and FMN as the prosthetic cofactors, delivering two electrons to the heme-moiety from NAD(P)H. Fungal species most commonly use CYP450 monooxygenase enzyme (Class II CYP450) that adds one oxygen atom to the substrate, thereby facilitating its detoxification and degradation (Durairaj et al. 2016).

CYP450 enzymes catalyze the stereospecific and regioselective oxidation reaction in case of non-activated hydrocarbons. This type of catalysis results in epoxidation, dealkylation, heteroatom oxygenation, and hydroxylation of C=C bonds (Deshmukh et al. 2016; Durairaj et al. 2016; Olicon-Hernandez et al. 2017). In the generalized mechanism of CYP450 monooxygenase enzyme, one of the atoms of molecular oxygen is added to the substrate molecule, while the other oxygen atom is reduced in this enzymatic reaction to form water (McLean et al. 2005; Urlacher and Eiben 2006). The chemical reaction involved is as follows:

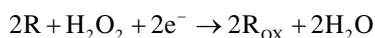


The X-H in the above reaction acts as a substrate for CYP450 enzyme. The electron donor in the above reaction is either NADPH or NADH that helps in reducing the second oxygen atom into water molecule (Kues 2015). The catalytic hydroxylation of substrate mediated by CYP enzymes involves the following steps: (1) binding of substrate is the first step in this process, where the substrate migrates to the active site of the enzyme and binds to the iron group of CYP in its oxidized state (Fe^{3+}); (2) CYP450 reductase transfers one electron to the ferric ion (Fe^{3+}), followed by addition of an oxygen atom to this ion resulting in its reduction of Fe^{2+} ion forming a ferrous-dioxy complex ($\text{Fe}^{2+}\text{-O}$); (3) formation of ferric hydroperoxy complex ($\text{Fe}^{3+}\text{-OOH}$) by the addition of an electron and a proton transferred either from CPR

or cytochrome b5; (4) breakdown of O-O bonds takes place, and formation of a ferryl-oxo intermediate ($\text{Fe}^{4+} = \text{O}$) takes place with the help of protonation and generation of water molecule; (5) formation of ROH, a hydroxylated product, as a result of radical recombination followed by removal of a hydrogen atom; and (6) finally, the oxidized substrate dissociates from the active site of CYP450 enzyme, and the enzyme returns to its original state (Fe^{3+}). There is another alternate pathway leading to the shunting of peroxide pathway by binding hydrogen peroxide to the ferric heme iron group (Podust and Sherman 2012; Munro et al. 2013; Durairaj et al. 2016).

5.5.3 Peroxidase

Lignin peroxidase and manganese peroxidase are the two commonly used peroxidase enzymes whose mechanisms have been elucidated in this section. In the presence of hydrogen peroxide as an electron acceptor, peroxidase enzyme catalyzes the oxidation of several organic and inorganic substrates. The generalized mechanism is shown in the reaction below:



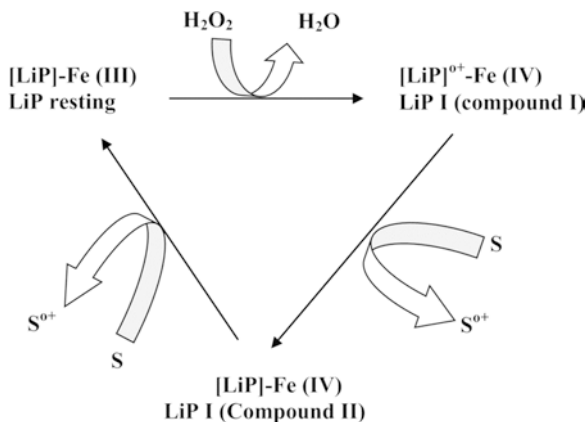
Where R is the substrate and R_{ox} is the oxidized substrate.

The oxidation of peroxidase enzyme takes place by the removal of electrons by hydrogen peroxide co-substrate. This oxidized peroxidase enzyme, thereby, abstracts an electron from the substrate and, hence, transforms the substrate (Mougin et al. 2009; Falade et al. 2017).

5.5.3.1 Lignin Peroxidase

Lignin peroxidase is a heme-containing diarylpropane oxygenase enzyme that carries out the hydrogen peroxide-mediated oxidative cleavage of lignin model. LiP enzyme is structurally a monomeric hemoprotein. LiP folds into a globular structure of dimension $50 \times 40 \times 50 \text{ \AA}$, which is subdivided into two domains by the heme group fixed on the protein: distal and proximal domains connected through two small channels. These two small channels facilitate the movement of iron group despite its fixed position on the enzyme (Falade et al. 2017). LiP can be distinguished from other peroxidases owing to its low optimum pH of 3.0. LiP oxidizes β -O-4 linkage in non-phenolic lignin model compounds (Kadri et al. 2017). The catabolism of different compounds by LiP involves three steps (Fig. 5.3). Firstly, formation of oxo-ferryl intermediate takes place as a result of oxidation of the ferric enzyme in its resting state $[\text{Fe(III)}]$ by the electron acceptor hydrogen peroxide acting as a co-substrate. Secondly, the non-phenolic substrate undergoing degradation transfers one electron to the oxo-ferryl intermediate, and thus reduces itself to form another intermediate (having deficiency of one electron). Lastly, the reduced substrate

Fig. 5.3 Catalytic oxidative cleavage of substrate by lignin peroxidase enzyme. (Adapted with permission from Abdel-Hamid et al. (2013))



donates another electron to bring back the enzyme (LiP) to its native state, thereby completing the process of catalytic oxidation (Abdel-Hamid et al. 2013).

LiP also has the capacity to catalyze phenolic compounds such as ring substituted anilines, catechol, vanillyl alcohol, syringic acid, and guaiacol (Baciocchi et al. 2001; Wong 2009). The most commonly adapted mechanism for the catalysis of phenolic compounds by LiP is through the presence of redox mediators such as veratryl alcohol (VA). LiP oxidizes the redox mediator VA to a cation radical (VA^+), which then oxidizes the compound under investigation through a redox reaction (Christian et al. 2005; Falade et al. 2017).

5.5.3.2 Manganese Peroxidase

It is a glycoprotein of molecular weight 38–62.5 kDa, containing ferriprotoporphyrin IX, which can be very easily segregated from the apoenzyme upon electrophoresis under non-denaturing conditions. In the presence of hydrogen peroxide, MnP catalyzes the oxidation reaction of Mn^{2+} to form Mn^{3+} . The similar cycle when carried out in the presence of chelating agents results in the formation of highly reactive chelator complex (Mn^{3+} -chelator complex), which further oxidizes various phenolic substrates (Fig. 5.4). The first step of the catalytic cycle involves the binding of hydrogen peroxide to the enzyme in its native state forming a complex containing iron-peroxide. The successive cleavage of O-O bonds results in the transferring of electrons and formation of complex containing Fe^{4+} -oxo-porphyrin radical. Next, the water molecule is formed after the bond is broken. Another porphyrin complex is formed, for which monochelated Mn^{2+} acts as an electron donor and is oxidized to Mn^{3+} . Finally, the porphyrin compound formed in the second step is reduced, and another Mn^{2+} is oxidized to Mn^{3+} , leading to the formation of one more water molecule and the enzyme regains its native state. Organic acids such as oxalate stabilize excess of Mn^{3+} ions, thereby acting as a

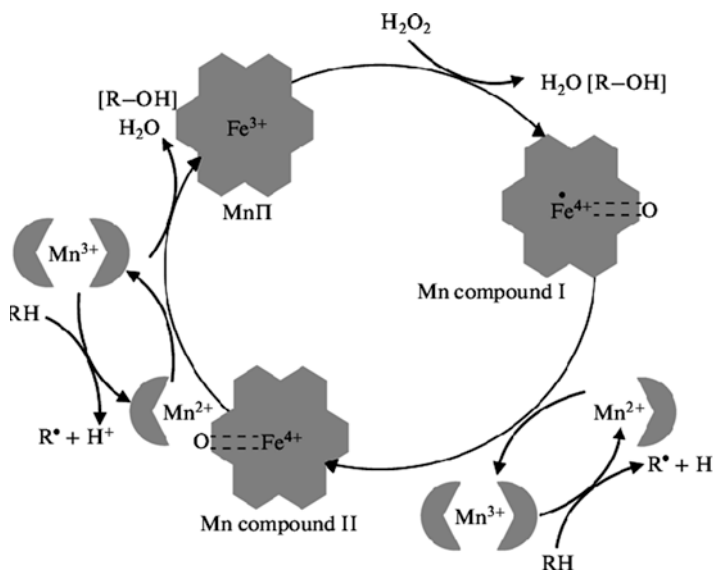


Fig. 5.4 Catalytic cleavage of substrate by manganese peroxidase enzyme. (Adapted with permission from Kulikova et al. (2011))

redox mediator that acts upon the substrate in a non-specific manner by cleaving the hydrogen bond and extracting an electron from the substrate (Kulikova et al. 2011).

5.6 Strategies for Enhancing Fungal Remediation of Recalcitrant Compounds

Fungi are an important part of our ecosystem that can be conveniently applied to degrade and detoxify different organic and inorganic pollutants contaminating the environment. Various technical advances have been made in the field of mycoremediation of pollutants. One such advancement is exploiting fungal enzymes for this process instead of using whole fungal cells with production of excess biomass as its major drawback (Hesham et al. 2021, Kumar et al. 2021, Sharma et al. 2021). Even fungal enzymes have their own limitations such as less stability, lower shelf-life, and high cost (Yadav et al. 2020d). However, various developments in the fields of enzyme immobilization and whole cell immobilization have resulted in increased stability and improved shelf-life of these enzymes with the advantage of reusing these enzymes, thereby reducing the overall remediation cost.

Laccase enzyme purified from *Trametes* sp. was immobilized on glass beads, showed 90% of its activity intact after several cycles, and was also found resistant toward protease enzyme (Dodor et al. 2004; Bilal et al. 2017). Nowadays, different

types of bioreactors are also being used to detoxify pollutants using immobilized preparations. Successful degradation of an azo dye Reactive Blue 222 was achieved using a two-phase reactor combining the Photo-Fenton treatment and aerobic treatment using two commonly available white-rot fungi *P. ostreatus* IBL-02 and *P. chrysosporium* IBL-03 (Kiran et al. 2013). In a continuous flow fungal membrane bioreactor, *Trametes versicolor* depicted significant removal percentage of two common pharmaceutical contaminants bisphenol-A and diclofenac within 2 days of incubation under sterile condition (Yang et al. 2013).

Fungal genomics and proteomics can serve as important tools in understanding different pathways involved in the degradation mechanism. The availability of whole genome sequence of the fungal species could enable functional and structural investigations in the catalysis of pollutants using different enzymes. Moreover, it could also help in devising molecular tools to engineer these enzymes to further improve the remediation process. In one such study, the whole genome sequence of fungi helped in understanding the metabolic diversities of cytochrome P450 enzyme (Ichinose 2013). Also 3D ligand docking models have been designed and explored to collect significant amount of information on the versatile nature of fungal CYP450 monooxygenase enzyme in oxidizing different polyaromatic hydrocarbons and have also been compared with the active domains of similar enzyme extracted from bacteria and mammals (Syed et al. 2013).

Construction of recombinants to engineer and manipulate fungal enzymes has helped improving their activity, thereby achieving efficient biodegradation rates. A robust expression platform was developed from *L. edodes* and was significantly investigated as a green approach to treat phenolic compound guaiacol (Wong et al. 2013).

A yeast, *Yarrowia lipolytica*, was exploited to construct highly efficient recombinant laccase for the hydrolysis of wood biomass. The laccase gene from *Y. lipolytica* was cloned in *Pichia pastoris*, further being used to demonstrate its application in treating phenolics produced from woody biomass (Kalyani et al. 2015). Similarly, laccase gene from *Pleurotus eryngii* ERY4 was cloned into *Saccharomyces cerevisiae*, which showed high substrate specificity over high pH and temperature range (Bleve et al. 2014). In another study, gene encoding laccase enzyme from *Phanerochaete flavidoalba* was cloned into *A. niger*, producing a very efficient recombinant enzyme system stable in the pH range 2–9 and was found capable of treating synthetic dyes Acid Red 299 and Remazol Brilliant Blue R from textile effluents (Benghazi et al. 2014). Continuous evolution in molecular biology techniques serves as a promising tool in improving environmental health (Rastegari et al. 2019a; Yadav et al. 2019). Also, the ethical issues need to be addressed before releasing genetically modified fungal species in the environment to degrade pollutants.

5.7 Conclusion

With the growing number of industries around the world, the amount of toxic wastes accumulating in the environment is increasing day by day. These toxic pollutants are a matter of grave concern as they pose serious threat to our terrestrial and aquatic ecosystems. Fungal species have a wide diversity of enzymatic machinery proving beneficial in treating these pollutants. Use of these fungal enzymatic systems in detoxifying recalcitrant compounds serves as a perfect green tool in environmental cleanup. Fungal enzymes such as laccase, peroxidases, and oxygenases, in particular CYP450 monooxygenase, have shown potential to degrade a wide range of environmental pollutants. These fungal enzymes have also been exploited to detect the quantity of xenobiotic compound present in the environment. Basidiomycota is the class of fungi capable of oxidative degradation and detoxification of different organic and inorganic pollutants considered toxic to the environment.

White-rot fungi belonging to Basidiomycota are capable of tolerating these toxic compounds, and some of them are capable of mineralizing it. Fungal enzymes extracted from extreme environments have shown potential to detoxify heavy metals from contaminated sites. All the primary enzymes involved in the detoxification process are extracellular in nature, which are secreted in less quantity during the initial stage of fungal metabolism. Genetic manipulation and immobilization techniques can be helpful in improving the quantity and quality of these enzymes, thereby enhancing the detoxification rates. These fungal enzymes can be modified to improve their catalytic activity along with their thermostability. Genetic tools help in creating enzymes having high redox potentials which might result in widening their substrate specificity. Production of different nanozymes is also being explored as an alternate to fungal enzymes. The structural analysis of these enzymes might be helpful in molecular docking to understand the substrate suitable for different enzymes. The need of the hour is to investigate novel potential fungal species and their enzymatic machinery to combat environmental pollutants creating havoc in nature.

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Chapter 6

Fungal Communities for the Remediation of Environmental Pollutants



Aditi Singh and Arpita Roy

6.1 Introduction

Environmental pollution is biggest challenge our planet has been facing in the present century. The increasing world population and development of socioeconomic, scientific, and technological advancements have directly or inadvertently resulted in ecological deterioration, loss of biological diversity and habitats, ozone layer depletion, and climatic changes, consequently leading us toward a global environmental crisis (Singh et al. 2020a, b, c). It is becoming worse with insurmountable amount of pollutants produced everyday due to some natural disasters and mostly through anthropogenic activities, which regularly contaminate the air, soil, and water supplies, both in developing and developed countries. There are many sources of pollution that drastically affect the environment, terrestrial, and aquatic organisms. The accumulation of toxic or hazardous compounds or some disease-causing agents leads to contamination. Some major source of contaminants constitutes uncontrolled discharge of solid and liquid wastes such as industrial wastes, automobile emissions, accidental spillages, sewage effluents, power generation, use of fossil fuels, construction and mining sites, domestic disposals, tanneries, agriculture fertilizers, herbicides, pesticides, etc. (Pandey and Singh 2019).

Pollution is also responsible for economic losses and, due to this, ~5% worldwide GDP loss in several developing countries (Reddy and Behera 2006). Among all different categories of pollution, water, land, and soil pollution are prevalent due to their severe long-term consequences associated with their contamination. A report by World Health Organization reveals that every 1 out of 3 people worldwide

A. Singh

Department of Biotechnology, Bennett University, Greater Noida, Uttar Pradesh, India

A. Roy (✉)

Department of Biotechnology, School of Engineering & Technology, Sharda University, Greater Noida, India

face inadequate access to safe drinking water, and at least 2 billion people are regularly using contaminated water. This water scarcity is estimated to affect ~50% of the total world population by 2025 reference. Soil pollution is the deterioration of land and soil quality, due to human activities or other plausible perturbations in the natural soil environment. Soil pollutants such as chemicals, fertilizers, pesticides, industrial effluents, sewage and municipal solid waste, electronic wastes, plastics, organic and inorganic, and radioactive wastes reduce the soil productivity and also influences physicochemical and biological properties of soil (Singh and Dhupal 2019; Kour et al. 2020b; Thakur et al. 2020).

Soil pollution recently gained attention due to the escalating problems associated with toxic levels of contaminants, for instance, loss of soil fertility, and unsuitability for agricultural purposes (Kour et al. 2019b). It significantly reduces food security and also causes crops cultivated in polluted soils to be unfit for consumption (Rai et al. 2020). Approximately 22 million hectares of land was estimated to be polluted, and increasing rate of urbanization and industrialization predicted the further increment in these numbers.

Pollution poses severe health impact on human. It also accounts for ~16% deaths and 25% of the most polluted regions across the globe (Landrigan et al. 2018). Another report by World Health Organization reveals that annually, ~1.7 million deaths of children aged below 5 years are attributed to polluted environment. Human exposure to pollution can cause asthma and other respiratory illnesses, neurodegenerative disorders, skin problems, heart diseases, and birth defects, weaken immune, endocrine, and reproductive system, and cause DNA damage and can even put people at a risk of Cancer (Khan and Ghouri 2011; Kour et al. 2020a). Therefore, it is important to develop strategies and adopt them to conserve the environment and reduce pollution. Conventional remediation methods possess various limitations, and therefore, process of bioremediation has been trending due to its advantages like economical approach and conversion of various pollutants into non- or less hazardous one. Use of fungal culture for remediation of various pollutants from environment is a new approach. Therefore, in this chapter, utilization of fungal culture for remediation of various environmental pollutants has been discussed.

6.2 Conventional Remediation Process

The use of conventional means of remediation is adapted according to the nature and concentration of the pollutants, their origin, and amendment type. A variety of physical, chemical, or biological processes are frequently implemented for treatment of pollutants. Some current methods of remediation involve physical approaches (such as pyrolysis, electrokinetic coagulation, separation by membrane filtration, irradiation, soil replacement, and vitrification), as well as chemical remediation approaches (such as Ozonation, Ion exchange, Fenton's reaction, immobilization, electrochemical destruction, leaching, adsorption using different adsorbents like activated charcoal, coal peat, fly ash, and wood chips, and nanoremediation)

(Robinson et al. 2001; Sharma et al. 2018). However, these methods are not sustainable due to less area availability for treatment, high cost, and unhealthy. These methods do not always provide satisfactory results for treatment of such toxic pollutants. Therefore, an alternative method is of great need and bioremediation is an emerging, alternative, economical, and recognized innovative method to combat against such contaminants.

Bioremediation is an emerging area of green biotechnology (Kumar et al. 2019). It refers to the use of biological systems (bacteria, fungi, algae, etc.) to reduce and transform the concentrations of toxic and hazardous wastes into non- or less-hazardous substances in the environment (Dahiya and Nigam 2020) (Fig. 6.1).

Utilizing plants or phytoremediation serves as a promising strategy. However, this process has constrained due to reasons such as slower plant growth rates or metal toxicity. Therefore, phytoremediation using microbe can be helpful in plant growth and stress tolerance.

Microbial bioremediation uses microbial metabolism to degrade pollutants under specific conditions (Rana et al. 2019b; Verma et al. 2017). Both the in situ or ex situ means of remediation are valuable approaches for microbial degradation of pollutants. In situ techniques involve microorganisms indigenous to the contaminated region for pollutant removal. Ex situ bioremediation requires the excavation of contaminated soil prior treatment, which involves bioreactors, biopile, and wind row techniques for better aeration and irrigation, which can aid in alleviation of the microbial activity (de Lima et al. 2018).

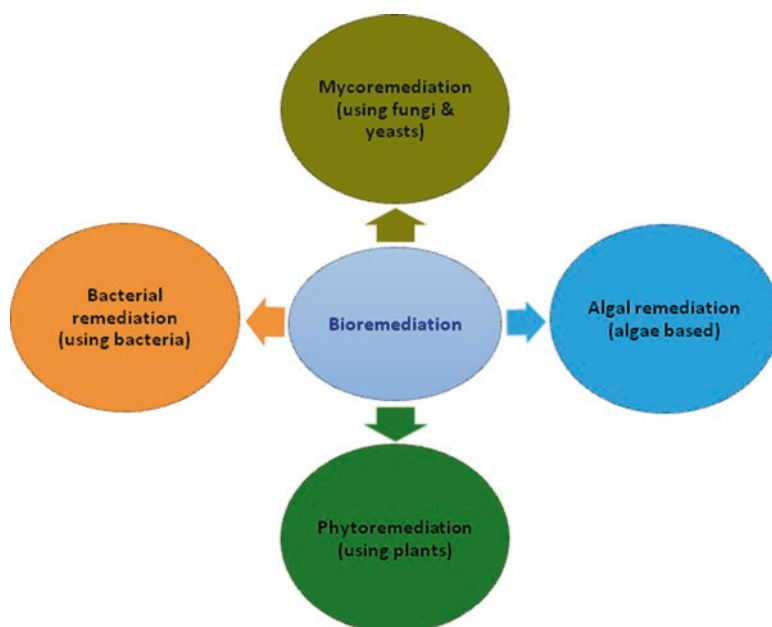


Fig. 6.1 Bioremediation using different biological sources

6.3 Fungi for Potential Bioremediation

Fungi are a powerful biotechnological tool capable of metabolizing and immobilizing many organic substances, metals, and radionuclides (Fig. 6.2). They store them in various parts of the cell or translocate through fungal hyphae. They also utilize several extracellular oxidoreductases for degradation of lignocelluloses along with a wide array of pollutants (Singh et al. 2020a, b, c; Devi et al. 2020a; Kour et al. 2019c). Mycoremediation has advantages over other means of remediation techniques of environmental contaminants such as cost-effective, nature friendly, diverse, safe, reusable, and highly efficient (Kumar 2017; Jain et al. 2017; Yadav 2020). Fungi can exploit their own product for their survival without any additional need of nutrition for their growth in adverse environments (Yadav et al. 2019). They can also degrade several pollutants such as dioxins and several drugs, which could not be detoxified by bacteria (Singh et al. 2020a, b, c).

Currently, the most reviewed phyla of fungi used in mycoremediation are *Ascomycota*, *Basidiomycota*, and *Zygomycota* (Varjani and Patel 2017; Yadav et al. 2020d). *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Neurospora crassa*, *Penicillium* sp., and *Trichoderma* sp. belong to *Ascomycota*. *Zygomycota* includes *Mucor* sp. and *Rhizopus* sp., which lacks septa in their hyphae. White-rot fungi (WRF) are ligninolytic fungi belonging to *Basidiomycota* including *Pleurotus*

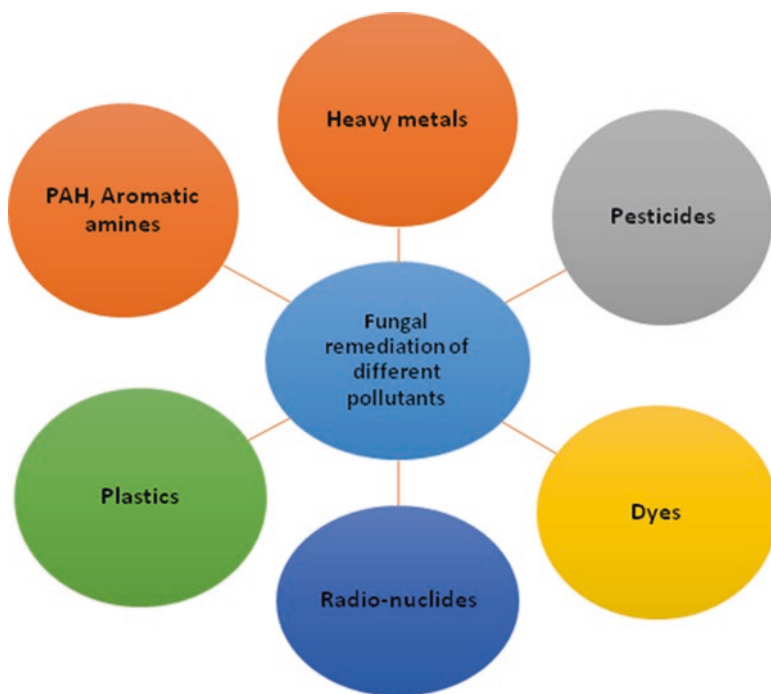


Fig. 6.2 Fungal remediation of major pollutants

sp., *Irpex lacteus*, *Lenzites betulinus*, *Phlebia* sp., *Perenniporia* sp., *Peniophora* sp., and *Trametes* sp. These fungi have been well exploited in the applications of bioremediation, dye degradation, biopulping, and bioleaching. The list of different fungi degrading different contaminants is mentioned in Table 6.1.

Fungus-associated phytoremediation is capable of increasing heavy metal uptake for host plants. Mycorrhizal fungi grow symbiotically with plant roots and possess a heavy tolerance and ability to degrade toxic metals and organic compounds in soil (Kumar 2017). Among them, certain arbuscular mycorrhizal fungi have resorted the growth of host plants under stress conditions. Some marine-derived fungi have also been utilized for bioremediation purposes due to their unique acquired adaptability to extreme environments, such as high salinity, pressure, temperature, low oxygen concentration, and low nutrient availability.

6.3.1 Mechanism of Actions

The mechanism of biodegradation of toxicants in a regular fungus takes place at several stages: The initial stage involves the state of growth of fungal culture, viz., nutrient capture, through establishment of a biomass or substrate. Fungi capture the nutrients from their habitat and expand themselves over a large area, and mycelium thickening takes place so that it can digest its substrate. On the verge of habitat depletion, fungi enter second stage by producing certain enzymes responsible for breakdown of toxic complex molecules and target the pathogens (Jain et al. 2017).

Fungi possess strong ability to remediate the pollutants through biosorption and production of biodegrading enzymes. Some fungi produce certain extracellular ligninolytic enzymes such as laccases, lignin peroxidase (LiP), and manganese peroxidase (MNP), which oxidize phenol and nonphenolic organic compounds (Khan et al. 2020). The presence of intracellular cytochrome P450 (CYP) monooxygenases in fungi also plays important role in degradation of lignin and many other organopollutants. These enzymes are universally present in microsomes of eukaryotic cells that are capable of detoxifying harmful chemicals in the environment (Prenafeta-Boldú et al. 2018). Low-substrate specificity of these enzymes enables oxidation of a wide range of compounds (Vieira et al. 2019).

These enzymes achieve a one-electron radical oxidation, producing cationic radicals from toxic compounds followed by the release of quinines (Kumar and Chandra 2020). Several cellulolytic enzymes such as cellulases, pectinases, amylases, proteases, and xylanases present in some fungi are responsible for degradation of cellulosic substrates by their hydrolytic degradation in agricultural and other wastes (Kumar 2017). In contrast, process of biosorption is independent of cellular metabolism, as it utilizes biomass for rapid binding of solutes in aqueous form present in wastewater (Rana et al. 2019a). Both dead and live biomass has been reported in this process. The role of these fungi in depletion of numerous toxic pollutants such as heavy metals and several other agricultural and industrial wastes such as textile,

Table 6.1 Bioremediation applications of fungi against various contaminants

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Allescheriella</i> sp.	PAH	Naphthalene	92.4%	D'Annibale et al. (2006)
	AA	2,4-dichloroaniline	59.6%	
		2,6-dichloroaniline	80.5%	
<i>Alternaria alternata</i>	PI	Low density polyethylene	75.9%	Ameen et al. (2015)
<i>Aspergillus</i> sp.				
<i>Aspergillus</i> sp.	HM	Cr (VI)	90%	Congeevaram et al. (2007)
		Ni (II)	55%	
	PAH	Benzo[a]pyrene	60%	Wu et al. (2009)
	PI	Low density polyethylene	3.89–4.16 g L ⁻¹ of CO ₂ released	Sindujaa et al. (2011)
			100%	Vignesh et al. (2016)
	HM	Ca	78%	Dhami et al. (2017)
		Pb	34%	
		Sr	48%	
<i>A. foetidus</i>	HM	Cr (VI)	97%	Prasenjit and Sumathi (2005)
<i>A. flavus</i>	Dye	Direct brown dye	92%	Abd El-Rahim and Moawad (2003)
		Polar red dye	73–81%	
		Congo red	98.86%	
	HM	Cu	20.75–93.65 mg g ⁻¹	
<i>A. fumigatus</i>	HM	Cd, Cu, Fe, Mn, Pb, and Zn	86%	Bhattacharya and Das (2011)
	PAH	Anthracene	~65%	Iram et al. (2015)
	HM	Cu	69.6%	Bano et al. (2018)
		Cr	40%	Ye et al. (2011)
		Pb	76.07%	Shazia et al. (2013)
<i>A. japonicus</i>	AA	Aromatic amines	15.9–76.1%	

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>A. niger</i>	Dye	Direct brown dye	93–95%	Abd El-Rahim and Moawad (2003)
		Polar red dye	89–94%	
	Oc	Endosulfan	100%	Bhalerao and Puranik (2007)
			59%	Hussaini et al. (2013)
	Oc	Lindane	29%	Hussaini et al. (2013)
	Dye	Basic fuchsin	81.85%	Rani et al. (2014)
		Nigrosin	77.47%	
		Malachite green	72.77%	
		Dye mixture	33.08%	
	HM	Pb	3.25–172.25 mg g ⁻¹	Iram et al. (2015)
<i>A. ochraceus</i>	PI	Low density polyethylene	26.14%	Alshehrei (2017)
	HM	Cu	327.81 mg kg ⁻¹	Ong et al. (2017)
	Dye	Direct brown dye	91%	Abd El-Rahim and Moawad (2003)
		Polar red dye	96%	
	HM	Cr (III)	97%	Nasser et al. (2002)
	Dye	Polar red dye	86%	Abd El-Rahim and Moawad (2003)
	Oc	Endosulfan α	95%	Goswami et al. (2009)
		Endosulfan β	97%	
	Op	Methyl parathion	80%	Alvarenga et al. (2014)
		Cu	228.81 mg kg ⁻¹	Ong et al. (2017)
<i>A. tamarii</i>	Dye	Direct brown dye	~32%	Abd El-Rahim and Moawad (2003)
		Polar red dye	76%	
	PI	Low density polyethylene	63%	Ameen et al. (2015)
	Pe	Triclosan	71.91%	Taştan and Dönmez (2015)

(continued)

Table 6.1 (continued)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Beauveria bassiana</i>	HM	[Zn(II), Cu(II), Cd(II), and Cr(VI) Ni(II)]	84.5%	Gola et al. (2016)
<i>Bjerkandera</i> sp.				
<i>Bjerkandera</i> sp.	Oc	Toxaphene	~85%	Romero et al. (2006a, b)
<i>B. adusta</i>	Dye	Cibacron Yellow C-2R, Cibacron Red C-2G, Cibacron Blue C-R, Remazol Black B, and Remazol Red RB	Up to 53.1%	Robinson and Nigam (2008)
<i>Cladosporium</i> sp.				
<i>C. sphaerospermum</i>	Oc	Benzo[a]pyrene	18%	Potin et al. (2004)
<i>Coniophora puteana</i>	HM	As	18%	Kartal et al. (2004)
		Cr	19%	
		Cu	67%	
<i>Cordyceps militaris</i>	PAH	Benzo[a]pyrene	Up to 42.2%	Mori et al. (2015)
		Phenanthrene	Up to 50.1%	
		Pyrene	22.5–22.8%	
	Dx	2,7-Dichlorodibenzo-p-dioxin (2,7-CDD)	11–55%	
		2,3,7-trichlorodi-5,6benzo-p-dioxin (2,3,7-triCDD)	<60%	
		2,4,8-triCDF	~ 80%	

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Coriolus versicolor</i>	Dye	Methylene Blue	–	Mazmanci et al. (2002)
		Fluorescein	85%	Itoh and Yatome (2004)
		4-Aminofluorescein	95%	
		5-Aminofluorescein	91.9%	
		Drimarene Orange K-GL	51%	Asgher et al. (2006)
		Remazol Brilliant Yellow 3GL	100%	
		Procion Blue PX-5R	100%	
		Cibacron Blue P-3RGR	87%	
<i>Cunninghamella elegans</i>	AA	Congo Red	~80%	Jayasinghe et al. (2008)
		Malachite Green	>40%	
		Vinclozolin	93%	Pothuluri et al. (2000)
<i>Cyclothyrium</i> sp.	PAH	Anthracene	59%	Da Silva et al. (2003)
		Benzo[a]pyrene	38%	
		Phenanthrene	70%	
		Pyrene	74%	
<i>Doratomyces</i> sp.				
<i>D. nanus</i>	PCB	Polychlorinated biphenyl mix	73.81 ± 2.78	Mouhamadou et al. (2013)
<i>D. purpureofuscus</i>			82.01 ± 3.97	
<i>D. verrucisporus</i>			70.02 ± 7.08	
<i>Eupenicillium crustaceum</i>	Organo chloride	n-hexane soluble ¹⁴ C-compounds (24.6%)	converted to nonextractable ¹⁴ C-residues (20.5%)	Vitali et al. (2006)
<i>Fomes</i> sp.				

(continued)

Table 6.1 (continued)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>F. fomentarius</i>	Dye	Congo Red	~80%	Jayasinghe et al. (2008)
		Methylene Blue	>40%	
<i>F. sclerodermeus</i>	Dye	Malachite green	—	Papinutti and Forchiassin (2004)
<i>Fomitopsis</i> sp.				
<i>F. palustris</i>	HM	As	100%	Kartal et al. (2004)
		Cr	87%	
		Cu	72%	
<i>F. rosea</i>	Dye	Congo Red	~80%	Jayasinghe et al. (2008)
<i>Funalia trogii</i>	Dye	Astrazon Blue	42–54%	Apohan and Yesilada (2005)
		Astrazon Red	93–100%	
<i>Fusarium</i> sp.				
<i>F. oxysporum</i>	HM	Pb ²⁺ and Cd ²⁺	Cd- and Pb-CO ₃ crystal formation	Sanyal et al. (2005)
<i>F. poae</i>	Oc	Lindane	56.7%	Sagar and Singh (2011)
<i>F. solani</i>	Oc	Lindane	59.4%	Sagar and Singh (2011)
		Cu	395.18 mg kg ⁻¹	Ong et al. (2017)
<i>Fusarium</i> sp.	Pl	Low density polyethylene	100%	Vignesh et al. (2016)
	HM	Ca	67%	Dhami et al. (2017)
		Pb	54%	
		Sr	31%	
<i>Ganoderma</i> sp.				

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>G. lucidum</i>	Dye	Drinarene Orange K-GL	66%	Asgher et al. (2006)
		Remazol Brilliant Yellow 3GL	100%	
		Procion BluePX-5R	54%	
		Cibacron Blue P-3RGR	44%	
	Dye	Congo Red	>90%	Jayasinghe et al. (2008)
	PAH	Methylene Blue	>70%	Punnapayak et al. (2009)
		Benzol[a]pyrene	100%	
		Anthracene	100%	
		Acenaphthene	95.4%	
		Acenaphthylene	90.1%	
		Fluorene	98.63%	Rozman et al. (2020)
		Benzol[a]anthracene	85.3%	
	HM	Pb, Cd	> 80%	
	PCB	Polychlorinated biphenyl mix	67.44 ± 6.84	
	HM	Cd	87.2%	
<i>Ipex lacteus</i>	Dye	Pb	88%	Malachová et al. (2006)
		Reactive Orange 16	95.2%	
		Disperse Blue	77.8%	
<i>Laetiporus sulphureus</i>	HM	Methylene Blue	100%	Malachova et al. (2013)
		As	85%	
		Cr	69%	
		Cu	50%	
<i>Lichtheimia corymbifera</i>	PCB	Malachite Green	57%	Mouhamadou et al. (2013)
		Polychlorinated biphenyl mix	61.34 ± 3.91	

(continued)

Table 6.1 (continued)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Monilinia</i> sp.	PAH	Anthracene	72 ± 2%	Wu et al. (2008)
		Benzo[a]pyrene	70 ± 8%	
		Endosulfan β	50%	
<i>Mucor</i> sp.				
<i>M. hiemalis</i>	HM	Al	Up to 90–98%	Hoque and Fritscher (2019)
		Cd	91%	
		Cr	89–99%	
		Cu	85–87%	
		Hg	99%	
		Ni	82–86%	
		Pb	93–97%	
		U	89%	
		Zn	71–83%	
<i>M. racemosus</i>	Oc	Dieldrin	92.7%	Kataoka et al. (2010)
		Endosulfan α	82.5%	
		Endosulfan β	78.9%	
		Endosulfan sulfate	95.2%	
		Heptachlor	94.4%	
		Heptachlor epoxide	67.5%	
	HM	Cu	60.13 mg g ⁻¹	El-Morsy et al. (2013)
		Pb	21.97 mg g ⁻¹	
		Zn	57.67 mg g ⁻¹	
<i>Myceliophthora thermophila</i>	PCB	Polychlorinated biphenyl mix	74.96 ± 1.10	Mouhamadou et al. (2013)
<i>Naematoloma fasciculare</i>	Dye	Congo Red	>95%	Jayasinghe et al. (2008)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Penicillium sp.</i>				
<i>P. aurantiogriseum</i>	PCB	Polychlorinated biphenyl mix	68.95 ± 0.94	Mouhamadou et al. (2013)
<i>P. decaturense</i>	Op	Methyl parathion	81 %	Alvarenga et al. (2014)
<i>P. duclauxi</i>	Pl	Low density polyethylene	56.2 %	Ameen et al. (2015)
<i>P. janthinellum</i>	PAH	Pyrene	57 %	Saraswathy and Hallberg (2002)
<i>P. oxalicum</i>	Op	Methamidophos	99.9 %	Zhao et al. (2010)
<i>P. terrestre</i>	PAH	Pyrene	75 %	Saraswathy and Hallberg (2002)
<i>Penicillium sp.</i>	Pl	Low density polyethylene	43.4 %	Alshehrei (2017)
	PAH	Decane	49 %	Govarthanan et al. (2017)
		Butyl benzene	42 %	
		Dodecane	33 %	
	AA	2,4-dinitroanisole	100 %	Schroer et al. (2017)
<i>Phanerochaete sp.</i>				

(continued)

Table 6.1 (continued)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>P. chrysosporium</i>	Explosive	RDX	Converted to CO ₂ , N ₂ O, and fungal biomass (52.9, 62 and 28.3% respectively).	Sheremata & Hawari (2000)
	Dye	Drimarene Orange K-GL	100%	Asgher et al. (2006)
		Remazol Brilliant Yellow 3GL	100%	
		Procion BluePX-5R	100%	
<i>P. velutina</i> <i>Phlebia</i> sp.		Cibacron Blue P-3RGR	100%	
	Dye	Methylene Blue	Up to 90%	Alam et al. (2009)
	PI	Polyvinyl chloride	178,292 Da ⁻¹	Ali et al. (2014)
	Dye	Nigrosin	90.15%	Rani et al. (2014)
		Basic fuchsin	89.8%	
		Malachite green	83.25%	
		Dye mixture	78.4%	
<i>P. velutina</i> <i>Phlebia</i> sp.	AA	Amido black 10B	98%	Senthilkumar et al. (2014)
		2,4,6-trinitrotoluene (TNT)	80%	Anasonye et al. (2015)
<i>Phlebia</i> sp.	PAH	Naphthalene	83.3%	D' Annibale et al. (2006)
	AA	2,4-dichloroaniline	84.3%	
		2,6-dichloroaniline	61.5%	
<i>P. acanthocystis</i>	Oc	Heptachlor	90%	Xiao et al. (2011)
<i>P. brevispora</i>	PCB	3,3',4,4'-tetraCB	~50%	Kamei et al. (2006)
		2,3,3',4,4'-pentaCB		
		2,3',4,4',5-pentaCB		
		3,3',4,4',5,5'-hexaCB		
<i>P. tremellosa</i>	Oc	Heptachlor	74%	Xiao et al. (2011)
	Oc	Heptachlor	71%	Xiao et al. (2011)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Phoma</i> sp.				
<i>P. eupyrena</i>	PCB	Polychlorinated biphenyl mix	81.59 ± 1.81	Mouhamadou et al. (2013)
<i>Phoma</i> sp.	HM	Pb ²⁺	98.42%	Zhao et al. (2020)
<i>Pleurotus</i> sp.				
<i>P. ostreatus</i>	PAH	Benzo[a]anthracene	58%	Baldrian et al. (2000)
		Benzo[a]pyrene	57%	
		benzo[ghi]perylene	50%	
		Pyrene	83%	
	Dye	Drinarene Orange K-GL	83%	Asgher et al. (2006)
		Remazol Brilliant Yellow 3GL	52%	
		Procion BluePX-5R	100%	
		Cibacron Blue P-3RGR	100%	
	Dye	B49	97.7%	Casieri et al. (2008)
		R243	44.8%	
		RBBR	97.2%	
	PCB	PCB mix (Delor 103)	98.4-99.6%	Čvančarová et al. (2012)
	PAH	Anthracene	90-100%	Pozdnyakova et al. (2018)
		Phenanthrene	100%	
		Fluorene	100%	
<i>P. pulmonarius</i>	Dye	Congo Red	~80%	Jayasinghe et al. (2008)
<i>P. sajor-caju</i>	HM	Hg (II)	81%	Apca et al. (2003)
<i>Pycnoporus</i> sp.				
<i>P. cinnabarinus</i>	Dye	Congo Red	>90%	Jayasinghe et al. (2008)
		Methylene Blue	~70%	
<i>P. coccineus</i>		Congo Red	>95%	

(continued)

Table 6.1 (continued)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>Rhizomucor tauricus</i>	HM	Cd (II)	75%	Kumar et al. (2009)
<i>Rhizopus</i> sp.				
<i>R. oryzae</i>	HM	Co(II)	13.56 ± 0.37 mg g ⁻¹	Gharieb et al. (2014)
		Pb(II)	69.73 ± 1.48 mg g ⁻¹	
<i>Rhodotorula glutinis</i>	HM	Ni (II)	63.53 mg g ⁻¹	Suazo-Madrid et al. (2011)
<i>Saprolegnia delica</i>	HM	Cd	80%	Ali and Hashem (2007)
		Pb	90%	
		Zn	44%	
<i>Schizophyllum commune</i>	Dye	Solar brilliant red 80	84.8%	Asgher et al. (2013)
	PAH	Anthracene	39.8%	Pozdnyakova et al. (2018)
<i>Simplicillium subtropicum</i>	HM	Cu	795.58 mg kg ⁻¹	Ong et al. (2017)
<i>Stachybotrys</i> sp.	PAH	9,10-anthracenedione	49.8%	D'Annibale et al. (2006)
		Naphthalene	91.9%	
	AA	2,4-dichloroaniline	75.2%	
		2,6-dichloroaniline	91.5%	
		2,3,4,5,6-Pentachloroaniline	37.6%	
<i>Stereum ostrea</i>	Dye	Congo Red	~60%	Jayasinghe et al. (2008)
		Methylene Blue	~40%	
<i>Sterigmatomyces halophilus</i>	HM	Cd, Cu, Fe, Mn, Pb, and Zn	83%	Bano et al. (2018)
<i>Streptomyces</i> sp.	Op	Chlorpyrifos	Up to 99.2%	Fuentes et al. (2013)
<i>Talaromyces helicus</i>	HM	Cu ²⁺	Up to 52%	Romero et al. (2006a, b)
<i>Trametes</i> sp.				

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>T. pubescens</i>	Dye	B49	97.8%	Casieri et al. (2008)
		R243	65.4%	
		RBBR	96.8%	
<i>T. suaveolens</i>	Dye	Congo Red	~70%	Jayasinghe et al. (2008)
<i>T. versicolor</i>		¹⁴ C-PCP	28%	
	HM	Hg (II)	73%	Arca et al. (2003)
	Dye	Crystal Violet	72%	Moturi and Charya (2009)
		Malachite Green	43%	
	Pe	Fipronil	96.5%	Wolfand et al. (2016)
<i>Trichoderma</i> sp.	PAH	Anthracene	~90%	Pozdnyakova et al. (2018)
<i>Trichoderma</i> sp.	Oc	DDD	100%	Ortega et al. (2011)
<i>T. asperellum</i>	PAH	Phenanthrene	74%	Zafra et al. (2015)
		Pyrene	63%	
		Benzo[a]pyren	81%	
	HM	Cd	88.9%	Maurya et al. (2019)
		Pb	81.30%	
<i>T. brevicompactum</i>	HM	Cd (II)	20.13%	Zhang et al. (2020a, b, c)
		Cr (VI)	31.83%	
		Cu (II)	64.46%	
		Pb (II)	97.5%	
		Zn (II)	4.62%	
<i>T. hamatum</i>	Pl	Low density polyethylene	Up to 3.9 ± 0.5%	Malachová et al. (2020)
<i>T. harzianum</i>	R	Uranium	612 mg U g ⁻¹	Akhtar et al. (2007)
			99.9% U recovery	
	Oc	Pentachlorophenol	100%	Vacondio et al. (2015)

(continued)

Table 6.1 (continued)

Fungi	Contaminants		Bioremediation efficiency	References
	Type	Name		
<i>T. virens</i>	AA	Benzidine	90%	Cocaign et al. (2013)
		3,4-dichloroaniline (3,4-DCA)	42%	
			79%	
		4-isopropylaniline (4-IPT)	55%	
<i>T. viride</i>	HM	As	31–58%	Babu et al. (2014)
		Cd	59–62%	
		Cu	25–35%	
		Pb	34–64%	
		Zn	38–48%	
<i>T. viride</i>	HM	Cd	74.2%	Ali and Hashem (2007)
		Pb	68.3%	
		Zn	61.7%	
<i>Trichophyton rubrum</i>	Dye	Remazol Tiefschwarz	83%	Yesiladali et al. (2006)
		Remazol Blue RR	86%	
		Supranol Turquoise GGL	80%	
<i>Trichosporon</i> sp.	Op	Chlorpyrifos	55%	Hussaini et al. (2013)
<i>Verticillium</i> sp.				
<i>Verticillium</i> sp.	Op	Chlorpyrifos	~ 80%	Yu et al. (2006)
<i>V. dahliae</i>	Op	Chlorpyrifos	64%	Hussaini et al. (2013)

Dye Textile or industrial dyes, DX Dioxins, HM Heavy metals, Oc Organochlorine Pesticides, Op Organophosphate Pesticides, PAH Polycyclic aromatic hydrocarbons, PCB Polychlorinated biphenyl, Pe Other pesticides, Pl plastics, and R Radionuclides

mining, tannery, oil refineries, food, and pharmaceutical has been enumerated (Akhtar and Amin-ul Mannan 2020).

6.4 Fungal Bioremediation of Heavy Metals

Heavy metals such as lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni), arsenic (As), and zinc (Zn) are present ubiquitously on earth's crust (Kaur and Roy 2020). Heavy metal contamination is a global concern, resulting in verdure disruption and subsequent crop yield (Roy and Bharadvaja 2020). Beside natural activities, the major heavy metal contaminant sources in environment constitute the industrial effluent discharges from mining, smelting, electroplating, leather tanning, textile, distillery, as well as through agricultural practices (Deb et al. 2020; Yadav et al. 2020a, b). Heavy metals beyond the permissible limits degrade the water quality and threaten all the living organisms (Muszynska and Hanus-Fajerska 2015). Due to their potential to produce reactive-oxidative species (ROS) in the body, heavy metals exert teratogenic and carcinogenic effect. Heavy metals cause respiratory, cardiovascular, renal, hepatic, neurological, gastrointestinal, and reproductive problems in human (Verma et al. 2020; Tonelli and Tonelli 2020). Hence, assessment and remediation of such toxic metals are necessary for toxicological, environmental, and occupational studies, especially when it concerns the health outcomes.

Fungi tolerant to toxic metal stress and immobilize them within their hyphae, and they also secrete various metabolites responsible for chelating metal ions. Some endophytic fungi have also been reported to hinder the transportation of metal ions into plant tissues and optimize their distribution within the host plant (Domka et al. 2019). Such strains may also facilitate growth of plants in metals contaminating environment. Gola and his team (2016) established bioaccumulated characteristics of an entomopathogenic fungal strain *B. bassiana*, for remediation of heavy metals [Pb (II), Cu (II), Zn (II), Cd (II), Cr (VI), and Ni (II)]. Bioremoval percentage for these contaminants ranged from 58% to 75%. A detailed investigation for Pb (II) degradation under optimal conditions demonstrated homogenous bioaccumulation of metal inside the fungal cell, while yielding 28% biomass reduction at 30 mg L⁻¹ lead (II) with 58.4% bioremoval efficiency. Domka et al. (2018) described the high tolerance of mycorrhizal fungus *Mucor* sp. to elevated levels of Pb, Zn, and Cd, promoting the plant-host growth under stress conditions.

The inoculation of *Mucor* sp. with the plant *Arabidopsis arenosa* acquired more nitrogen from soil during metal stress and showed significant downregulation of catalase activity, therefore suggesting the alleviation of toxic metal-induced oxidative stress. An Hg (II)-volatilizing *Lecythophora* sp. was combined with biochar to effectively reduce mercury contents in soil and plants (Chang et al. 2019). Treatment of both bioagents in soil exhibited low mercury uptake in *L. sativa* plants. This fungus altogether with biochar yielded an economical and eco-friendly remediation of mercury-contaminated sites. Efficient bioremoval of Pb (68%) and Ni (81.25%)

using fungus *Pleurotus ostreatus* was demonstrated by Bharath and his colleagues (2019) for bioremediation of soil contaminated with municipal solid waste, when incubated with appropriate nutrients for 25 days.

Haradean et al. (2019) investigated Zn removal using certain vesicular arbuscular mycorrhizal fungal (VAM) species in association with *Lactuca sativa* plants. Albert et al. (2019) demonstrated the metal tolerance and biosorption capacities of several fungal isolates against Cu, Cd, and Pb. Among them, *Absidia cylindrospora* recorded the biosorption efficiencies of Cd (45%) and Pb (65%); Cu (35%), Cd (41%), and Pb (46%). Biosorption by *Chaetomium atrobrunneum* and *Perenniporia fraxinea* was more than 42% in the case of Cd, Cu, and Pb. Best-performing strain *Coprinellus micaceus* was able to biosorb Pb (100%). In another study, *Penicillium citrinum* and *Trichoderma viride* were isolated from tannery effluents and they exhibited Cr (VI) tolerance and growth adaptability up to the concentration of 250 and 500 mg/L, respectively (Zapana-Huarache et al. 2020). The Cr (VI) stress tolerance in these fungal strains was due to laccase enhanced expression, which makes them highly potential for bioremediation of tannery effluent. Another study demonstrated the bioleaching capacity of fungal strains *A. fumigatus* M3Ai, *A. niger* M1DGR, and *Penicillium rubens* M2Aii for Cd and Cr removal from contaminated soil (Khan et al. 2019). *A. fumigatus* revealed a comparatively lower cumulative bioremoval ability of Cd (79%) and Cr (69%).

Chatterjee et al. (2020) demonstrated the green synthesis of superparamagnetic iron-oxide nanoparticles using mangrove fungus *A. niger* and its successful utilization in bioremoval of Cr (VI) in aqueous form, with an efficiency of 79.7% till five regeneration cycles with minimal losses, thus suggesting potential of fungal-based nanoparticles in nanoremediation of waste and toxic materials. A novel Cd (II) resistant-fungus *A. fumigatus* F2 was isolated from polluted sites and was assessed for biosorption of Cd (II) metal at high concentrations with the removal efficiency of 74.76% with a high uptake rate, suggesting a good source for treating such toxic metal ion effluents (Talukdar et al. 2020).

6.5 Fungal Bioremediation of Dyes

Dyes are frequently used in various industries such as food, textiles, pharmaceuticals, cosmetics, photographic, paper, tannery, and plastics. Dyes can be categorized under the groups of direct, reactive, acidic, basic, disperse, and pigment dyes (Raina et al. 2020; El Enshasy et al. 2017). Even at low concentrations, dye contaminated water is visible due to their pigmentation (Roy and Bharadvaja 2019). Wastewater effluents from textile and dye manufacturing industries are discharged into water bodies, which disturb the ecosystem. It also raises certain health issues to aquatic life, flora and fauna. Most of these dyes are highly cytotoxic, mutagenic, and even carcinogenic to animals and humans (de Lima et al. 2018). The color of these dyes indicates the presence of conjugated systems in their chromophores. Some of these dyes possess auxochromes (such as azo, amino, sulfo, hydroxyl, carbonyl, and

carboxylic acid groups) attached to chromophores, which are responsible for the color shift and recalcitrant to microbial degradation (Xiang et al. 2016). The discoloring effect can be accomplished by breaking electronic bonds within these chromophores.

Over the last few decades, many physicochemical treatments have been employed to combat against dye pollution. However, their implementation is not economical due to high input costs, large amount of sludge generation leading to handling and disposal difficulties, and synthesis of harmful by-products. Some commonly used methods for dye removal include adsorption, oxidative remediation, coagulation, membrane separation, and ion-exchange methods (Vikrant et al. 2018).

Few fungi have been studied to decolorize and degrade the dye containing effluents. The mechanism of dye degradation using fungi involves the processes of bio-sorption, enzymatic biodegradation, or a combination of both. The factors affecting these processes include molecular structure of dye, concentration, temperature, pH, agitation, etc. (Khan et al. 2020). Gola et al. (2018) demonstrated bioremediation of some industrial dyes such as Indanthrene blue, Remazol red, Vat novatic gray, and Yellow 3RS using fungi *B. bassiana* isolated from synthetic wastewater, resulting in a very high dye removal rate (88–97%) for decolorization of industrial dyes. Munck and his colleagues (2018) also demonstrated the application of filamentous fungi *Corioloropsis* sp. by using its biofilms to treat Cotton Blue (CB) and Crystal Violet (CV).

A significant decolorization activity by filamentous biofilm for both these triphenylmethane dyes (with efficiency rates of 79.6 and 85.1%, respectively) was successfully recorded. Bankole et al. (2018) revealed an effective decolorization process of cibacron brilliant red 3B-A dye using some WRF (full form) consortium of *Daldinia concentrica* and *Xylaria polymorpha*, where both these strains were cocultured in static as well as shaking (optimal) conditions using solid-state fermentation technology. Further studies indicated the role of enzymes LiP, MnP, and lac-case present in *P. prosopidis*, and this strain is a cost-effective and eco-friendly option for biodegradation of dye, dye mixture, and dye effluent.

Li et al. (2019) demonstrated excellent abilities of *A. niger* for remediation of certain dyes: Acid Blue 40, Acid Orange 56, and Methyl Blue, with a high biosorption efficiency of 98% in in vitro process. Remediation performance was enhanced by adjusting pH and incorporating the sheets of graphene oxide into a fungus solution. A white-rot fungal strain *Phlebia brevispora* was studied by Harry-asobara and Kamei (2019) for its potential of dyes Congo red and Crystal violet at pH 4.5 and 7, respectively, when grown individually. However, coculturing with bacteria *Enterobacter* sp. resulted in higher removal of crystal violet at all pH and degradation of dye mixture at pH 9 only. Thus, it can be inferred that pH impacts the fungal cell growth, fungal biomass surface dye binding sites, and several biochemical and enzymatic mechanisms.

A novel fungus species *Lasiodiplodia* sp. exhibited the ability to degrade malachite green in a wide range of temperatures and pH. The maximal degradation of this dye was found to be 96.9% under following process conditions: 50 mg/L of dye concentration, 30 °C, and pH 7, thus making it a potential candidate for eco-friendly

remediation of dye polluted environment (Arunprasath et al. 2019). In a study, two fungal strains, *A. lentulus* and *A. fumigatus*, were investigated for bioremoval of wide concentration range of reactive remazol red, reactive blue, and reactive yellow dyes (25–2000 mg L⁻¹). The biosorption process was successful only at concentrations below 250 mg L⁻¹. Bioaccumulation of 500 mg L⁻¹ dye concentration was up to 67–85% using *A. lentulus*. Whereas *A. fumigatus* was also effective, but due to its nonviability after 3-day incubation, its action was limited. Bioaccumulation of reactive remazol red dye by *A. Lentulus* was 76% followed by its biosorption at a high concentration of 2000 mg L⁻¹. Therefore, it can be concluded that dye concentration clearly influences the color removal efficiency of fungal strains.

6.6 Fungal Bioremediation of Aromatic Compounds

The elevating concentration of aromatic compounds, mainly, polycyclic aromatic hydrocarbons (PAHs) and aromatic amines as chemo-pollutants, is becoming a major reason for cytotoxic, mutagenic, and carcinogenic development for most species in the environment (Manzetti 2012). PAHs are organic compounds formed by fusion of two or more aromatic rings by thermal decomposition of organic molecules (Kadri et al. 2017). PAHs occur in nature by volcanic eruptions and generic combustion of biomasses, but incomplete combustion of organic matters is caused by human activities such as oil spills, industrial catastrophes, and industrial waste accumulation accounting for substantial increase of PAHs in environment (Johansson and van Bavel 2003). The high thermodynamic stability, high hydrophobicity, and low volatility make them highly bioaccumulative and persistent in soil sediments. PAH deposition has always resulted in devastating damage to ecosystem, especially in the affected regions for decades. PAHs, being allergic, cytotoxic, and oncogenic, in high concentrations may cause some other chronic effects such as liver and renal abnormalities, skin inflammation, weakened immune system, and teratogenicity during pregnancy (Abdel-Shafy and Mansour 2016). Hence, it is necessary to bioremediate the PAH compounds from the ecosystem. Nowadays, researchers are focusing on the biodegradation of PAHs using fungi, due to their PAH-removal capability by their cometabolic pathway (Agrawal et al. 2018).

Birolli et al. (2018) illustrated anthracene degradation by different marine-derived fungal strains such as *Aspergillus sydowii*, *Cladosporium* sp., *Mucor racemosus*, *Penicillium citrinum*, and *Trichoderma harzianum* in artificial seawater under optimal growth conditions. *Cladosporium* sp. was found to be most efficient for anthracene (71%) biodegradation after 21 days of incubation and was employed for biodegradation of several other PAHs such as anthrone (100%), acenaphthene (78%), fluorene (70%), nitropyrene (64%), pyrene (62%), fluoranthene (52%), phenanthrene (47%), and anthraquinone (32%). Agrawal et al. (2018) also illustrated phenanthrene and pyrene degradation using a novel WRF fungus *Ganoderma lucidum* with efficiency rates of 99.65% and 99.58%, respectively, in a mineral salt broth during 30-day incubation. This strain produced a significant amount of

ligninolytic enzymes (Laccase, LiP, and MnP) in phenanthrene and pyrene-containing media, thus making itself a highly potential PAH degrader candidate. Bioaugmentation approach was adapted by Fayeulle et al. (2019) for biodegradation of benzo[a]pyrene (BaP) by potential saprotrophic fungi present in different PAH-contaminating soil samples.

The mycelia of these BaP degrading novel fungal strains, *C. cladosporioides*, *F. solani*, *P. canescens*, and *T. helicus*, were preestablished on clay particles for their utilization for bioaugmentation purposes. These strains displayed the degradation rates exceeding 30% of BaP (500 μg) within 9 days of incubation. After 30 days, *T. helicus* showed highest BaP degradation capacities, i.e., 26% of the initial amount of total PAH (321.7 mg kg^{-1}) content. This study also confirmed the BaP degrading efficiency of fungal strain *F. solani* as previously reported (Wu et al. 2010). Transformation of several PAHs, namely, anthracene, fluoranthene, phenanthrene, and pyrene by a white rot fungus, *Dentipellis* sp. KUC8613, has been reported by Park et al. (2019). Further genomic and transcriptomic study of this strain revealed that upregulation of P450 genes and downregulation of some PAH-transforming enzymes might be responsible for removal of PAH by nonligninolytic enzymes. This study gives a better insight for screening and utilization of this fungal strain as a host for PAH bioremediation. Some fungi have also oxidized polycyclic aromatic hydrocarbon (PAH) compounds in a cytochrome P-450 monooxygenase-catalyzed reaction, in which one atom of O_2 molecule is incorporated into water and another atom into the PAH, consequently leading to formation of unstable arene oxides (Ghosal et al. 2016).

Many nonligninolytic fungi oxidize PAHs to phenols, quinones, tetralones, trans-dihydrodiols, dihydrodiol epoxides, and several other conjugal intermediates; a few of them were also capable of biodegradation of PAHs to CO_2 (Singh et al. 2020a, b, c). Lee et al. (2020) described the stepwise assessment of bioassay, tolerance, and biodegradation of four PAHs, namely, anthracene, fluoranthene, phenanthrene, and pyrene, against some selective PAH-degrading white rot fungi, including *Peniophora incarnata*, *Perenniporia subacida*, *Phanerochaete sordida*, *Phlebia acerina*, *Phlebia radiata*, and *Microporus vernicipes* (Yadav et al. 2020c, e). The enzymes laccase, MnP, and LiP present in fungus *P. incarnata* were responsible for highest PAH degradative activity (from 40 to >90% efficiency). The genes *pilc1* and *pimp1* encoding enzymes laccase and MnP were expressed, reflecting the phenomena of extracellular enzyme-driven PAH degradation using potential fungi.

Aromatic amines are organic structures with an amine or imino group that are bound to one or more aromatic (benzene) rings. These compounds are ubiquitously present in dyes, textiles, oil refining, plastics, rubber, cosmetics, in pharmaceutical and agrichemical products, and in manufacturing of synthetic polymers, pigments, adhesives, explosives, and also in grilled meats (Manzetti 2012; de Lima et al. 2018). They also cause carcinogenic and mutagenic effects in organisms. The inactivated 4-aminobiphenyl molecule (nonmutagenic in nature) present in aromatic amines has a region (LUMO+2 orbitals) around its amine group, which undergoes reduction by P450 and oxidative enzymes in the organism, enabling the 4-aminobiphenyl structure to gain a particularly reactive electronic potential,

leading to large modifications in LUMO+2 and HOMO-1 electron orbitals upon its metabolic activation. This reduction phenomenon takes place in the immune system of mammals, hence resulting in the activation of nitrenium ion, and those are carcinogenic in nature (Manzetti 2012).

Fungi tolerate aromatic amines by the mechanism of action of enzymes called N-acetyltransferases (NAT) accountable for the transfer of acetyl groups to arylamines present in pesticides and herbicides' toxic residues (de Lima et al. 2018). Among them, some fungal species includes *Aspergillus* sp., *F. oxysporum*, *Trichoderma* sp., *Beauveria bassiana*, and *P. anserine*, which have been reported to degrade some aromatic amines such as p-bromoacetanilide and 3,4-dichloroaniline (3,4-DCA). A study by reported degradation of six aromatic amines substrates, namely, aniline, 4-methylaniline, 4-methoxyaniline, 2-aminophenol, 4-aminophenol, and 1-(4-aminophenyl)ethan-1-one, through N-acetylation up to the bioremediation potential of 15.9–76.1%. The presence of NAT-encoding genes has also been reported in soil sediments (Glenn et al. 2010), making such fungal community a potential tool for bioremediation of aromatic amine-polluted soils.

6.7 Fungal Bioremediation of Pesticides

Pesticides are a diverse group of chemicals constituting both organic and inorganic groups. They are used to control unwanted weeds, pests, and insects as well as act as crop productivity booster. Classification of pesticides is done as per their origin, chemical composition, and target entities. The broad categories of pesticides constitute synthetic pesticides and biopesticides. Pesticides can further be grouped into following chemical families: organochlorine, organophosphate, carbamates, and pyrethroids (Zaman et al. 2020). Although large amount of pesticides is utilized in production of crops worldwide, their recurrent use has led to the contamination of food, air, soil, and ground water (Devi et al. 2020b; Rastegari et al. 2019). Being inherently toxic in nature, it also exhibits detrimental consequences on humans. Long-term exposure to pesticides can cause neurological disorders, reproductive complications, cancer, and immunological and pulmonary diseases (Srivastava et al. 2020). Since pesticide is one of the major concerns worldwide nowadays, exploiting fungi to biotransform a variety of harmful chemicals into the non- or less-toxic forms has been an emerging research interest (Rudakiya et al. 2019).

Wang and his coworkers (2020) demonstrated biodegradation of chlorpyrifos via immobilization of white rot fungi strains *Irpex lacteus*, *Lenzites betulinus*, and *Phlebia* sp., in soil. Corn stover, wood chips, peanut shells, wheat straw, and corn cobs were employed as carriers for immobilization of these fungal strains. *Phlebia* sp. and *I. lacteus* were depicted to be fit for biodegradation purposes while using corn stover and wheat straw as potential carriers. The degradation rate for chlorpyrifos was up to 74.35% in 7 days when *Phlebia* sp. was immobilized in corn stover. Another similar study was carried out by where the same immobilization carriers for the biodegradation of carbofuran were used in the case of fungal strains *Irpex*

lacteus, *Lenzites betulinus*, and *Phlebia* sp. However, the biodegradation rate of carbofuran went up to 69.83% within 5 days after *I. lacteus* was immobilized in corn stover. Both the studies proved that pH and temperature primarily affected biodegradation efficiency of the respective pesticides.

Carbendazole [common name: methyl-benzimidazol-2-yl carbamate (MBC)] is a benzimidazole-based pesticide that is not easily degradable in the environment. The major mechanism for MBC decomposition involves its ring cleavage, which is accomplished by action of both fungal cells and hydrolysis. An experiment by Ahmad (2019) exhibited the adsorption and subsequent bioremoval of MBC from water and soil samples, viz., activated carbon from sugarcane husk. MBC-adsorbed soils were remediated up to 51–68% efficiency rate through the application of concentrated sulfuric acid-activated *S. officinarum* biomass, whereas MBC degradation using fungi *A. niger* and *P. chrysogenum* was successful for MBC removal in water samples (89%). Bisht et al. (2019a, b) reported efficient degradation of endosulfans and chlorpyrifos by using pesticide tolerant fungal strains: *C. cladosporioides*, *I. lacteus*, *P. decumbens*, *P. fimeti*, *T. harzianum*, *P. frequentans*, *S. commune*, *T. hirsuta*, *T. viride*, and *T. virens*. Maximum degradation of chlorpyrifos was observed using *C. cladosporioides*.

The complete degradation of both α - and β -endosulfan isomers to produce endosulfan sulfate was also observed. All tested fungi degraded endosulfan more efficiently than chlorpyrifos, except *P. chrysosporium*, *T. harzianum*, and *T. virens*. Another study conducted by Bisht and her colleagues (2019a, b) illustrated efficient degradation of endosulfan in soil using artificial bed when contaminated soil was bioaugmented with fungal strains *C. cladosporioides* (60.37% degradation), *P. frequentans* (56.18%), *T. versicolor* (67.35%), and *T. hirsuta* (86.23%) up to 30-day incubation period. Russo et al. (2019) investigated the acquired tolerance of DDT on fungi *T. hamatum* and *R. arrhizus* and evaluated their catabolic activity upon 95 carbon sources in the presence or the absence of this pesticide. The presence of DDT promoted the formation of higher amount of reactive oxygen species (ROS) in the fungal cells. These fungal strains represent an attractive potential means for bioremediation of soil contaminated with DDT.

Dey et al. (2020) studied the bioremediation of pesticide lindane, along with bioaccumulation and biosorption of multimetals (mixture of Cr, Cu, Ni, Pb, Zn, and Cd) using fungal strain *A. fumigatus* from composite medium. An enhanced uptake of metals Pb and Zn in fungus was deduced in the presence of lindane. However, the bioremediation of other metals was impeded on addition of the pesticide. Novel fungal strain *Fusarium proliferatum* isolated by Bhatt et al. (2020) from contaminated agricultural fields was utilized for degrading 50 mg·L⁻¹ of pesticide allethrin as the sole source of carbon and energy. This fungus was also studied for its tolerance at high concentrations (up to 1000 mg·L⁻¹) of allethrin. The culture conditions of fungus were optimized and able to effectively degrade allethrin over broad pH and temperature ranges. Kinetics analysis showed that half-life of allethrin decreased substantially from 533.2 to 26.1 hours upon further addition of fungus. These results highlighted the propitious potential of *F. proliferatum* in the bioremediation of sites contaminated with allethrin.

6.8 Fungal Bioremediation of Organic Compounds

Organic compounds consist of aromatic (phenolic and benzene) rings in their structure. Phenols and cresols are one among such compounds with hydroxyl and methyl + hydroxyl groups, respectively, and are attached directly to benzene ring. They adversely affect the aquatic environment (for instance, surface and ground waters and drinking water reservoirs) through rapid reduction of dissolved oxygen levels, limit the exchange of oxygen, and thus exert toxic effects on aquatic animals (Singh et al. 2020a, b, c). Xenobiotic organic compounds (XOCs) such as PAHs, organic pesticides, persistent organic pollutants, and other organic micro pollutants have become a worldwide concern (Ceci et al. 2019). Most common sources include azo dyes, fertilizers, pesticides, preservatives, plasticizers, personal care products, non-steroidal anti-inflammatory drugs, pharmaceutical products, and softeners (Noman et al. 2019; Malyan et al. 2019; Yadav 2019).

6.9 Fungal Bioremediation of Oil Contaminants

Many fungal strains like Arbuscular mycorrhiza, *Aspergillus* sp., *Candida* sp., *Penicillium* sp., *S. cerevisiae*, WRF, and certain mushrooms have been utilized for petroleum-contaminant bioremediation in an artificial environment. Degradation of oil and petroleum contaminants was carried out by fungal ligninolytic enzymes—lignin peroxidases, laccases, and manganese peroxidases. The biodegradation process involves direct/indirect oxidation of aromatic rings (formation of ether peroxide), with spontaneous production of muconic acid derivatives, followed by decarboxylation of the formed carboxyl groups to CO₂. Solubility and bioavailability of oil pollutants are important factors in their remediation (Dickson et al. 2019).

Bioaugmentation approach was adapted by Agarry et al. (2019) for fungal remediation of 10% crude-oil contaminated microcosms containing metals like Ni, Cd, and Zn in mixed form, by utilizing certain fungal strains (*A. niger*, *A. carmari*, and *P. notatum*), or in a coculture system with the bacterial consortia. Total petroleum hydrocarbons in microcosm, when bioaugmented with a mixture of fungal and bacterial consortia, displayed highest rate of biodegradation constant with lowest half-life of these hydrocarbons. Two fungal strains *A. clavatus* and *C. tropicalis* were isolated by Mbachu and his colleagues (2019) from soil samples polluted with spent-engine oil and cocontaminated with heavy metals (Zn, Pb, Cd, and Cu). Although high concentrations of metals inhibited the pollutant biodegradation, the study revealed that pH 5.5 had positive influence in biodegradation of spent engine oil cocontaminated with metals (Cd and Cu) by the pure and mixed culture of fungal isolates. These strains, therefore, can be utilized in the bioremediation of soil contaminated with spent engine oil at appropriate pH levels.

6.10 Fungal Bioremediation of Plastics

Plastics are mostly synthetic polymers made up of long chain of hydrocarbons with a high molecular weight. Plastics rapidly gained popularity due to their high durability, flexibility, light weight, low production, and processing cost. On the basis of chemical structure of their backbone, plastics are classified into thermoplastics (recyclable in nature) and mainly include high- and low-density polyethylene (HDPE & LDPE), polystyrene, polypropylene, and polyvinyl chloride (PVC) and thermosets (nonrecyclable, duroplastic in nature, for example, polyurethane, silicone, epoxy, etc.) (Sánchez 2020). Since plastics are nonbiodegradable, their accumulation in nature causes plastic pollution (Jenkins et al. 2019). Formation of microplastic (the tiny fragments of <5 mm diameter) takes places due to a phenomenon called weathering, which involves oxidation, hydrolytic degradation, photodegradation, and mechanical disintegration of plastic polymer, depending upon their type and morphology (Lehner et al. 2019).

Such microplastics are ubiquitously present in large water bodies, readily ingested and egested by birds, fish, turtles, mammals, and invertebrates in these aquatic systems, whereas nanometer-sized plastics can easily penetrate the cell membranes. Intake of contaminated water and sea food causes exposure of microplastics into humans, causing harmful health effects such as weakened immune system, reproductive ailments, and even cancer (Lehner et al. 2019). The most common methods for disposal of macrosized plastic wastes include landfilling, dumping in water bodies, incineration, fuel production, recycling, road construction, bioplastic production, and biodegradation (Zhang et al. 2019). However, biodegradation using bacteria and fungi has raised interest in the last few years. Plastic biodegradation can be calculated by utilizing different parameters such as loss of weight and tensile strength, as well as erosion of surface (Shahnawaz et al. 2019).

In an investigation by Sangale et al. (2019), many elite polythene-degrading fungal strains were isolated from mangrove, *Avicennia marina* rhizosphere soil, growing near plastic waste dumping sites in the marine environment. Among them, *Aspergillus terreus* and *Aspergillus sydowii* strain P exhibited the maximum percent reduction of $50.00 \pm 4\%$ (pH 9.5) in loss of weight and $94.44 \pm 2.40\%$ loss in tensile strength of the treated polythene at pH 3.5, respectively, thus showing their potential efficiency for polythene biodegradation when incubated at ambient temperature with continuous shaking for 60 days. In contrast, another polythene-degrading fungus *A. flavus* was isolated by Zhang et al. (2020b) from the gut of *Galleria mellonella*, a wax moth. The strain was reported to degrade high-density polyethylene into microplastic particles, and two laccase-like multicopper oxidase (LMCO) enzymes were also retrieved for potential biodegradation of polythene.

6.11 Challenges of Fungal Bioremediation

Due to high tolerance against organic contaminants and heavy metals, many fungi are extensively investigated for degradation of pollutants. However, it is a time-consuming process and found to be more effective only for contaminants that are biodegradable in nature. Despite having such extraordinary bioremediation capabilities, many fungi face interruption in their metabolism and consequently death, due to the saturation of accumulated toxicants or nutrient depletion in the mycoremediation process. The final products obtained by biodegradation can sometimes be more persistent and toxic than the parent metabolites. Many fungi cannot directly assimilate these pollutants as a source of carbon and energy like bacterial and/or several other nonligninolytic fungal strains (Covino et al. 2016). Other challenges include the diversity in chemical structures of untreated toxicants, their interaction with each organism in the ecosystem, and entry of these contaminants into the food chain. Moreover, bioremediation capacity of many fungal strains is still very much understudied. Lack of comprehensive knowledge of biodegradation mechanisms and awareness of process methodologies by different fungal species restricts the application of mycoremediation in a more specific manner (Jain et al. 2017).

A recent study by Zhang et al. (2020a, b, c) validated the improvement of arbuscular mycorrhizal fungal diversity by addition of sodium sulfide (Na_2S) in Pb–Zn polluted soil, accountable for better growth, stronger metal-resistance, and a high uptake rate of heavy metals in the host plant. Without Na_2S , these pollutants had an adverse impact on the fungal diversity and on remediation performance of this host plant. Therefore, factors affecting fungal growth, including media composition, C/N ratio, pH, temperature, aeration, incubation time, agitation, initial concentration of substrate, addition of additives, and inoculum size play a crucial role in bioremediation process (El Enshasy et al. 2017) and can possibly be optimized at large scale for an efficient mycoremediation.

6.12 Conclusion and Future Prospects

Mycoremediation process is not often practiced because of its certain limitations. The isolation and screening of different resilient fungal species from their natural consortia have been carried out since decades to attain distinct sustainable methodologies against diverse pollutants. More studies on coculturing of bacteria-fungi have been carried out to understand the interactions and their impact on development of novel, ecologically stable bioremediation approaches. Moreover, these bench-scale studies can also be upgraded to large scale to provide the insights into mycoremediation efficiencies and applications at industrial level. Mycoremediation efficiency can also be increased through the application of bioreactors to remediate the pollutants such as agricultural, pharmaceutical, and industrial wastes. The remedial efficiency of the bioreactor depends upon the viability of biosorbent and

optimal culture conditions required for fungal growth and biomass adaptability. Optimal processes can be batch, fed-batch, or continuous culture, depending upon the substrate concentration, nature, and amount of bioadditives, inoculum, substrate, and product formed, process feasibility, scale up conditions, and other process parameters.

Various functional genomics tools, such as proteomics, metabolomics, and transcriptomics, are some efficient approaches for understanding the regulation of gene expression present in fungus and the enzyme production aiding in the fungal degradation process, in response to the fungal adaptabilities. Recent advances in metabolic engineering offer unprecedented opportunities for development of transgenic fungi with superior capabilities to retrieve polluted sites and make our environment greener, cleaner, and even sustainable for the future generations. Biotechnological interventions and molecular biology techniques can be implemented for improving the bioremediation capabilities of fungi.

The emerging field of green nanobiotechnology has a prospective to improve the mycoremediation process. The metal nanoparticles can be used as a biosensor for detecting pollutants to control their release in the environment. The microbial system can aid in synthesis of stable nanoparticles, so that fungal sources as the biofactories can further explore the nanoremediation possibilities against dyes, heavy metals, pesticides, etc. to regain and maintain soil fertility. Further new research can highlight the mechanism actions, opportunities, and process methods to improve the fungal bioremediation applications. By adapting fungi for remediation of various polluted environments, contamination of water through runoff and entering of pollutants into the food chain can be prevented. It is also possible to retrieve contaminated regions and to provide a protected and safe region for our future generations.

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Chapter 7

Microbial Consortia for Effective Degradation and Decolorization of Textile Effluents



D. Vijayalakshmi, B. V. Sivaprasad, P. Veera Brahmma Chari, Madhu Kumar Reddy, and Durbaka V. R. Prasad

7.1 Introduction

Dyes perhaps are defined as substances that when applied to substrates provide color by a process that alters, at least temporarily, any crystal structure of the colored substances. It has been estimated that more than 10,000 different types of dyes and pigments are used in the industries and over 7×10^5 metric tons per year of synthetic dyes are produced globally. The most common areas where these synthetic dyes are widely employed are textile, food, pharmaceutical, plastics, cosmetics, and photographic and paper industries. In India alone, the dyestuff industry produces around 60,000 metric tons of dyes, which is approximately 6.6% of total colorants used worldwide. The largest consumer of the dyes is the textile industry accounting for two-thirds of the total production of dyes.

Generally in textile industry, continuous, semi-continuous, or batch processes are the methods for dyeing textile materials. Among these, batch process is the most common method and produces large volumes of effluents, often rich in color, and chemicals and also generates waste as sludge, fibers, and chemically polluted waters. This effluent requires proper treatment before being discharged into the natural resources. If these effluents are discharged into the natural resources without proper treatment, they degrade the quality of the soil and water and its dependent habitats and environment also. Hence, these industries are now facing major problems in environment pollution. To mitigate this problem, industries and researchers are focusing on the reduction of textile wastewater and the formulation of alternative efficient treatment techniques.

In general, the dye removal methods have been classified into three principal categories: physical, chemical, and biological methods. The physical methods

D. Vijayalakshmi (✉) · B. V. Sivaprasad · P. Veera Brahmma Chari · M. K. Reddy
D. V. R. Prasad
Department of Microbiology, Yogi Vemana University, Vemana Puram, Kadapa,
Andhra Pradesh, India

include precipitation (coagulation, flocculation, and sedimentation), adsorption (on activated carbon and biological sludge), filtration, or reverse osmosis membrane processes. Unfortunately, most of the dyes used in textile industry escape these conventional effluent treatment processes and persist in the environment, as a result of their high stability to light, temperature, detergents, chemicals, soap, and other parameters such as bleach and perspiration. Moreover, some limitations in this process can be pointed out, such as cost, low efficiency, labor-intensive operation, and lack of selectivity of the process. These limitations made scientific community in order to search for cost-effective, environmental-friendly alternative methods for effective conversion of toxic textile dyes into nontoxic compounds.

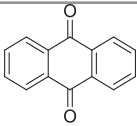
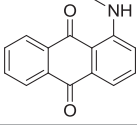
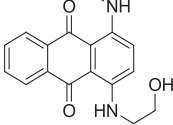
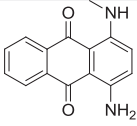
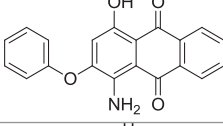
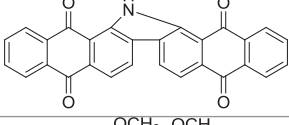
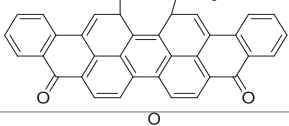
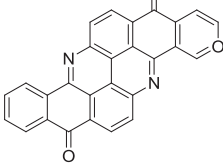
A number of biotechnological approaches have been suggested by researchers as of potential interest in combating with this problem in an eco-efficient manner. Microorganisms are more capable of dye decolorization in general; bacteria either in pure cultures or in consortia in particular have been reported. The applications of microbial consortia in synthetic dye degradation process offer considerable advantages over the use of pure cultures (Yadav et al. 2020c, d). Different strains may interact with dye molecule at different positions. Decomposition of complex organic materials to simple produced by one bacterium, further these simple components may use by another bacterial strains. Therefore, bacterial consortium was developed using selected (five) bacterial strains which are isolated from the textile effluents and also investigated the biodegradation efficiency of toxic anthraquinone dyes, i.e., Acid Blue 25 turned into environmentally nontoxic metabolic compounds which may be confirmed by toxicological analysis.

7.2 Structure and Classification of Textile Dyes

New dyes are continuously being developed to meet the demands of current technology, in fabrics, use of several advanced dyeing equipment, and to overcome the serious environmental concerns associated with some existing dyes. Generally, textile dyes are classified either in accordance with their chromophore group or in their application to textile fibers and other coloring applications. There are some commonly available chromophore groups, namely ethylenic, nitro, azo, keto, nitroso, thioketo, quinonoids, and acridine. Among these, anthraquinone dyes come under quinonoid chromophore group.

Generally, anthraquinone dyes comprise two carbonyl groups on both the sides of a benzene ring (Table 7.1, A). Its dye variants are formed by changing the substituent (amino, hydroxyl, halogen, and sulfonic acid), and the properties and locations of the substituent affect the dye color. Electron-donating substituent and electron-accepting substituent have different effects, with the introduction, and therefore have little influence on color. In contrast, the electron-donating substituent (i.e., amino or substituted amino groups) has a significant impact on dyes with color changing from yellow to red, purple, or blue (Table 7.1, A–D).

Table 7.1 Basic structures of several Anthraquinone dyes

S.No	Name of the dye	CAS	Chemical structure
A	Anthraquinone (Yellow)	84-65-1	
B	1-(Methylamino)Anthraquinone (Red)	82-38-2	
C	1-[(2-Hydroxy amino)-4-(methylamino) Anthraquinone (Purple)	86722-66-9	
D	1-Amino-4-(methylamino) anthracene-9,10-dione (Blue)	1220-94-6	
E	Disperse Red-3B	84-65-1	
F	Vat Yellow-28	4229-15-6	
G	Vat Green-1	128-58-1	
H	Pigment Yellow-24	475-71-8	

Sources: Li et al. (2019)

Anthraquinone dyes are further divided into four categories as follows: (1) anthraquinone derivatives, such as Disperse Red 3B (Table 7.1, E) and Acid Green 25; Acid Blue 25; (2) heterocyclic anthraquinone dyes, such as Vat Yellow 28 (Table 7.1, F); (3) fused ring anthrone dyes, such as Vat Green 1 (Table 7.1, G); and (4) heterocyclic anthrone dyes, such as Pigment Yellow 24 (Table 7.1, H). Due to the presence of different substituents in anthraquinone dye, the color of the dye

varies greatly, and this is the main reason why the same strains have different degradation efficiencies for different anthraquinone dyes.

7.3 Dye Degrading Microorganisms

Bacteria play far more important ecological role in natural environments with their small size which suggests, as an ecological perspective, these bacteria are the part of microbial communities. Each organism in a part of an ecosystem interacts with its surroundings and in some cases greatly modifies the profile characteristics of the environment with their biological process. This is particularly true with bacteria, where significant chemical changes can occur because of their vast metabolic activities.

Microbial ecologists are primarily interested in two aspects in microbiology of ecosystem concerned to bacteria, i.e., (i) the bacterial presence and (ii) the nature and extent of their metabolic activity. The knowledge of the qualitative and quantitative aspects of these two concerns and yields valuable information about the potential function in ecosystem for biogeochemical cycles. Just because the presence of an organism not only enough to overcome the problem necessarily, but they might be metabolically active. Since the only active organisms have an ecological impact. The measurable occurrence of a biochemical transformation thus serves as good evidence that a specific group of bacteria has to be present as well as it should metabolically active. Environmental factors affect the growth of bacteria in nature, as they do in culture. Thus, temperature, pH, water availability, and oxygen all play a role in governing the composition and number of bacteria in a particular habitat.

The microbes can degrade, assimilate, or absorb a wide range of textile dyes (Singh et al. 2020). The biological treatment is more effective than conventional modes of treatment, which is low cost and less accumulation, and produces harmless sludge. Hence, the biological treatment was an eco-friendly and less toxic and causes mineralization of dyes in an easy way. The majority of microbial enzymes currently used in industry are extracellular hydrolytic enzymes (Devi et al. 2020; Kour et al. 2019a). The main theme of decolorization and degradation of anthraquinone dyes was the breakdown of bonds, leading to removal of color, and also produces its metabolites. Anthraquinone dyes are known to undergo reductive cleavage, where aromatic amines are metabolized under aerobic conditions. These enzymes can be produced by a large number of microorganisms which include bacteria (*Aeromonas hydrophila* DN322, *Bacillus cereus* strain DC11, *Escherichia coli* DH5 α , *Staphylococcus* sp. K 2204, and *Staphylococcus hominis* subsp. *hominis* DSM 20328), fungi (*Schizophyllum commune* IBL-06, *P. eryngii* F032), algae (*Lyngbya* sp. BDU 9001), and yeast strains (*C. guilliermondii* Y011, *C. dubliniensis* Y014, *C. guilliermondii* Y004, *C. famata* Y003, and *Kluyveromyces marxianus* IMB3) (Rana et al. 2019; Rastegari et al. 2019; Yadav 2020; Yadav et al. 2020a, b).

7.4 Enzymes Involved in Dye Degradation

There are two major steps that have been involved in degradation of anthraquinone dyes, which consist of cleavage of aromatic amine ring and mineralization of intermediates. The biodegradation ability of bacteria is associated with its intracellular and extracellular oxidoreductive enzyme system such as laccases, lignin peroxidase, manganese peroxidase, tyrosinases, and anthraquinone reductase. However, a single bacterial species may produce more than one enzyme that can actively participate in the biodegradation process (Table 7.2).

7.4.1 Laccases (*Phenol Oxidase*)

Laccase (EC.1.10.3.2) is a low molecular weight multi-copper oxidase family enzyme with less substrate specificity and is potent to degrade a wide range of xenobiotic compounds and aromatic and nonaromatic substrates. It has great importance in biotechnological approaches as it has bioremediation capacity and does not use readily available oxygen as an electron acceptor. Several low molecular weight compounds act as the efficient redox mediator in electron transfer steps of laccase reaction. It is able to degrade and decolorize the phenolic compound, aromatic azo compounds, etc. It oxidizes the aromatic amine using Cu^{2+} as the mediator. Majority of laccases are either fungal origin or plant origin, and very few have bacterial origin having great importance in biotechnological approaches (Kour et al. 2019b).

7.4.2 Lignin Peroxidase

Lignin peroxidase (Lip) (EC.1.11.1.14) is an extracellular glycosylated heme protein, topical owing to their high redox potential and prospective industrial applications. The potential applications of Lip span through sectors such as bio-refinery, textile, energy, bioremediation, cosmetology, and dermatology industries. The huge number of potentials attributed to lignin peroxidase is occasioned by its versatility in the degradation of xenobiotics and compounds with both phenolic and nonphenolic constituents. Several iso-enzymes of Lip are produced; each of them is very specific and distinct in terms of pH, sensitivity, and substrate specificity.

Table 7.2 List of some industrially important microbial enzymes responsible for dye degradation along with its reaction specificity

Name of the Enzyme	Producing organisms	Dye degraded	% of degradation	Reference
Laccase	<i>Geobacillus stearothermophilus</i>	Indigo carmine, Congo red, Remazole, Brilliant Blue R, Brilliant Green	99, 98, and 60, respectively	Mehta et al. (2016)
	<i>Micrococcus luteus</i>	CI Acid Black 234, CI Acid Black 210	92.2, 96.4	Kanagaraj et al. (2015)
	<i>Aeromonas</i> sp. DH-6	Methyl orange	100 (in 12 h in optimum conditions)	Du et al. (2015)
	<i>Providencia</i> sp. SRS82	Acid Black 210, Triazodye	Maximum in 90 min under optimum condition	Agrawal et al. (2014)
Azoreductase	<i>Shewanella</i> sp. Strain IFN4	Reactive Black 5, Acid Red 88, Direct Red 81, Acid yellow 19, and Disperse Orange 3	Showing maximum degradation using Reactive Black 5 as a substrate	Imran et al. (2016)
	<i>Pseudomonas entomophila</i> BSI	Reactive Black 5	93 after 120 h of incubation	Khan and Abdul (2015)
	<i>Aeromonas</i> sp. DH-6	Methyl orange	100 (in 12 h in optimum conditions)	Du et al. (2015)
	<i>Lysinibacillus</i> sp.	Reactive orange 16	–	Bedekar et al. (2014)
	<i>Enterobacter</i> sp SXCR	Congo Red	Wide Range	–
	<i>Alishewanella</i> sp. strain KMK6	Reactive Blue 59	More than 90	Kolekar et al. (2013)
Peroxidase	<i>Pseudomonas putida</i> MET94	Anthraquinonic or azo dyes, phenolics, methoxylated aromatics	–	Santos et al. (2014)
	<i>Providencia</i> sp. SRS82	Acid Black 210	Maximum in 90 min under optimum condition	Agrawal et al. (2014)
Tyrosinase	<i>Providencia</i> sp. SRS82	Acid Black 210	Maximum in 90 min under optimum condition	Agrawal et al. (2014)

7.4.3 Manganese Peroxidase

Manganese peroxidase (MnP) (E.C. 1.11.1.13. Mn^{2+} : H_2O_2 oxidoreductases) belongs to the family of oxidoreductases, to specifically act on peroxide as acceptor (peroxidases), is an extracellular heme protein which catalyzes the H_2O_2 -dependent oxidation of lignin derivative-based polymers. MnP is a specific enzyme that can oxidize Mn^{2+} to Mn^{3+} , which diffuses from the enzyme surface and in turn oxidizes the phenolic substrate, including lignin model compounds and some organic pollutants.

In nature, MnP catalyzes plant lignin depolymerization as component of ligninolytic enzyme complex. So it is one of the most common lignin degradation enzymes and has great and potential application in the field of agriculture for degradation of some cellulose, hemicellulose and lignin, etc. To protect the environment, it was widely used in many industrial fields for the degradation of some recalcitrant organic pollutants such as polycyclic aromatic hydrocarbons, chlorophenols, industrial dyes, and nitroaromatic compounds, which are very harmful to human health. Recently, more and more attention has been paid to the value of bioremediation of MnP enzyme. Previous research had been conducted extensively on azo dyes which could be efficiently degraded by the purified MnPs isolated from *P. chrysosporium*, *Lentinula edodes*, *Trametes versicolor*, *Dichomitus squalens*, *Stereum ostrea*, and *Irpex lacteus* (Yadav et al. 2018, 2019).

7.4.4 Tyrosinase

Tyrosinase (EC 1.14.18.1) is formally termed monophenol monooxygenase and is copper-containing enzyme. It is also known as phenolase, monophenol, oxidase, and cresolase. It is functionally similar to oxidoreductase enzyme.

7.4.5 Anthraquinone Reductase

Reductases are the enzymes which catalyze the reductive cleavage of sulfonated bond to produce colorless aromatic amine products. Several studies have been reported that bacterial cytoplasmic reductases have a suitable application for the purpose of environmental biotechnology.

7.5 Isolation and Identification of Dye Degrading Microorganisms

7.5.1 Sample Collection

Textile industrial units are using lots of water for dyeing process, and this untreated wastewater effluents are directly discharged into the canals. These effluents were collected in sterile containers which were rinsed with the same effluents and then collected, stored at low temperature (4 °C), and transported to the laboratory for analysis. The following process of laboratory analysis path way is used in isolation, decolorization and degradation (Fig. 7.1).

7.5.2 Physicochemical Properties

7.5.2.1 Temperature

Temperature is the most familiar ecological factor and is also easily measured. An ordinary mercury thermometer was used for recording the temperature at sample sites.

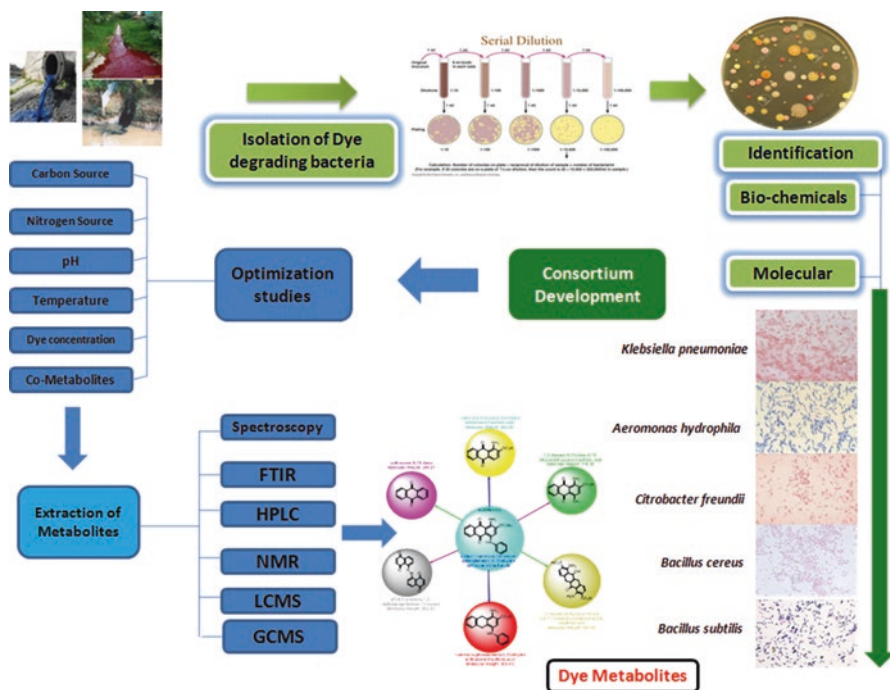


Fig. 7.1 Schematic representation of optimization of Acid Blue 25 degradation process and its metabolites analysis

7.5.2.2 pH

Collected effluents were used to test for pH with the help of calibrated pH meter.

7.5.2.3 Conductivity

Conductivity is a measure of a material's ability to conduct an electric current which is a numerical expression also applied for dye effluents. The ability of ions depends on the presence of total ions, their total concentration, mobility, valency, and relative concentrations including temperature. It has been measured by using a simple device "conductivity meter," and their values were expressed in "micro mho/cm."

7.5.2.4 Turbidity

Turbidity is the clarity of the effluent water. It is caused by dye silt and soil particles, therefore limiting the growth of an organism which is adapted for textile effluent conditions. A nephelometer was used to measure the turbidity of effluents. An appropriate amount of sample was placed in the test tubes, and the turbidity at different ranges was measured, and the values were recorded as "NTU" (nephelometric turbidity units).

7.5.2.5 Dissolved Oxygen

Dissolved oxygen levels in effluent water depend on the physical, chemical, and biochemical activities of microorganisms amended. The amount of dissolved oxygen is also resulted from photosynthetic activity of aquatic plants. Usually, the purification of water system depends on the presence of sufficient amount of oxygen in dissolved nature. In general, the effluent water met with organic pollutants that ultimately deprive the dissolved oxygen content.

7.5.2.6 Biochemical Oxygen Demand

The biochemical oxygen demand (BOD) is an empirical test determined by using dilution method in which the relative oxygen requirements are present in natural waters. It measures the oxygen required for the biochemical degradation of organic material and the oxygen used to oxidize inorganic materials, such as sulfates and ferrous ions.

7.5.2.7 Chemical Oxygen Demand

The chemical oxygen demand (COD) is a reflux method used as a measure of the oxygen equivalent of the organic matter content of water, that is, susceptible to oxidation by a strong chemical oxidant.

7.5.2.8 Total Dissolved Solids

Total dissolved solids (TDS) is denoted mainly by the acclimatization of various minerals present in water. TDS does not contain any gas and colloids. Perhaps, they can be determined by the residue left after evaporation of the filtered sample.

7.5.2.9 Total Suspended Solids

The suspended impurities present in the water mostly are of organic nature and pose severe problems of water pollution. The total suspended solids (TSS) is determined by the difference between total solids and total dissolved solids.

7.5.2.10 Phosphates

Phosphorus is available in nature only as phosphates. It is one of the major nutrients and is necessary for the production of nucleic acids, phospholipids, and variety of phosphorylated compounds. The main sources of phosphates are phosphorous-bearing rocks, discharge of domestic and industrial wastes, detergents, and agricultural runoff. Phosphates are mainly involved in biological productivity and enrichment of nutrients by 'eutrophication.

7.5.2.11 Nitrates

Different forms of nitrogen are available in the nature which has greatest interest in soil or water, in order to facilitate oxidation state, nitrate, nitrite, ammonia, organic nitrogen. All these forms of nitrogen are biochemically interconvertible. Nitrate generally occurs in trace quantities in effluent water but may attain high levels of groundwater.

7.5.2.12 Chlorides

Chloride is a free ion, one of the major inorganic anions in water. The salty taste produced in potable water is due to the presence of chloride ions. In a neutral alkaline condition, slightly alkaline condition, the use of potassium chromate can indicate the presence of chloride.

7.5.3 *Isolation of Dye Degrading Bacteria From Textile Effluents*

Microorganism assimilates the nutrients as the basic requirements of growth. But there is diversity in the use of organic and inorganic compounds. Thus, culture media vary in composition depending on the microorganisms which are to be cultivated. Culture media also provide inorganic salts and may be supplemented with one or more organic diversified compounds for enrichment. The most commonly used medium for dye degradative bacteria is as follows:

Nutrient broth: peptone 5.0 g; beef extract 3.0 g pH 7.0; NaCl 2.5 g; and distilled water 1 L.

Glucose broth: peptone 10 g; glucose 5 g; NaCl 5 g; distilled water 1 L.

Zhou and Zimmermann medium: (g/l glucose 0.5%, yeast extract 0.5%, $(\text{NH}_4)_2\text{SO}_4$ 0.5 g/l, KH_2PO_4 2.66 g/l, Na_2HPO_4 4.32 g/l, distilled water 1 L. The above ingredients were mixed thoroughly, and its pH was adjusted and sterilized at 121 °C, at 15 lbs for 15 min.

7.5.3.1 Serial Dilution Method

A amount of 10 ml of effluent was suspended in 90 ml of sterile water to make a microbial suspension. Serial dilutions (10^{-2} – 10^{-7}) were made. Finally, one ml of aliquot of various dilutions is placed in Petri dishes, by incorporating the sterile, cooled, and molten media; after 24 h of incubation, the culture plates were analyzed for bacterial colonies.

7.5.3.2 Secondary Screening

The selected bacterial isolates are then enriched in culture having Zimmerman media along with different dyes separately. All the strains were individually inoculated into the Zimmerman medium amended with 50 mg/L of each dye (Acid Blue 25, Reactive Blue 19, and Brilliant Blue R). The medium should be sterilized, and

10 ml of this medium is dispensed into different sterilized test tubes. The test tubes were inoculated with one loop full of selected organisms and incubated at 37 °C for 3 days under static and shaking conditions. The color change was noted at regular time intervals. The organisms that show maximum decolonization were selected and used for further biodegradation studies. Among the isolates, the selection of optimal organisms found who possess maximum decolonization of all three anthraquinone dyes.

7.5.4 Identification of Selected Bacterial Strains

7.5.4.1 Biochemical Methods

The “Bergey’s Manual of Determinative Bacteriology” served as a practical guide for the identification of bacteria. The identification was based on the five different criteria: (i) energy and carbon sources, (ii) mode of location, (iii) morphology and gram’s stain reaction, (iv) gaseous requirement, and (v) ability of spore formation. These five common features used for its identification and the information gathered for an unknown bacterium and their identification is as follows:

1. Morphological: shape, arrangement, capsule, gram’s stain reaction, spore stain, and motility.
2. Cultural characters: nutritional state, colonies, temperature, growth, form, margin, elevation, and density.
3. Growth: surface growth, cloudy, sediment, etc.
4. Biochemical tests: The biochemical analysis is usually made to separate the microorganisms easy. The main biochemical aspect of the bacterial identification is the fermentations (glucose, sucrose, lactose, fructose, maltose, mannitol, etc.).

7.5.4.2 Molecular Identification

The selected potential strains were identified by comparing their 16S r DNA sequence with 16S r RNA sequence data reference from the gene bank database using BLAST. The PCR products obtained from amplification with the universal primers and targeting r RNA gene was sequenced by using upstream and downstream primers. All the bacterial isolates and their sequences were confirmed by the using NCBI / BLAST. Program www.ncbi.nlm.nih.gov/blast. Phylogenetic analysis method was used for comparative analysis of genes in order to show the evolutionary relationships which is mainly performed by aligning the sequences by using ClustalW; then, the phylogenetic tree was constructed by MEGA 5 Software.

7.5.4.3 Scanning Electron Microscopy

The scanning electron microscope (SEM) analysis is mainly focused on the identification studies. The selected bacteria were fixed on the surface of glass piece with 2.5% W/V glutaraldehyde overnight. Fixed samples were dehydrated by successive treatment with 30, 50, and 75% (v/v) ethanol and finally with pure ethanol for 1 h. The surface of particles was examined using a scanning electron microscope.

7.5.5 Optimization of Decolorization Process

7.5.5.1 Decolorization Assay

In order to know the decolorization activity of different microorganisms, the percentage of decolorization was calculated by decolorized broth and compared to the corresponding control. After decolorization, the contents were centrifuged at 10,000 rpm for 10 min in a cooling centrifuge. The supernatant was measured at maximum wavelength of each dye selected. Decolorization was expressed in terms of percentage and was calculated by using the following formula::

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

7.5.5.2 Development of Bacterial Consortia

After initial screening of the bacterial cultures with the dye decolorization ability, different potential bacterial strains were selected, namely *Klebsiella pneumonia*, *Aeromonas hydrophila*, *Citrobacter freundii*, *Bacillus cereus*, and *Bacillus Subtilis*. A loop full (0.01 ml) of each selected culture suspensions were inoculated individually into Nutrient broth and incubated for 24 h to form consortium. 2% inoculum containing approximately 2×10^8 cells/ml in different combinations in 250 ml flask. The selected bacterial strains were used in different combinations to construct the different consortiums.

7.5.5.3 Optimization of Dye Decolorization

The decolorization of Acid Blue 25 textile anthraquinone dye by bacterial consortium was optimized with various parameters such as pH, temperature, carbon and nitrogen sources, and dye concentration for efficient dye decolorization.

The optimization of dye decolorization with developed consortium was analyzed by changing the different parameters like carbon source (glucose, sucrose, fructose, maltose, mannitol), different nitrogen source (yeast extract, beef extract, and

peptone), different temperatures (25–45 °C), different PH (5–9), dye concentrations (100 mg to 500 mg/l), in Zimmermann's medium, and the flasks were incubated at 37 °C at static conditions.

7.5.5.4 Extraction of Biotransformed Products

In order to extract the biotransformed metabolites from the decolorized medium, the supernatant was collected, and the degradation products of Acid Blue 25 were extracted with ethyl acetate and concentrated under reduced pressure with the help of rotator evaporator and dried to powder form. Dried extract was dissolved in 10 ml of methanol. After solvent evaporation, collected residue is examined for Uv-Vis spectrophotometer, Fourier Transform Infra-Red Spectrophotometric, Liquid chromatography – Mass Spectrophotometer and Nuclear Magnetic Resonance.

7.5.6 Physicochemical Analysis of Effluents

The physico-chemical parameters of textile industrial effluents were analyzed. The pH ranged between 7.2 and 10.5, and the temperature was at 28–37 °C. Electrical conductivity showed minimum 2.42–6.31 values which is very toxic to plants and microorganisms due to high salt concentration; the total suspended solids of the effluents was observed at a range of 130–650 mg/L. Total dissolved solids is recorded high 8090 mg/L. The high range of TSS and TDS of industrial effluents shows effects on growth and metabolism of plants and microorganisms due to lack of soil permeability and aeration. There was a high load of BOD (500 mg/l), which indicates the low or zero levels of oxygen available. Biochemical oxygen demand and chemical oxygen demand showed high value 500 and 2548 mg/l which implies the toxicity by chemicals which indicates that the dissolved oxygen of the effluents was depleted due to cause of chemical pollution. Phosphates, nitrates, and chlorates showed 10.65, 247, and 2750 mg/L, respectively, which are more than the permissible range of WHO. The chloride extent was 2750 mg/l, and a very high range was recorded in textile effluents.

Selected microbial strains were confirmed for their decolorization efficiency of three dyes at 50 mg/l (Acid Blue 25, Reactive Blue 19, and Brilliant Blue R). Usually, maximum decolorization was showed by bacteria within 1 week of incubation. Screening experiments assessed the potential of selected bacterial strains for decolorization ability of anthraquinone dyes in liquid medium. The decolorization ability of the selected bacterial isolates were ranging from 11.32% to 92.38% (Fig. 7.2).

Five potential bacterial strains were labeled as B1, B2, B3, B4, and B5 according to their maximum decolorization efficiency. The selected bacterial strains were identified by using biochemical and molecular analysis (16S rRNA sequencing) and

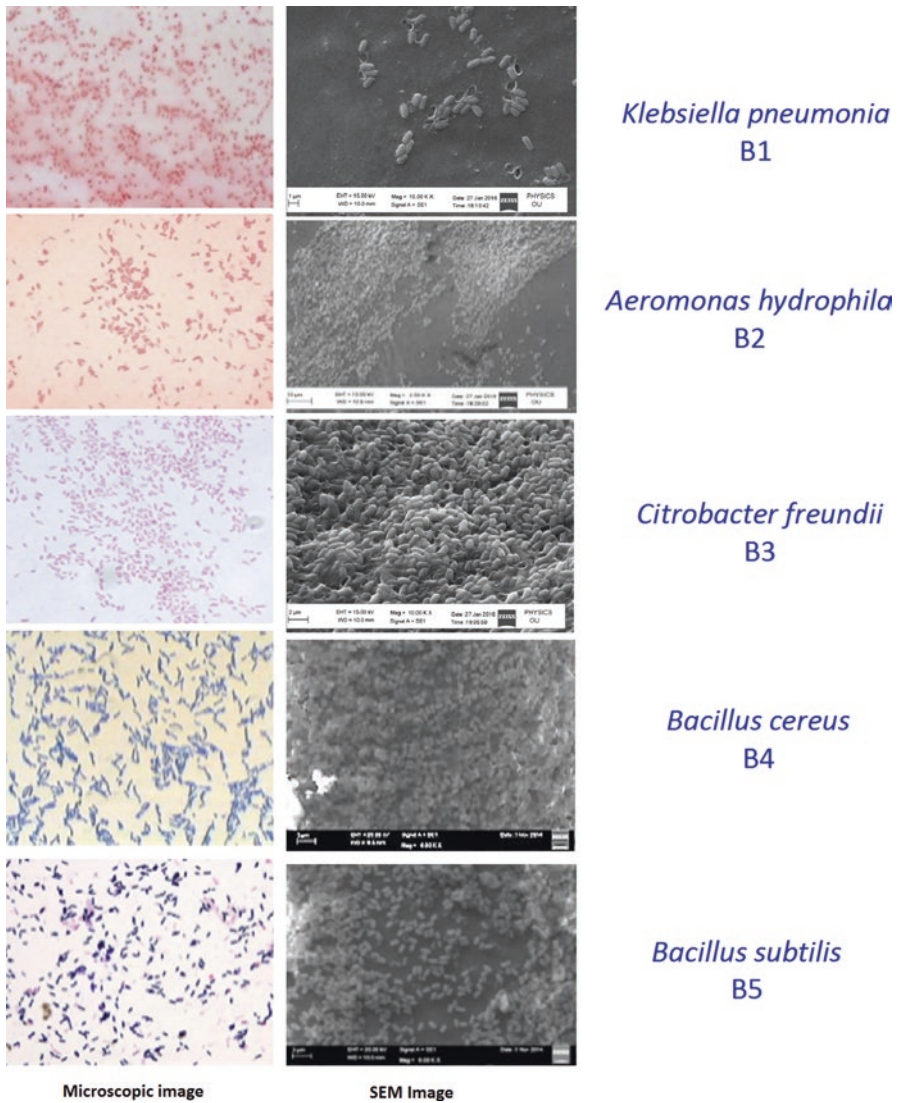


Fig. 7.2 Microscopic and SEM images of selected bacterial isolates

identified: B1 as *Klebsiella pneumonia*, B2 as *Aeromonas hydrophila*, B3 as *Citrobacter freundii*, B4 as *Bacillus cereus* and B5 as *Bacillus subtilis* (Table 7.3).

The maximum decolorization extent of Acid Blue 25 was observed with *Klebsiella pneumonia* (92.36%) within 48 h of incubation followed by *Aeromonas hydrophila* (84.48%), *Citrobacter freundii* (89.31%), *Bacillus cereus* (68.05%), and *Bacillus subtilis* (69.13%) within 96 h of incubation. From the present study, the *Klebsiella pneumonia* was proved to be highly potential bacteria for its

Table 7.3 Characterization of data of compounds A, B, C, D, E, and F forms during microbial degradation of Acid Blue 25

Compound	Name	Mass (m/z)	IR(cm^{-1})	$^1\text{H-NMR}(\delta)$
A	(E)-4-((5,8-dihydroxy-5,8-dihydro naphthalen-1-yl) diazenyl)-5,8-dihydroxy-5,8-dihydronaphthalene-2-sulfonic acid	Calculated $m/z = 430.43$ [A]; Found: $m/z=429[\text{A-H}]^-$	3429(OH), 2924, 2854(Ar-H), 1457(N=N)	7.49–8.39 (m, 5H, Ar-H), 6.81 (s, 4H, C=C-H), 5.19 (dd, 4H, CH), 3.30 (s, 4H, OH), 2.24 (s, 1H, SO_3 of OH)
B	Sodium 1,4-diamino-10-hydroxy-9-oxo-9,10-dihydro anthracene-2-sulfonate	Calculated $m/z = 342.30$ [B]; Found: $m/z=341[\text{B-H}]^-$	3430(OH), 2925, 2853(Ar-H), 1654(C=O)	6.81–7.39 (m, 6H, Ar-H), 5.35 (s, 1H, OH), 4.02 (s, 1H, NH), 4.30 (s, 4H, NH_2).
C	5-amino-6-hydroxy-8-(phenylamino)-2,3-dihydronaphthalene-1,4-dione	Calculated $m/z = 282.29$ [C]; Found: $m/z=281[\text{C-H}]^-$	3428(OH), 2923, 2851(Ar-H), 1655(C=O)	7.15–8.29 (m, 5H, Ar-H), 5.32 (s, 1H, OH), 5.09 (s, 1H, CH), 4.29 (s, 4H, NH_2), 2.27–3.16 (m, 4H, CH_2).
D	Anthracene-9,10-dione	Calculated $m/z = 208.21$ [D]; Found: $m/z=207[\text{D-H}]^-$	2923, 2854(Ar-H), 1657(C=O)	7.85–8.29 (m, 8H, Ar-H).
E	3-hydroxy bicyclo[4.2.0] octa-1(6),2,4-triene-7,8-dione	Calculated $m/z = 150.13$ [E]; Found: $m/z=149[\text{E-H}]^-$	3427(OH), 2921, 2854(Ar-H), 1655(C=O)	6.87–7.39 (m, 3H, Ar-H), 5.33 (s, 1H, OH), 5.10 (s, 1H, CH), 3.79 (s, 1H, OH).
F	3-hydroxybicyclo[4.2.0] octa-1(6),2,4-triene-7,8-dione	Calculated $m/z = 148.12$ [F]; Found: $m/z=147[\text{F-H}]^-$	3429(OH), 2924, 2854(Ar-H), 1658(C=O)	7.17–7.93 (m, 3H, Ar-H), 5.33 (s, 1H, OH).

The Acid Blue 25 dye appears to form six major compounds during microbial degradation. This is indicated in the LCMS analysis of the analyte; the major peaks of its LCMS (Fig. 7.1) are labeled as A, B, C, D, E, and F. These structures were characterized on the basis of negative-ion mass spectral analysis, IR, and $^1\text{H-NMR}$ spectral analysis, and identified their names as (A)-(E)-4-((5,8-dihydroxy-5,8-dihydronaphthalen-1-yl) diazenyl)-5,8-dihydroxy-5,8-dihydronaphthalene-2-sulfonic acid, (B)-sodium 1,4-diamino-10-hydroxy-9-oxo-9,10-dihydroanthracene-2 sulphonate, (C)-5-amino-6-hydroxy-8-(phenylamino)-2,3-dihydronaphthalene-1,4-dione, (D)-anthracene-9,10-dione, (E)-3-hydroxybicyclo[4.2.0] octa-1(6),2,4-triene-7,8-dione and (F)-3-hydroxybicyclo[4.2.0]octa-1(6),2,4-triene-7,8-dione

decolorization efficiency for Acid Blue 25. The results confirmed that the five bacterial isolates can decolorize Acid Blue 25 anthraquinone dye with high decolorization efficacy.

Biodecolorization of textile effluents by using microorganisms is gaining importance as it is eco-friendly, cost-effective, and nontoxic and produces less sludge or

sediment. The bioremediation has a wide range of applications and prospects, because it is mediated by suitable microbial communities which completely decolorize and degrade the contaminated dyes on site. The main advantage of these processes is initiated from the fact that it is an aerobic treatment process, where the toxic aromatic substances formed as intermediates are completely mineralized by bacteria. Previously, several scientific reports showed that microbial degradation of dyes used in industrial level and the released products were characterized effectively.

Optimization studies were conducted with various parameters influenced on decolorization activity of Acid Blue 25 dye. Among these, the dye concentration significantly affects the decolorization potential of bacterial consortium. It was very clear that the decolorization of Acid Blue 25 dye by all groups of bacterial consortium increased with the increase in dye concentration up to 100 mg/l; hence, the maximum decolorization was observed at 100 mg/l of dye concentration; then, the rate of decolorization was reduced while increasing dye concentration up to 600 mg/l (600 ppm). Consortium-1(C-1) showed maximum 96% at 100 ppm, but enthusiastically C-1 showed the decolorization at very high dye concentration also (500 ppm). Among the 11 consortia except C-1, other combination of consortia from C-2 to C-11 showed moderate to less decolorization potential. With the increase in dye concentration to 200 ppm, all of them showed high percentage of decolorization. But in the range of 300 mg/l dye concentration and above up to 500 mg/l only C-3, C-6 showed good decolorization, i.e., 72.36% and 66.24%. In general, C-1 and C-2 are more effective.

The effect of different carbon sources (glucose, fructose, mannitol, maltose, sucrose, and starch) on dye decolorization efficiency of consortium was monitored. Highest decolorization was observed with C-1 when glucose was used as sole carbon source, though this C-1 consortia showed significant decolorization with all the carbon sources. Except C-1, remaining all other combinations of consortiums are not up to the level. It is also highlighted that in the presence of mannitol and starch used as a co-substrates, the decolorization efficacy of C-1 was observed very less than the C-2 and C-6.

Similarly, the effect of different nitrogen sources (Yeast extract, Beef extract, and Peptone) has been used on decolorization efficiency with different consortia. However, the maximum decolorization was observed with all the consortia with all nitrogen sources. The maximum decolorization efficacy (86.34%) with C-1 observed in the presence of yeast extract used as a sole source of nitrogen. The remaining nitrogen sources does not have any influence in the decolorization process. Instead of yeast extract, incorporation of other nitrogen sources (Beef extract and Peptone) are resuming the low decolorization efficacy of other consortium groups (C2-C11).

Out of the 11 consortia, C-1 is found to be the better combination of mixture of different bacterial species that has the optimum compatibility with each other and stringently shares the co-metabolites for their emergency need. Similarly, other influencing factors like 0.1% glucose and 0.5% yeast extract showed almost 90% similar activity in terms of degradation and decolorization.

It was noticed that almost all the individual bacterial strains performed dye degradation activity at pH range from 7 to 8. In contrast to the individual strains

optimized by various pH ranges, the consortium-1 recorded high decolorization (90.73%) activity at the same range of pH significantly. Very less decolorization activity observed at pH 9. Hence, it was concluded that the neutral pH supports the decolorization activity in the medium. Due to the presence of mild alkaline, pH range mediated the enzymatic activity involved in dye decolorization in addition to the cellular growth.

The temperature played an important role in decolorization efficiency of consortium, over a temperature range (25–45 °C). Consortium 1 showed maximum of 88% decolorization; remaining all the other bacterial consortium groups showed the less decolorization efficiency from 60% to 75% when the temperature increased to 37 °C. C-1 recorded 96%. But during the high temperature (45 °C), there is a significant decrease in decolorization of all bacterial consortiums.

Degradation is a complex process that involves the tremendous multiple steps, though there are varieties of bacteria, which can bring about dye degradation efficiency, and each of the species is very obligate and particular to the group of dyes. But the treatment of textile effluent cannot be the easy process where the effluent contains different groups of dyes. Under these circumstances, the constructed microbial consortia with different specification can perform the dye treatment process very efficiently, which is very difficult or not even possible for individual strain.

7.5.7 Monitoring of Biotransformed Compounds of Acid Blue 25

The bioremediation process is receiving increased attention across the globe as eco-friendly and cost-effective for the remediation of dye-contaminated effluents. Generally, anthraquinone dye degradation exhibits intermolecular difference and their recalcitrance to biodegradation due to their complex structures and xenobiotic nature and also is considered as mutagenic and carcinogenic.

After the degradation, the biodegradable products were observed at different stages, which can help to propose and establish in order to predict the detailed mechanism and degradation pathways. Different microorganisms have different degradation pathways for variety of dyes, depending on the structure strategy of microbial system that used for degradation. But even small structural differences can affect the decolorization process. The major mechanism behind the biodegradation of synthetic dyes in microbial systems is the biotransformation of enzymes (Li et al. 2016; Rastegari et al. 2020a, b; Zeng et al. 2001).

It is generally emphasized that reductases play an important role in bacterial dye decolorization. Among the synthetic dyes, anthraquinone dyes constitute second most important class of dyes with a wide variety of shades. The mechanism of biodegradation of anthraquinone dyes involves the reductive cleavage of C=O bond, C-N, and C-OH with the help of anthraquinone reductase enzyme under aerobic treatment process resulting to form colorless aromatic amines. In this regard, to

explain the possible mechanism of dye decolorization, different analytical methods were used to identify the metabolites liberated from anthraquinone dyes after the bacterial treatment.

Usually, the chemical interaction of dye with the metabolic functioning state of cell was principally assessed by UV–Visible spectroscopy. The total disappearance of the absorption maxima after 48 h of Acid Blue 25 dye indicates the completion of decolorization process. Spectral analysis revealed a continuous decrease in the intensity of the original dye absorption peak 600 nm with significant decolorization efficiency in 72 h. After the chromophoric group cleavage, most of the intermediate metabolites seem to be trapped by nucleophilic or electrophilic reaction. This entire event is the crux of further pathway of degradation, where the metabolic burden may divert to activate accumulation or degradation.

The structural analysis of the intermediates is established by FTIR, H NMR, and LC-MS. FTIR is a very useful technique to determine the optimal levels of factors and their interactions with each other. The anthraquinone dye, i.e., Acid Blue 25, degradation showed very interesting results by the facilitation of different and characteristic peaks. The FTIR spectrum of untreated dye (control) showed the specific peaks between 3371.32 cm^{-1} and 1043.93 cm^{-1} for the substituted benzene rings. The peak at 1162.30 cm^{-1} denotes C-N stretch amines, 1216.40 cm^{-1} with C-N stretch amines, 2852.93 cm^{-1} and 2921.99 cm^{-1} for O-H stretch carboxylic acids, and 3371.32 cm^{-1} for the O-H stretch phenol and also for the hydrogen bond with sulfonic acid. The FTIR spectra of treated dye sample showed at 1224.62 cm^{-1} for C-N stretch amines, an aliphatic amines peak at 1454.70 cm^{-1} of C-H bending alkanes, whereas 1654.97 cm^{-1} for C=C stretch alkenes, both 2854.15 cm^{-1} and 2954.93 cm^{-1} for O-H stretch carboxylic acids and 3429.46 cm^{-1} for O-H stretch phenols.

The peaks at O-H stretching of substituted benzene ring derivatives showed that the aromatic nature of the base dye compound treated with bacterial consortium was remained same. Usually the change observed that the surface participation of specific functional groups ensuring the peaks at 1216.40 cm^{-1} . A broad peak was observed at 3371.32 cm^{-1} of the biosorbent before treatment (control) which changed into sharp peak at 3429.46 cm^{-1} for the treated dye by the formation of C=O, O-H, carboxylic acid groups in the biotransformation of dye compounds (Fig. 7.3). In the FTIR spectra the Acid blue 25 degrades to 10 compounds, out of which 6-8 compounds are remaining. Another two compounds are C=C, O-H stretching aromatic structures with NH_2 and OH substitutes of sulfonic acids. At the same time, there are two more peaks remaining, but are reabsorbed or disappeared in the matter.

The H NMR spectrum of intermediates derived from the purified dye compounds during the degradation with retention time of 13 min (Fig. 7.4). The chemical shift region of the acid protons for the compounds 1 and 2 showed double singlets (one proton each) located at 12.343 pm and 13.850 pm. Typical ring aromatic protons of Acid Blue 25 skeleton peaks were observed from 7.20 to 8.40 pm. At the same time, the singlet is recorded at 7.80 pm which corresponds to aromatic ring carbon protons. There are two –OH protons and two – NH_2 protons, observed at 5.10 pm to

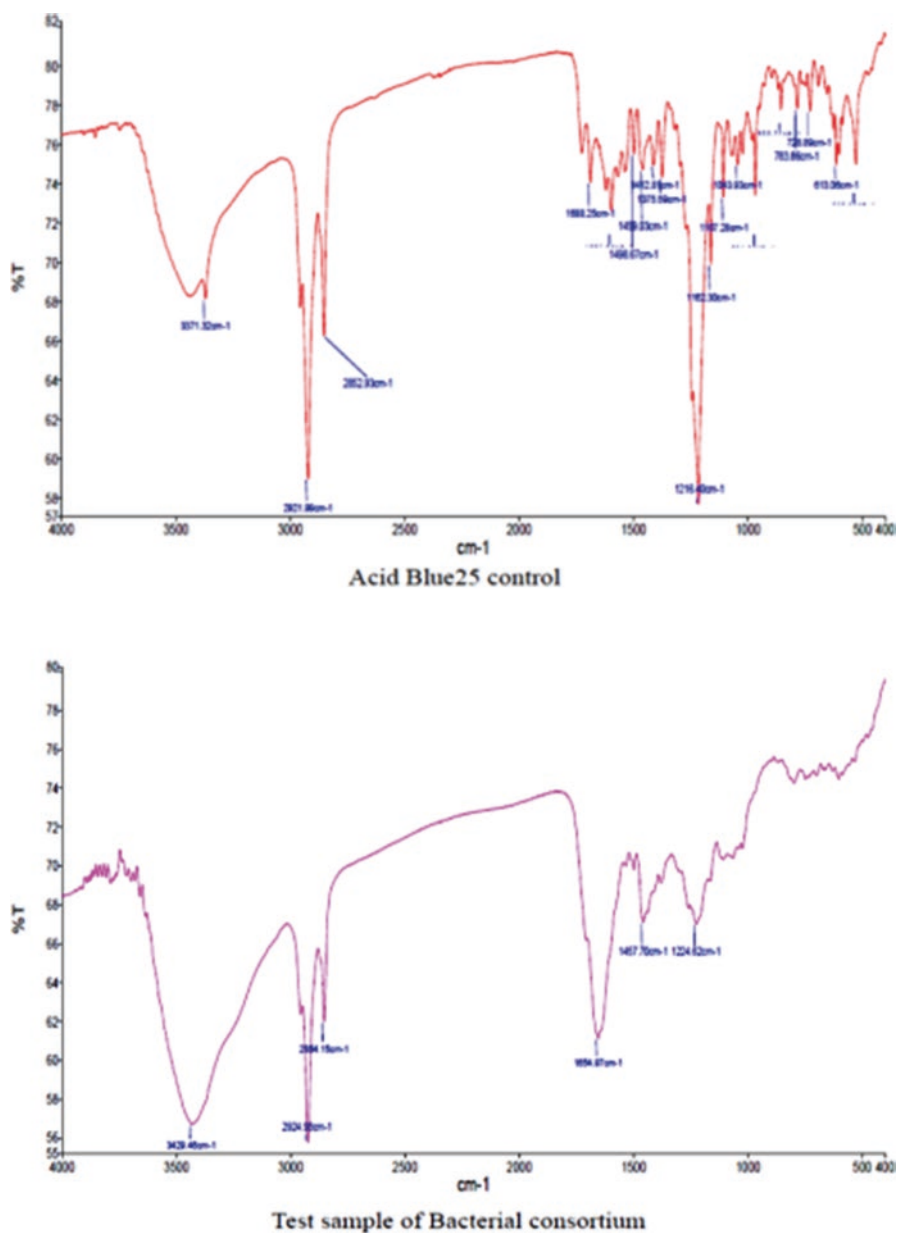


Fig. 7.3 FTIR spectrum of Acid Blue 25 before and after degradation

Similarly, the eluted compounds were extracted and purified, and their biotransformed components were analyzed by LC-MS. The chromatogram was with four retention times (15, 1.995, 6.573, and 4.120), and their molecular mass was observed. The products after degradation mass spectrum peak at retention time of 4.103 showed a significant molecular mass ratios of 207 which corresponds to anthracene-9, 10-dione. No perfect and prominent peaks were at retention time of 3.113. The significant peaks with molecular mass details were recorded at the retention of 19.813, the molecular mass of 208 (M-H) corresponds to anthracene-9, 10-dione, and the molecular mass 303 (M-Na) gives 1-amino-9, 10-dioxo-9, 10-di hydroanthracene-2-sulfonic acid. Similarly at the retention time of 18.263, the peaks observed with the molecular mass of corresponding structures viz., anthracene-9, 10-dione & 1-amino-9, 10-dioxo-9, 10-di hydroanthracene-2-sulfonic acid are showed with 207 and 281 are same with the retention time of 4.103. At the retention time of 19.813, the molecular mass of the compound 342.30 (M-Na) corresponding to structure 5, 5-diazene-1, 2-diyl) bis (naphthalene 1, 4-dione) was derived after the dimerization of compounds was formed. Similarly, at the same retention time, molecular mass of 366.43 (M-Na) of a compound, also formed from the dimerization of phenylamine with 9,10 dihydroxy anthracene-2-sulfonic acid, corresponds to the structure 5 (Fig. 7.5) of (1-amino-4-(phenylamino)-9,10-dihydro-anthracene-2-sulfonic acid), and it was also confirmed from HNMR with negative mode of ionization.

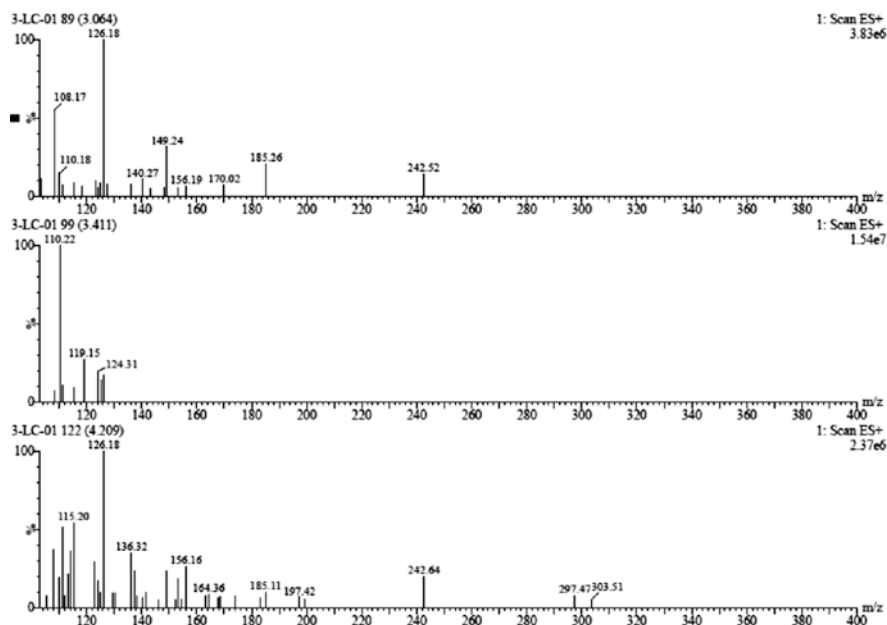


Fig. 7.5 (LCMS) Chromatogram and mass spectra of Acid Blue 25 by bacterial consortium

The compound 4 derived from the degradation of Acid Blue 25 by bacterial consortium showed the molecular mass of 452.46 at 18.113 retention time. It is corresponding to the structure formed from the dimerization of the liberated components; its corresponding structure is 1, 7, diamino-6-hydroxyl-12-oxo-5, 6, 11, 12-tetrahydrotetracene-2, 8-disulfonic acid. The compounds 2 and 3 are synthesized by splitting of phenylamine from the base compound. Their mass values were 303.29 and 318.30, and their corresponding structures were 1-amino-9, 10-dioxo-9, 10-dihydro anthracene-2-sulfonic acid and 1, 4-diamino-9, 10-dioxo-9, 10-dihydroanthracene-2-sulfonic acid. Acid Blue 25 forms abundant deprotonated molecule (M-H) (M-Na) upon ionization via negative-ion ESI. All deprotonated acid dyes fragment upon Collision Activated Dissociation (CAD) fragment ion loss of SO₂ and minor fragment ion loss of the secondary aromatic amine (Fig. 7.6).

The molecular mass value is the monoisotopic mass deprotonated molecule; sodium (Na⁺) cation under negative ion ESI conditions will not be added to the m/z value. The m/z value of deprotonated molecule will differ by one or two Na⁺ ions and/or one hydrogen weight of the dye. It is suggested that the lowest energy of fragmentation is the rearrangement of the sulfonate group to lose SO₂ as compared to the loss of secondary aromatic amine. Therefore, fragmentation pathway is very useful in determining possible structural substituents present on the anthraquinone backbone. The catabolic production of degraded components was identified; some of them are absorbed and reinteracted with derivatives resulted to form dimerized compounds (Table 7.3 and Fig. 7.6). However, these carbonyls, phenol oxides, and amino derivatives were transformed under static conditions during the biodegradation process. Very less information is available on molecular mechanism of transformation studies, particularly on anthraquinone Acid Blue 25 dyes. However, the biotransformation studies on anthraquinone Acid Blue 25 dye knowledge are very important not only for the development of efficient bioremediation, but also in search of harmless dyeing compounds being synthesized during degradation.

7.5.8 Toxicological Studies

The assessment of the ecological and genetic impact of dyes and their metabolites on agriculturally important microorganisms and plant populations is of great importance. Microbial toxicity was studied on *Pseudomonas aeruginosa*, and zone of inhibition was observed with undegraded Acid Blue-25, whereas its biodegradation products did not show growth inhibition. The dye Acid Blue-25 was found to be toxic, whereas the dye metabolites found to be stimulatory to the microorganisms.

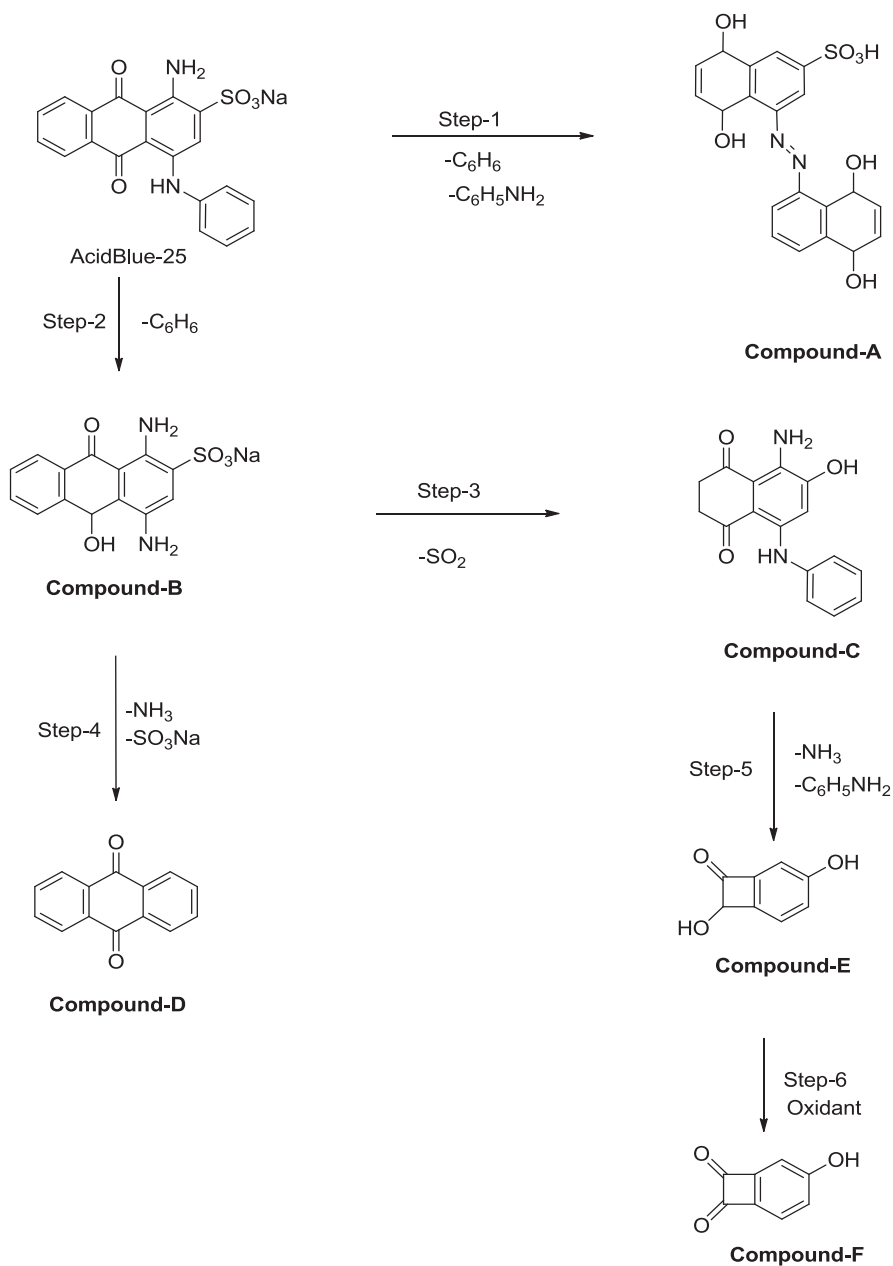


Fig. 7.6 Biodegradation pathway (structures and pathway of metabolites released during the decolorization) of Anthraquinone dye (Acid Blue 25)

7.5.9 Phytotoxicity Studies

After the degradation process the bio-transformed metabolites released were tested for toxicity analysis which is helpful for the safe discharge of effluents from the textile industry. There are two studies in practice, phytoremediation and *microbial remediation*. *Phaseolus mungo* seeds were used to evaluate the phytotoxic effects of Acid Blue 25 and its degraded products. Acid Blue 25 dye and its degraded products in different concentrations (100–10,000 ppm) were along with the appropriate controls. Germination (%), plumule, and radicle lengths are considered as parameters to verify the toxicity levels of metabolites in the biotreated effluents. It was proved that the extracted degradation products are nontoxic in nature, resulting in good germination as well as plumule and radicle length of *Phaseolus mungo* when compared to the untreated dyes.

7.6 Conclusions

From the above information summarized and for growing evidences from literature, and of the work performed by researchers, the following conclusions were made.

1. Bioremediation is a cost-effective, better environmental-friendly, and alternative method for the color removal from the textile industry effluents.
2. Biological treatment has been effective in reducing the textile industry effluents, which has lower operating cost than other remediation systems used for decolorization process.
3. Microbial consortium has become a very good source for the textile industries, in getting rid of problems by biodegradation and decolorization. Because of their low cost and efficiency, they are highly recommended for the industries by making use of consortium for the proper disposal of textile effluents.
4. For the technology transformation there is a need to design a mini reactor or bio filtration unit for the better treatment of textile effluents before they discharge in to the environments showed significant decolorization recorded with bacterial consortium. Maintenance and applicability of this bioreactor were very simple and easy-to-use onsite for small-scale industries.
5. In short, the main advantages of bacterial decolorization process are as follows:
 - (a) Bacterial treatment uses no chemicals.
 - (b) Bacterial consortia dispose no harmful matter into the environment, and the process eliminates chemical processing and unusual vapor.
 - (c) No energy is required; consequently, it is zero-carbon process; no complicated technology is required.
 - (d) Therefore, we conclude that using bacterial consortia is the most effective way of treating industry effluents

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Chapter 8

Fungi in Remediation of Hazardous Wastes: Current Status and Future Outlook



Manali Singh, Dipti Singh, Pankaj Kumar Rai, Deep Chandra Suyal, Satyajit Saurabh, Ravindra Soni, Krishna Giri, and Ajar Nath Yadav

8.1 Introduction

Plants interact with diverse group of microorganisms in order to digest the metal contents in the soil, and the phenomenon is known as phytoremediation. Plant root metabolites released are a good source of minerals and energy which provide ambient environment for the growth of microbes. Plant growth-promoting microorganisms (PGPMs), such as fungi, bacteria, and yeast, enhance the growth of plant by the uptake of minerals and metals in with the help of enzymes like catalases,

M. Singh (✉) · P. K. Rai

Department of Biotechnology, Invertis Institute of Engineering and Technology (IET),
Invertis University, Bareilly, Uttar Pradesh, India

D. Singh

Department of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

D. C. Suyal

Department of Microbiology, Akal College of Basic Sciences, Eternal University,
Baru Sahib, Sirmaur, Himachal Pradesh, India

S. Saurabh

DNA Fingerprinting Laboratory, Bihar State Seed and Organic Certification Agency,
Patna, Bihar, India

Department of Bioengineering, Birla Institute of Technology,
Mesra, Ranchi, Jharkhand, India

R. Soni

Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi
Vishwa Vidyalaya, Raipur, Chhattisgarh, India

K. Giri

Rain Forest Research Institute, Jorhat, Assam, India

A. N. Yadav

Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal
University, Baru Sahib, Sirmaur, Himachal Pradesh, India

laccases, peroxidases, and cytochrome P450 monooxygenases (Tomer et al. 2016; Rawat et al. 2019; Kour et al. 2019b; Mondal et al. 2020; Rai et al. 2020). The metal–microbe interactions are also affected by mechanisms like biotransformation, bioaccumulation, biomineralization, metal chelation, acidification, chemisorption, bioleaching, and bioseparation, thus affecting phytoremediation (Subrahmanyam et al. 2020). Plants respond to different types of biotic abiotic stress like salt, temperature, and pathogens. Microbes and plants coexist for the bioconversion of toxic metals into less toxic forms which is well studied, but not much information is available as far as the underlying, genetic, and physiochemical mechanisms are concerned. Thus, the present chapter focuses on the understanding of the underlying molecular mechanisms of the plant–metal–microbe interaction for sustainable survivability of living organisms and their subsequent impact on phytoremediation.

Phytoremediation is a biological mechanism of recycling wastes into another form that can be used and reused by other microorganisms. These days, the world comes under the serious problem of different types of environmental pollution that is being laden with a large quantum of contaminants and unmanageable compounds like polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), polyaromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs), and heavy metals. Due to their variations in morphology, fungi adapt themselves and play chief role in bioremediation and varied metabolic ability. Microorganisms are omnipresent and thus dwell in varied habitats in all places on the biosphere; because of their remarkable metabolic activity, it is surprising that it enables their survival in all array of environmental conditions. The nutritional capacity of microorganisms is entirely diverse and thus is utilized for the purpose of phytoremediation of environmental pollutants. The chief law is degrading and transforming toxic pollutants such as heavy metal, pesticides, hydrocarbons, oil, and dyes into less toxic compounds.

This enzyme-based process is carried out for metabolizing the hazardous waste into simpler and useful form, so it has immense contribution in resolving many environmental problems. There are two types of factors which govern conditions and establish the rate of degradation, biotic, and abiotic. Diverse strategies and methods, such as stimulation with some microorganisms known as biostimulation, improving the quality of soil through bioaugmentation, bioventing, biopiles, and bioattenuation, are being applied globally (Fig. 8.1). With specific application and limitations, all the types of bioremediation techniques have their own pros and cons. Bioremediation by fungi is the most sustainable and environment-friendly way to manage the unmanageable toxic organic contaminated sites. Here, we attempt to review the role of fungi for detoxification of different toxic and unmanageable compounds including prominent fungal enzymes, viz. catalases, laccases, peroxidases, and cytochrome P450 monooxygenases (Durairaj et al. 2015; Jebapriya and Gnanadoss 2013; Morel et al. 2013).

Microbial organisms are fundamental components to maintain the ecological integrity of any ecosystem (Singh et al. 2020a, b). Microbes have often been considered as pests by farmers and ranchers which are harmful to the crops, cattle, and human as well, but most of the microorganisms are equally beneficial (Suyal and

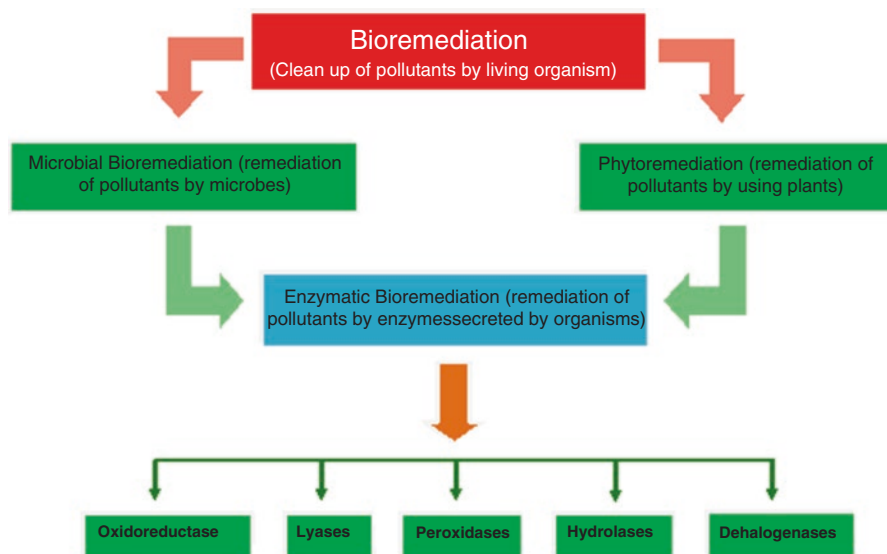


Fig. 8.1 An overview of types of bioremediation

Tewari 2013a, b). Those found in soil (bacteria or fungi) degrade the harmful compounds present in soil and recycle plant waste materials. These microbes help to form symbiotic and nonsymbiotic relationships with the roots of higher plant (Mycorrhiza) using the nitrogen-fixing process and benefit each other in terms of survival and protection (Rajwar et al. 2018; Joshi et al. 2019). Some of microbes are in mutual relationship with the host plant to provide resistance in extreme environmental conditions, i.e., drought, heat, lack of oxygen at high altitude, and insects. Microbial diversity is greatly influenced by types of climatic conditions, altitude, and soil physiochemical properties. High-altitude ecosystems are generally characterized by low temperature, variable precipitation, decreased atmospheric pressure, and soil nutrient stress which have major impact on flora and fauna (Mora n-Tejeda et al. 2013; Soni et al. 2016; Tomer et al. 2017). Cold-adapted microorganism survives well in lower temperatures, due to metabolic balance in subzero temperatures.

There are a number of plant species and microorganisms which are adapted to the harsh environmental and climatic conditions including low temperature, dryness, intense UV radiation, and heavy metal toxicity (Yadav et al. 2020e, f). Now, the question arises how these trees tend to survive even in high altitudes in spite of low temperature and lack of available moisture? If microbes are helping these plants, then what makes microbes survive and how do these microbes reached 6480 m above the sea level and got specialized in increasing crops yield, plant sustainability, and plant growth at higher altitudes. Such mind-bursting questions were a major challenge for the scientist since decades. Several researchers reported that fungi and bacteria arrive first because they disperse more easily and are more

stress-tolerant. They develop spores which become dormant and thrive in adverse conditions. These microorganisms manipulate soil fertility and allow plants to grow by various biological processes and enrich infertile and metal contaminated soil with nutrients and minerals. Microbes thrive underneath snow, thereby creating a way for plants and other life (Edward et al. 2006).

8.2 Bioremediation

Bioremediation is measured as cleaner, cost-effective, and eco-friendly technologies for disinfecting regions infected with varied range of pollutants. Various biological processes are involved for the removal/reduction of hazardous chemicals present in the environment (Kour et al. 2019a, b; Gianfreda and Rao 2004). “Bioremediation” defines the overall enzymatic reactions of biochemical reduction naturally for conversion of harmful compounds into less harmful. This included the use of various biological agents such as bacteria, algae, yeast, fungi, higher plants, and enzymes to degrade environmental pollutants. Bioremediation can be further divided into phytoremediation and microbial bioremediation (Fig. 8.2).

Phytoremediation is an in situ consumption process of plants, roots, their enzymes, and related microorganisms to degrade pollutants present in soil, water, and air. Microbial bioremediation involved various kinds of microorganisms for the removal and alteration of pollutants from a range of environmental systems. The technique of bioremediation based on the release of microbes producing enzymes which are helpful in detoxification of contaminants to harmless products.

Bioremediation techniques can be categorized as breakdown and transfer of hazardous wastes in situ and ex situ (Fig. 8.3). In case of in situ bioremediation, contaminated material is treated at the site of contamination itself, whereas ex situ involves transferring the contaminated material from one site to another site where it has been treated biochemically. It was found that there was a great difference in the rate of biodegradation, and regularity of process outcome differs between in situ and ex situ methods. The ex situ bioremediation cost is reasonably high as compared to in situ due to the cost of uncovering of contaminated samples for treatment (Kumar et al. 2019a). Both the bioremediation methods are basically determined by microbial metabolism, but in situ methods are superior over ex situ for ecological reestablishment of contaminated site (Vidali 2001).

Microbes can live in extreme conditions of environment, but effective bioremediation with enzymatic processes usually favors optimal conditions which are complicated to attain outside the laboratory (Bernhard-Reversat and Schwartz 1997; Vidali 2001; Dua et al. 2002; Dana and Bauder 2011; Kour et al. 2019a). Optimal conditions can be achieved easily in ex situ technique than in situ technique. Therefore, in situ bioremediation by enzymes may be measured as less efficient and taken more time and requires a high concentration of enzyme as compared with ex situ bioremediation.

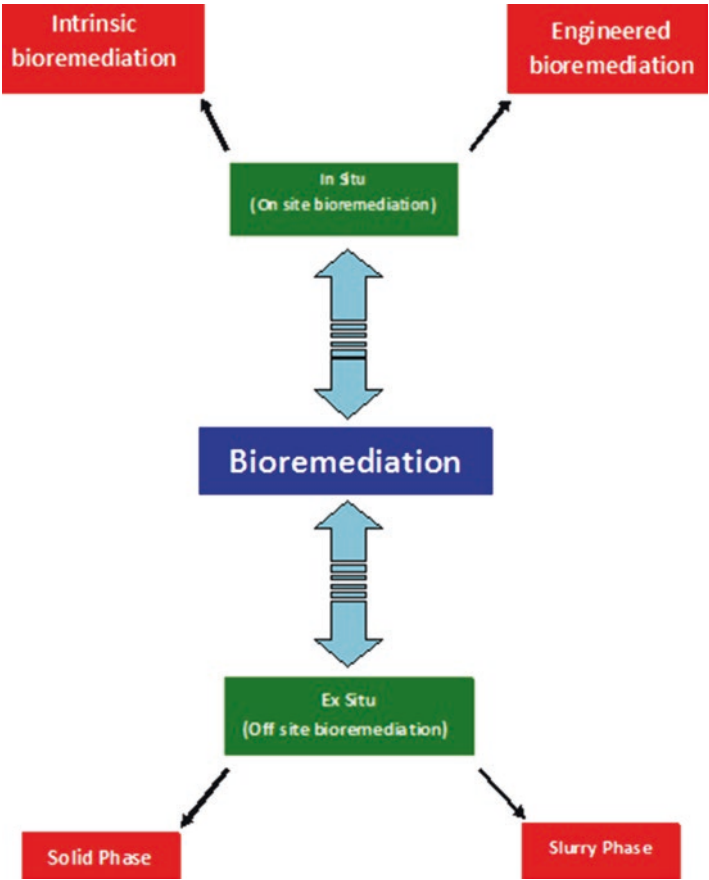
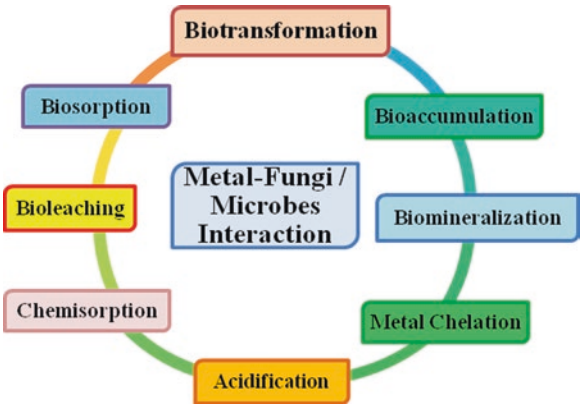


Fig. 8.2 An outline of different methods of bioremediation

Fig. 8.3 Metal–microbe interaction which affects bioremediation



8.2.1 *Types of Bioremediation*

The basic bioremediation methods involve techniques like biostimulation, attenuation, and augmentation.

8.2.1.1 Biostimulation

This kind of strategic is associated through the insertion of precise nutrients at the site (soil/groundwater) to encourage the activity of indigenous microorganisms. In this method, it is required to prompt native or naturally existing bacteria and fungus community—firstly by providing fertilizers, growth supplements, and trace minerals and secondly by maintaining other environmental requirements like temperature, pH, and oxygen to enhance their metabolism rate and pathway (Adams et al. 2015; Kumar et al. 2011). In this technique, there is continuous supply of minerals in the presence of oxygen to the microorganisms for their growth and development. These nutrients fulfill the nutritional demand of the microbes and are measured as the fundamental crucial for life because by this microbial growth get flourished by gaining the basic requirement like cell biomass, enzymes, and energy to decontaminate the polluted site. They also require some vital components like nitrogen, carbon, and phosphorous (Madhavi and Mohini 2012).

8.2.1.2 Bioattenuation (Natural Attenuation)

Bioattenuation or natural attenuation is the removal of pollutant assemblage from surrounding. It is categorized as biological processes; it contains aerobic and anaerobic breakdown through plant and animal uptake, physical phenomena (advection, diffusion, dispersion, dilution, volatilization, and sorption/desorption), and chemical reactions (abiotic transformation, ion exchange, and complexation). In general, natural attenuation definition is related to terms such as internal remediation or bioconversion (Mulligana and Yong 2004). Nature can also plays a role in decontamination of environment through chemicals. First, tiny bugs or microbes living in the soil and groundwater utilize these decontaminants in order to fulfill their food requirement and thereby convert these contaminants into less harmful materials by digesting the harmful compounds in the soil.

Second, a soil has a capability to hold these chemicals through sorption process which restricts their movement and eventually does not cause groundwater pollution. This process restricted the flow of chemicals to other sites. Third, when contaminants move through soil and groundwater, their dilution occurs as it mixes with clean water. This resulted in reduction in the level of contamination. Fourth, the pollutants like oil and various solvents easily convert their state from liquid to gas and are finally ruined by the sunlight. When this method of natural attenuation does not result in rapidly or completely removal of contamination, then bioremediation

can be speeded up by the process of either biostimulation or bioaugmentation (Li et al. 2010).

8.2.1.3 Bioaugmentation

It is the mechanisms of biodegradation. It is an addition of additional pollutant degrading potential microorganism (natural/exotic/engineered) in existing soil which has already its natural microbial populations on the contaminated area to enhance the indigenous microbial capacity; this process is known as bioaugmentation. This process is helpful in quickly enhancing the automatic growth of microbes resulting in speedy degradation of the infected or polluted region. Microbes are assembled from the contaminated site which is separated, cultured, and genetically modified to enhance their capacity and retransferred to the contaminated site. For their favorable growth environments, essential microorganisms are found in their sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene. The in situ microbes can eliminate and change the harmful compounds into ethylene and chloride, which are less harmful comparatively (Niu et al. 2009).

Bioaugmentation is the technique of accumulation of recombinant microorganisms as biological agents of bioremediation for quickly and fully removing the complex pollutants. Furthermore, genetically modified microorganisms resulted in improved breakdown efficiency of contaminants, due to the presence of biochemical ability to break down the complex compounds into lesser complex ones (Malik and Ahmed 2012; Alwan et al. 2013; Gomez and Sartaj 2014). Natural species have not a capacity to break down the certain compounds so quickly and effectively, but this can be achieved by their genetic manipulation. Genetically engineered microbes degrade pollutants much quicker than the natural species and highly compete with the indigenous species, predators, and also various abiotic factors. Recombinant microbes have tremendous ability for bioremediation of different contaminated sites for varied types of pollutants (Sayler and Ripp 2000).

8.2.2 Advantage of Bioremediation

It is a naturally occurring mechanism, less time-consuming, and satisfactory method of decontamination for contaminated sites. Microbes break down the complex pollutants into simpler one, which is considered as less toxic or harmful substances, for living organisms. When the pollutants are degraded, the residual material, which comes out, as by-product is not harmful such as water, carbon dioxide, and cellular biomass. It is natural process requiring less efforts and can directly be performed on the contaminated site, without disturbing normal activities of neighboring regions. It is also cost-effective as it is a biological process to decontamination comparative to other methods used for removal of pollutants. It is also a significant technique for

removal of oil spills (Montagnolli et al. 2015). These methods resulted in complete degradation of pollutant to less toxic materials and reduce the probability of any further risks linked to the decontamination procedure of the contaminants. Moreover, this process does not use any hazardous chemicals. Fertilizers are added to the soil to enhance the supply of nutrients to the soil microbes for their fastidious growth (Kour et al. 2020b). Bioremediation is the conversion of harmful chemicals and their complete elimination (Sharma 2012). This method is easy, lesser manpower is required, no expense is required financially, and it is eco-friendly and sustainable (Dell Anno et al. 2012).

8.2.3 Limitations of Bioremediation

It is restricted to those compounds that are biodegradable. Some compounds are not easily degradable. Some data suggested that the products of biodegradation may be more constant or hazardous as compared to the pollutants. Biological processes are often highly specific. Degradation by microbes requires enzymatically active microbes, favorable conditions to thrive, and sufficient supply of nutrients. It is complicated to do all the possible arrangements for bioremediation. Research is desirable to expand and do betterment as far as possible for bioremediation techniques that are suitable for regions with harmful mixtures of pollutants collected at a particular site. Pollutants can be of various types in either gaseous, aqueous, and solution form or hard crystalline form. Most of the time, a longer duration is required for the treatment procedures, such as uncovering and removal of soil or burn up. It requires very experienced human power (Kumar et al. 2011).

8.2.4 Fungi as Agents of Bioremediation

Breakdown of toxic/complex materials that cannot be broken down easily with the help of fungi and other microbes into smaller and lesser toxicity results in return making bioavailability of important nutrients to the soil for the further growth and development of the plants and microbes symbiotically. This microbial-based decontamination known as bioremediation is very useful for agricultural practices. Thus, the elimination of such toxic compounds can be due to the requirement of living organisms itself or degradation procedure or can be by enzymatic or nonenzymatic methods (Loss and Yu 2018; Yadav et al. 2020c, d).

Recalcitrant compounds are new to the environment which are not biodegradable and introduced by the human activities and their interaction with the surroundings (Fetzner 2002). Different types of hazardous and untreated chemicals are released into the environment by the human activities by different channels of wastewater (Cabana et al. 2007). Recalcitrant compounds are released from untreated hazardous waste, human activities, solid waste from industries, wastewater agricultural

wastes, pesticides and insecticides, polycyclic aromatic hydrocarbons (anthracene, benzo[a]pyrene, chrysene, naphthalene, and pyrene) (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane [DDT]). These harmful chemicals are not easily degraded even by the fungi or microbes (Fernando and Aust 1994; Devi et al. 2020). Fungi dwell and thrive in various regions of earth comprising of different soil textures where they form colonies with the higher plants; as a result of this association, the chemical exudates are released from plant; the heavy metals are metabolized by the fungi, and the process is known as phytoremediation (Kour et al. 2020a). Fungi survive in complex environmental conditions by developing spores (Anastasi et al. 2013). Fungi occupy a large area on the earth crust and are distributed all over in the different habitats on the earth surface (Odegaard 2000; Blackwell 2011). They approximately constitute 75% of total soil surface dwelling in varied soil matrix (Ritz and Young 2004), but till date only a few species have been studied in detail and described (Stajich et al. 2017). Fungi grow well with the help of filaments known as fungal hyphae which resemble the roots of higher plants. The fungi grow slow in comparison to bacteria inhabiting the areas of low nutrition (Harms et al. 2011). Though their length is approximately 2 ~ 10 μm , they also can cover wide areas.

The turgor pressure inside the hyphae (20 μm — $^{\text{J}}$ long) is about 600 kPa, equivalent to the tire of bike due to the presence of glucans and chitin which make up the cell wall (Lew 2011), while in bacteria due to the presence of peptidoglycans (Scott Cayley et al. 2000; Deng et al. 2011). Thus, the tremendous turgor pressure helps the filamentous fungi in penetrating the hard surfaces reorganizing the soil matrix (Bornyasz et al. 2005). Both fungi and bacteria possess a wide range of metabolically active digestive enzymes that aid both the microbes in degradation of complex molecules into simpler ones. These also produce substances that help in reaching those least exposed compounds for degradation (Cooper and Paddock 1984; Desai 1987). Several studies have proved that enzymes from white-rot fungi are effectual degraders of so many organic pollutants like dyes, PAHs, phenols, polychlorinated biphenyls (PCBs), and TNT (Yadav et al. 2019). Numerous enzymes such as cellulase, lipase, and protease prove to have the capability to lessen solid contents and number of pathogens and enhance deflocculation in the sludge. Enzymes like hydrolases and oxidoreductases are used widely in bioremediation. Extracellular enzymes are those which are either secreted by the microorganisms (e.g., lignin peroxidase (LiP) from white-rot fungi) or which go through the aqueous stage during an aerobic submerged fermentation procedure (Bhargava et al. 2003). Some strains of white-rot fungi express LiP and MnP, while some express MnP and laccase; thus, these enzymes work either together or independently (Ellouze and Sayadi 2016).

The genome of fungi is bigger as compared to the bacteria, (8000–180,000 kbp) (Mohanta and Bae 2015) having greater number of transcription and other related factors governing the existence of microbes in harsh and unfavorable environmental conditions. Fungi can withstand typical environmental factors owing to the presence of highly regulated genes which help fungi in survival. The well-understood nutrient utilization, defense mechanism of fungi in the stress conditions, and well-organized multiplication are all factors which help fungi to grow and reproduce in harsh conditions. The enzymes peroxidase and laccase are capable of catabolizing a

wide range of toxic compounds which are not easily broken down. These enzymes are tightly under controlled genetic regulation and thus are used in decontamination of industrial areas which have been contaminated with huge amounts of recalcitrant compounds (Rastegari et al. 2020a, b). The fungal strain of white-rot fungi produces extracellular enzymes manganese peroxidase and lignin peroxidase, which break down lignocellulosic material (Loss and Yu 2018). Common enzymes of fungal origin used in bioremediation include amylases, catalases, cellulases, laccases, lipases, peroxidases, proteases, and xylanases, which have potential industrial applications, and are used in managing organic waste (Marco et al. 2013; Rastegari et al. 2019; Yadav 2020; Yadav et al. 2019). These fungal enzymes may be utilized for hydrolysis of polymeric materials, for example, cellulose, protein, lipid, starch, and xylan from organic wastes such as kitchen waste, vegetable market waste, leaf, and litter. The hydrolyzed substances can be further used for composting or for manufacturing significant products such as volatile fatty acids and biogas (Khardenavis et al. 2013; Hattori et al. 2015).

The group of white-rot fungi synthesizes various types of ligninolytic enzymes which rely on the fungal species and environmental conditions, which cannot only be used for lignin degradation of lignocellulosic materials but also in the remediation of different recalcitrant pollutants including dyes. These enzymes alter azo dye configuration by damaging the chromophoric linkages which lead to the synthesis of phenoxyl radicals in the reactions (Gul and Donmez 2013). Ligninolytic enzymes produced by the group of white-rot fungi have been divided into two groups: peroxidases (lignin peroxidases and manganese peroxidases) and laccases (Jebapriya and Gnanadoss 2013; He et al. 2015). These are considered as important agents for biodegradation of lignin-containing material in environment and therefore also contribute largely to the carbon recycling. Previous studies showed the bioremediation prospective of white-rot fungi like *Bjerkandera adusta*, *P. chrysosporium*, *Pleurotus* sp., and *Trametes versicolor* in the degradation of recalcitrant compounds by secreting various ligninolytic enzymes like laccases and peroxidases, which results in bioaccumulation, toxicity to aquatic life, and deleterious effects to human beings (dos Santos Bazanella et al. 2013). Mechanisms are adopted by fungi for bioremediation of toxic, recalcitrant compounds, and this review assesses the multifaceted role of fungi in the bioremediation of xenobiotic compounds with reference to features employed by the fungi for detoxification and subsequent bioremediation of toxic waste.

Breakdown of lignocellulosic material comprises of extracellular enzymes, namely manganese peroxidases, H_2O_2 -producing enzymes, veratryl alcohol, and oxalate. Such enzymes are glycosylated heme proteins that break down hydrogen peroxide to water. Both LiP and MnP have high redox potentials and low pH (Piontek et al. 2001; Erden et al. 2009). As a result, they can easily break down those compounds which cannot be broken by microbes like polycyclic aromatic hydrocarbons (PAH), phenol and its derivatives, cyanide, and TNT (Cameron et al. 2000; Oyadomari et al. 2003). Lignin peroxidases (LiP) are oxidoreductases, found in white-rot basidiomycetes (Pointing et al. 2005; Hammel and Cullen 2008;

Martinez et al. 2005) which for the first time were studied in the basidiomycete *P. chrysosporium* in 1983 (Glenn et al. 1983). These enzymes are of immense significance in industrial treatment processes (Erden et al. 2009). Manganese peroxidases (MnPs) are also oxidoreductases (Hammel and Cullen 2008). MnP is also produced by basidiomycete *P. chrysosporium* (Paszczynski et al. 1985). MnP catalyzes the oxidation of phenolic structures to phenoxyl radicals (Hofrichter 2002). Laccases act on xenobiotic compounds, thus eliminating the toxic compounds or those which cannot be easily broken down (Ellouze and Sayadi 2016).

Fungi have been shown to play a significant role in bioremediation of variety of pollutants such as POPs, textile dyes, petroleum hydrocarbons, pulp and paper industry effluents, leather tanning effluents, PAHs, pesticides, and PPCPs (Table 8.1). Filamentous fungi like *Aspergillus*, *Curvularia*, *Acremonium*, and *Pythium* have been studied for their metal tolerance ability (Akhtar et al. 2013). Members of the basidiomycota, such as *T. versicolor* and white-rot fungi *Pleurotus ostreatus*, are capable of breaking down PAHs in solid-state fermentation (SSF) growing on agro-industrial wastes, such as orange peels (Rosales et al. 2013). Bioremediation/decolourization of colored effluents from sugar industry, textile dye, bleached kraft pulp mill, and leather tanning effluents has been reported in case of fungi belonging to various groups including *Aspergillus*, *Penicillium*, and alkali-philic white-rot fungi indicating diverse substrate preference of these fungi (Jebapriya and Gnanadoss 2013; Huang et al. 2014; Buvaneswari et al. 2013; Duarte et al. 2013; Reya et al. 2013).

Bioremediation with fungal strain *A. niger* and *P. chrysosporium* eliminates petroleum hydrocarbons from toxicated soil even when exposed to smaller duration as indicated by enhanced total organic carbon (TOC) removal (Maruthi et al. 2013). Silambarasan and Abraham 2013 observed elimination of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) by *A. niger* JAS1 from infected regions completely removing both the metabolites. The degradation of TCP by chlorpyrifos-degrading strain was a significant finding considering the antimicrobial nature and catabolite repression property exhibited by TCP.

8.3 Sources of Heavy Metal Contamination

Heavy metals (e.g., cobalt, copper, iron, manganese, molybdenum, and vanadium) are required by the living beings in traces or very minute quantities. But when these metals exceed in their quantities, then they become dangerous for the survival of living beings. There are different sources of these heavy metals like waste disposal generates metals like arsenic, cadmium, chromium, copper, lead, mercury, and zinc, while agriculture practices generate metals like arsenic, cadmium, copper, lead, selenium, uranium, and zinc. Industrial wastes cause contamination of metals like arsenic, cadmium, chromium, cobalt, copper, mercury, nickel, and zinc, whereas mining and smelting produce arsenic, cadmium, lead, and mercury, and atmospheric

Table 8.1 Fungi involved in bioremediation

SN	Compounds	Fungal communities
1	Persistent organic pollutants (POPs) Polychlorinated biphenyls Polychlorinated dibenzofurans Phenylurea herbicide diuron	<i>Doratomyces nanus</i> , <i>D. purpureofuscus</i> , <i>D. verrucosissporus</i> , <i>Myceliophthora thermophila</i> , <i>Phoma eupyrena</i> , and <i>Thermoascus crustaceus</i> <i>Aspergillus Niger</i> , white-rot fungi, <i>Phanerochaete sordid</i> , <i>Mortierella</i>
2	Textile dye decolourization	<i>Aspergillus niger</i> , <i>A. foetidus</i> , <i>T. viride</i> , <i>A. sojae</i> , <i>Geotrichum candidum</i> , <i>Penicillium</i> sp., <i>Pycnoporus cinnabarinus</i> , <i>Trichoderma</i> sp., White-rot fungi, <i>Bjerkandera adusta</i> , <i>Ceriporia metamorphosa</i> , <i>Ganoderma</i> sp.
3	Petroleum products, crude oil, Gasoline	<i>A. niger</i> , <i>Rhizopus</i> sp., <i>Candida</i> sp., <i>Penicillium</i> sp., <i>Mucor</i> sp., <i>Exophiala xenobiotica</i>
4	Bleached Kraft pulp mill effluent	<i>Rhizopus oryzae</i> or <i>Pleurotus sajor caju</i>
5	Effluent from leather tanning	<i>Aspergillus flavus</i> , <i>Aspergillus</i> sp. and <i>A. niger</i> , <i>Aspergillus jegita</i>
6	Polyaromatic hydrocarbons (PAH) Diphenyl ether, anthracene, naphthalene	White-rot fungi, <i>Pleurotus ostreatus</i> , <i>Trametes versicolor</i> , <i>Armillaria</i> sp., white-rot fungi, <i>Pleurotus eryngii</i>
7	PPCP (pharmaceuticals and personal care products) Caffeine, citalopram, fluoxetine, Sulfamethoxazole	<i>Chrysosporium keratinophilum</i> , <i>Gliocladium roseum</i> , <i>Fusarium solani</i> , <i>A. restrictus</i> , <i>Penicillium</i> and <i>Stemphylium</i> , <i>Bjerkandera</i> sp. <i>R1</i> , <i>Bjerkandera adusta</i> and <i>Phanerochaete chrysosporium</i>
8	Fungicide, Metalaxyl, and Folpet	<i>Gongronella</i> sp. and <i>R. stolonifer</i>
9	Pesticide chlorinated hydrocarbons: Heptachlor Chlorpyrifos	<i>P. ostreatus</i> , <i>Aspergillus terreus</i>
10	Heavy metals	<i>Aspergillus</i> , <i>Curvularia</i> , <i>Acremonium</i> , <i>Pythium</i> , <i>Aspergillus flavus</i>

pollution contains metals like arsenic, cadmium, chromium, copper, lead, mercury, and uranium contamination (Fig. 8.4). Thus, there are varied sources which cause heavy metal contamination of the soil that needs to be eliminated by the process of phytoremediation. Although there have been a lot of studies conducted on the phytoremediation with the aid of microbes, we have listed a few of the important microbes which have been studied on large scale (Table 8.2).

Fig. 8.4 Sources of metal contamination of soil

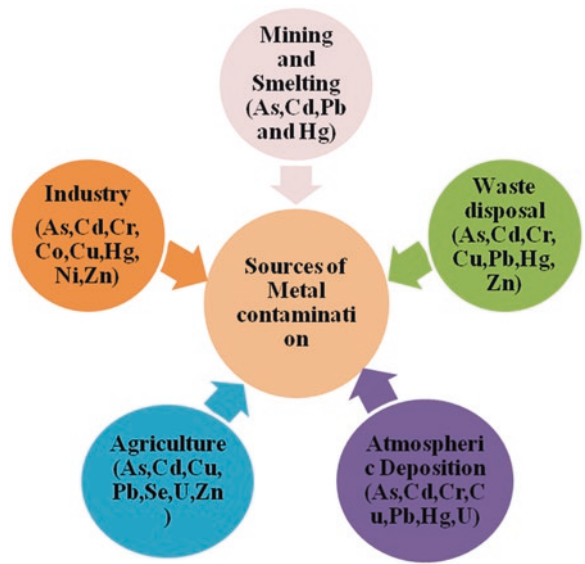


Table 8.2 Phytoremediation of heavy metal contamination by the microbes

Organism	Genus/Species
Fungi	<i>Aspergillus tereus</i>
	<i>Penicillium chrysogenum</i>
Bacteria	<i>Arthrobacter</i>
	<i>Bacillus sp.</i>
	<i>Citrobacter</i>
	<i>Cupriavidus metallidurans</i>
	<i>Cyanobacteria</i>
	<i>Enterobacter cloacae</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Streptomyces sp.</i>
	<i>Zoogloea ramigera</i>
Yeast	<i>Candida utilis</i>
	<i>Hansenula anomala</i>
	<i>Rhodotorula mucilaginosa</i>
	<i>Rhodotorula rubra</i> GVa5
	<i>Saccharomyces cerevisiae</i>

8.4 Effect of Climatic Variations Microorganisms Mediated Bioremediation

Changing in climatic condition has led substantial glacier to thaw which has increased the risk of drought, flash flooding, and soil erosion (Yang et al. 2014). Soil microbes drive a variety of biogeochemical processes such as carbon, nitrogen,

phosphorous, and sulfur cycling, thereby imparting service through soil fertility and greenhouse mitigation (Suyal et al. 2014a, b, 2017; Soni et al. 2015). Yang et al. (2014) have studied to analyze vegetation and soil biogeochemical process in Alpine grassland (covering 35% of Tibetan plateau) which is biologically most threatened region by global warming. Tibetan plateau has the fragile and loose ecosystem, and to study microbes thriving in that region in such ecosystem is itself challenging. The major causes that attributed in the distribution of types of microbial community in different geographical areas are temperature, soil pH, carbon and nitrogen content, climate variability, and plant diversity (Suyal et al. 2019a, b). Biogeographic modifications of microbes up to microbial taxonomic level have already been studied. Now, scientists have started examining microbial diversity, structural and functional gene assisting in metabolic pathways, energetic and regulatory circuits involved in microbial functional potential related to maintenance and sustainability of ecosystem (Suyal et al. 2018, 2020).

Yang et al. (2014) have extensively studied to prove the potential microbial response in changing climate with the study of functional gene. They made use of Gene Expression Omnibus GEO CHIP 4,0 (advance version of GEO chip) to discover microbial gene diversity. GeoChip is a microarray-based tool used in detection of microbial functional genes. It contains a probe whose sequence is complimentary to the target functional gene including those linked with stress response, elemental cycling, and metal resistance. The following hypothesis has been postulated from the studies: (i) The morphology and function of microbes vary among elevations and altitudes, due to differences in soil matrix and changes in vegetation under changing climatic conditions, which are of great significance in the functional morphology; (ii) variation in stress genes of microbes at higher elevation; (iii) variation of C- or N-cycling genes are significantly linked to soil C and N dynamics; (iv) pattern of endemic gene abundance varies among elevation gradient. Wang et al. (2009, 2010) have postulated the following hypothesis: (i) microbial colonization as per the contaminated sites; (ii) huge differences were observed in stress, nitrogen, and carbon cycling genes; (iii) at higher altitudes, cold shock genes were found to be more apparent; (iv) *gdh* gene which converts ammonium into urea was in higher number at higher altitudes as compared to *ureC* which converts urea into ammonium; (v) both N-cycling genes (*ureC*, *gdh*, and *amoA*) and nitrous oxide flux were correlated. On the basis of these observations, scientist predicts that climate changes are responsible for change in soil microbial community functional structure, with particular impacts on microbial N-cycling genes and consequently microbe-mediated soil N dynamics. A total of 49,520 genes were detected via GEO RNA-Seq microarray chip-4.0 metagenomic tool. Although there are many genes which response to stress, here we have mentioned a few of them which were found to be lowest at 3200 site elevation while in double concentration at 3400 m in microbial community (Table 8.3).

Table 8.3 Microbial genes involved in stress response and C/N cycling

SN	Name of the gene	Genes	Role
1.	Cold shock genes	<i>cspA</i> , <i>cspB</i> , <i>desK</i> and <i>desR</i>	Adaptation to cold environment
2.	Heat shock genes	<i>dnaK</i> , <i>groEL</i> , <i>groES</i> , <i>grpE</i> and <i>hrcA</i>	Adaptation to hot environment
3.	Nitrogen cycle	<i>ureC</i> and <i>gdh</i> <i>napA</i> <i>nosZ</i> <i>amoA</i>	Ammonification N ₂ reduction Denitrification Nitrification
4.	Carbon cycle	<i>mcrA</i> <i>pmoA</i> and <i>mmoX</i>	Methane producing gene Methane oxidising gene

8.5 Rhizosphere Engineering

A rhizosphere is a complex association of roots, soil, and diverse microbial consortium (bacteria, archaea, viruses, and microeukaryotes). The complex association enables us to control and modify plant and microbe's interaction to increase crop yield, plant longevity, restore plant ecosystem, and degradation of toxic heavy metals to lesser toxic minerals. The rhizosphere is greatly influenced by root activity (Suyal et al. 2015a, b). The intimacy of plants and their environment is essential for beneficial interactions with soil-borne microorganisms along with the acquisition of water and nutrients (Singh and Yadav 2020). The association also enhances the vulnerability of plants to various types of stresses either biotic or abiotic. Due course of time, plants have evolved a variety of strategies to modify the rhizosphere to lessen the impact of these heavy metals and environmental stresses as phytoremediation and an understanding of the processes involved will suggest ways in which the rhizosphere can be modified for the betterment of plant health and productivity. The rhizosphere is composed of three distinct zones: (i) endorhizosphere—consisting of root cortex and endodermis occupied by microbes and mineral ions, the free space between the cells known as apoplastic space; (ii) rhizoplane—middle zone consisting of root epidermal cells, found next to root mucilage; (iii) ectorrhizosphere—outermost zone extending from rhizoplane out into the soil (Yadav et al. 2020g).

Root exudate and plant metabolism impose a strong impact on rhizosphere. Root exudates include phytohormones, act as a messenger molecule to start biological and physiological interaction between soil microbes and roots of the plant, and facilitate symbiotic associations. It also inhibits the growth of competent plant species. Besides phytohormones, roots also secrete certain phosphatases which are very vital enzyme for mineralization. In the recent past, the changes in climatic condition, such as rising temperature or increase in carbon dioxide concentration, affect rhizosphere ecology, thereby disturbing ecosystem function. Abiotic stresses like drought, salinity, and frost increase the level of ethylene which inhibits root growth and affects plant metabolic pathway. Several reports suggest that there was a rapid decrement in the yield of major cereals between 1981 and 2002 due to the increase in global temperature (Lobell and Field 2007).

Plant growth-promoting microorganisms (PGPMs) are a special type of microbial strain that stimulates plant growth by nutrient solubilization, biological nitrogen fixation, and production of plant growth regulators (Yadav et al. 2020a, b). There are certain plant growth-promoting microbes that are found on the surface of roots which enhance plant growth by the release of microbial exudates in the form of metabolites. These PGPMs have been reported to increase productivity of a variety of agriculturally important crops such as wheat, apples, soybean, and lettuce. A good rhizosphere is an overall indication of good quality of soil, appropriate water content, healthy microbial community, and plant physiology as well. For better crop yield and plant growth, the agronomic management dealing with the components of rhizosphere can be modified over short periods of a plant's growth cycle (Bowen and Rovira 1999). Farmers influence the microclimate of roots of their crops and pasture species every time they do the cultural practices like irrigation of field, weeding, earthing, and application of fertilizer. The rhizosphere tends to acidify by applying ammonium-based fertilizers, whereas the application of fertilizers composed of nitrates results in alkalinity of rhizosphere. Thus, changing the pH can modify the composition of soil of the rhizosphere zone influencing the growth rate and consortia of microbes. More prolonged changes in the rhizosphere that persist through the growth cycle can be generated with plant breeding and biotechnology.

Microbes can be genetically engineered that in turn can manufacture micro- and macronutrients for the developing plant. Genome of the plants can also be altered to harbor novel trait of interest. Certain microbes have already been engineered to inhibit the syntheses of stress-inducing hormone such as ethylene and produce antibiotics against soil-borne root pathogen. The rhizobacteria *Burkholderia phytofirmans* strain has been reported for colonization and promoting the growth of switchgrass under varied conditions, especially in the early growth stages (Kim et al. 2012). Modification in crop plants through root exudation gives better result by manipulating the efflux of H^+ and organic anion from the roots in transgenic plants. Many genes/QTLs have been found controlling the formation of exudates; it is feasible to alter expression level of these genes to engineer the rhizosphere. Synthetic microbial communities in rhizosphere promote disease resistance, insect resistance, and abiotic stress tolerance and enhance biomass and productivity. While designing a microbial community for artificial rhizosphere, several importance regarding microbial traits must be considered prior to selection of microbes: (i) Can these microbes colonize the target host plant? (ii) Can these microbes compete with other microbe in consortia? (iii) Can this microbe attach well to the root surface? (iv) Can this microbe be helpful for enhancing plant yield, stress resistance, and shelf life of the plants?

Increasing demand for food supply has forced researchers to shift from plant breeding programs to PGBM. Synthetic PGBM has allowed human to eradicate all those problems which were frequently faced by farmers such as pest attack, viral attack, abiotic stressed condition, and infertile metal contaminated soil. The emerging field of synthetic rhizosphere provides excellent opportunity to overcome food scarcity. Future research program will apply synthetic biology approach to enhance major food and bioenergy crops even under stressed condition. Strategies to

engineer rhizosphere are quite safe, beneficial to productivity, and improve the sustainability of agriculture system (Verma et al. 2017).

8.6 Genetics Involved in Biotransformation of Heavy Metals

Rhizosphere comprises of the roots of the plant, the plant metabolites released from roots of the plant known as root exudates, and microorganisms like fungi and bacteria, which interact with each other. All of them are significant components of plant–metal–microbe interaction. Thereby altering the composition of metals, minerals and nutrients are available for the plant–microbe–metal interaction with the soil. Plant metabolites released from the roots provide ambient environment to the microbes and help in their growth by providing them ample amount of minerals, nutrients, and energy. On the other hand, microorganisms increase the production of root exudates. As a result, there exists a mutual relationship between the microorganisms and the plant. The plant metabolites tend to increase the bioavailability of metals, minerals, and nutrients from the soil. They stimulate the uptake of metals and nutrients by various methods like (i) by formation of metal complexes by the removal of H^+ by the process of acidification; (ii) by phytochelatin, i.e., chelation of metal ions by the plant protein phytochelatin; (iii) by reduction or oxidation (redox) reactions with the help of enzymes present in the root zone of plants; and (iv) by enhancing the microbial colonization, thereby upregulating the phytoremediation (Strom et al. 2002; Perez Esteban et al. 2013; Sessitsch et al. 2013) (Fig. 8.5).

8.7 Genetically Engineered Bioinoculants

In recent years, Recombinant DNA Technology (RDT) has witnessed a remarkable success by allowing manipulation/modification of prokaryotic and eukaryotic genomes. Transgenic plants, animals, and microbes produced through genetic engineering are boon to human society because of their several advantages. Genetically engineered microbes are used in agriculture, forensic science, weather forecast, and phytoremediation. Engineered bioinoculants such as fungi or bacteria are used in fields to boost crop productivity. Even common farmers make use of biological inoculants and had minimized the use of chemical fertilizers and pesticides. Biotechnology has an immense potential to make significant advances through the use of genetically modified microbial inoculants. Rhizosphere is the most diverse habitat of the planet. Rhizosphere is being modified to solve environmental issues such as improving forest and agriculture sustainability, conservation of bio diversity, and prevention of soil erosion. Different soil microorganisms have been used as bioinoculants such as rhizobia, *Azospirillum*, mycorrhizal fungi, and biocontrol agents.

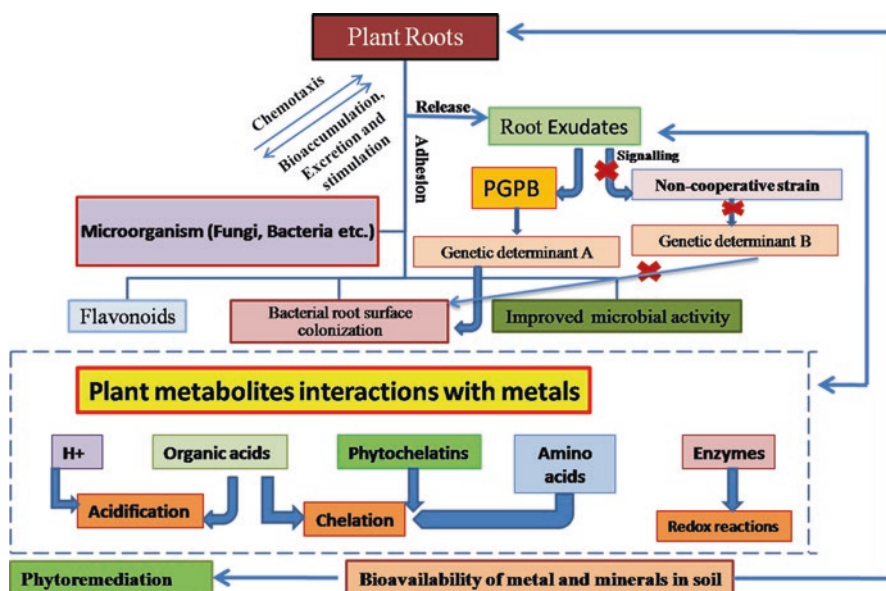


Fig. 8.5 A schematic overview of mechanisms of plant–metal–microbes interaction

8.7.1 *Rhizobia Inoculant*

Rhizobia influence crop yield and productivity by fixing atmospheric nitrogen to the soil, producing plant hormone and reducing the risk of bacterial or fungal infection to plants. Rhizobia inoculant is used in a condition when rhizobia are present in surplus amount with high level of mineral nitrogen. Inoculation should be carried out if (i) population density of species rhizobia, is low, (ii) wasteland have to be converted in grasslands, and (iii) source of nitrogen is poor in native soil.

8.7.2 *Azospirillum Inoculant*

Bacterium *Azospirillum* are nitrogen-fixing bacteria which grow in proximity with higher plants in rhizosphere. It has certain specialized function to perform in rhizosphere such as (i) helps in root development/establishment; (ii) increase in water and mineral absorption rate by root hairs; (iii) nitrogen fixation ability. *Azospirillum* has a tendency to enhance the yield of major food crop plants in different soil and climatic region. Metabolites produced by *Azospirillum* promote host root rate of respiration, metabolism, and growth of roots vigorously.

8.7.3 *Mycorrhizal Fungi Inoculant*

Mycorrhizae association induces plant growth. They form interlinked meshwork between the soil and roots of higher plants which in turn provide plant root making water and oxygen accessible. Mycorrhizal fungi live in association with either the roots of higher plant. They form long filamentous network spread over the soil surface and enhance plant nutrition by providing phosphorous. Unfortunately, till date, large-scale use of mycorrhizae as inoculum is not widely used because of several limitations in obtaining a large amount of inoculum. Growth of plant and its protection may not be fully associated with inoculated microbe but might be due to expression or suppression of residing microbial communities. There may be also synergistic/antagonistic interactions between target and nontarget effects. Such modifications are of great significance to soil in performing different biochemical processes like N_2 fixation.

8.8 Advanced Technologies of Microbial Sensing

Human and plants are sharing healthy relationships with microbial world since ages. Microbes sometimes are proved to be very lethal to human society, on the other hand are equally beneficial. Microbes are playing significant role in human activity and health. Rate of mutation in microbes is very high; each day a new species evolves, but lack of innovative technology makes us lame to identify the type of evolved microbe, its genera and species, and its advantage and disadvantage. Presently, there is handful of technologies to sense new microbial world, but they are not cost-effective and suffer from low sensitivity. These technologies are currently being used in clinical and pathological laboratory, and some advance scientific laboratory also uses this approach. Some of these techniques are described below:

- (i) *Laboratory-on-a-chip (LOC) devices*: Research laboratory-on-a-chip (LoC) devices, or micrototal analysis systems (μ TAS), offer great scopes in terms of their material (e.g., reaction substrates) and time requirements (Abbasian et al. 2018). Currently, these are the most accurate biosensors being used. LoC systems have multiple reaction chambers and channels for the preparing and delivering samples (e.g., fungi, bacteria, and DNA) toward miniaturized embedded sensing sites. The LoC system comprises of engineered microbial biosensor, which has a recognition element and a digital display unit (Charbon 2008). The biological component which functions as recognition part of the biosensor can be fungal components, antibodies, bacteriophages, antimicrobial peptides, and bacteriocins, which convert the biological phenomenon to physiochemical reaction. The digital display unit utilizes microelectromechanical system (MEMS) technology.

- (ii) *Complementary metal Oxide semiconductor (CMOS)*: CMOS is specially designed for the analysis of millions of variations in the nanoscale/microscale levels. It consists of interconnected digital and analog circuit system for sample analysis. This method has an advantage as it is cheap and less time-consuming. This method offers industry-based manufacture of such CMOS devices at large-scale integrated with high-throughput data processing and is user-friendly that makes it inexpensive. Till today, studies have been conducted on the benefits of CMOS for different biological purposes, like DNA sequencing and microbial growth analysis.
- (iii) *Genomic-Based Analysis*: With the advancement in genomic research, currently, microbial genomic studies are the most popular molecular identification and classification methods of microorganisms in different types of samples. Genomic approaches are based on the selection of one or more specific molecular target(s), such as DNA, RNA, and protein, without the requirement to grow in their culture media (Logares et al. 2014). The detection of microbes at genetic level can be accomplished through GC% (Guanine + Cytosine) contents of genome, pattern amplification (polymerase AFLP) and chain reaction, PCR, and loop-mediated isothermal amplification (LAMP), DNA/RNA hybridization, genome polymorphism (such as restriction fragment length polymorphism (RFLP)).
- (iv) *Protein-Based Methods*: The protein-based methods work via either immunological assays or gel electrophoresis. The antigen–antibody-based methods, like enzyme-linked immunosorbent assay (ELISA), agglutination reactions, and western blotting, are most commonly used for recognizing microbes based on their epitopes and are very specific and sensitive.

8.9 Future Outlook

Agricultural and manufacturing industry's rapid intensification has resulted in an increased release of a wide range of xenobiotic compounds. Bioremediation is a biotechnology division responsible for using biological agents, mainly microorganisms such as yeasts, fungi, or bacteria, to clean up polluted soil and water (Strong and Burgess 2008). Microorganisms use contaminants as nutrients or as an energy source in bioremediation processes (Kumar et al. 2011, 2019b, c). In order to compensate for the extinction of microbial communities due to soil pollution, the biodegradation potential of the various groups of fungi is important and is therefore a key aspect of ecosystem recovery. Wunch et al. 1999 carried out the first research detailing the ability of the fungi to degrade anthropogenic compounds. The group reported the degradation of a polycyclic aromatic hydrocarbon by *Marasmiellus troyanus* in a liquid culture. To date, various authors and research groups have been involved to further explore the potential of fungi in bioremediation. Álvarez et al. 2017 reported three genera of fungi, i.e., *Trichoderma*, *Beauveria*, and *Paecilomyces*,

that play a direct and important role in the degradation of complex compounds through a metabolic and enzymatic degradation.

Phenolic compounds, a major environmental pollutant, are noted to be intermediates in generating several other chemicals. Being ubiquitous in waters generated from different industries, phenols generally are arduous to remediate. Thus, developing new methods and technologies is of utmost importance for the proper and complete destruction of these organic pollutants. Administration and application of white-rot fungus, *Trametes pubescens*, have been identified as an effective bioremediation tool for the treatment of phenolic wastewaters. Research studies have predicted white-rot fungi to be the most efficient in removing phenolic compounds from wastewater (Ryan et al. 2008). Air-lift reactor (ALR) has been found to be best suited for the fermentation of white-rot fungi as visible by increased rate of growth, enzyme production, and the accompanying highest removal of phenolic compounds by microbes grown in this ALR. Additionally, being economical and feasible, this bioremediation process is a method of choice for the removal of phenolic compounds from wastewaters.

Aromatic amines (AAs) and their derivatives, being toxic in nature, are believed to adversely affect the human health and animals. These organic compounds act as intermediate for the production of different harmful chemicals. Fungal bioremediation offers an ability to degrade these pollutants effectively, generating compounds which are less toxic and usually inert. Since fungi produce metabolites, they are thus able to metabolize AAs by undergoing hydrolysis or redox and N-acetylation reactions. Fungal bioremediation of AAs is a biofriendly approach, but choosing the right fungal species is essential since some of the fungal species such as *Fusarium* spp. and *Aspergillus* spp. may be pathogenic to animals and plants. Moreover, it is extremely crucial to check the toxicity of the end products of fungal bioremediation since the formed products may elevate the precursor in its toxicity. Creosote, a carbonaceous chemical, is a deadly contamination of soil. Research study has revealed that fungi may have a potential role in treating creosote-contaminated soil.

In a research study (Atagana et al. 2006), five fungal isolates, *Cladosporium*, *Fusarium*, *Penicillium*, *Aspergillus*, and *Pleurotus*, were separately inoculated onto sterile barley grains and were further inoculated onto creosote-contaminated soil and left for incubation at 25 °C for almost 70 days. It was observed that creosote and polyaromatic hydrocarbon (PAH) removal was higher when a mixed population of fungi supplemented with nutrients (barley) was used for treatment. This experiment made evident that fungi (with *Pleurotus* sp. having maximum creosote removal rate and *Aspergillus* isolate, the least) are found to be more efficient than any other microorganisms, mainly bacteria, on the same contaminated soil. The ability of fungal mycelia to reach to the substrate is one of the major reasons for efficient and successful fungal bioremediation of creosote from the soil. The production of olive oil generates olive mill wastewaters (OMWWs), as a by-product, which is known for their high chemical oxygen demand (COD) (50–150 g L⁻¹). The presence of phenol and long-chain fatty acids in OMWWs exhibits phytotoxic and microbial

inhibitory action which renders them unsuitable for agricultural purposes ruining soil porosity and pH.

OMWW varies in composition as per their method of production, processing, and usage and often faces the problem of efficient effluent treatment and disposal due to technical and economical and geographical barriers. Fungal bioremediation is believed to rescue from this situation. To observe the effect of fungal bioremediation, an experiment was set up, whereby 29 fungi were observed for radial growth on minimal medium in combination with 5 varying concentration of OMWWs (5–15%) (Bevilacqua et al. 2016). The results showed that *Aspergillus ochraceus* shows promising features to be effectively used for OMWW treatment. Radioactive waste, being rich in heavy metals and acidity, is potentially hazardous to humans. Incapability of routine physiochemical process in mitigating these radioactive sites has shifted the human attention toward the use of biological processes. In this regard, 27 different environmental yeasts have been characterized offering the advantage of being resistant to extreme conditions, viz. ionizing radiation, heavy metals, pH, and temperature (Tkavc et al. 2018). Among these, *Rhodotorula taiwanensis* MD1149 is the yeast of choice because of being able to form biofilm and withstand radiations, low pH, and high concentrations of mercury and chromium compounds.

Although the fungal biocatalysts have shown promising results in treating wastewaters, the actual gap of translating the technological and biological knowledge from laboratories to industrial or commercial scale still needs to be fulfilled. There are a number of challenges that need to be faced for filling this gap such as maintenance of fungal sterility to avoid cross contamination, minimal use of micropollutants, operative conditions pertaining to WTPs, enhancement of stability, and efficiency of biocatalysts, developing reactor configurations that that can withstand long operation times and integrating the newly developed technologies with the existing ones (Spina and Varese 2016). Nowadays, further efforts are needed to better understand how the fungi work as a whole, and a better knowledge of the complexity of their heterogeneous environment, and the interactions between the different microorganisms, will make it possible to develop more effective bioremediation strategies.

Microorganisms are fundamental basic components to maintain the ecological integrity of any ecosystem. Microbial communities are the research of choice because of certain adaptive traits. Screening of microbial communities for different abiotic stresses and establishing the microbial tolerance to different environmental conditions should be focused for microbial researcher for estimating the potentiality of survival and dispersal abilities in the different species (Tiwari et al. 2020; Verma et al. 2016). Microbiota respond to stresses and disturbances brought by the global change. The recent advancement in biotechnology has led to the identification of soil microbial structural genes, aiming to explore the potential microbial responses to climate changes. Metagenomics-based approached coupled with microarray-based technology will substantially lead to more precise characterization of cold shock genes, carbon cycling genes, and other vital genes.

8.10 Conclusion

Biodegradation is very productive and good-looking option to remediating, cleanup, running, and recovering technique for solving polluted environment through microbial activity. In the future it can be improved by exploring novel species which have great prospectives. The rate of degradation depends upon the microbial communities, availability of essential nutrient, abiotic environmental conditions (temperature, aeration, moisture, pH), and accessibility of contaminants and biotic factors like growth of microbes and bioavailability of microbial stain. Thus, natural decontamination due to these factors is not so easy. But keeping in mind the advantages, this technique is being applied with increased popularity.

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Chapter 9

Applications of Myconanoparticles in Remediation: Current Status and Future Challenges



Suyog A. Joshi, Sagar P. Salvi, Chanda Parulekar- Berde,
and Vikrant B. Berde

9.1 Introduction

Bioremediation is a biological mechanism of recycling waste into another form that can be used and reused by other organisms. Bioremediation is involved in degradation, eradication, immobilization or detoxification of diverse chemical waste and physical hazardous materials from the surrounding by the action of microorganisms. The main principle followed is degrading and transforming pollutants such as hydrocarbons, oil heavy metal, pesticides and dyes. Ex situ bioremediation methods require excavation of contaminated material or toxic substances before they can be treated. There are different examples of pollutants which affect human and animal health like chlorinated hydrocarbons that mostly affect the respiratory tract and cause irritation in eyes; phenols that affect the skin and become chronic by affecting the central nervous system and kidneys; arsenic exposure that causes hyperkeratosis and lung cancer; lead exposure that causes blood toxicity; chromium exposure that causes epigastric pain, diarrhea and chronic ulcer; cadmium that causes renal tubular damage and affects bones (Xie et al. 2016).

Nanotechnology is the branch that deals with the dimension and tolerance of less than 100 nm (Mukherjee et al. 2010; Gholami-Shabani et al. 2016a, b, c). While nanoparticles are considered as a development of new science, nanoparticles were used by craftsmen as far back as the nineteenth-century Mesopotamia, for

S. A. Joshi · S. P. Salvi
Department of Microbiology, Gogate Jogalekar College,
Ratnagiri, Maharashtra, India

C. P. Berde (✉)
Marine Microbiology, School of Earth, Ocean and Atmospheric Sciences (SEOAS),
Goa University, Taleigao Plateau, Goa, India

V. B. Berde
Department of Zoology, Arts, Commerce and Science College,
Lanja, Maharashtra, India

producing a glittering effect on the external of pot. Copper and gold nanoparticles were also used during Roman times (Holister et al. 2003; Shankar et al. 2004; Amin et al. 2011). Nanotechnology is involved in the design, characterization, production and application of particles by controlling their size and shape at nanoscale. Nanostructures exist in different shapes and sizes commonly being the spheres, rods, wires, and tubes. Nanotechnology is the fast and effective solution for environmental cleanup, especially in providing unpolluted water from polluted water resources and cleanup of natural and unnatural contaminants contained in waste and toxic material (Jansen et al. 1994; Pourabadehei and Mulligan 2016).

Mycoremediation is the use of fungi to remove toxins from the environment. There are evidences of the role of specific fungi that neutralizes toxic waste (Yadav 2020; Yadav et al. 2020a, b). It is a process by which various natural agents, such as microorganisms and their products, are used to reduce the environmental contaminants into a reduced amount of toxic or nontoxic forms (Bai et al. 2009; Khan et al. 2015; Kour et al. 2019). Microorganisms utilize intracellular/extracellular enzymes for the synthesis of nanoparticles with different chemical composition, size, shapes and controlled monodispersity (of similar size and shape). It is a cheap, economically promising and eco-friendly means for biosynthesis of nanoparticles. The advantage of green bioremediation, using nanoparticles, beyond economic aspects is high capability, minimization of biological and chemical toxins, selectivity to exact metals, no extra nutrient requirements, renewal of biosorbent and the metal recovery (Cheng et al. 2004; Purnomo et al. 2014).

9.2 Pollution Control Using Nanotechnology

Green-synthesized metal nanoparticles are proficiently used in bioremediation and biofuel generation. They are used to produce hydrogen via oxidizing the carbon monoxide which can be used as fuel cell and to decrease several organic chemical pollutants including *para*-nitro phenol. Titanium dioxide acts as an oxidizing agent in the presence of UV light, so it is also used in contamination control. Another example is the use of silicon and titanium dioxide in photovoltaic cell formation (Serrano et al. 2009).

Nanomaterials might be significantly more reactive than larger particles because of their much greater surface area per unit of mass which makes them suitable in environmental remediation (Hochella et al. 2008). Zerovalent iron (ZVI) is stated to be used in reactive barriers at various sites worldwide, for the eradication of organic and inorganic pollutants (Fu et al. 2014). The reaction rates of nanoscale nZVI are 25–30 times more rapid than the reaction rates of granular iron in the micrometer to millimeter range, and the sorption capability is also much higher (O'Carroll et al. 2013).

Nanotechnology may provide solution to many of the current problems involving water quality. Nanoparticle enhanced filtration products and processes developed with nanotechnology that would greatly help to get potable drinking water. The development of nanoscale materials and processes for treatment of surface water, groundwater and industrial wastewater contaminated by toxic metal ions, radionuclides, organic and inorganic solutes, like pesticides, bacteria and virus, are major environmental contributions of nanotechnology (Devi et al. 2020; Rana et al. 2020). Recent studies prove that many of the issues involving potable water quality could be resolved using nanoparticles, nanofiltration or other nanomaterials. The use of nanotechnology reduces cost, time and labour for the industries and increases their eco-friendliness. Most of the remediation technologies available at present, while being effective, very often are costly and time-consuming, particularly pump-and-treat methods (Rastegari et al. 2020a, b).

Removal of contaminants and recycling of the contaminated water would provide significant cutbacks in cost, time and labour for the industries and increase their eco-friendliness. Aquifer and groundwater remediation are very critical issues, becoming more important as potable water supply is gradually decreasing. The use of metallic nanomaterials as remediating agents is based on the redox reaction concept in which a neutral electron donor (usually a metal) reduces an electron acceptor. This property of metals leads to their potential use as reducing agents for site remediation.

9.2.1 Poly Chlorinated Hydrocarbons (PCH) Bioremediation

Polychlorinated hydrocarbons (PCH), the organic compounds with multiple chlorine atoms attached to benzene rings, are major recalcitrant pollutants in the environment. PCHs/PCBs are widely used for many applications, especially as dielectric fluids in transformers, capacitors and coolants. Electron transfer can break it down into methane, carbon monoxide and formate. Organic contaminants like benzoquinone, trichloroethene and several other chlorinated aliphatic hydrocarbons can be converted to compounds having less toxicity (Nurmi et al. 2005). TiO_2 nanotubes have been used in laboratory-scale degradation of pentachlorophenol (PCP) through a photoelectrocatalytic reaction (Quan et al. 2005). Nanoparticles are also being used to increase the bioavailability of hydrophobic organic compounds for their enhanced bioremediation (Singh et al. 2020; Yadav et al. 2019).

Organic contaminants like benzoquinone, trichloroethene and several other chlorinated aliphatic hydrocarbons can be changed to compounds having less toxicity (Nurmi et al. 2005). The use of nanoparticles as biocatalyst for reductive dechlorination of organic contaminants has also been demonstrated by De Windt et al. (2005). In one of the studies, palladium $\text{Pd}(0)$ is deposited on the cell wall and inside the cytoplasm of *Shewanella oneidensis* and charged with H^* radicals by

using various substrates such as hydrogen, acetate and formate as electron donors in a bioreductive assay containing Pd(II). When these charged Pd(0)-bearing cells are brought in contact with chlorinated compounds, the H^* radical on Pd(0) catalyzes the dechlorination of the chlorinated compounds (De Windt et al. 2005).

9.2.2 *Hydrophobic Compounds Bioremediation*

Nanoparticles are also being used to increase the bioavailability of hydrophobic organic compounds for their enhanced bioremediation. Polymeric nanoparticles prepared from a poly(ethylene) glycol-modified urethane acrylate (PMUA) precursor were applied to enhance the availability of polynuclear aromatic hydrocarbons (PAHs) in soil and aqueous solutions. Subsequently, the released PAHs can be treated by natural weakening or pump-and-treat process in which polymeric nanoparticles can be recovered and recycled after microbial degradation of PAHs (Kumar et al. 2019a; Yadav et al. 2020d).

9.3 *Mycoremediation of Metals/Metalloids*

Heavy metal pollution in wastewater, groundwater, and streams is the cause of serious long-term health issues in human beings. The enormous industrialization makes the conventional methods unsuitable for the treatment due to their nonspecificity, decrease in efficiency and being cost-intensive. To reduce these difficulties, few studies have been reported by combining the biological methods with other remediation techniques such as biophysical methods, biochemical methods, physico-chemical methods and nano-based physicochemical methods. In recent studies, it was reported that the metal oxide-based nanoparticles can be used as an effective nanoadsorbents for the removal of heavy metals and organic pollutants from water. The applications of polymer-templated nanoparticles and functionalized nanoparticles for the removal of heavy metals are gaining much attention owing to their promising superiority over the other methods of water treatment.

There is urgency for the development of innovative treatment technologies because new water quality standards have been promulgated for water treatment. Heavy metals that are predominantly considered as toxic materials need immediate remediation such as lead, mercury, copper, arsenic, cadmium and chromium. Emerging applications of nanomaterials will endeavor to find an effective remediation solution for removing these heavy metals. Magnetite nanoparticles (Fe_3O_4) impregnated with a silica compound are used for the removal of a large amount of toxic substances usually found in the environment. It is also used for the biological separation of cells and remediation purposes. The bioavailability of heavy metal fractions in the soil is largely conditioned by soil pH and determined by many

factors like the magnitude of heavy metals, exposure time, presence or absence of nutrients and other chemical species.

Mycoremediation (fungal remediation) is an evolutionary approach that can provide environmental benefits in addition to a cost-efficient alternative to other remediation methods. Mycoremediation encompasses the use of fungi in the substantial or partial remediation of certain heavy metal contaminants in surface and groundwater, industrial wastewater, soil, sediment and sludge. The diverse metabolism capabilities of fungi play an important role in the detoxification of organic pollutants and are involved in remediating heavy metals in the environments (Gadd 2009). Unlike bacteria, fungi are robust organisms that have the ability to tolerate high levels of toxic chemicals including heavy metal/ metalloid(s) (Joshi 2014; Dixit et al. 2015). Fungi have been extensively studied by researchers since 1980 for their bioremediation potential; metal-tolerant filamentous fungi have been isolated from heavy metal-contaminated soils and sediments, for instance, *Fusarium* (Saxena et al. 2006; Ezzouhri et al. 2009), *Penicillium* (Xu et al. 2012; Bhargavi and Savitha 2014), *Rhizopus* and *Aspergillus* (Maheswari and Murugesan 2009).

Tolerance to different concentrations of toxic heavy metals is also supported by the ability of fungi to produce extracellular degradative enzymes that reduce the toxicity of the heavy metals when taken inside the cell (Danesh et al. 2013). Fungal cell wall composition is unique and responsible for providing excellent metal-binding properties (Gupta et al. 2000). The membrane transport systems can play a part in suppressing cellular influx or active efflux of metals and metalloids. Metals and metalloids are present in the cells; they can be sequestered via intracellular precipitation, chelation and localization in organelles or can be biotransformed to less toxic forms (Rajendran et al. 2003; Malik 2004; Gadd 2009, 2010).

The cell surface of fungi consists of mostly polysaccharides, peptides, melanin and chitins. They help in providing excellent metal-binding sites such as the carboxyl, hydroxyls and amides (Wang and Chen 2009, 2014). Such structural composition actually is responsible in affording fungi first-line defense against metal/ metalloid (Tamás et al. 2005; González-Guerrero et al. 2009; Wysocki and Tamás 2010; Pócsi 2011). The cell surface sorption of metals and metalloids generally occurs as a result of physicochemical interaction between the metal ions and the functional groups such as cell surface proteins. This reaction is rapid, reversible and metabolism-independent (Dhankhar and Hooda 2011). Cell surface is metabolically independent; metal precipitation depends on metabolic reactions; the fungal cells produce compounds in response to the presence of metal/metalloids that aid the precipitation process (Dhankhar and Hooda 2011).

The mycoremediation method is comparatively economical and requires expertise in the design of field projects with technical strategies. The success of the strategy depends upon the selection of right fungal culture for the degradation or removal of specific contaminant. During the last two decades, lot of work on the removal of metals by macrophytes has been reported (Denny and Wilkins 1987; Tangahu et al. 2011; Vishnoi and Srivastava 2008). Several species of plants and fungi coexist and survive in such contaminated soils. *Glomeromycota* is a group of soil microfungi that form a symbiotic relationship with the root system of terrestrial plants. More

than 80% of terrestrial plants are in a symbiotic relationship with *arbuscular mycorrhizal* (AM) fungi (Lee et al. 2013). A study was conducted on the physiological role of a native *Funneliformis geosporum* on the uptake, translocation of Zn and other nutrients in *Triticum aestivum*. Inoculation of *T. aestivum* with mycorrhizae improved the photosynthetic pigment that was affected due to Zn treatments. It was observed that with increased concentration of Zn, mycorrhizal colonization decreased. When native mycorrhizal taxon, i.e. *F. geosporum*, was used as inoculum, improved yields of wheat were obtained under higher levels of Zn. These mycorrhizae thus have application in the bioremediation of zinc contaminated soils (Abu-Elsaoud et al. 2017).

9.4 Mycoremediation of Toxic Weapons

Wood-degrading fungi are tremendously useful in decomposing toxic aromatic contaminants from petroleum and also persistent chlorinated pesticides (Rhodes 2013). Mycofiltration is a similar way, in which mycelia are used as a filter to remove toxic materials as well as microorganisms from water in the soil (Stamets 2005). It has been recommended that edible mushrooms might be grown for the purposes of mycoremediation (Kulshreshtha et al. 2014). Recent reports show myconanoparticles being applied in the area of water purification in order to reduce the bulk concentrations of toxic substances, such as radionuclides, metal ions and organic and inorganic compounds to the ppb levels. Magnetite nanoparticles impregnated with a silica compound are used for the removal of a large amount of toxic substances usually existing in the environment, also for the biological separation of cells and remediation purposes.

9.5 Myconanoparticles: the Nanoparticles from Fungi

Fungi are an important part of the list of microorganisms used for metal nanoparticle synthesis. Fungi are being extra advantageous than other microorganisms in several approaches. The ability of filamentous fungi to grow on readily available and cheap substrates, in addition to their ability to produce an extensive range of commercially interesting metabolites, has attracted considerable interest to exploit them as production microorganisms in biotechnology (Dhanasekar et al. 2015; Yadav et al. 2020c, e).

Several fungal strains have been used as promising resources for nanoparticles fabrication, for example, *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. Different fungal species are proficient candidates for the production of metal nanoparticles both intracellularly and extracellularly. Mycosynthesis of silver, gold, gold–silver alloy, platinum, selenium, palladium, titanium, zirconium, magnetite, uraninite and

Table 9.1 Biosynthesis of myconanoparticles

Metal nanoparticle	Nanoparticle-producing fungi	Reference
Silver	<i>Trichoderma reesei</i>	Nanda and Saravanan (2009)
	<i>Cladosporium cladosporioides</i>	Kalishwaralal et al. (2010)
	<i>Neurospora crassa</i>	Calderon and Fullana (2015)
	<i>Alternaria alternata</i>	Sarkar et al. (2011)
	<i>Cladosporium cladosporioides</i>	Balaji et al. (2009)
	<i>Fusarium oxysporum</i> , <i>Fusarium solani</i> , <i>Fusarium semitectum</i> , <i>Fusarium acuminatum</i>	Ahmad et al. (2003a), Ingle et al. (2009), Basavaraja et al. (2008), Ingle et al. (2008)
	<i>Aspergillus clavatus</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , <i>A. niger</i> , <i>A. tamarii</i> , <i>A. terreus</i>	Verma et al. (2010), Vala et al. (2014), Ranjbar-Navazi et al. (2010), Bhainsa and D'souza (2006), Gade et al. (2008), Kathiresan et al. (2010), Kumar et al. (2012), Li et al. (2011)
	<i>Helminthosporium tetramera</i>	Shelar and Chavan (2014)
	<i>Mucor hiemalis</i>	Aziz et al. (2016)
	<i>Penicillium citrinum</i> , <i>P. fellitanum</i> , <i>P. nalgiovense</i> , <i>P. purpurogenum</i> NPMF, <i>P. glabrum</i>	Honary et al. (2013), Kathiresan et al. (2009), Maliszewska et al. (2014), Nayak et al. (2011), Nanda and Majeed (2014)
	<i>Phoma glomerata</i> , <i>Phoma gardenia</i> , <i>Phoma sp.</i>	Birla et al. (2009), Rai et al. (2014), Chen et al. (2003)
	<i>Phanerochaete chrysosporium</i>	Vigneshwaran et al. (2006)
	<i>Pleurotus sajor-caju</i>	Nithya and Ragunathan (2009)
	<i>Trichoderma asperellum</i> , <i>Trichoderma viridae</i>	Mukherjee et al. (2008), Fayaz et al. (2010)
Cadmium tellurite	<i>Yeast</i>	Bao et al. (2010)
Magnetite	<i>Verticillium sp.</i> , <i>Fusarium oxysporum</i>	Bharde et al. (2006)
Gold	<i>Thermomonospora sp.</i>	Ahmad et al. (2003b)
	<i>Cylindrocladium floridanum</i>	Malarkodi et al. (2013)
	<i>Aspergillus oryzae</i>	He et al. (2007)
	<i>Nigrospora oryzae</i>	Kar et al. (2014)
	<i>Hormoconis resinae</i>	Varshney et al. (2009)
	<i>Neurospora crassa</i>	Castro-Longoria et al. (2011)
	<i>Penicillium brevicompactum</i>	Mishra et al. (2011)
	<i>Trichoderma sp.</i>	Mishra et al. (2014)
	<i>Aspergillus clavatus</i>	Saravanan and Nanda (2010)
	<i>Trichothecium sp.</i>	Ahmad et al. (2005)
	<i>Fusarium oxysporum</i>	Mukherjee et al. (2002)
Zirconia	<i>Fusarium oxysporum</i>	Bansal et al. (2004)
Platinum	<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>	Riddin et al. (2006)

(continued)

Table 9.1 (continued)

Metal nanoparticle	Nanoparticle-producing fungi	Reference
Iron	<i>Plerotus</i> sp.	Kaul et al. (2012)
Zinc	<i>Candida albicans</i>	Mazumdar and Haloi (2011)
Copper	<i>Fusarium oxysporum</i>	Majumder (2012)
	<i>Hypocrea lixii</i>	Salvadori et al. (2014)
	<i>Penicillium citrinum</i>	Honary et al. (2012)
	<i>Sterium hirsutum</i>	Cuevas et al. (2015)
	<i>Penicillium aurantiogriseum</i> , <i>Penicillium citrinum</i> , <i>Penicillium waksmanii</i>	Abboud et al. (2014)
Titanium	<i>Aspergillus tubingensis</i>	Tarafdar et al. (2013), Bansal et al. (2005)
Palladium	<i>Fusarium oxysporum</i>	Subramanyam and Siva (2016), Raliya et al. (2013)
Lead	<i>Aspergillus</i> spp.	Pavani et al. (2012)

cadmium telluride nanoparticles has also been reported by different researchers (Table 9.1).

Fungal cell walls and cell wall sugars are likely to play an important role in the absorption and reduction of metal ions. The intracellular synthesis of nanoparticles can be explained using a stepwise mechanism. In the first step of bioreduction, trapping of metal ions takes place at the fungal cell surface. This is due to the electrostatic interaction of the positively charged groups in enzymes present on the cell wall of mycelia. In the next step, the metal ions are probably reduced by the enzyme within the cell wall, which leads to the accretion of metal ions and formation of nanoparticles (Mukherjee et al. 2002; Gholami-Shabani et al. 2016c).

9.6 Future Prospects of Fungal Bioremediation

Metal reduction resulting in immobilization has applications in bioremediation as well as production of novel biomaterials and catalysts. Biosorption-based biosynthesis of nanoparticles is mainly used for removing heavy metals from wastewaters and soils. Fungi have several advantages over other microorganisms for nanoparticle synthesis as they are easy to handle, require simple nutrients and possess a high cell wall-binding capacity and high intracellular metal uptake capabilities (Dias et al. 2002; Sanghi and Verma 2009a, b). Fungi bioremediation provides a plausible and sustainable solution for heavy metal/metalloid-contaminated land as it fulfills a number of the key criteria in rectifying and reestablishing the natural condition of soil (Kumar et al. 2019b).

Despite the challenges in the use of molecular approaches to enhance the remediation prospects, fungal bioremediation remains an attractive and promising alternative to the conventional physicochemical methods such as chemical precipitation,

oxidation or reduction, filtration, ion exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment. With the advent of nanotechnology and improved understanding of the nanoparticle's life cycle, the application of metal-resistant fungi offers a promising solution for metal/metalloid pollution. Metal nanoparticles will be used as transporters for delivery of pesticides and chemical fertilizers for efficient, less and equal distribution in soil. Soil microflora can be used to synthesize metal nanoparticles and further can be used as green remediatees to elucidate the role of microbes in biosorption of heavy metals from contaminated area and extract for industrial purpose. Microbes can be used as a factory for various purposes to explore the nanoremediation along with fertility of the soil and maintaining the balance in ecosystem.

9.7 Conclusions

With the use of microbial systems to synthesize nanoparticles, an added advantage of clean and green nanotechnology in environmental cleanup has been obtained. Nanoparticles are also being widely applied in environmental research in degradation/transformation and recovery of toxic/nontoxic chemical compounds. Understanding potential environmental hazards of nanomaterials will require the ability to determine not only the chemical nature of the nanoparticles but also their size, shape and number released in the environment. Several types of nanoparticles have proven to induce oxidative stress, suggesting that particles of different types could initiate and aggravate inflammation and tissue damage leading to neurodegenerative and cardiovascular disease, carcinoma and other disorders (Biswas and Wu 2005; Scalbert et al. 2005). Though the field is rapidly increasing, the exact mechanism of biosynthesis and bioremediation through metal nanoparticles remains unknown which needs to be more explored.

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Chapter 10

Marine Fungal Communities: Metabolic Engineering for Secondary Metabolites and Their Industrial Applications



Mohamad Hesam Shahrajabian, Ram B. Singh, Anathi Magadlela, Wenli Sun, and Qi Cheng

10.1 Introduction

Fungi are the most diverse and adaptable group of living organisms. Fungi have established a beneficial association with numerous hosts and have the capability to degrade environmental pollutants such as hydrocarbons and by-products. Fungi and fungal products offer eco-friendly and cost-effective strategies to address pollution, especially with respect to bioremediation of oil spill polyaromatic hydrocarbons (Yakop et al. 2019; Ren and Gao 2019). Apart from playing a key role in the ecosystems, fungi are also beneficial for industrial and other uses such as pigment, drug production, food industry, and bioremediation (Cerniglia and Sutherland 2001; Gupta et al. 2016; Nwankwegu and Onwosi 2017). Mycorrhizae and endophytes are representative symbiotic association of the plants and fungi and are capable of modulating the physiological response of plants to water stress and overcome yield barrier (Verma et al. 2017). Water and nutrients available to plant are determined by the rhizospheric water potential which can be manifested to some extent by the AMF (Devi et al. 2020a).

The fungal community forming symbiosis with plant may exert their effect through phytohormone production, solubilization of nutrients, and induction of

M. H. Shahrajabian (✉)

Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Department of Agronomy and Plant Breeding, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

R. B. Singh

Halberg Hospital and Research Institute, Civil Lines, Moradabad, Uttar Pradesh, India

A. Magadlela

School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

W. Sun · Q. Cheng

Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

pathogen resistance or increasing abiotic stress tolerance through increased antioxidant levels in plants (Devi et al. 2020b; Kour et al. 2019c; Rana et al. 2019a). While so many researches support the fungal endophytes and mycorrhizae as an eco-friendly alternative to combat drought stress, an appropriate perceptive of physiological effects of the microbes to stress can develop a stronger and resilient agroecosystem (Rana et al. 2019b). Rhizosphere fungi are critical component of the rhizosphere microbial communities and play an important role in plant growth and health. In turn, plants largely control rhizosphere fungi through the production of carbon- and energy-rich compounds and bioactive phytochemicals (Kour et al. 2019b). Some of the beneficial fungi are directly involved in the cycling of nutrients and function as an essential link for soil nutrient availability (Kour et al. 2020). Some fungi are known for having biocontrol activity against pathogenic microorganisms (Sharma et al. 2019; Thakur et al. 2020). These fungi positively influence plant productivity by enhancing plant growth, but certain rhizosphere fungi can negatively influence plant productivity by infecting roots and causing serious disease.

Retter et al. (2019) reported that fungi are heterotrophic, unicellular, or filamentous organisms that exhibit a wide range of different lifestyles, e.g., symbionts, parasites, and saprotrophs. Mycologists have traditionally considered fungi to be a nearly exclusively terrestrial group of organisms, but it is now known that fungi have a significant presence in aquatic environments as well. Lazarova et al. (2019) concluded that soil fungi are a key component of soil biota having an important role in many ecological processes, and as pathogens, decomposers, and plant mutualists, they can affect plant and soil health in agro-ecosystems significantly. Selbmann (2019) reported that fungi, the most versatile phylogenetic lineage for their stunning ecological and morphological plasticity, may easily explore and persist in novel environments, virtually dwelling in all types of habitats, even those normally precluded to most. Khan et al. (2014) deduced that fungi are somewhere in between the microorganism and macroorganism which is a good source of producing biologically active secondary metabolites.

Fungi have been used as a tool for producing different types of secondary metabolites by providing different nutrients at different laboratory conditions. They have noticed that fungi survive in a wide-ranging of habitats, such as in water, in land/soil, in air, and also in/on animals and plants, simply including both terrestrial and marine environment (Singh et al. 2020b; Yadav et al. 2020a, b). However, majority of them are terrestrial, living in/on soil or surviving on dead bodies of the multicellular organisms including both plants and animals and are contributing to the natural recycling of the dead bodies into organic compounds. It is also clear that fungi are the significant source for new and/or biologically active secondary metabolites. Baker et al. (2019) reported that enzymatic efficiency and longevity are, however, dependent on many variables such as pH, temperature, and environmental availability of various minerals and cosubstrates. As a result, further investigation into fungal enzyme bioremediation and enzyme optimization is warranted to realize the tremendous biotechnical potential of these enzymes in the field of xenobiotic bioremediation. Genetic engineering for enzyme optimization and new enzyme immobilization approaches to generate stable and efficient enzyme-based detoxification systems are exciting prospects in development.

10.2 Biodiversity of Fungal Communities

Fungi are important soil components as both decomposers and plant symbionts and play a major role in ecological and biogeochemical process. However, little is known about the richness and structure of fungal communities. DNA sequencing technologies allow for the direct estimation of microbial community diversity, avoiding culture-based biases. Fungi are important in soil as decomposers and plant symbionts; it is thus important to understand the composition of their complex communities, and they play essential roles in many aspects of ecosystem development, function, and stability (Vetrovsky and Baldrian 2013; Taylor et al. 2014; Morris and Robertson 2005). Understanding fungal diversity, community structure, and spatial patterns is one of the central issues in soil microbial ecology (Green et al. 2008; Singh et al. 2020a). Some common secondary metabolites produced commercially from fungi are shown in (Table 10.1).

Liu et al. (2015) used 454 pyrosequencing to investigate the fungal communities in the rhizosphere of Xinjiang jujube, they obtained no less than 40,488 internal transcribed spacer (ITS) rDNA reads, the number of each sample was 6943, 6647, 6584, 6550, 6860, and 6904, and they used bioinformatics and multivariate statistics to analyze the results. They have reported that the index of diversity showed greater richness in the rhizosphere fungal community of a 3-year jujube than in that of an 8-year-old jujube, and most operational taxonomic units belonged to Ascomycota, and taxonomic analyses identified Hypocreales as the dominant fungal order. Their results also demonstrated that the fungal orders are present in different proportions in different sampling areas. Redundancy analysis (RDA) revealed a significant correlation between soil properties and the abundance of fungal phyla. Their results also indicated lower fungal diversity in the rhizosphere of Xinjiang jujube than that reported in other studies. Relative abundance of the dominant fungal phyla in 6 soil samples is shown in (Fig. 10.1).

Alzarhani et al. (2019) stated that the composition and structure of plant root-associated fungal communities are determined by local abiotic and biotic conditions. However, the relative influence and identity of relationships to abiotic and biotic factors may differ across environmental and ecological contexts, and fungal functional groups. They have mentioned that levels of root-associated fungal diversity were comparable with forests and temperate grassland, quadrupling previous

Table 10.1 Secondary metabolites producing fungal communities

Metabolites	Fungal source	Applications
Cephalosporins	<i>Acremonium chrysogenum</i>	Antibacterial
Cyclosporins	<i>Tolypocladium spp.</i>	Immunosuppressants
Fucidin	<i>Fusidium coccineum</i>	Antibacterial
Gibberellins	<i>Gibberella fujikuroi</i>	Plant hormone
Griseofulvin	<i>Penicillium griseofulvum</i>	Antifungal
Penicillins	<i>Penicillium chrysogenum</i>	Antibacterial
Zearalenone	<i>Gibberella zeae</i>	Cattle growth promoter

Source: Khan et al. (2014)

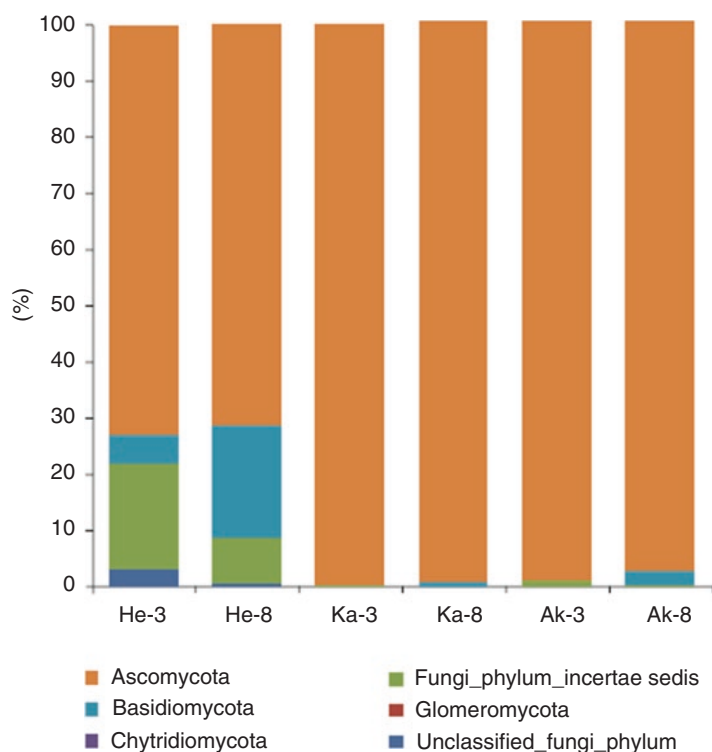


Fig. 10.1 Relative abundance of the dominant fungal phyla in six soil samples

estimates of salt-marsh fungal diversity, while abiotic variables were generally most important. In their conclusion, the site spatial scale and functional group are key factors that, it accounted for, may lead to more predictive understanding of fungal community ecology. Plant root-associated fungi are one group known to significantly affect ecosystem process and functions (Zak et al. 2003; Clemmensen et al. 2013), as they influence individual plants and entire plant communities via a spectrum of plant–fungal interactions, ranging from highly phytobeneficial to highly phytodetrimental (Johnson et al. 1997; Veresoglou and Rillig 2012). The development of molecular methods has significantly improved the understanding of how root-associated fungal communities are influenced by the environment (Dumbrell et al. 2010; Cotton et al. 2015).

Shi et al. (2019) revealed that intercropping of wheat changed the cucumber seedling rhizosphere fungal community composition but had no significant effects on Cucumber seedlings fungal community richness and diversity indices; specifically, intercropping of wheat decreased the relative abundances of fungal taxa that contained potential plant pathogens in cucumber rhizosphere and increased the relative abundances of fungal taxa with antifungal and plant growth-promoting potentials taxa with phenolic compound-degrading capability in cucumber rhizosphere. Opik et al. (2003) characterized communities of arbuscular mycorrhizal (AM) fungi

in roots of rare *Pulsatilla patens* and common *P. pratensis* native adults and seedlings growing in soils from Estonian boreal forest and grassland habitats. Since establishment of *Pulsatilla* species predominantly occurs in vegetation-free gaps, seedling baiting experiments were aimed at gap simulation. The AM fungal small subunit ribosomal RNA gene (SSU rDNA) sequences amplified from roots were subjected to denaturing gradient gel electrophoresis (DGGE) cloning, restriction fragment length polymorphism (RFLP) grouping, sequence phylogenetic and multivariate analyses. Nineteen identified sequence groups comprised 14 putative *Glomus*, two *Acaulospora*, two *Scutellospora*, and one *Gigaspora* groupings. Four and six groupings, respectively, contained previously described species and root-derived AM fungal sequences. Sequences groups were identified in seedling roots that were more abundant in grassland (*Glomus* sp. MO-G3) or a forest soil (*Glomus* spp. MO-G2 and MO-G5). These data showed site-dependent differences in AM fungal community composition, but they failed AM fungi specifically or preferentially colonizing the rare plant species.

Qiao et al. (2019) reported that understanding the dynamics of the rhizosphere fungal community structure of cotton during different developmental stages will not only provide basic information on the dynamics of the cotton rhizosphere fungal community structure but also help lay a foundation for understanding the mutual influence between rhizosphere fungal communities and the plant health of cotton. Gomez-Cornelio et al. (2016) did a research; in their study, the fungal communities immersed in biofilms were different at each stage of development, defined by the time of exposure of the limestone substrate to the environment. Although the mineral composition of rock substrates has been found to have a certain degree of influence on the structure of fungal communities, in their study limestone samples with similar characteristics of bioreceptivity, such as rock color, roughness, and porosity, were selected. Environmental conditions were also similar across sites in the city of Campeche, Mexico. In addition to time of exposure, the differentiation in the community structure and diversity of fungi in their study may be determined by the interactions among the species of each biofilm; this should be confirmed by subsequent studies. A particular species composition was isolated in each biofilm corresponding to a different developmental stage, although a common pool of hyaline and melanized fungi appears to colonize rock with great success and may have specific functions on the rock substrate.

Hassan et al. (2017) has designed a study, the first study of recovery and characterization of fungi from ice, sediments, and water samples collected from Siachen glacier, Himalaya Range, Pakistan. The isolation and total viable count (CFU/ml or g) was carried out by spread plate technique at 4 °C and 15 °C. Seventeen fungal isolates were obtained and identified by analysis of 18S rRNA ITS region. Most frequently isolated fungal isolates were *Leotiomycetes* sp., followed by *Thelebolus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Periconia*, *Geomyces*, *Cryptococcus*, and *Pueraria*. All isolates were found halophilic, and they were able to tolerate NaCl concentration up to 10–20%. Some isolates showed viability at 45 °C, and most of the isolates were able to grow at pH 1–13. All isolates were screened for their antimicrobial activity against clinically isolated bacterial and fungal strains, but they

showed good antimicrobial activity against Gram (+) bacteria. None of the fungal isolates inhibited Gram-negative clinically isolated *Escherichia coli* and *Klebsiella pneumonia*, but few were able to inhibit Gram-positive bacterial and fungal strains. Fungal isolates were also screened for the production of extracellular enzymes (amylase, cellulase, deoxyribonuclease, lipase, phosphatase, and protease). Various isolates were good producers of cellulase, lipase, and protease, whereas only 2 out of 17 produced DNase and 4 produced phosphatase.

Bärlocher et al. (2011) reported that fungal colonization of *Eucalyptus viminalis* exposed in three streams (two sites each) near Armidale (NSW Tablelands, Australia) was characterized by measuring reproduction from recovered in aerated and static water. Spore production for zoosporic and mitosporic fungi increased up to 220% and 310%, respectively, in aerated water. Percentage similarities of aquatic hyphomycetes communities between pairs of aerated and static samples from the same stream averaged 67.5%; similarities among samples from different streams averaged 50.3%. Canonical analysis of principal coordinates (CAP) revealed no significant difference between fungal communities of aerated and static treatments summarized over all sites.

The fungi communities of substrates from an additional nine streams, primarily from the coast, were characterized in 2010, and they were compared to those of *E. viminalis* leaves incubated for 4 weeks at the original six sites. CAP revealed a significant difference between tableland and coastal fungal communities. Percentage similarities correlated significantly with geographic distance of the streams ($R^2 = 0.13$), their temperature ($R^2 = 0.46$), and their altitude ($R^2 = 0.65$). Cantrell (2013) demonstrated that microbial mats are a laminated organic sedimentary ecosystem, found in a wide range of habitats. Fluctuating diel and seasonal physico-chemical gradients characterize these ecosystems, resulting in both strata and microenvironments that harbor specific microbial communities. Their study was undertaken to compare two types of microbial mats across seasons to further understand the structure of fungal communities in hypersaline microbial mats and their seasonal dynamics. The structure and diversity of fungal communities were documented in young transient and mature hypersaline microbial mats from a tropical region (Puerto Rico) using one culture-dependent and three culture-independent molecular techniques based on the internal transcribed spacer (ITS) region of ribosomal DNA: terminal restriction fragment length polymorphism (TRFLP), denaturing gradient gel electrophoresis (DGGE), and clone libraries. Two microbial mats (one young and transient, one mature) were sampled in Nov 2007 (wet season), Jan 2008 (intermediate season), and Mar 2008 (dry season) in the Cabo Rojo Solar Salterns on the southwestern coast of Puerto Rico.

Traditional and molecular techniques revealed strong spatial and temporal heterogeneities in both microbial mats. Higher abundance of isolates and phylotypes was observed during the wet season, and diversity decreased from the top (oxic) to the bottom (anoxic) layers in both seasons. Some of the species isolated belong to the genera *Aspergillus*, *Cladosporium*, *Hortaea*, *Pichia*, and *Wallemia*, which often are isolated from hypersaline environments. The most abundant clones belong to *Acremonium strictum* and *Cladosporium halotolerans*, which were not isolated in

pure culture. The differences observed using culture-based and molecular techniques demonstrate the need of combining methods to study the diversity of fungi in a given substrate. Gao et al. (2019) concluded that continuous cropping of sweet potato led to the most dominant fungi phylum Ascomycota, while richness in rhizospheric soil of sweet potato increased significantly. They have found that continuous cropping altered the fungal community structure of the sweet potato rhizospheric soil, such that the content of beneficial fungi decreased, while that of harmful fungi increased, thereby increasing soil-borne diseases and reducing the yield and quality of sweet potato. These effects were different for different sweet potato varieties. Thus, during actual production, attention should be paid to maintain the stability of sweet potato rhizospheric soil microecology through rotation or application of microbial fertilizers and soil amendments to alleviate continuous cropping obstacles.

Wu et al. (2017) announced that microbial diversity in any soil is considered as bioindicator toward the maintenance of soil ecosystem service. Citrus is one of the most widely grown commercial fruit crops and is heavily dependent on arbuscular mycorrhizal fungi (AMF). In their researches, in the species levels, the clone, *Glomus Glo20*, had the highest relative and read abundance in all the root and soil samples than other clones. Their results provide the new perspectives at the molecular level to highlight the community of AMF in citrus. Chen et al. (2019) reported that *Gastrodia elata* is a widely distributed achlorophyllous orchid and is highly valued as both medicine and food. *Gastrodia elata* produces dust-like seeds and relies on mycorrhizal fungi for its germination and growth. In its life cycle, *G. elata* is considered to switch from a specific single-fungus relationship (*Mycena*) to another single-fungus relationship (*Armillaria*). However, no studies have investigated the changes in the plant–fungus relationship during the growth of *G. elata* in the wild. In their experiment, their data indicated that *G. elata* interacts with a broad range of fungi beyond the *Mycena* genus. These fungi changed with the growth phases of *G. elata*. In addition, their data suggested that the development of the fungal community during the growth of *G. elata* was more complex than previously assumed and that at least two different fungi could be involved in the development before the arrival of *Armillaria*.

Wang et al. (2017) revealed that long-term integrated use of chicken manure and legume straw with chemical fertilizer (IF) had larger amounts of organic C and N in soils and higher yields of tea as compared to those in the other fertilizer treatments. Moreover, they also showed that IF strongly modified the composition in acid tea soils. Meanwhile, the IF-treated soils had a clear difference in the phylum and genus-level composition of fungi compared with that in the nonfertilization control-treated and chemical fertilizers only-treated soils. The fungal phylum Ascomycota and the genus *Fusarium* were mainly found in the IF treatment. Results of correlation analysis further revealed that soil pH, moisture, soil organic C, total N, and silt were the most influential factors in determining the structure of soil fungal communities under different fertilization treatments. On the basis of their results, it is recommended that IF should be utilized to improve soil physical–chemical properties as well as fungal community composition and thus contribute to soil fertility management of tea plantations toward sustainability in the subtropical region.

Wilkinson et al. (2019) found that aphids increased the evenness of the entire fungal community within plant roots and also tended to increase the level of vesicles and abundance of the AMF family Gigasporaceae. Whether these increases are due to increased nutrient uptake or the active selection of fungal taxa in response to herbivory requires elucidation. Whether these changes in the below-ground soil community feed back into altered aphid performance is currently unclear, but the response of agriculturally relevant fungal communities to top-down effects of herbivory suggests that above-below-ground community feedback could occur in agricultural systems. Broeckling et al. (2008) described that plants are in constant with a community of soil biota that contains fungi ranging from pathogenic to symbiotic. A few studies have demonstrated a critical role of chemical communication in establishing highly specialized relationships, but the general role for root exudates.

He et al. (2017) used high-throughput sequencing to investigate the diversity and composition of soil fungal communities and their latitudinal and seasonal variations in five forest ecosystems in eastern China. Soil fungal diversity was greater, with more distinguishable seasonal variation in temperate deciduous forests than in subtropical evergreen forests, which was mainly affected by the spatiotemporal variation of soil pH, cation contents (mainly Mg^{2+} , Ca^{2+} , and Mn^{2+}), and nutrient contents (TP, TOC, and TN). Both diversity and composition variations were greater across latitude than among seasons, indicating the dominant roles of spatial heterogeneity in shaping soil fungal communities. At different organizational levels (e.g., overall community level or functional guild level), the latitudinal distribution patterns of fungal diversity differed in their responses to environmental variations, which points to a scale dependency of environmental selection of soil fungi in forests of eastern China. Relative abundance of the major taxonomic groups (a) and functional guilds (b) of soil fungal community in five latitudinal forests of eastern China at different seasons are shown in (Fig. 10.2).

Teixeira et al. (2017) reported that the tailings piles in rehabilitation with molasses grass exhibit greater Arbuscular mycorrhizal fungi (AMF) species in relation to the other environments evaluated and can facilitate introduction of new plants in the rehabilitation process. The greater potential of mycorrhizal inoculums in the canga environment can contribute to resilience in the area. The use of Trap Cultures in both locations of Brazil increases the diversity of AMF species captured insofar as different species tend to develop better under distinct environmental conditions. The distribution of some AMF species is better explained by chemical properties of the soil, such as pH and exchangeable contents of S and P. The iron mining area and its surroundings are hotspots of AMF diversity, and noteworthy in this study is the first report of the occurrence of *Acaulospora nivalis* and *Acaulospora alpine* in Brazil.

Brinkmann et al. (2019) concluded that soil fungi are key players in nutrient cycles as decomposers, mutualists, and pathogens, but the impact of tropical rain forest transformation into rubber or oil palm plantations on fungal community structures and their ecological functions are unknown. Rain forest conversion was not associated with strong diversity loss but with massive shifts in soil fungal community composition. Fungal communities clustered according to land use system and loss of plant species. Network analysis revealed characteristic fungal genera

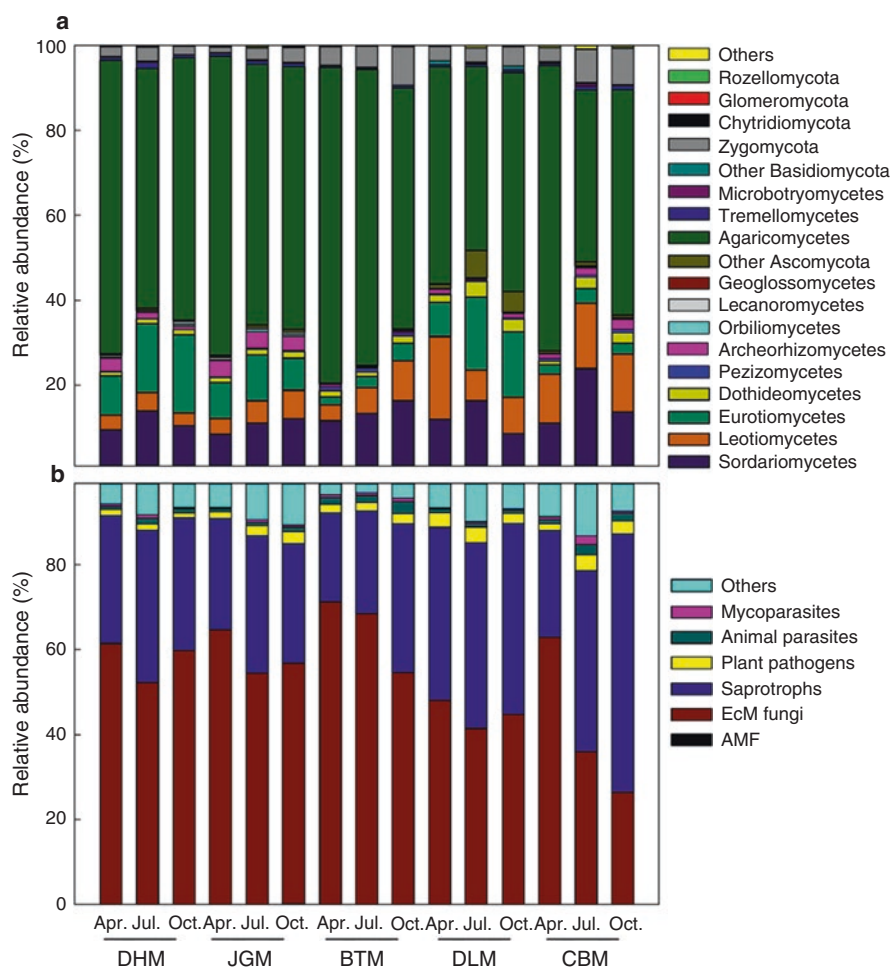


Fig. 10.2 Relative abundance of the major taxonomic groups (a) and functional guilds (b) of soil fungal community in five latitudinal forests of eastern China at different seasons. (Source: He et al. 2017)

significantly associated with different land use systems. Shifts in soil fungal community structure were particularly distinct among different trophic groups, with substantial decrease in symbiotrophic fungi and increases in saprotrophic and pathotrophic fungi in oil palm and rubber plantations in comparison with rain forests. They concluded that conversion of rain forests and current land use systems restructures soil fungal communities toward enhanced pathogen pressure and, thus, threatens ecosystem health functions.

Wang et al. (2018) revealed that soil productivity correlated with N mineralization rate, P_2O_5 , and maize growth, but not with decomposition activity, which is characterized by C turnover rate, soil organic C, and microbial mass; soil productiv-

ity showed a significant association with community composition, but not with richness and mass of soil microbial communities. They have found that soil productivity also correlated with the abundance of several specific taxa, both in bacteria and fungi. Root communities did not show any clear correlations with soil productivity. Their results demonstrate that community composition and abundance of soil microbial communities play important roles in determining soil productivity.

Hopkins et al. (2018) in their experiment revealed that fungal richness was not significantly different between trees in drought-affected plots compared with minimally affected and unaffected plots. However, soil beneath trees affected by drought had different fungal community composition and changes in the abundance of key functional groups, namely higher levels of Am and saprotrophs, and fewer ECM mycorrhizal fungi. They have reported that fungal communities were also different beneath living and dead plants within the drought-affected sites with more AM fungi, saprotrophs and pathogens, and fewer ECM beneath dead trees found in the drought-affected plots. If the differences in rhizosphere communities seen in their study are due to drought-affected trees, and not due to microtopographic and inherent plot differences, their results mirror other studies from different ecosystem types across the world, suggesting common global trends in shifts in fungal composition in response to climate change. If such changes in fungal communities are being driven by drought-induced forest die-off, these may have consequences for ecosystem resistance, resilience, and stability, which is increasingly concerning in regions that are projected to be hotter and drier in the future.

Brinkmann et al. (2019) stated that soil fungi are key players in nutrient cycles as decomposers, mutualists, and pathogens, but the impact of tropical rain forest transformation into rubber or oil palm plantations on fungal community structures and their ecological functions are unknown. They have concluded that fungal communities clustered according to land use system and loss of plant species. Network analysis revealed characteristic fungal genera significantly associated with different land use systems. Shifts in soil fungal community structure were particularly distinct among different trophic groups, with substantial decreases in symbiotrophic fungi and increases in saprotrophic and pathotrophic fungi in oil plant and rubber plantations in comparison with rain forests. They have concluded that conversion of rain forests and current land use systems restructures soil fungal communities toward enhanced pathogen pressure and, thus, threatens ecosystem health functions. The contribution of fungi or fungal enzymes to volatile organic compound production is presented in (Table 10.2).

Sommermann et al. (2018) in his study demonstrated alterations in fungal communities of agricultural soils in a long-term field trial due to different tillage and fertilization strategies in two distinct winter wheat fields with maize and rapeseed as preceding crops. They have concluded that crop rotation is widely accepted to stabilize soil structure and fertility and to positively affect pathogen and weed control; furthermore, it exerts strong selective power on soil mycobiome structures. In-depth analysis of putative phytopathogenic and plant beneficial fungal groups indicated distinct responses; for example, *Fusarium* was significantly enriched in the intensively fertilized conservation tillage variants with the precrop maize, while *Phoma*

displayed significant association with conventional tillage and precrop rapeseed. Many putative plant beneficial fungi also reacted differentially to farming practice with the most distinct responders identified among the Glomeromycota (arbuscular mycorrhizal fungi, AMF).

Yang et al. (2019) found that soil aggregate size significantly impacts microbial communities and soil respiration. Soil total porosity and pH can regulate the distribution of soil bacteria and fungal communities within aggregates, thereby influencing soil respiration. However, it is unclear how it affects the microbial community composition distributed in soil aggregates, especially for fungal communities. The roles of soil total porosity and pH in controlling the microbial composition of soil aggregates are also unknown. In their study, they used high-throughput sequencing of the 16S rRNA and ITS gene regions to target bacterial and fungal members of aggregate samples of four sizes (2–4 mm, 1–2 mm, 0.25–1 mm, and <0.25 mm). Their results showed that high respiration occurred in soil aggregate of 2–4 mm and 1–2 mm when there was high soil total porosity and low soil pH than in aggregates of 0.25–1 mm and <0.25 mm. Moreover, soil aggregates of 2–4 mm and 1–2 mm were dominated by four bacterial families (*Oxalobacteraceae*, *Sphingomonadaceae*, *Cytophagaceae*, and *Gemmatimonadaceae*) and two fungal families (*Lasiosphaeriaceae* and *Rhizophlyctidaceae*), while the 0.25–1 mm and <0.25 mm aggregates were dominated by two bacterial families (*Bacillaceae* and *Clostridiaceae*) and one fungal family (*Nectriaceae*). Their results suggest that soil organic carbon and total porosity positively influenced the bacterial Shannon index, which led to a further positive influence on soil aggregate respiration, while soil pH positively affected the soil fungal Shannon index, leading to increased negative control of the respiration of soil aggregates.

Table 10.2 Contribution of fungi or fungal enzymes to volatile organic compound production

Fungi	Volatile organic compounds
<i>Aspergillus niger</i>	Methyl ketones (creamy-type odor)
<i>Aureobasidium pullulans</i>	
<i>Penicillium</i> sp.	
<i>Aspergillus niger</i>	Vanillin (vanilla odor)
<i>Phanerochaete chrysosporium</i>	
<i>Pycnoporus cinnabarinus</i>	
<i>Beauveria bassiana</i>	Raspberry ketone (berry-type odor)
<i>Diutina rugosa</i> (reported as <i>Candida</i>)	(–)-Menthol (mint flavor)
<i>Ischnoderma benzoinum</i>	Benzaldehyde (bitter almond flavor), 4-Methoxybenzaldehyde (coumarin-like odor)
<i>Kluyveromyces</i> sp.	Phenylethanol (rosary-like odor)
<i>Pithomyces</i> sp.	Nor-patchoulenol (woody-type odor)
<i>Yarrowia lipolytica</i>	γ-Decalactone (peach flavor)
<i>Zygosaccharomyces rouxii</i>	Furaneol (caramelly-type odor)

Source: Hyde et al. (2019)

Witherden et al. (2017) showed that the fungal communities unsurprisingly show significant variation between different body habitats and with changes in disease status. Voriskova et al. (2019) demonstrated rapid response of soil fungal and bacterial communities to short-term climate manipulation simulating increased winter precipitation at two tundra sites. They provide evidence that fungal community composition was more affected by increased snow cover compared to prokaryotes indicating fast adaptability to changing environmental conditions. They have concluded that since fungi are considered the main decomposers of complex organic matter in terrestrial ecosystems, the stronger response of fungal communities may have implications for organic matter turnover in tundra soils under future climate.

10.3 Marine Fungi

Marine fungi have attracted attention in recent years due to increased appreciation of their functional role in ecosystems and as important sources of new natural products. The concomitant development of various omic technologies has boosted fungal research in the fields of biodiversity, physiological ecology and natural product biosynthesis. Each of these research areas has its own research agenda, scientific language, and quality standards, which have so far hindered an interdisciplinary exchange. Interdisciplinary and transdisciplinary interactions are, however, vital for (1) a detained understanding of the ecological role of marine fungi, (2) unlocking their hidden potential for natural product discovery, and (3) designing access routes for biotechnological production (Reich and Labes 2017). Marine fungi are also natural resources rich in various antimicrobial compounds such as cephalosporins, anthrones, anthraquinones, polyketides, alkaloids, peptides, sulfoalkylresorcinols, aflatoxins, diterpenoids, steroids, and glycosphingolipids. Marine fungi have a broad diversity in terms of species richness, phylogenetic distribution, and their natural products (NPs) (Reich and Labes 2017). Compared to other marine organisms, the diversity of marine fungi and their NPs had been largely neglected and only recent has started to provide in-depth data on this understudied organism group (Imhoff 2016; Rama et al. 2016; Taylor and Cunliffe 2016).

Cultivation-dependent and molecular studies demonstrate that marine macroorganisms, such as sponges and algae, are a rich source for fungi (Debbab et al. 2010). The diversity of marine fungi is not adequately represented in investigations on their secondary metabolites; most of the researches on secondary metabolites of marine fungi have focused on just a few genera, mainly *Penicillium*, *Aspergillus*, *Fusarium*, and *Cladosporium* (Imhoff 2016). Rojas-Jimenez et al. (2019) showed that salinity is a significant factor for structuring microbial communities, but little is known for aquatic fungi, particularly in the pelagic zone of brackish ecosystems. In their study, they explored the diversity and composition of fungal communities following a progressive salinity decline (from 34 to 3 PSU) along three transects of ca. 2000 km in Baltic Sea, the world's largest estuary. Based on 18S rRNA gene sequence analysis, they detected clear changes in fungal community composition along the salinity

gradient and found significant differences in composition of fungal communities established above and below a critical value of 8 PSU. At salinities below this threshold, fungal communities resembled those from freshwater environments, with a greater abundance of Chytridiomycota, particularly of the orders Rhizophydiales, Lobulomycetales, and Gromochytriales. At salinities above 8 PSU, communities were more similar to those from marine environments and, depending on the season, were dominated by a strain of the LKM11 group (Cryptomycota) or by members of Ascomycota and Basidiomycota. Their results highlight salinity as an important environmental driver also for pelagic fungi and thus should be taken into account to better understand fungal diversity and ecological function in the aquatic realm. The current knowledge and potential marine fungi for producing anticancer compounds have been reviewed, and the ability of the sea-derived *Streptomyces helimycini* for the production of actinomycins is presented.

Singh et al. (2019) did gathered information in research topic Microbial Secondary Metabolites: Recent Development and Technological Challenges explore the role of microorganisms from different sources showing biological activities, which will further enhance the present knowledge on the potential of microbial secondary metabolites in health and industry. Retter et al. (2019) confirmed that there is a disproportionately large number of fungal lineages waiting to be discovered and described, especially in marine environments. They found many terrestrial lineages also in the marine samples, showing that aquatic mycology cannot be pursued in isolation from traditional mycology. Their data showed the improved collaboration among scientific disciplines is needed to tackle the explosion of new data emerging from fungal community metabarcoding studies. Their data show that there are predominantly endophytic, parasitic, and pathogenic fungi in their marine samples, which hints at the presence of interesting and currently poorly understood fungus-driven ecological processes in the marine environment.

Wang et al. (2019) assessed the diversity and distribution of fungal communities in thirteen marine seawater samples from four sites (L1, L3, L4, and L7) of the Mariana Trench, with a depth range of 1000–4000 meters, using Illumina Hiseq sequencing with fungal-specific primers targeting the internal transcribed spacer (ITS) region of the ribosomal rRNA gene. Sedimentary fungal communities showed high diversity with 209,880 reads belonging to 91 operational taxonomic units (OTUs). Of these OTUs, 45 belonged to the Ascomycota, 37 to Basidiomycota, 3 to chytridiomycota, 1 to Glomeromycota, 1 to Cryptomycota, and 4 to unknown fungi. In their experiment, the major fungal orders included Saccharomycetales and Sporidiobolales. The commonly found fungal genera were *Candida*, *Malassezia*, and *Cryptococcus*. Their results suggest the existence of diverse fungal communities in the Mariana Trench marine seawater, which can be considered as a useful community model for further ecological and evolutionary study of fungi in the trench.

Witherden et al. (2017) in their study analyzed fungal diversity and how the biotic and abiotic factors affected their diversity. Compared with edaphic properties controlling the soil fungal community patterns, plant growth stage was the dominant factor in determining soil fungal community dynamics. They have concluded

Table 10.3 Most important points about marine fungi, their ecology, and molecular diversity

S. No.	Marine fungal communities
1.	Currently known fungal diversity in marine environments represents a tiny fraction of that from terrestrial environments.
2.	Fungal sequences detected in marine environments span a large diversity of forms and lineages, including chytrids, filamentous hyphal forms, and multicellular forms.
3.	Dikarya yeast forms appear to dominate the known diversity of marine fungi.
4.	Marine environments, like freshwater environments, harbor a number of highly divergent, deep-branching, and uncultured fungi, of which future study will greatly improve our understanding of fungal cell diversity and evolution.
5.	There appears to have been frequent marine–terrestrial and terrestrial–marine colonization events during the radiation of the fungi.
6.	Fungi play diverse ecological roles in marine ecosystems and have frequently been associated with parasitism of marine animals, plants, and algae.
7.	Many ecological niches inhabited by true fungi in terrestrial and freshwater habitats are occupied by diverse fungal analogs in marine habitats.
8.	Fungal-specific molecular studies in marine environments are so far relatively few, yet many reveal a marine fungal diversity that is significantly higher than other methods suggest but much less diverse than terrestrial environments.

Source: Richards et al. (2012)

that fungal community diversity was closely related to base fertilizer application, and fungal community richness was associated with rhizobium inoculation. Buee et al. (2011) showed a significant negative correlation between fungal species richness and nitrogen mineralization, indicating that increases in mineral N availability are associated with decline in saprotrophic and ECM community richness. Amend et al. (2019) revealed that terrestrial fungi play critical roles in nutrient cycling and food webs and can shape macroorganism communities as parasites and mutualists. Fungi have been found in nearly every marine habitat explored, from the surface of the ocean to kilometers below ocean sediments. Despite their varied roles, remarkably little is known about the diversity of it. The most important points about marine fungi, their ecology, and molecular diversity are shown in (Table 10.3).

10.4 Biotechnological Applications of Fungal Secondary Metabolites

In view of population growth and the need for sustainable economies, it has become urgent to dramatically improve the productivity of agricultural crops and other food sources. In addition, market demands for energy, medicine, and other aspects related to bioproducts are growing at an accelerated rate. Fungi are known to be a treasury of medical compounds since the discovery of penicillin. Subsequent reports of taxol-producing endophytes inspired the hope that fungal endophyte-based biotechnology could be a promising alternative way to produce valuable biological products (Yan et al. 2018; Rastegari et al. 2019a, b; Yadav et al. 2019). Andryukov et al.

(2019) reported that marine habitats are a rich source of molecules of biological interest. In particular, marine bacteria attract attention with their ability to synthesize structurally diverse classes of bioactive secondary metabolites with high biotechnological potential.

Many compounds isolated from marine bacteria are unique in their structure and biological activity. Modern biotechnologies of invention and development of novel antibiotics having a medical value are based on application of natural strategies of nonribosomal peptide synthesis (Kour et al. 2019a). Revealing the mechanisms and potential of this type of metabolite production in terrestrial and marine bacteria is not only of fundamental, but also of great practical importance. In human pathogens, this biosynthetic pathway probably causes the emergence of microbial isolates with multidrug resistance. Under these conditions, the activity peptide synthetase and clusters of the genes responsible for nonribosomal synthesis becomes a new target for the strategy of treatment of infections caused by drug-resistant from bacteria. With regard to marine bacteria and their secondary metabolites, the targeted control of biosynthesis mechanisms by using the metabolite engineering methods to create hybrid peptides or obtain hybrid peptide synthetases by disrupting the target gene of nonribosomal synthesis is now one of the noteworthy trends in modern biotechnology. The range of active antimicrobial agents derived from marine bacteria, discovered and tested in recent years, indicates a high pharmacological potential of their secondary metabolites, and the study of these peptides is expected to be an interesting and fruitful activity in the coming years. Gerke and Braus (2014) concluded that fungi are industrial important producers of drugs and food additives, but they also have a devastating impact on human and animal health and economy.

The current state of increasing resistances against established antibiotics, drastic crop losses, and human mortality rates due to fungal contaminations and infections emphasizes the importance of an exhaustive understanding of the underlying genetic mechanisms in fungi (Rastegari et al. 2020a, b). New bioactive SMs need to be discovered, for which the huge fungal kingdom with its numerous members is well suitable. The CSN and velvet protein complexes act at the interface between secondary metabolism and fungal development. The fact that both complexes are conserved among fungi makes them a perfect target to address issues in agriculture and health, which are highly important for the society. Patil et al. (2016) concluded that endophytes are the microorganisms which are present inside the living tissues of the plant by establishing a variety of relationships such as symbiotic, mutualistic, or parasitic with their host plants. In their relationship with the host plant, endophytes contribute to the plant by producing a plethora of substances that provide protection and survival benefits to the host plant.

Endophytic fungi have been recognized as a novel source of bioactive secondary metabolites with diverse biological activities such as novel antibiotics, antimycotics, immunosuppressant, and anticancer compounds (Rana et al. 2019c; Singh and Yadav 2020). Their bioactive potential was first recognized when the first multibillion dollar anticancer drug, paclitaxel (Taxol), was obtained from *Taxomyces andreanae*, an endophytic fungus isolated from the yew plant, *Taxus brevifolia*. However, it has been estimated that less than 1% of endophytic microorganisms are currently

known, suggesting that a vast majority of them are yet to be discovered. Thus, endophytic microbes represent a potential source for the discovery of new and useful compounds, or new platforms for the organic synthesis of such compounds, or new platforms for the organic synthesis of such compounds for the benefit of mankind. Boruta (2018) stated that fungi produce a variety of secondary metabolites (SMs), low-molecular-weight compounds associated with many potentially useful biologic activities. The examples of biotechnologically relevant fungal metabolites include penicillin, a β -lactam antibiotic, and lovastatin, a cholesterol-lowering drug. The discovery of pharmaceutical lead compounds within the microbial metabolic pools relies on the selection and biochemical characterization of promising strains (Yadav et al. 2020d).

Not all SMs are produced under standard cultivation conditions; hence, the uncovering of chemical potential of investigated strains often requires the use of induction strategies to awake the associated biosynthetic genes. Triggering the secondary metabolic pathways can be achieved through the variation of cultivation conditions and growth media composition. The alternative strategy is to use genetic engineering to activate the respective genomic segments, e.g., by the manipulation of regulators or chromatin-modifying enzymes. Firakova et al. (2007) concluded that the mutual relationship between endophytic microorganisms and their host plants, taxonomy, and ecology of endophytes are being studied. Some of these microorganisms produce bioactive secondary metabolites that may be involved in a host–endophyte relationship. They have also reported that many endophytic bioactive metabolites, known as new substances, possessing a wide variety of biological activities as antibiotic, antitumor, anti-inflammatory, antioxidant, etc., have been identified. Deveau et al. (2018) noted that a large number of fungal and bacterial families engage in complex interactions that lead to critical behavioral shifts of the microorganisms ranging from mutualism to antagonism.

The importance of bacterial–fungal interactions (BFI) in environmental science, medicine and biotechnology has led to the emergence of a dynamic and multidisciplinary research field that combines highly diverse approaches including molecular biology, genomics, geochemistry, chemical and microbial ecology, biophysics, and ecological modeling. Yadav (2018) concluded that endophytic fungal associations with plants confer protection against adverse environmental conditions such as tolerance to heavy metal and increased drought resistance. Endophytes are ubiquitous and have been reported from different host plant studies, which are valued for their ability to synthesize various bioactive compounds and extracellular enzymes (Yadav 2020; Yadav et al. 2020c). The plant inoculated with endophytic fungi results in significant increase in biomass and improved commercial plant production. Endophytic fungi are of biotechnological interest due to their potential of being used as biological control agents, source of secondary metabolites, antimicrobial agents, antitumor compounds, antibiotics, immunosuppressant, production of natural antioxidant, antiviral compounds, insecticidal products, and antidiabetic agents. Zhi-lin et al. (2007) revealed that a group of secondary metabolites are accumulated inducibly or enhanced constitutively in plant tissues during the process. Symbiotic fungi usually perform compatible and friendly interactions with host plants, which

contribute to growth promotion and secondary metabolites accumulation simultaneously, such as alkaloids and terpenoid with pharmacological characteristics. Especially, some secondary metabolites derived from root exudation act as signal molecules, which induce the spore germination and hypha branching in mycorrhizal fungi. Selim et al. (2012) discovered that endophytic fungi that are residing asymptotically in internal tissues of all higher plants are of growing interest as promising sources of biologically active agents. They also considered their medicinal applications especially in the production of anticancer, antimicrobial, antioxidant, and antiviral compounds. Endophytic fungi are one of the most creative groups of secondary metabolite producers that play important biological roles for human life. They are potential sources of novel natural agents for exploitation in the pharmaceutical industry, in agriculture, and in environmental applications.

10.5 Conclusion and Future Prospects

Fungi and fungal products offer eco-friendly and cost-effective strategies to address pollution, especially with respect to bioremediation of oil spill polyaromatic hydrocarbons. Apart from playing a key role in the ecosystems, fungi are also beneficial for industrial and other uses such as pigment, drug production, food industry, and bioremediation. Fungi are used in many industrial fermentative processes, such as the production of enzymes, vitamins, pigments, lipids, glycolipids, polysaccharides, and polyhydric alcohols. Fungi are extremely useful in making high-value products like mycoproteins and act as plant growth promoters and disease suppressor. Fungal secondary metabolites are important to the health and nutrition and have tremendous economic impact. Fungi are also useful in carrying out biotransformation processes. Fungi are heterotrophic, unicellular, or filamentous organisms that exhibit a wide range of different lifestyles, e.g., symbionts, parasites, and saprotrophs. Fungi are important soil components as both decomposers and plant symbionts and play a major role in ecological and biogeochemical process. However, little is known about the richness and structure of fungal communities. Marine fungi are an ecological, not a taxonomic, grouping, known primarily as saprotrophs from intertidal zones where they represent an important food source for invertebrates. Marine fungi perform important and unique ecosystem functions in diverse environments from coral reefs to marine sediments at the bottom of the ocean. Marine fungi have unique adaptations to salinity and intense pressures found in the deep ocean. Marine fungi are also natural resources rich in various antimicrobial compounds such as cephalosporins, anthrones, anthraquinones, polyketides, alkaloids, peptides, sulfoalkylresorcinols, aflatoxins, diterpenoids, steroids, and glycosphingolipids. Marine fungi have more salt efflux pumps in their cell membranes, and they create compounds called osmolytes that allow them to function in saltwater. There is potential for marine fungi to help combat the oil spills and microplastics crisis. More studies are need to be done in this field.

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Chapter 11

Industrially Important Fungal Enzymes: Productions and Applications



Periyasamy Dhevagi, Ambikapathi Ramya, Sengottiyan Priyatharshini, Kalyanasundaram Geetha Thanuja, Sakthivel Ambreetha, and Ambikapathi Nivetha

11.1 Introduction

Enzymes are group of complex proteinoid organic compounds produced by living organisms which catalyze specific organic and also inorganic reactions. Every enzyme has its own applications and these enzymes are used in the fields of food industry, pulp and paper industry, textile industry, pharmaceutical industry, agriculture field, biofuel production, and bioremediation. Compared to the conventional chemical methods, enzymatic processes have more advantages like gentle reaction circumstances, advanced selectivity and specificity, lead to the production lower amount of by products and waste material during the reaction because of its superior properties (de Souza et al. 2020). It also gives more efficient and clean industrial processes and contributes to the sustainable growth concept.

Enzymes can be derived from various sources of plants, animals, and microorganisms by wide range of catalytic activities and genetic manipulation (Mamo et al. 2020; Kour et al. 2019b, 2020). Nowadays several enzymes are well-defined, crystalline substances with extremely reproducible catalytic activities and physical properties. Among the enzyme-producing organisms, fungi-derived enzymes attained significant industrial applications due to high production yields at

P. Dhevagi (✉) · A. Ramya
Department of Environmental Sciences, Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu, India

S. Priyatharshini
Department of Crop Management, Vanavarayar Institute of Agriculture,
Pollachi, Tamil Nadu, India

K. Geetha Thanuja · S. Ambreetha
Department of Agricultural Microbiology, Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu, India

A. Nivetha
Department of Chemistry, Bharathiar University, Coimbatore, Tamil Nadu, India

large-scale, ease of cultivation, and simultaneous biomass production (Bala and Singh 2019), thereby half of the commercially utilized enzymes are from fungal origin. The filamentous, thermophilic, psychrotrophic, and white-rot fungus has a capability of producing various enzymes such as amylase, glucosidase, glucose oxidase, protease, pectinase, cellulose, invertase, laccase, ligninase, lipase, chitinase, and xylanase at optimum reaction conditions (Zhao et al. 2017; Kour et al. 2019a; Verma et al. 2019; Yadav et al. 2015). As a result, overall scenario favors and describes fungal enzymes gaining more profit at large-scale industrial application, hence the need of diverse enzyme production from fungal organism has been increased nowadays (Yadav et al. 2017). In the current scenario, the present chapter describes the industrially important fungal enzymes and highlighted with enzyme source, properties, mechanism, production, and its various industrial applications.

11.2 Why Do Fungi Synthesis Enzymes?

Fungi are chemo-organotrophic heterotrophs which could obtain nutrients only through breakdown of external organic source. They might be either parasitic or saprophytic depending over live host or dead substrates respectively for their nourishment (Devi et al. 2020b; Suman et al. 2015). In both cases, a fungi synthesis multitude of hydrolytic (glycolytic, lipolytic, and proteolytic) and oxidative enzymes breakdowns complex organic materials into simple forms (Kour et al. 2019a). The main role of these enzymes is to predigest the food outside the cell prior to absorption as they lack digestive units within their body. Fungal enzymes are synthesized during their rapid growth (log phase) and hence grouped under primary metabolites (vital for growth of the organisms). Majority of secreted enzymes get diffused into the environment (extra-cellular enzymes or exoenzyme) and breakdown complex polymeric compounds. Intracellular enzymes or endoenzymes in turn are retained within the cell wall (periplasmic space) after synthesis and aid in further assimilation of absorbed nutrients.

11.3 Enzymes for Carbon and Nitrogen Assimilation

The type of enzyme released by parasitic fungi depends on their choice of host (plant, animal, human, insect, or other fungi), while saprophytic fungi regulate their enzyme based on the availability of organic materials. Multitudes of enzymes are synthesized by different genera of fungi to meet out their carbon requirement. For instance, *Trichoderma*, *Aspergillus*, and *Penicillium* release cellulase (endo- β -1, 4-glucanase, cellobiohydrolase, and β -glucosidase (BGL) to hydrolyze cellulose-based plant litters into glucose (Panchapakesan and Shankar, 2016; Sharma et al. 2019). Similarly, certain fungi release hemicellulose-degrading enzymes such as arabinanases, galactanases, mannanases, and xylanases to rapidly breakdown the

hemicellulose content of plant cell wall into xylose and glucose (Dekker and Richards 1976). Woody trees are rich in lignin and cannot be degraded by any other microbial enzymes. Brown-rot fungi (*Gloeophyllum*, *Fomitopsis*, *Laetiporus*, etc.) and white-rot fungi (*Ceriporiopsis*, *Phlebia*, etc.) exude ample amount lignin-degrading enzymes including manganese peroxidase, lignin peroxidase, and laccase to decay lignin-based woody substrates into phenolic compounds (Kantharaj et al. 2017). Pectinase and polygalacturonase from *Rhizopus* and *Trichoderma* potentially disrupt pectin in fruits into galacturonic acid. Extracellular amylase from filamentous fungi hydrolyzes starch (storage material in plants) into glucose (Anisa and Girish 2014; Rana et al. 2019b). *Candida*, *Rhizopus*, *Mucor*, *Geotrichum*, etc. generate lipase enzyme to hydrolyze lipid molecules into fatty acids (Gurung et al., 2013). Chitin is an aminopolysaccharide predominantly occupying the fungal and insect cell wall. Fungal chitinase (glycoside hydrolase family) and N-acetylglucosaminidases can breach out the chitin-rich cell wall to access the nutrients (Hartl et al. 2012; Yadav 2020). In addition, fungal chitinase effectively hydrolyzes the chitin in nematode eggs into N-acetylglucosamine which prevents hatching (Gortari and Hour 2008). Fungal proteases further classified into exopeptidase (aminopeptidase and carboxypeptidase) and endopeptidase (serine proteases, cysteine proteases, aspartic proteases, and metalloproteases) are involved in hydrolysis of plant and animal proteins. This action converts high-molecular weight proteins into simple amino acids which can be assimilated by fungal cells (de Souza et al. 2015). Nitrogen nourishment is aided by the enzymes such as urea aminohydrolase, nitrate reductase, and nitrite reductase which hydrolyze urea subsequently into nitrate, nitrite, and ammonia which could be finally assimilated by fungal cells through GOGAT pathway. The properties and structures of important fungal enzymes are depicted in (Table 11.1; Fig.11.1).

Although fungal cells synthesize and release enzymes to digest or breakdown huge array of complex metabolites for their own benefit, those enzymes are exploited for various human applications. It has been reported that more than 50% of industrially important enzymes are extracted from fungal sources (Anisa and Girish 2014). Industrial production of different fungal enzymes and their unique applications are elaborated in upcoming units of the chapter.

11.4 Industrial Production of Fungal Enzymes

In the era of sustainable environment, enzymes play crucial role in enhancing the quality of life by replacing energy intensive processes. Most of the enzymes find role in the technical and nontechnical applications and are recruited to make more than 700 commercial products. Surprisingly, most of the commercial enzymes are hydrolytic with fungal origin. Fungi being the source of wide extra cellular enzymes, many researches are focused on the cost-effective production of efficient enzyme formulations.

Table 11.1 Fungal enzymes, sources, and their properties

Enzymes	Co-enzymes	Fungal organism	Source	Properties	Reference
Amylase	α -amylase β -amylase γ -amylase	<i>Lichtheimia ramosa</i> , <i>Thermoascus</i> <i>aurantiacus</i>	Sugarcane bagasse and leaf litter	Molecular weight: 10–210 kDa pH: 2–12 Temperature: 30–130 °C	Saranraj and Stella (2013) and de Oliveira et al. (2016)
		<i>Aspergillus niger</i>	Soil	Inhibitors: heavy metal ions, sulphydryl group reagents, N-bromosuccinimide, hydroxyl mercuribenzoic acid, iodoacetate, EDTA, and EGTA	Gopinath et al. (2017) Benabda et al. (2019)
		<i>Rhizopus</i> <i>oryzae</i> CH ₄	Culture collection (Laboratory of Ecology and Microbial Technology, University of Carthage, Tunisia)		
		<i>Aspergillus niger</i> RBP7	Soil		Mukherjee et al. (2019)
Glucosidase	α -glucosidase β -glucosidase	<i>Myceliophthora</i> <i>heterothallica</i>	Compost	Molecular weight: 60–100 kDa pH: 5.2–6.0	Teixeira et al. (2016)
		<i>Gongronella butleri</i>	Soil	Temperature: 60 °C	Santos et al. (2016)
		<i>Fusarium oxysporum</i>	Wood	Inhibitors: HgCl ₂ , heavy metal ions, and sulphydryl-binding compounds	Olajuyigbe et al. (2016)
		<i>Penicillium</i> sp. LMI01	Soil		Santa Rosa et al. (2018)
Glucose oxidase		<i>Aspergillus tubingensis</i> CTM 507	Cereal	Molecular weight: 150–186 kDa pH: 4–7	Kriaa and Kammoun (2016)
		<i>Aspergillus niger</i>	Soil	Temperature: 20–50 °C Inhibitors: Ag ⁺ , Hg ²⁺ and Cu ²⁺ ions	Dubey et al. (2017)

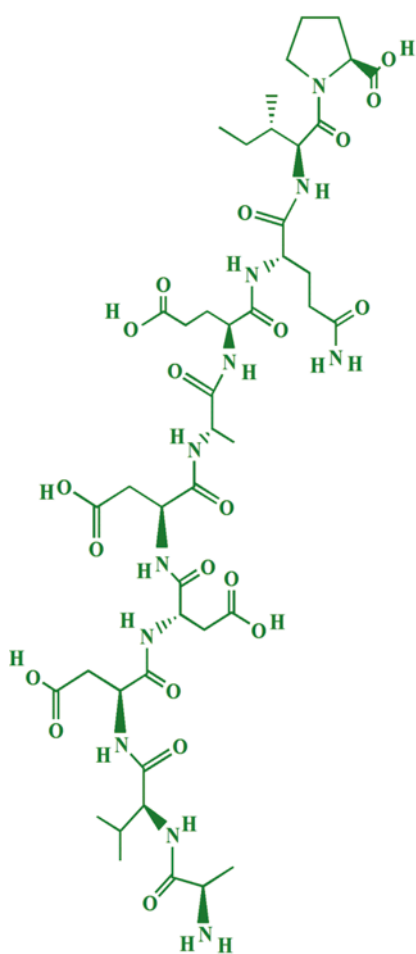
Enzymes	Co-enzymes	Fungal organism	Source	Properties	Reference
Protease	Acid proteases, Neutral proteases, Alkaline proteases	<i>Aspergillus niger</i> LBA 02	Culture collection (Laboratory of Food Biochemistry, School of Food Engineering, University of Campinas)	Molecular weight: 32 - 120 kDa pH: 6–7 Temperature: 45–55 °C Inhibitors: Cu ²⁺ and Hg ²⁺	de Castro et al. (2015)
		<i>Penicillium citrinum</i>	Fish sauce		Xiao et al. (2015)
		<i>Aspergillus flavipes</i> , <i>Aspergillus brasiliensis</i> , <i>Aspergillus oryzae</i> , and <i>Penicillium roquefortii</i>	Culture collection (bioprocess laboratory, Institute of Biosciences of Botucatu (UNESP), Brazil)		Novelli et al. (2016)
		<i>Aspergillus flavus</i>	Soil		Franco et al. (2017) Singh et al. (2016a)
Pectinase	Pectolyase (pectinlyase) Pectozyme	<i>Fusarium</i> sp. PSTF1	Agriculture waste	Molecular weight: 66 kDa.	Reddy and Saritha (2015)
		<i>Penicillium oxalicum</i> PJ02	Rotten oranges	pH: 5.8 Temperature: 30 °C	Li et al. (2015)
		<i>Aspergillus</i> sp.	Citrus fruit	Inhibitors: EDTA, beta- mercaptoethanol (BME) and L-cysteine	El Enshasy et al. (2018)
		<i>Aspergillus</i> spp	Soil		Sudeep et al. (2020)
Cellulase	Endo Cellulase, Exo Cellulase Cellobioase	<i>Oudemansiella radicata</i>	Mushroom	Molecular weight: 87 kDa.	Balaraju et al. (2010)
		<i>Sporotrichum</i> <i>thermophile</i>	Compost	pH: 4 Temperature: 50 °C	Bala and Singh (2017)
		<i>Aspergillus niger</i> and A. <i>fumigatus</i>	Sugarcane bagasse	Inhibitors: Ca ²⁺ , Co ²⁺ , Mg ²⁺ , and Fe ²⁺	Dias et al. (2018)

(continued)

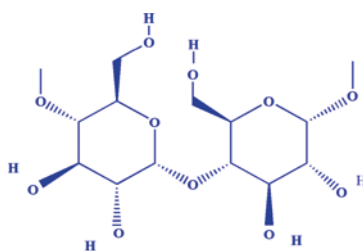
Table 11.1 (continued)

Enzymes	Co-enzymes	Fungal organism	Source	Properties	Reference
Invertase		<i>Cladosporium herbarum</i> ER-25	Soil	Molecular weight: 47–430 kDa, pH: 2.9–6.2	Nadeem et al. (2015)
		<i>Aspergillus niger</i> IBK1	Orange peel	Temperature: 30–75 °C	Taskin et al. (2016)
		<i>Aspergillus sp.</i> ,	Soil	Inhibitor: Na ⁺ , K ⁺ , Ca ²⁺ , and EDTA Mg ²⁺	Oyedele et al. (2017)
Laccase					Chelliappan and Madhanasundareswari (2013)
		<i>Trametes versicolor</i> CICC 14001	Culture collection (Center of Industrial Culture Collection, China)	Molecular weight: 50 to 100 kDa. pH: 4.5–6.0	Adekunle et al. (2017)
		<i>Penicillium chrysogenum</i>	Tree barks	Temperature: 25 °C to 30 °C	
		<i>Ganoderma lucidum</i> EF 31	Culture collection (macrofungi culture collection of Embrapa Florestas, Colombo, Paraná, Brazil)	Inhibitors: EDTA, thioglycolic acid, sodium azide (NaN ₃), ethanol diethyl dithio carbamic acid (DDC), Co, Sn, Hg, Fe, K, Zn, Mg, Mn, Na, Ba, Cr, and Ca	Senthivelan et al. (2019)
		<i>Aspergillus fumigates</i>	Rice straw		Rodrigues et al. (2019)
Ligninase		<i>Aspergillus niger</i>	Agricultural waste		Jin et al. (2020)
		<i>Pleurotus ostreatus</i>	Mushroom	Molecular weight: 38–46 kDa, pH: 4.5–6.0	Santos et al. (2016a)
		<i>Xylaria sp.</i> BCC 7794	Culture collection (biotech culture collection, Thailand)	Temperature: 60 °C	Ergun and Urek (2017)
				Inhibitors: Hg ²⁺ , Pb ²⁺ , EDTA, Ag ⁺	Wattanakitjanukul et al. (2019)
Lipase		<i>Trichoderma sp.</i>	Soil	Molecular weight: 52.9 kDa	Musa et al. (2017)
		<i>Penicillium sp.</i>	Soil	pH: 4.0–6.0	Nema et al. (2019)
		<i>Aspergillus niger</i>	Oil seeds	Temperature: 70 °C Inhibitors: Mn ²⁺ , Mg ²⁺ , NH ₄ ⁺ , Na ⁺ , and Co ²⁺	Alabdallal et al. (2020)

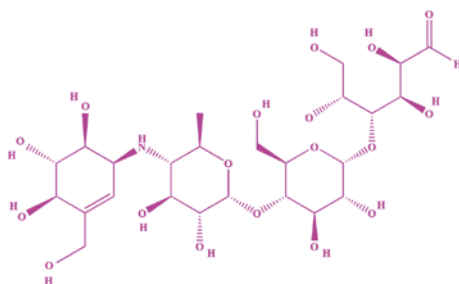
Enzymes	Co-enzymes	Fungal organism	Source	Properties	Reference
Chitinase	Endochitinases, Exochitinases, N-acetylglucosam	<i>Trichoderma viride</i>	Mangrove soil	Molecular weight: 62 kDa pH: 6.5	Abu Tahon and Isaac (2020)
		<i>Penicillium chrysogenum</i>	Sea water	Temperature (°C): 40 °C	Atalla et al. (2020)
		<i>Trichoderma koningiopsis</i> UFSMQ40	Bed bugs	Inhibitors: Hg ²⁺ , Zn ²⁺ , Cu ²⁺ , Co ²⁺ , dodecyl sulphate, and EDTA	Baldoni et al. (2020)
		<i>Aspergillus niger</i>	Soil	Molecular weight: 32 kDa pH: 5.5	Ajjolokewu et al. (2017) Bhardwaj et al. (2019)
Xylanase	Endo-1,4-β-D-xylanases, β-D-xylosidases	<i>Rhizopus oryzae</i>	Oil palm fruit bunch	Temperature: 50 °C	Ezeilo et al. (2020)
		<i>Aspergillus fumigatus</i> JCM 10253	Soil	Inhibitors: Ca ²⁺ , Zn ²⁺ , Mg ²⁺ , Fe ²⁺ , Cu ²⁺ , Fe ³⁺ , SDS, and EDTA	Saroj et al. (2020)



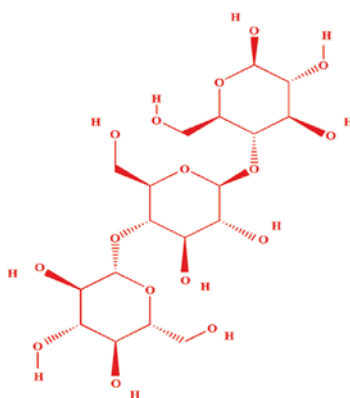
a) Laccase



b) Amylase



c) Glucosidase



d) Cellulase

Fig.11.1 Chemical structures of fungal enzymes

The general techniques employed in enzyme production are (I) selection of microorganism, (II) medium formulation with appropriate substrate, (III) production process, and (IV) recovery and purification of enzymes.

11.4.1 Selection of Microorganisms and Production Process

The significant criteria to be considered for selecting the organism are that the organism should produce utmost quantities of preferred enzyme in a short period of time. It is then followed by strain improvement which is the keystone in fermentation process by various methods like mutagenesis, protoplast fusion, and direct genetic manipulation to enhance the metabolic capacities. Culture medium should ensure adequate growth to microorganisms for optimized productivity. In the commercial production of enzymes, fermentation is the prime process which can be either submerged fermentation (SmF) or solid-state fermentation (SSF).

11.4.1.1 Submerged Fermentation (SmF)

It is the technique of cultivating microorganisms in nutrient-rich broth. Depending upon operational scale, different grades of vessels and volumes are designed. The operational scales are usually a laboratory, pilot, or industrial level. In the industrial scale, SmF is widely used due to its developed techniques for the efficient control of pH, temperature, stirring, with varying reactor under different modes of fermentation. The two major operational conditions include batch and continuous mode of fermentation. In general, crude media is used for enzyme production which can be obtained from various industrial wastes such as corn, whey, etc. Some of the operational conditions of SmF technique include extensive sterilization, high-energy expenditure, and extensive purification process. In addition, some of disadvantages of this technique cover high possibility of contamination rate, organic solvent toxicity, and high wastewater production. (Table 11.2) summarizes the overview of fungal enzymes production through submerged fermentation process in different studies.

11.4.1.2 Solid-State Fermentation (SSF)

SSF is the technique taking place in solid matrix in the near absence of free water. There is a noteworthy attention towards using SSF owing to their ability to produce wide variety of enzymes, resistance of microorganisms to catabolic repression, efficient use of agro-industrial residues. It is a low-cost alternate and energy intensive technology to reduce the overall cost of the enzymes. SSF method of fermentation has several advantages; the aeration can be attained by air spaces present in the media and agitation can be provided intermittently. Further, the

Table 11.2 Fungal enzymes production through submerged fermentation

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Amylase	<i>Penicillium chrysogenum</i>	Linseed oil cake	550.0 U/ml	pH: 9 Incubation period: 6–18 days Temperature: 30 °C	Dar et al. (2015)
	<i>Aspergillus flavus</i> AUMC 11685	Mandarin peel	26.90 U/ml	pH: 4–5.5 Incubation period: 4–5 days Temperature: 40 °C Inoculum: 10%	Ali et al. (2017)
	<i>A. tamarii</i>	Growth medium containing cassava and potato peel flour (2.5%)	3.00 U/ml	pH: 7.0 Incubation period: 72 h Temperature: 37 °C Inoculum: 10%	Fonseca et al. (2018)
Glucose oxidase	<i>A. niger</i>	Beet molasses	550 U/ml	pH: 7.2 Incubation period: 72 h Temperature: 30 °C Inoculum: 10%	Sarrafzadeh and Jafari (2008)
	<i>A. niger</i> A247	Glucose	145.22 U/ml	pH: 7.2 Incubation period: 72 h Temperature: 30 °C Inoculum: 10%	Haq et al. (2014)
	<i>A. tubingensis</i>	Fermentation medium containing waste residue (wheat bran, fish meal, wheat gluten, soybean meal)	43.73 U/ml	pH: 5.0 Incubation period: 32 h Temperature: 30 °C Inoculum: 1%	Kriaa and Kammoun (2016)
Lipase	<i>A. nidulans</i> (mbl-S-6)	Rice bran	102 U/ml	pH: 7.0 Incubation period: 72 h Temperature: 40 °C Inoculum: 10% Additional carbon source of 1% starch	Niaz et al. (2014)
	<i>A. tamarrii</i>	Mineral salt medium supplemented with 2.5% (v/v) coconut oil	23.25 × 10 ⁴ U/ml	pH: 5.0 Incubation period: 7 days Temperature: 25 °C Inoculum: 10%	Das et al. (2017)

(continued)

Table 11.2 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Chitinase	<i>Trichoderma harzianum</i> TUBF 966	Colloidal chitin (1.5%w/v)	14.7 U/ml	pH: 5.5 Incubation time: 96 h Temperature: 30 °C Inoculum: 10%	Sandhya et al. (2004)
	<i>Hunicola grisea</i> ITCC10, 360.16	Colloidal chitin	17.25 × 10 ⁴ U/ml	pH: 6.5 Incubation period: 72 h Temperature: 45 °C Inoculum: 10%	Kumar et al. (2017)
Cellulase	<i>A.flavus</i>	Czapek Dox medium and wheat bran as substrate	1.1 U/ml	pH: 6.0 Incubation period: 3 days Temperature: 30 °C Inoculum: 4%	Gomathi et al. (2012)
	<i>A.syndowii</i>	Culture medium induced with lactose	Endoglucanase –1.32 U/ml	pH: 5.5 Incubation period: 6 days Temperature: 40 °C Inoculum: 10%	Matkar et al. (2013)
			Exoglucanase -3.99 U/ml		
			β-glucosidase -9.24 U/ml		
	<i>T.reesei</i> QM9414	Pretreated pea hulls	13.8 U/ml	pH: 5.0 Incubation period: 91 h Temperature: 30 °C Inoculum: 10%	Sirohi et al. (2019)
Invertase	<i>A.caespitosus</i>	Wheat bran	301 U/ml	pH: 5.0 Incubation period: 72 h Temperature: 30 °C Inoculum: 10%	Alegre et al. (2009)
	<i>Cladosporium cladosporioides</i>	Pomegranate peel	197.5 U/ml	pH: 4.0 Incubation period: 4 days Temperature: 40 °C Inoculum: 10%	Uma et al. (2012)

(continued)

Table 11.2 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Protease	<i>A.spp</i>	Cheese whey and molasses, supplemented with 1% rice flour	3.16 U/ml	pH: 5.0 Incubation period: 120 h Temperature: 32 °C Inoculum: 10%	Radha et al. (2011)
	<i>Thermomucor indicae-seudaticae</i> N31	Wheat bran	60.5 U/ml	pH: 5.5 Incubation period: 72 h Temperature: 45 °C Inoculum: 10%	Silva et al. (2014)
	<i>A. nidulans</i> LCJ 249 and <i>A. flavus</i> LCJ 253	Fermentation medium containing peptone, malt extract, ammonium sulphate, chloride, and nitrate	1178 U/ml and 950.6 U/ml	pH: 7.0 Incubation period: 6 days Temperature: 37 °C Inoculum: 30 mg	Gnanadoss and Devi (2019)
Xylanase	<i>Cochliobolus sativus</i> Cs 5	Wheat straw	98.25 U/ml	pH: 4.5–5.0 Incubation period: 120 h Temperature: 55 °C Inoculum: 10%	Bakri et al. (2008)
	<i>A.terreus</i>	Wheat bran	5124 U/ml	pH: 5–6.5 Incubation period: 48 h Temperature: 55 °C Inoculum: 10%	Sorgatto et al. (2012)
	<i>Cladosporium oxysporum</i> GQ-3	Wheat bran	55.92 U/ml	pH: 7.0–8.5 Incubation period: 2 h Temperature: 50 °C Inoculum: 10%	Guan et al. (2016)
	<i>Fusarium</i> sp. BVKT R2	Birch wood xylan	4560 U/ml	pH: 5 Incubation period: 72 h Temperature: 32.5 °C Inoculum: 10%	Ramanjaneyulu and Rajasekar Reddy (2016)

(continued)

Table 11.2 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Laccase	<i>Pleurotus ostreatus</i> ATCC 32783	Liquid medium	13×10^6 U/ml	pH: 6 Incubation period: 456 h Temperature: 25 °C Inoculum: 10%	Tellez-Tellez et al. (2008)
	<i>Ganoderma lucidum</i>	Pomelo peel	$11,842 \times 10^6$ U/ml	pH: 3.0 Incubation period: 7 days Temperature: 60 °C Inoculum: 10%	Ding et al. (2012)
Pectinase	<i>A.niger</i> ABT-5	Wheat bran	15.5 U/ml	pH: 6.0 Incubation period: 72 h Temperature: 30 °C Inoculum: 10%	Abdullah et al. (2018)
	<i>Piriformospora indica</i>	Apple pectin	10.47 U/ml	pH: 5.0 Incubation period: 6 days Temperature: 50 °C Inoculum: 10%	Heidarizadeh et al. (2018)
	<i>A. fumigates</i> MS16	Banana peels	0.25 U/ml	Incubation period: 6 days Temperature: 25–35 °C Inoculum: 10%	Zehra et al. (2020)

condition of low-moisture content has additional benefits such as inhibits bacterial growth and avoids effluent treatment processes. Solid agro-industrial wastes can be utilized as substrate, minimum sterilization, very low-energy expenditure, less susceptible to contamination rate, minimum wastewater production, and low-capital investment meet more advantage. (Table 11.3) summarizes the overview of fungal enzymes production through solid-state fermentation process in different studies.

11.4.2 Recovery and Purification of Fungal Enzymes

Enzyme purification is vital in acquiring knowledge on structural and functional properties and determines its unique biochemical and functional applications in industries. The current industrial interest vests in fungal extracellular enzymes which are excreted into the culture medium. The crude enzymes mixtures are

Table 11.3 Fungal enzymes production through solid-state fermentation

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Amylase	<i>Thermomyces lanuginosus</i>	Wheat bran, molasses bran, rice bran, maize meal, millet cereal, wheat flakes, barley bran, crushed maize, corncobs, and crushed wheat	534 U/ml	pH: 6.0 Incubation period: 120 h Temperature: 50 °C Inoculum: 10%	Kunamneni et al. (2005)
	<i>A.fumigates</i> NTCC1222	Pomegranate peel, wheat bran	164.1 U/ml	pH: 6.0 Incubation period: 60 minutes Temperature: 55 °C Inoculum: 10%	Singh et al. (2014)
	<i>A. terreus</i> NCFT4269	Pearl millet	19.19 U/ml	pH: 7.0 Incubation period: 96 h Temperature: 30 °C Inoculum: 10%	Sethi et al. (2016)
Glucose oxidase	<i>A.tubingensis</i>	Fermentation medium containing industrial waste residue (wheat bran, fish meal, wheat gluten, soybean meal)	170.59 U/ml	pH: 7.2 Incubation period: 72 h Temperature: 30 °C Inoculum: 10%	Kriaa and Kammoun (2016)
Invertase	<i>Paecylomyces variotii</i>	Soy bran and wheat bran	197.9 and 115.0 U/ml	pH: 4.0–4.5 Incubation period: 96 h Temperature: 40 °C Inoculum: 10%	Giraldo et al. (2012)
	<i>A.niger</i> GH1	Molasses and sugarcane bagasse	5231 × 10 ³ U/ml	pH: 7 Incubation period: 24 h Temperature: 30 °C Inoculum: 10%	Veana et al. (2014)

(continued)

Table 11.3 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Lipase	<i>A.fumigatus</i> MTCC 9657	Defatted rice bran	8.13 U/ml	pH: 8.5 Incubation period: 7 days Temperature: 30 °C Inoculum: 10%	Rajan and Nair (2011)
	<i>Trichoderma harzianum</i>	Wheat bran supplemented with 2% olive oil	14.3 U/ml	pH: 8.03 Incubation period: 120 h Temperature: 40 °C Inoculum: 10%	Toscano et al. (2013)
	<i>Penicillium camemberti</i> AM83	Rapeseed cake supplemented with lactose and calcium chloride	0.247 U/ml	pH: 8.0 Incubation period: 5 days Temperature: 30 °C Inoculum: 10% Moisture content: 80%	Boratynski et al. (2018)

(continued)

Table 11.3 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Protease	<i>A.oryzae</i> MTCC 5341	Defatted soy flour	8.47×10^5 U/ml	pH: 5.0 Incubation period: 120 h Temperature: 30 °C Inoculum: 10%	Vishwanatha et al. (2010)
	<i>A.tamarii</i>	Wheat bran	401.42 U/ml	pH: 8 Incubation period: 72 h Temperature: 40 °C Inoculum: 10%	da Silva et al. (2016)
	<i>P.camemberti</i> AM83	Rapeseed cake supplemented with lactose and ammonium sulfate	3.556 U/ml	pH: 9.0 Incubation period: 5 days Temperature: 30 °C Inoculum: 10% Moisture content: 90%	Boratynski et al. (2018)
	<i>A.brasiliensis</i> BCW2	Wheat bran and 10% orange peel as carbon source	1734 U/ml	pH: 9.0 Incubation period: 72 h Temperature: 30 °C Inoculum: 10% Moisture content: 60%	Chimbekujwo et al. (2020)

(continued)

Table 11.3 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Pectinase	<i>A.niger</i>	Orange waste peel	0.097 U/ml	pH: 5.5 Incubation period: 5 days Temperature: 30 °C Inoculum: 10%	Ahmad et al. (2016)
	<i>A.giganteus</i> NRRL 10	Wheat bran, orange, and lemon peels	180 U/gds	pH: 5.8–7.0 Incubation period: 60 h Temperature: 25 °C Inoculum: 10%	Ortiz et al. (2017)
	<i>A. nominus</i> MR 103	Orange peel	0.0048 U/ml	pH: 7.0 Incubation period: 7 days Temperature: 35 °C Inoculum: 10%	Ketipally et al. (2019)
	<i>A.awamori</i> NRC-F18	Sugar beet pulp	264.4 U/ml	pH: 5.0 Incubation period: 72 h Temperature: 35 °C Inoculum: 10%	Roshdy et al. (2020)

(continued)

Table 11.3 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Chitinase	<i>P. ochrochloron</i> MTCC 517	Wheat bran	2443.23 U/ml	pH: 7.0 Incubation period: 96 h Temperature: 30 °C Inoculum: 10% Moisture content: 70%	Patil and Jadhav (2014)
	<i>M.anisopliae</i> E6	Sugarcane bagasse	6.78 U/ml	pH: 5 Incubation period: 96 h Temperature: 33 °C Inoculum: 10% Moisture content: 60%	Reis et al. (2018)
	<i>Metarhizium anisopliae</i> IBCB 348	Sugarcane bagasse	12.07 U/ml	pH: 5 Incubation period: 8 days Temperature: 28 °C Inoculum: 10% Moisture content: 40%	Aita et al. (2019)
Chitosanase	<i>Trichoderma koningii</i> sp.	Wheat bran and chitosan	4.84 U/gds	pH: 5.5 Incubation period: 48 h Temperature: 50 °C Inoculum: 10%	da Silva et al. (2012)

(continued)

Table 11.3 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Cellulase	<i>Trichoderma</i> sp.	Apple pomace supplemented with lactose and corn steep liquid	5.8 U/gds	pH: 4.8 Incubation period: 120 h Temperature: 32 °C Inoculum: 10% Moisture content: 70%	Sun et al. (2010)
	<i>A.fumigatus</i> Z5	Corn stover	526.3 U/gds	pH: 5.0 Incubation period: 4 days Temperature: 50 °C Inoculum: 10%	Liu et al. (2011)
	<i>A.niger</i> NRRL-567	Apple pomace induced with lactose	133.68 U/gds	pH: 4.8 Incubation period: 48 h Temperature: 32 °C Inoculum: 10%	Dhillion et al. (2012)
	<i>Trichoderma reesei</i>	Municipal solid waste	20 U/ml	pH: 4.8 Temperature: 30 °C Incubation period: 168 h Moisture content: 60% Inoculum: 10%	Abdullah et al. (2016)
Xylanase	<i>A.awamorii</i>	Grape pomace	40.4 U/gds	pH: 5.4 Temperature: 50 °C Incubation period: 24 h Inoculum: 10%	Botella et al. (2005)
	<i>A.niger</i> NRRL-567	Apple pomace induced with veratryl alcohol	1412.58 U/gds	pH: 4.8 Incubation period: 48 h Temperature: 32 °C Inoculum: 10%	Dhillion et al. (2012)

(continued)

Table 11.3 (continued)

Fungal enzymes	Organisms	Substrate used	Enzyme activity	Optimized conditions	Reference
Laccase	<i>Ganoderma</i> sp.	Wheat bran	2400 U/gds	pH: 3 Incubation period: 7 days Temperature: 25 °C Inoculum: 10% Moisture content: 70%	Revankar et al. (2007)
	<i>Corioloropsis caperata</i> RCK2011	Wheat bran induced with 0.5 mM xyloidine	1623.55 U/gds	pH: 5.0 Incubation period: 5 days Temperature: 30 °C Inoculum: 10%	Nandal et al. (2013)
	<i>Pleurotus eryngii</i>	Peach waste	43,761 × 10 ³ U/ml	pH: 6.0 Incubation period: 20 days Temperature: 28 °C Inoculum: 10%	Akpınar and ÖztürkÜrek (2017)
	<i>Trametes versicolor</i>	Dry fermented tea residue	31.2 U/gds	pH: 5.0 Temperature: 26°C Incubation period: 7 days Inoculum: 4% Moisture content: 85%	Xu et al. (2020)

Enzyme units expressed in U/gds (units per gram of dry substrate) and U/ml (units per milliliter)

complex, multielemented containing nonenzymatic proteins and secondary metabolites. Maximum productivity, purity, and catalytic activity can be achieved through appropriate purification method. The cells are separated from media in order to concentrate either by filtration or centrifugation depending on the nature of enzyme which may be intracellular or extracellular. Followed by cell concentration, purification is performed viz., various ways which are depicted in (Table 11.4).

11.5 Applications of Fungal Enzymes in Industries

A fungal enzyme has been utilized for wide range of enzymatic processes in industries since prehistoric time. Application of the most common fungal enzymes and its role in various industries are discussed in the section (Figs.11.2 and 11.3).

Table 11.4 Purification techniques of various fungal enzymes

Enzyme	Source	Recovery and/Purification method	Reference
Amylase	<i>A.niger</i>	Precipitation, size exclusion chromatography, and anion exchange chromatography	Sahnoun et al. (2012)
Glucose oxidase	<i>A.tubingensis</i>	Concentration by rotavaporation and centrifugation followed by column chromatography	Kriaa et al. (2015)
Invertase	<i>A.terreus</i>	Ion exchange chromatography followed by gel filtration	Almeida et al. (2018)
Chitinase	<i>P.aculeatum</i> NRRL 2129	Size exclusion chromatography	Binod et al. (2005)
Protease	<i>A.foetidus</i>	Gel filtration chromatography	Souza et al. (2017)
Lipase	<i>A.tamarii</i>	Ion exchange and column chromatography	Das et al. (2016)
Xylanase	<i>T. inhamatum</i>	Ion exchange chromatography followed by subsequent molecular-exclusion chromatography	Silva et al. (2015)
Laccase	<i>Thieavia</i> sp.	Tangential flow filtration followed by ion exchange chromatography	Mitibaa et al. (2018)
Pectinase	<i>A.niger</i>	Ammonium sulfate precipitation and ion exchange chromatography and further purification by gel filtration	Ahmed et al. (2016)

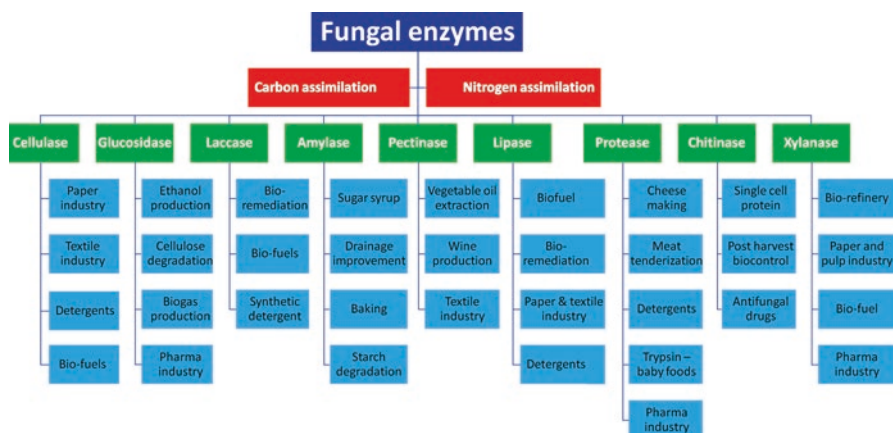


Fig.11.2 Industrial important fungal enzymes and their potential applications

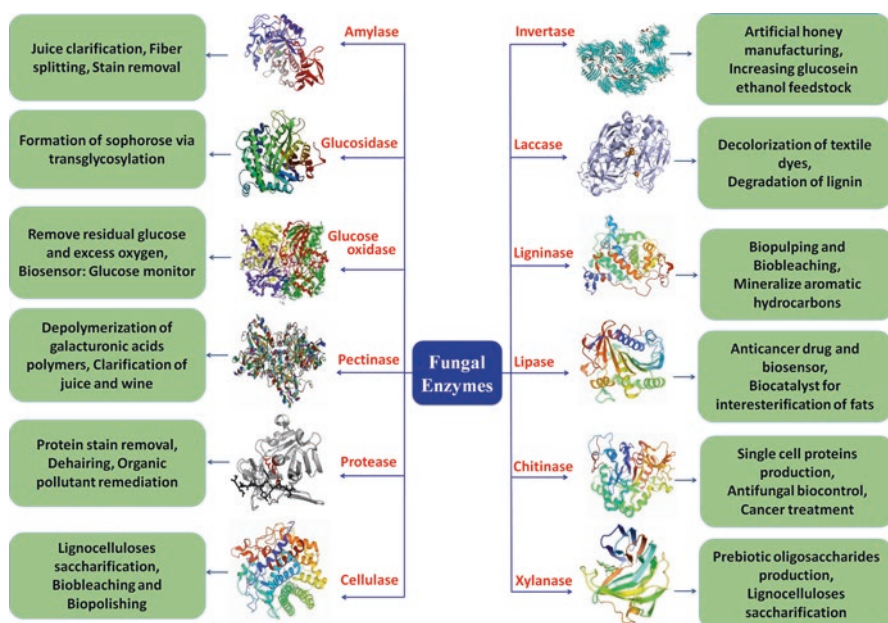


Fig.11.3 Role of fungal enzymes in various industrial applications

11.5.1 Amylase

Amylases application in food, textile, detergent, paper, and pharmaceutical industries is very common since nineteenth century due to its potential of hydrolyzing starch into simple sugars such as dextrans and oligosaccharides (Mehta and Satyanarayana 2016; Singh et al. 2016; Yadav et al. 2016a). In fact, fungal amylases have been utilized in baking industry since 1955 in USA. Amylase is produced from the fungal sources like *Aspergillus oryzae*, *A. niger*, *A. terreus*, *A. awamori*, *A. fumigatus*, *A. flavus*, *Thermomyces lanuginosus*, *Paecilomyces variotii*, *Penicillium brunneum*, *P. chrysogenum*, *Rhizopus oryzae*, *Mucor* sp., and *Trichoderma pseudo koningii* (Samanta 2020).

The α -Amylases, β -amylases, and γ -amylases catalyze the hydrolysis at α -1,4-*O*-glycosidic bonds, α -1, 4-glucan bonds, and α -(1–6) glycosidic bonds, respectively in amylose and amylopectin (Liu and Kokare 2017). These functions of amylase possessing several applications in fermentation, sugar syrups production (glucose/maltose), starch liquefaction, and saccharification process (Liu and Kokare 2017). In baking industry, addition of α -amylases enhances the dough fermentation rate, improves texture, color, taste, generates extra sugar, antistaling effect, and reduces the dough viscosity (de Souza 2010).

A cold-adapted α -amylase (AmyA1) gene cloned from fungal species *Geomyces pannorum* and expressed in the *Aspergillus oryza* showed potential value in food and starch industrial applications such as increased specific volume and

cohesiveness of bread and decreased gumminess. In addition, immobilized enzyme AmyA1 showed improved thermostability, reusability, and enhanced pH tolerance which supports industrial production (He et al. 2017). Similarly, psychrotrophic fungi, *Penicillium chrysogenum* HTF24 producing cold-active enzymes including amylase have wide range of application in biotechnological, health, industrial, and environmental sectors (Rafiq et al. 2020). According to Pasin et al. (2020), glucoamylase-producing fungus *Aspergillus carbonarius* cultivated from wheat and brewing residues on large-scale illustrated the possible reuse of the wastes, potential enzyme production, and generation of ethanol and lactic acid for sustainable industrial use.

Further, the amylase application desizing process removes the starch particles from the fabrics and do not attack the fibers (Mojsov et al. 2018). *Paecilomyces variotii* ATHUM 8891 which α -amylase with higher stability as well as spontaneous catalytic activity under varying temperature increases its potential use in starch-based industries (Apostolidi et al. 2020). Environmental applications of amylase from *Pleurotus florida* have been studied for as starch degradation in potato waste and desizing of cotton waste (Devi et al. 2020). Additionally, amylolytic treatment using α -amylase-mediated saccharification and alcoholic fermentation processes has potential application in alcohol and biofuel production (Kumar et al. 2014). Digestive medicines containing α -amylase obtained from *A. oryzae* used for indigestion treatment in syrup and capsule formulation. A detergent formulation containing α -amylases showed potential application in dish-washing due to its characteristics of alkalophilic, chelator resistance, and oxidant insensitive nature (Samanta 2020).

The extracellular amylase production through wheat-based biorefinery by *Neurospora intermedia*, an edible fungus can be advantageous in protein-rich biomass production and feed industries. Further, *N. intermedia* amylases have more stability and substrate affinity and hydrolyzing rate (Shahryari et al. 2019). Similarly, α -amylase gene (RmAmyA) was cloned from thermophilic fungus *Rhizomucor miehei* and expressed in *Pichia pastoris* displayed excellent properties with high pH stability, thermostability, and saccharification activity, Ca^{2+} -independence, and high maltose activity. Also, RmAmyA acts as potential candidate for improving Chinese steamed bread quality and antistaling property (Wang et al. 2020).

11.5.2 Glucosidase

β -glucosidases, an important glycoside hydrolases play a major role in cleavage and synthesis of glycosidic bonds. It catalyzes transglycosylation, hydrolyzes ligosaccharides, and mediates cellulose fiber saccharification, undergoes crucial biological pathways including cellular signaling and degradation of polysaccharides, provides number of biotechnological applications in cellulosic ethanol production, beverage, flavors enhancement, cassava detoxification, etc. (Singh et al. 2016). *Paecilomyces thermophila*, *Penicillium oxalicum*, *Trichoderma reesei*, *T. viride*,

Coprinopsis cinerea, and *Aspergillus niger* are the filamentous fungi known to produce great number of β -glucosidases (Xia et al. 2018).

In biofuel production, the activity of β -glucosidase is essential to hydrolyze the lignocellulosic biomass (Florencio et al. 2017). An efficient cellulolytic and hemicellulolytic enzyme, β -glucosidase produced by fungal *Thermoascus aurantiacus* (thermophilic) and *Trichoderma reesei* (mesophilic) helps in depolymerization and saccharification of sugarcane bagasse at high-temperature with low enzyme load (Frassatto et al. 2020). In cellulosic ethanol production, β -glucosidases from *Talaromyces thermophilus* (thermophilic fungus) yield maximum reducing sugars during waste paper saccharification (Mallek-Fakhfakh et al. 2017).

β -glucosidase secreted from *Meyerozyma guilliermondii* has ethanol-glucose tolerant characteristics showed valuable application during final saccharification process in winemaking. It releases terpene groups by cleavage of glycoside-terpene complexes to improve the flavor and quality of wine (da Silva et al. 2019). Further, glucose-tolerant β -glucosidase isolated from fungi-mediated release of aromatic compounds present in fruit juices (glycosidic structures) and fermenting products which enhances the wine quality. According to Karami et al. (2020), immobilized β -glucosidase produced from *Aspergillus niger* increased thermostability, storage stability, recyclability, and higher hydrolytic activity possessed more efficient application in food industry. In food industry, β -glucosidase acts as an important candidate in conversion of sugar derivatives into glucose (Alarid-Garcia and Escamilla-Silva 2017). β -glucosidase (AfBglA) from *Aspergillus flavus* displayed good thermostability, substrate specificity, pH stability, and excellent hydrolytic ability to the soybean meal suspension attracted the food and feed industry in saccharification process (Chen et al. 2019). The inactive form of glucosidic isoflavones such as glycitin and daidzin in soy-based foods can be hydrolyzed into aglycones by supplementing β -glucosidase for enhancing biological activity during digestion (Hati et al. 2015).

β -glucosidase obtained from *Chaetomium thermophilum* (thermophilic fungus) having CtBGL crystal structure provided more effective use in cellulose degradation (Mohsin et al. 2019). Similarly, β -glucosidase from *Myceliophthora thermophila*, a biocatalyst is capable of synthesizing alkyl glycoside at industrial level. Production of β -glucosidase from *Aspergillus niger* applied for pretreatment of municipal solid waste-enhanced biogas production potential by improved enzymatic hydrolysis of organic fraction even at high solid concentration (Mlaik et al. 2020). The extracellular β -glucosidases (BGL1 and BGL2) isolated from *Aspergillus* sp. YDJ216 showed potential application in pharmaceutical industries. Hydrolysis of flavone glycosides had inhibitory effect on monoamine oxidase suggests best treatment for neurological disorders (Oh et al. 2018).

11.5.3 Glucose Oxidase

GOD is widely utilized in baking, beverages, and gluconic acid production industry. Glucose oxidase (GOD) catalyzes the β -D-glucose into D-glucono- δ -lactone by oxidation method using atomic oxygen as an electron acceptor. *Aspergillus niger*, *A. tubingensis*, *A. flavus*, *A. terreus*, *A. oryzae*, *A. carbonarius*, *A. nidulans*, *Penicillium amagasakiense*, *P. variable*, *P. chrysogenum*, *P. notatum*, *P. funiculosum*, *P. adametzii*, *P. glaucum*, and *Penicillium vitale*, *Talaromyces flavus*, *Phanerochaete chrysosporium*, *Alternaria alternata*, *Pleurotus ostreatus*, *Rhizopus stolonifer*, and *Flavodon flavus* produce GOD at a large scale (Kriaa and Kammoun 2016).

In Baking Industry, GOD enhances the quality, viscoelasticity, strengthening of dough and bread volume (Kouassi-Koffi et al. 2014). In beverage industry, GOD applied to reduce the low-alcohol compounds in wine by removing residual glucose. It would generate H_2O_2 affects the growth of fermentative organisms that leads to decrease in liquor aging (Valencia et al. 2017). In dry egg powder making process application of GOD diminished residual glucose content and excess oxygen that would provide prolonged shelf life and tolerance to microbial contamination (El-Hariri et al. 2015).

P. chrysogenum producing GOD-mediated production of gluconic acid plays extensive role in cheese, curd formation, stability of milk, and cleaning of aluminum cans. Sodium gluconate has immense potential to chelate metal ions removed bitterness from food materials (Pal et al. 2016). In the pharmaceutical sector, GOD-derived calcium gluconate used for the treatment of calcium-associated deficiencies (Khurshid et al. 2013). GOD produced by *A. niger* and *P. chrysogenum* showed the antibacterial and antifungal activity against different fungal pathogens, respectively (Zia et al. 2013). A biosensor containing GOD efficiently applied on determining glucose content in beverages such as juices and energy drinks (Lopes et al. 2012). Further, its high selectivity for glucose and ionic resistance have advantages in developing continuous glucose monitor. Due to its potential functionality even at extreme temperature and pH, these biosensors widely employed to measure blood glucose level (Wang and Lee 2015).

11.5.4 Protease

Protease-mediated degradation of proteins into smaller components possesses various industrial applications. A wide range of protease application in industrial level has been studied which includes dairy, beverage, detergent industry, bakery, leather industry, and medical application covers treatment for AIDS, cancer, and diabetes. The best fungal source for protease enzyme production includes *Aspergillus fumigatus*, *Aspergillus oryzae*, *Aspergillus versicolor*, *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus flavus* (Yadav et al. 2017, 2020a, b).

Fungal proteases are very well utilized in food processing industries that are in brewing haze clarification in beer production, coagulation step in cheese making, and baking in food processing industries (de Souza et al. 2015). The hydrolytic properties of proteases have advantages in degradation of protein in juice and alcoholic liquors, hydrolysis of gelatin, soy and whey protein, meat protein recovery, and tenderization. Application of milk-clotting proteases as a rennin substitute in cheese production becomes popular nowadays due to high stability and activity at acidic pH and flavor enhancers properties (Mamo et al. 2020). The protease enzyme extracted from *Aspergillus* species is recognized as safe (GRAS), hence applied in dairy industries as milk-coagulating agents (Kumura et al. 2017) and for accelerating cheese ripening (Qureshi et al. 2015). Similarly, *Rhizomucor miehei* NRRL 2034-based protease utilized for white soft cheese production which was free from aflatoxins and *A. niger* FFB1-releasing proteases were free from ochratoxin A (Fazouane-Naimi et al. 2010). According to Mamo et al. (2020), proteases derived from *Aspergillus oryzae* DRDFS 13 were appropriate than bacterial enzyme due to organoleptic danbo cheese production using fungal enzyme.

In the pharmaceutical industries, proteases are used in skin ointments for healing skin ulcers, removal of keratin in acne, and degradation of keratinized skin (Shubha and Srinivas 2017; Devi et al. 2020a). In cosmetic industries, proteases enzyme-based products hydrolyzed the peptide bonds of keratin and collagenolytic proteases have been exploited in wound healing and adenovirus-mediated cancer therapy (Chao et al. 2007). Alkaline protease-based detergents formulations augment the degradation ability to remove proteinaceous material from tough stains (Savitha et al. 2011). In leather making industry, alkaline protease replaces the hazardous chemicals involved in soaking and dehairing process. Further, alkaline proteases from *Conidiobolus coronatus* and *Aspergillus flavus* were successfully used in leather tanning process (Franco et al. 2017).

11.5.5 Pectinase

Among the pectinases, fungal pectinases are important industrial enzymes. *Aspergillus niger*, *A. oryzae*, *A. versicolor*, *A. pulverulentus*, *A. flavus*, *Fusarium oxysporum*, *Mucor racemosus*, *Mucor hiemalis*, *Rhizopus stolonifer*, *Penicillium Jensenii*, *P. citrinum*, and *Trichoderma viride* were explored recently to produce pectinases and found to be suitable for industrial pectinase production (El-Rahim et al. 2020). Pectinases have crucial roles in fruit juice extraction, vegetable oil extraction, wine clarification, cocoa, and coffee fermentation (Kubra et al. 2018). Furthermore, application in bleaching of paper, retting of plant fibers, bio-scouring of cotton, oil extraction, bioenergy production, and wastewater treatment (Kubra et al. 2018).

In the textile industry, pectinases are used in the degumming process of plant fibers such as sunn hemp, flax, and jute (Sudeep et al. 2020). In pulp and paper

industry, application of pectinases depolymerized the galacturonic acids polymers and used to improve physical properties of paper (Rebello et al. 2017). In poultry feed, pectinase used as enzyme supplement to reduce feed viscosity (Hoondal et al. 2002). In food industry, *Aspergillus* sp. producing pectinase enzyme showed its ability in juice clarification due to higher stability from acidic to alkaline range (Sudeep et al. 2020). It also boosted the aroma and flavor of vegetable and fruit juices. Vacuum infusion of pectinase technology has been applied to soften the peel of citrus fruits. In beverage industry, it also contributes to clarification of wines, improving chromaticity, stability, and aroma of red wines. Pectinase from *A. niger* and *Mucor circinelloids* is used for coffee fermentation and to remove mucilaginous coat (coffee beans) (Rosales et al. 2018; Sudeep et al. 2020).

11.5.6 Cellulase

Cellulases, a key enzyme for efficient hydrolyzes of polysaccharides and act upon cellulosic chains found in biomass for degradation. Cellulases are used in many industries, i.e., pulp and paper, detergent, textiles and laundry, food and agricultural, and bioethanol production. Among the fungal species *Aspergillus fumigatus*, *Aspergillus niger*, *Myceliophthora thermophila*, *Rhizopus oryzae*, *Trichoderma reesei*, and *Thielavia terrestris* have been characterized to produce cellulases (Kupski et al. 2014; Kour et al. 2019c; Sahay et al. 2017; Yadav et al. 2019).

In pulp and paper industry, crude cellulases applied for biomodifying the fiber properties and pure cellulases applied for reducing pulp viscosity, improving fiber brightness, strength properties, and bleach ability of kraft pulps, and deinking the paper wastes (Kuhad et al. 2010). In food processing industry, cellulases were used for extracting and clarifying fruit and vegetable juices to enhance the texture and cloud stability by decreasing the viscosity (Carvalho et al. 2008). In agricultural sector, application of cellulase controls plant diseases by the way of degrading the cell wall of pathogens (Verma et al. 2016; Yadav et al. 2016b). Further, cellulase supplements on crop residues in soil accelerated the straw disintegration rate which in turn improved soil fertility (Han and He 2010). In detergent industry, cellulases as additive in liquid laundry detergent remove dirt in the interfibril spaces and maintain the garments quality (Karmakar and Ray 2011).

In textile industry, cellulases are applied in wet processing steps to attain improved cellulose-based textiles. Presently, biopolishing and biostoning of cellulosic fabrics and jeans are successfully achieved using cellulases. Further, acidic cellulases enhanced the softness of the fabric (Karmakar and Ray 2011). In finishing process of knitted cotton fabrics, fungal cellulases from *Acrophialophora nainiana* and *Penicillium echinulatum* were used (Imran et al. 2019). In wine and brewery industry, usage of cellulases during malting and fermentation process significantly increased the wine stability and viscosity. In feed industry, pretreating of grain and agricultural silage with cellulases improved the feed value, digestibility

of grasses, and energy values. In bioethanol industry, cellulase enzyme production by fungus *Penicillium funiculosum*, *Fusarium verticillioides*, and *Cladosporium cladosporioides* showed potential application in bioethanol production from moringa wastes (Vázquez-Montoya et al. 2020). *Aspergillus niger* MK543209-producing cellulases utilized for biofuel production from paper wastes (Darwesh et al. 2020). Further, cellulolytic thermophilic filamentous *Myceliophthora thermophila* fungus used for the production of bioethanol which reached up to 11.3 g/L on cellobiose (Li et al. 2020).

In pharmaceutical industry, cellulases act as biocatalysts which could help to release therapeutic compounds. A digestive aids containing cellulases used for treating metabolic disorders. A prebiotics with nondigestible fibers depolymerized by cellulases boost the growth of beneficial bacteria in the intestine tract (Karmakar and Ray 2011).

11.5.7 Invertase

Invertase (beta-fructofuranosidase) hydrolyzes sucrose, i.e., break down of α -1,4-glycosidic linkage between D-glucose and D-fructose of sucrose (Toledo et al. 2019). *Aspergillus*, *Cladosporium*, *Fusarium*, *Aureobasidium*, *Trichoderma*, *Eremothecium*, *Ceratocystis*, *Metarhizium*, *Sclerotinia*, *Penicillium*, *Rhizopus*, and *Paecilomyces* are some fungal species that produce invertase.

Invertase has been applied in various industrial applications, i.e., food, beverages, bakery, and calf feed production. Invertase application in manufacturing of sugar syrup leads to avoid crystal aggregation which can be recommended for diabetic patients and children for more iron absorption (Linde et al. 2012). Invertase-produced noncrystallizable sugar has been used in gingerbread dough and baking industry. Other applications include preparation of candies, chocolates, jams, artificial honey, and cookies (Andjelkovic et al. 2015). In brewing industry, applications of invertase used in hydrolyzing saccharose to fructose and glucose and invertase activity is important at watering and germination processes of beer production.

Invertase producing fructooligosaccharides (FOS) is an important nondigestible carbohydrate composed of fructose chains has a wide range of application in pharmaceutical industry. The FOS can be recommended for diabetic patients due its property of lower caloric value and short-chain fatty acids (Bhalla et al. 2017). Invertase application in ethanol feedstock increasing the glucose amount of molasses possesses greater importance in biofuel production (Hutsch et al. 2014) and used for rapid detection of sucrose and (Shankar et al. 2014).

11.5.8 Laccase

Laccases act as biocatalysts and its potential to degrade lignin and capability to oxidize phenolic compounds received much attention in industrial applications (Singh and Gupta 2020). Among the fungus used for enzyme production *Aspergillus tubingensis*, *Aspergillus flavus*, *R. oryzae*, *Mucor indicus*, *Mucor hiemalis*, *Fusarium udum*, *Peniophora*, and *Trichoderma viride* were confirmed higher laccase production (El-Rahim et al. 2020). A wide range of industrial applications covers food processing industries, paper and pulp industry, pharmaceutical and nanobiotechnological applications, textile industries, and biodegradation of xenobiotic compounds due to its broad range of substrate specificity (Periasamy et al. 2019).

In pulp industry, white-rot fungus producing laccases has advantages in degrading lignin compounds and biopulping process. Laccases-producing *Marasmius* sp. has been applied to degrade lignin in empty fruit bunches (Risdianto and Sugesty 2015). Application of lignolytic laccases also used for pulp biobleaching. In food processing industry, laccases applied for changing color appearance of beverage of food. Its capacity of removing phenolic compounds and oxidizing polyphenols groups possesses application in darkening, haze formation, and clarifying fruit and vegetable juices, beer and wine (Bilal et al. 2019). In beer industries, laccases used to reduce oxygen concentration thereby increasing stability and shelf life (Brijwani et al. 2010). Laccases from *Trametes hirsute* (white-rot fungi) applied to reduce expandability in flour and gluten-based dough. In textile and dye industry, laccases used in dichlorination techniques for improving brightness of cotton fiber and biostoning of denim fabrics. Laccases-producing white-rot fungus utilized to decolorize the Reactive Blue 15, Remazol Brilliant Blue R, and Congo Red (Mishra and Maiti 2019). Laccase catalyzing oxidation-reduction reactions involved in biodegradation of hazardous compounds such as recalcitrant synthetic substances, herbicides, and fungicides have potential application in environmental sector. Further, chitosan beads immobilized with laccase have proven its degradation ability on bisphenol-A (Bilal et al. 2019).

11.5.9 Ligninase

Ligninase is a heme-protein which oxidizes phenolic, nonphenolic, and aromatic compounds by H_2O_2 (Vandana et al. 2018). It acts as a key enzyme in lignin degradation and showed a high catalytic site on the protein surface (Falade et al. 2017). Lignin peroxidase (LiP) widely produced by white-rot fungi. Some of the lignin peroxidase-producing fungal organisms are *Phanerochaete chrysosporium*, *T. versicolor*, *Trichoderma viride*, *Aspergillus niger*, *Trichoderma reesei*, *Phlebia radiata*, *Trametes suaveolens*, and *Pleurotus ostreatus*. Manganese peroxidase

(MnP) can be secreted by *Pleurotus nebroleusis*, *T. versicolor*, and *Grifola frondosa* (Xu et al. 2013).

These fungal ligninases are used in biopulping process (Walia et al. 2017). The fungal ligninases from *Aspergillus sp.* LPB-5 were efficient during biobleaching and biopulping processes lead to increase in pulp digestibility. Effective removal of lignin and hemicelluloses achieved high yield and soften the product (Colonia et al. 2019). Lignin peroxidase from *P. chrysosporium* effectively decolorized structurally different polymeric dyes, triphenyl methane, anthraquinone, and indigo and azo dyes in the presence of mediator (veratryl alcohol) by oxidation process (Singh et al. 2015). LiP is reported to mineralize recalcitrant aromatic hydrocarbons comprises of three- and four-ring structures and chlorophenols released from coal industries (Achten et al. 2011). *Sporotrichum pruinosum* and *Ceriporiopsis sp.* producing LiP have ability to synthesis human skin melanin. The capability of degrading human hair and skin melanin improved skin texture and roughness. Skin-lightening effect of fungal LiP with no observable adverse effect proved the potential advantages in cosmetic industry compared to hydroquinone cream (Draeos 2015). MnP catalyzed the phenolic lignin compounds and it has both peroxidase and oxidase properties. The advantages of MnP have applied in biobleaching, demethylation, and delignification of kraft pulp processing (Xu et al. 2013).

11.5.10 Lipase

Lipases are able to hydrolyze the insoluble triacylglycerols, catalyzing reverse reactions, i.e., esterification, alcoholysis, and acidolysis and have high specificity for substrates (Javed et al. 2018). The most common fungal lipase producers are *Aspergillus*, *Fusarium*, *Mucor*, *Geotrichum*, *Penicillium*, and *Rhizopus sp.* In recent years, *Penicillium simplicissimum* has been studied to produce lipases. A psychrotrophic fungi, i.e., *Penicillium chrysogenum* HTF24 producing cold-active lipase has several advantages in industrial and environmental sectors (Rafiq et al. 2020). It is also used in various industries such as textiles, detergents, pharmaceuticals, pulp and paper, and biofuels (Borowiecki et al. 2017). Lipase-based biosensors are used to detect pollutants like pesticides in the environment while in presence of triacylglycerols, it can be used to control food quality (Ma et al. 2018).

In food industry, lipase from *Penicillium roquefortii* was used for synthesizing methyl ketones in order to improve flavor in blue cheese. Lipases-mediated production of trans-FA free margarines has several applications in food industry (Ornla-Ied et al. 2016). Immobilized *Thermomyces lanuginosus* lipase used as biocatalyst for interesterification of fats obtained from camel hump (Shekarchizadeh and Kadivar 2012). Lipase can able to produce different flavors based on the reaction medium such as improving the organoleptic characteristics, quality improvement in mayonnaise, development of aroma in drinks, cheese milk, and butter (Dhake et al.

2013). For example, immobilizing lipases from *Thermomyces lanuginose* showed higher catalytic activity and utilized for ethyl valerate (green apple flavor) synthesis (Sadighi et al. 2017). In biodiesel production, immobilized lipase on activated biochar showed higher efficiency in transesterification, i.e., high yield of fatty acid methyl esters of 92.23% was obtained because of its lipase-induced stability and catalytic activity (Vargas et al. 2018).

The capability of lipase to synthesize biodiesel even with high water content is a useful approach when waste oils are utilized for biodiesel production (Yu et al. 2016; Kaur et al. 2020; Prasad et al. 2020; Yadav et al. 2020d). Further, in medical industry, lipase-mediated production of Polixatel (taxol 1) was used in the treatment of cancer cells (Fukaya et al. 2016). Lipase-based biosensor can be used to detect triglycerides, cholesterol, and phospholipids levels in blood (Herrera- López 2012). In wastewater treatment, pretreatment of the effluent can be done by lipase produced from *Aspergillus niger* prior to anaerobic digestion to improve treatment efficiency. It is widely applied in fat-containing effluent released from food processing, tanneries, and paper and cellulose industries (Sarmah et al. 2018).

11.5.11 Chitinase

Fungal Chitinase hydrolyzes the chitin and it can be used in the production of single-cell proteins, N-acetyl d-glucosamines, chitooligo saccharides, and various medical fields. Some of the chitinase-producing fungal organisms are *Saccharomyces cerevisiae*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Ustilago maydis*, *Coprinopsis cinerea*, *Trichoderma harzianum*, and *Xenorhabdus nematophila* (Liu et al. 2019; Rastegari et al. 2020a, b; Yadav et al. 2020e).

Fungal chitinases can act as biocatalyst for chitobiose, have several applications in various industries (Le and Yang 2019). Chitinase obtained from *Penicillium monoverticillium*, *Aspergillus flavus*, and *Fusarium oxysporum* used to achieve certain oligomer chain length and also used to produce N-acetylglucosamine (GlcNAc) and chitooligosaccharides from α -chitin has various healthcare applications (Zhou et al. 2018). *S. marcescens* producing chitinases generated single-cell proteins with the maximum yields of 45% protein by hydrolyzing chitin waste.

The antifungal activity of chitinase obtained from fungi has potential biocontrol application (Le and Yang 2018). *Trichoderma harzianum*-generated chitinase efficiently inhibited the phytopathogen growth (*Botrytis cinerea*) (Deng et al. 2019). Liu et al. (2019) demonstrated that fungal chitinases inhibited the spore germination by *Coniella diplodiella* and *Verticillium dahlia* achieved possible application in postharvest biocontrol. Chitinase containing antifungal drugs attained promising therapeutic application for various infections (Gutiérrez-Román et al. 2015). In addition, *S. marcescens* and *S. griseus*-derived chitinase used for cancer treatment by destroying cancer cells (MCF-7 and B11-2) (Pan et al. 2005).

11.5.12 Xylanase

Xylanases act on linear polysaccharide found in lignocellulosic biomass and hydrolyze hemicelluloses beta-1,4-xylan into xylose (Vogel 2018). *Aspergillus tubingensis*, *A. terreus* (Fss 129), *A. niger* (SS7), *R. oryzae*, *Mucor indicus*, *Mucor hiemalis*, *Peniophora*, and *Trichoderma viride* were proven to produce xylanases in recent years (Bakri et al. 2008). *N.intermedia* is a potential candidate for production of xylanase through a wheat-based biorefinery (Shahryari et al., 2019). Xylanases used in bioprocessing of textiles, value addition in grain feed and silage, biobleaching process in wood pulp and paper industries, extraction of plant oils, starch, and coffee, and pharmaceutical industries (Walia et al. 2017). Biotechnological potential of xylanase has several advantages in the processes of ethanol and xylitol synthesis, liquid fuel extraction, cellular protein synthesis, and chemical production (Yadav et al. 2017).

T. reesei QM9414 produced higher amount of xylanase and can be applied in biobleaching process of kraft pulp (Campioni et al. 2019). Application of xylanase in biobleaching process decreased chlorine requirement from 10 to 50% and lower the overall production cost. Thermostable alkaline xylanase has advantages in pulp bleaching process at high-temperature and pH and it also enhanced brightness to paper due to reduction in hexenuronic acid release (Cunha et al. 2018). In deinking of waste paper process, xylanase-based enzyme cocktail obtained from *Trichoderma longibrachiatum* MDU-6 has showed 52% brightness (Chutani and Sharma 2016). In textile industries, xylanase is used for processing of plant fiber, especially for linen and hessian making. In bread making industry, wheat flour hemicelluloses (arabinoxylans) break down by xylanolytic enzymes results in redistribution of water enhanced the dough softness, volume, quality, and quantity of leavened pan bread (Cunha et al. 2018; Rana et al. 2019a; Yadav et al. 2020c).

In juice and wine industries, xylanases improved stabilization of the fruit pulp, clarification and liquefaction of fruit and vegetable juice, reduction of viscosity, and clearing of juice. Fungus strain *Fusarium* sp. 21 produced highly alkali-stable xylanases, in which Xyn11C potential application in clarification of orange fruit juice due to degradation of xylan reduces the viscosity. Moreover, hydrolysis of xylan by Xyn11C increased the reducing sugar level of the orange juice (Li et al. 2020). Xylanase produced from *Aspergillus niger* SH3 isolated from Himalayan region has application in biofuel production (Tiwari et al. 2015). Application in pretreatment of lignocellulosic biomasses has great advantages in biofuel production (Bala and Singh 2019). Bala and Singh (2017) reported that xylanase enzyme cocktail used in generation of xylooligosaccharides for biofuel production. Thermostable xylanase produced from *Malbranchea pulchella* and expressed in *Aspergillus nidulans* applied for second generation ethanol production from pretreated sugarcane bagasse to produce high amount of fermentable sugar (Ribeiro et al. 2014).

11.6 Conclusion and Future Perspectives

Enzymes act as a catalyst to every chemical reaction and fasten the conversion process. Hence, the demand for enzymes keeps on increasing day by day and global market for industrial enzymes is beyond \$10.6billion (Market analysis report USA 2020). As microbial enzymes are easy to produce, more stable and feasible than plant and animal enzymes, they are preferred in multiple sectors. Among microbial sources for enzyme production, fungi stand highly significant as they secrete multiple novel enzymes that can effectively breakdown any complex material. Till date, more than 200 industrially useful fungal enzymes have been identified and reported (Green and Beezhold 2011).

Regular use of stable enzymes for bakery, confectionery, bioremediation, detoxification of xenobiotic, generation of bio-fuels, textile and paper production, treatment of waste water, detergents, production of beverages, dairy products, animal feed, leather manufacture, and pharmaceuticals are in practice for decades. To be precise, every daily use product is industrially manufactured through enzymatic process and fungal enzymes are inevitable in agriculture, food, and pharmaceutical sectors. Most importantly, fungal enzymes are suggested as essential food supplements, particularly to people with genetic deficiency of certain enzymes. As per recent reports, nearly 65–70% of world population is lactose intolerant (inability to synthesize required amount of lactase enzyme) and they depend on external lactase supplement to digest the lactose-based dairy products (Bayless et al. 2017). Fungal enzymes are employed in the production process of life saving drugs such as penicillin and human insulin and act as chemotherapeutic agents for treatment of cancer and autoimmune diseases (Vimal and Kumar 2017).

The future demand and market for fungal enzymes are beyond imagination as they have become an integral part of our life. Unfortunately, most of the naturally occurring enzymes are not suitable for industrial applications and hence wide range of parameters has to be considered. It is desirable to engineer enzyme in such a way for maximum activity/stability under appropriate conditions. Recombinant DNA technology and engineering of proteins are currently encouraged to improve the industrial production of microbial enzymes and meet out the raising demands. Although, industrial importance of fungal enzymes was identified earlier, recent biotechnological progress has added novelty to their applications.

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Chapter 12

Fungal Exopolysaccharides: Production and Biotechnological Industrial Applications in Food and Allied Sectors



Pavidharshini Selvasekaran, Mahalakshmi, Felicia Roshini,
Lavanya Agnes Angalene, Chandini, Tushar Sunil,
and Ramalingam Chidambaram

12.1 Introduction

Carbohydrates are naturally occurring biological macromolecules and the most critical constituents of the biosphere. Carbohydrates are generally grouped into four categories- monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Among those, polysaccharides occur in all forms of life and are significant natural products that can be obtained from plants, microorganisms, and animals. Polysaccharides derived mostly from the plants, and the seaweed sources are being used industrially on a large scale (Mahapatra and Banerjee 2013). Microbial polysaccharides are polymers of carbohydrates with higher molecular weight. The cell wall of microorganisms, both prokaryotes and eukaryotes, is majorly comprised of polysaccharides (Yildiz and Karatas 2018). Microbial polysaccharides based on their cellular location can be classified as intracellular polysaccharides (IPS); structural polysaccharides that are present on the cell wall, e.g., lipopolysaccharides (LPS); exopolysaccharides (EPS) that are secreted from capsular polysaccharides or peptidoglycans which are covalently attached to the cell surface (Patel et al. 2010). Exopolysaccharides possessing the same mono polymer repeats linked with glycosidic bonds are called homopolysaccharides (e.g., cellulose, pullulan, curdlan, and levan) and those with different monopolymers are called heteropolysaccharides (e.g., Xanthan gum and gellan) (Nwodo et al. 2012; Kour et al. 2019; Rastegari et al. 2020a, b).

P. Selvasekaran · R. Chidambaram (✉)
Instrumental and Food Analysis Laboratory, School of Bio-Sciences and Technology,
Vellore Institute of Technology, Vellore, Tamil Nadu, India
e-mail: cramalingam@vit.ac.in

Mahalakshmi · F. Roshini · L. A. Angalene · Chandini · T. Sunil
Postgraduate students, School of Bio-Sciences and Technology, Vellore Institute of
Technology, Vellore, Tamil Nadu, India

Apart from the structural difference between the homopolysaccharides and heteropolysaccharides, the difference can be attributed to the site of synthesis and the enzymes utilized for synthesis. The precursors required for the synthesis of heteropolysaccharides are present intracellularly, whereas the homopolysaccharides require a suitable substrate such as sucrose for the synthesis. However, the quantity and structural complexities of exopolysaccharides synthesized by microorganisms differ significantly (Seviour et al. 1992; Sutherland 1994). The physicochemical factors influencing the synthesis include temperature, pH, laboratory conditions such as incubation period, and the nutrient medium composition (Moxon and Kroll 1990). The obtained exopolysaccharides are soluble in water (e.g., Xanthan gum, gellan, carrageenan, alginate), whereas few are insoluble in water (e.g., chitin, cellulose). Owing to their unique and complexity of the chemical structure the benevolent effects of exopolysaccharides can be applied in agriculture, food, packaging, pharmaceutical, and cosmetic industries (Ates 2015). In food industries, exopolysaccharides are used as hydrocolloids including various functions such as gelling agents, binders, thickening agents, fat substitutes, coagulants, humectants, carrier matrix, sequestrant, and stabilizer (Ramalingam et al. 2014).

Fungi are eukaryotic heterotrophs that occur in oceans, coastal areas, mangroves, swamps, and land, etc. Nearly 1.5 million species of fungi are discovered, and more than 1500 are of marine origin (Devi et al. 2020b; Yadav et al. 2020a, b). After the nineteenth century, scientists found the ability of fungi to synthesize a wide range of profitable primary and secondary metabolites, including vitamins, alcohol, antibiotics, pigments, immuno suppressants, immunomodulatory agents, enzymes, and proteins which are of more considerable significance. Fungi and humankind are in an integral relationship since the discovery of *Penicillin* (a secondary metabolite obtained from *Penicillium* moulds) by *Alexander Fleming* both in beneficial as well as harmful aspects. In terms of food, fungi are an excellent source of direct food such as edible mushrooms (*truffles and morels*), and indirectly it acts as a significant leavening and fermenting agents to produce various foods and beverages such as bread, cheese, and wine, e.g., use of *Saccharomyces cerevisiae* in bread production (Azizan et al. 2016; Devi et al. 2020a). Also, fungi are an excellent source of exopolysaccharides, hence in the last two decades, research community and food industries focused on the fungal exopolysaccharides which are of higher value for biotechnological food applications (Mahapatra and Banerjee 2013; Jaroszuk-Scise and Kurek 2012; Donot et al. 2012).

As mentioned above, various exopolysaccharides such as xanthan gum, pullan, dextran, chitin, chitosan, etc., play a significant role in the food industry. Among them, the most common exopolysaccharides obtained from the fungal species are chitin, chitosan, pullulan, pleuran, scleroglucan, schizophyllan, lentinan, elsinan, and grifolan. Although these exopolysaccharides can be obtained from many other resources, the fungal source is advantageous as they have uniform particle size and antimicrobial properties (Singh and Yadav 2020; Thakur et al. 2020). The distinctive biological and functional properties of these exopolysaccharides render them suitable for various applications in the food industry, such as natural film-forming

agents, food preservatives, and coating agents (No et al. 2007). The undermentioned subjects discussed include common exopolysaccharides that are synthesized from diverse fungal species, and their applications in the food and allied sectors are emphasized.

12.2 Fungal Exopolysaccharides

12.2.1 Chitin and Chitosan

Chitin is one of the abundant polysaccharides and natural biopolymer after cellulose with high beneficial applications in the food industry. Chitin was first identified as the major component that is present in the mushrooms. Chitin is a linear copolymer of β -(1 $\rightarrow$ 4)- linked D-glucosamine and is similar to the structure of cellulose but differing in the group at the C-2 position, which is acetamido in chitin and hydroxyl in cellulose (Rana et al. 2019a). Further, the chitin is categorized into three polymorphic crystalline structures: α - chitin, β - chitin, and γ - chitin with the structure of the antiparallel chain, parallel chain, and parallel as well as antiparallel chain, respectively. Therefore, the γ - chitin is a combination of α - chitin, and β - chitin. The physicochemical and biological properties of chitin are determined based on differences in the structure. The properties of chitin and chitosan are listed in Table 12.1. As of now, chitin has been applied as functional food material of biological origin (Cabib 1981; Jang et al. 2004). In nature, they are majorly found in the cell walls of fungi, crustacean’s shells, and also in the cuticles of insects. The commercial fungal source of the chitin is the mycelia and spores of Ascomycetes, Blastocladiaceae, Chytridiaceae. Fig. 12.1 represents the chemical structure of chitin (a) and chitosan (b). Chitosan is a polysaccharide consisting of N-acetyl d-glucosamine and d-glucosamine units. It is commonly obtained by the deacetylation of chitin, which results in the formation of N-acetyl-glucosamine and d-glucosamine and is a soluble form of chitin (Table 12.2).

Table 12.1 Properties of chitin and chitosan

Properties	Chitin	Chitosan
Structure	Poly-(2-acetamido-2-deoxy-D-glucose	Poly-(2-amino-2-deoxy-D--glucose
Solubility	Less soluble	Highly soluble
Molecular weight	100–1000	20–750
Deacetylation degree	<50	80–95
Reactive groups	Hydroxyl and carboxyl	Amino and hydroxyl

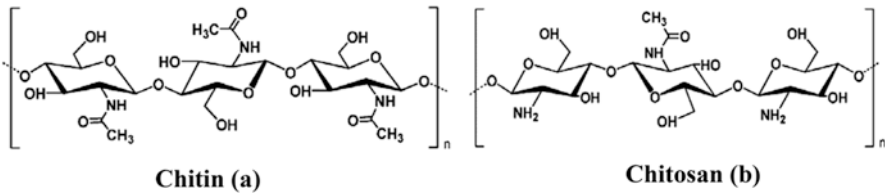


Fig. 12.1 Structure of chitin (a) and chitosan (b)

Table 12.2 Advantages of chitin produced from fungal source over other sources

Factors	Fungal production	Production from other source
Extraction methods	Easy and feasible	Depends on the use of harsh solvents
Availability	There is no seasonal and geographical limitation	Seasonal limits
Treatment based on demineralization	Does not require demineralization	Depends on demineralization
Inorganic materials	Lower level	Higher level
Waste management cost	Less	High
Plant immune response induction	Highly effective	Less effective
Physicochemical properties	Constant	Inconstant and varies

12.2.1.1 Fungal Species Producing Chitin and Chitosan

Chitin and chitosan, one of the most abundant exopolysaccharides, are present in the cell wall of various fungi. In 1811, chitin was first isolated from *Agaricus volva-ceus*, *Agaricus piperatus*, *Agaricus acris*, *Agaricus cantarellus*, *Boletus viscidus*, *Hydnum repandum*, and *Hydnum hybridum*. Mycelia of few *Penicillium* species were reported to contain 20% chitin. Another important fungal source of chitin is *Aspergillus niger* (Whistler 2012; Campana-filho et al. 2007). Table 12.1 lists the advantages of fungal production of chitin and chitosan over the crustacean source. The biosynthesis pathway of chitin is represented in Fig. 12.2, and deacetylation of chitin produces chitosan. To maintain the strength, cell structure integrity, and shape, many classes of fungi (Basidiomycetes, Deuteromycetes, Ascomycetes, and Zygomycetes) contain chitin in their cell wall and septa (Abo Elsoud and El Kady 2019; Rana et al. 2019b; Yadav et al. 2020c).

The various classes of fungi that have been investigated for the production of exopolysaccharides, chitin and chitosan (Chatterjee et al. 2005) include *Lentinus edodes*, *Absidia blakesleeana*, *Absidia coerulea*, *Absidia glauca*, *Trichoderma reesei*, *Mucor rouxii*, *Rhizopus oryzae*, *Aspergillus niger*, *Colletotrichum lindemu-thianum*, *Pleurotus sajor-caju*, *Phycomyces blakesleeanus*, and *Gongronella butleri* (Teng et al. 2001; Balasubramanian et al. 2019). Table 12.3 lists few chitin- and chitosan-producing fungi. The quantity of the chitin present in the cell wall depends

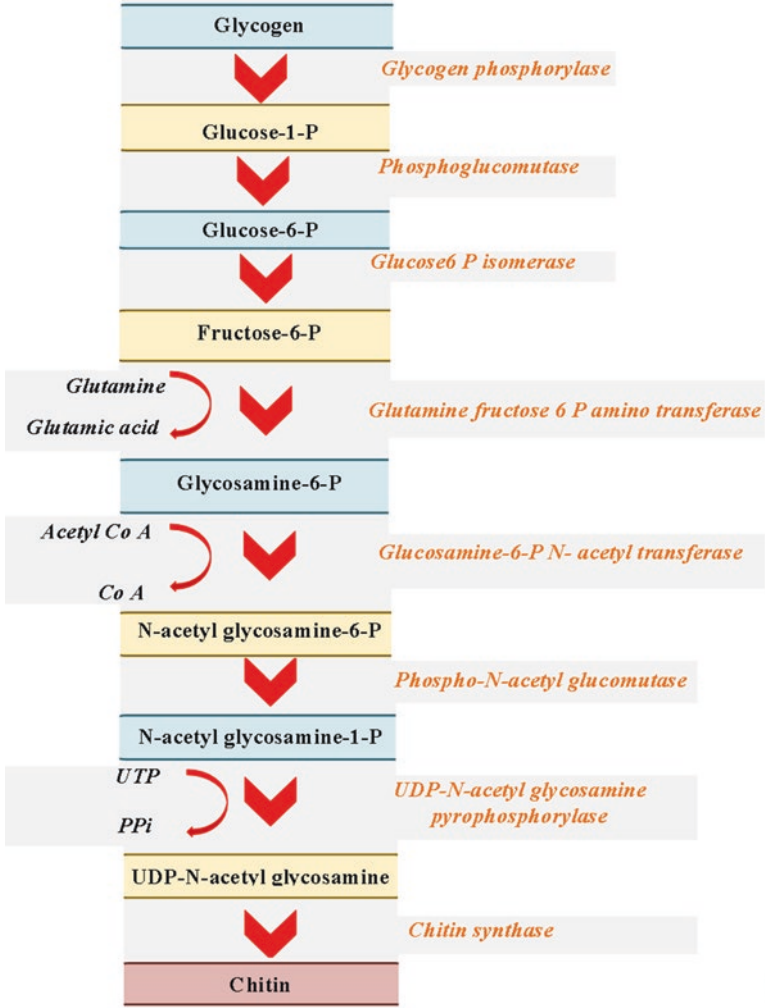


Fig. 12.2 Biosynthesis of chitin which upon deacetylation forms chitosan

on fungal species and environmental conditions. Also, the quantity of chitosan present in the fungal cell wall varies based on the fungal taxonomy. As an example, it was reported that Euascomycetes contain a chitin-glucan matrix, whereas Zygomycetes contain chitosan-glucan complex (Lenardon et al. 2010). The cell wall of *M. rouxii* belonging to the class Zygomycetes constitutes 35–40% chitin and chitosan of the dry matter. *Agaricus bisporus* and *A. niger* are a significant source of raw materials for the production of chitin and chitosan (Wu 2004). *Absidia* and *Mucor* have been frequently studied for the production and characterization of chitosan (Pochanavanich and Suntornsuk 2002).

Table 12.3 List of few fungi-producing chitin and chitosan

Fungi	Enzymes	References
<i>Hericium erinaceus</i>	Chitin	Liao and Huang (2019)
<i>Saccharomyces cerevisiae</i>	Chitin	Sun et al. (2018)
<i>Agaricus bisporus</i>	Chitin	Hassainia et al. (2018)
<i>Aspergillus niger</i>	Chitosan	Muñoz et al. (2015)
<i>Komagataella pastoris</i>	Chitin–glucan complex	Farinha et al. (2015)
<i>Rhizopus oryzae</i>	Chitosan	Kleekayai and Suntornsuk (2011)
<i>Aspergillus terreus</i>	Chitosan	Cheng et al. (2014)
<i>Pleurotus ostreatus</i>	Chitin	Vetter (2007)
<i>Lentinula edodes</i>	Chitin	Vetter (2007)
<i>Mucor rouxii</i>	Chitin & Chitosan	Chatterjee et al. (2005)
<i>Lactarius Vellereus</i>	Chitin & Chitosan	Erdogan et al. (2017)
<i>Phyllophora Ribis</i>	Chitin & Chitosan	Erdogan et al. (2017)
<i>Absidia coerulea</i>	Chitosan	Nwe et al. (2011)
<i>Gongronella butleri</i>	Chitosan	Nwe et al. (2011)
<i>Cryptococcus neoformans</i>	Chitin	Baker et al. (2007)
<i>Ganoderma lucidum</i>	Chitin	Ospina Álvarez et al. (2014)
<i>Schizophyllum commune</i>	Chitin–glucan complex	Smirnou et al. (2011)
<i>Lentinus edodes</i>	Chitosan	Crestini et al. (1996)
<i>Absidia glauca</i>	Chitosan	Hu et al. (1999)
<i>Cryptococcus neoformans</i>	Chitin	Banks et al. (2005)
<i>Candida albicans</i>	Chitin	Munro et al. (2001)
<i>Aspergillus nidulans</i>	Chitin	Specht et al. (1996)

12.2.1.2 Biosynthesis of Chitin and Chitosan

To attain the maximum yield of chitin and chitosan, fungal species are harvested at the end of the growth phase. Yeast peptone glucose, molasses salt medium, and potato dextrose broth are used for the culturing of the fungi to isolate the chitin and chitosan. Compared to the extraction of chitin and chitosan from other sources, the fungal source is beneficial as it does not require demineralization treatment due to the lower mineral content in fungal mycelia. The extraction process includes three major stages (Fig. 12.3): (i) removal of protein and soluble polysaccharides through the alkaline treatment, (ii) separation of chitin and chitosan through the acid reflux, and (iii) precipitating the chitin or chitosan subjecting to alkaline conditions (Pochanavanich and Suntornsuk 2002; Zhao et al. 2019; Ward 2015). Generally, 1N NaOH (121 °C for 0.25–1 h) is used as an alkaline treatment to remove proteins and polysaccharides. Acetic acid (2–10%) and hydrochloric acid (2–10%) are used (95 °C, 3–14 h) to separate chitin and chitosan through acid treatment (Muslim et al. 2018). The alkaline and acid reflux treatment for the separation of chitin and chitosan from *Aspergillus flavus*, *Botryodiplodia theobromae*, *Cladosporium cladosporioides*, *Fusarium* sp., and *Phoma* sp. was performed with NaOH (121 °C for 20

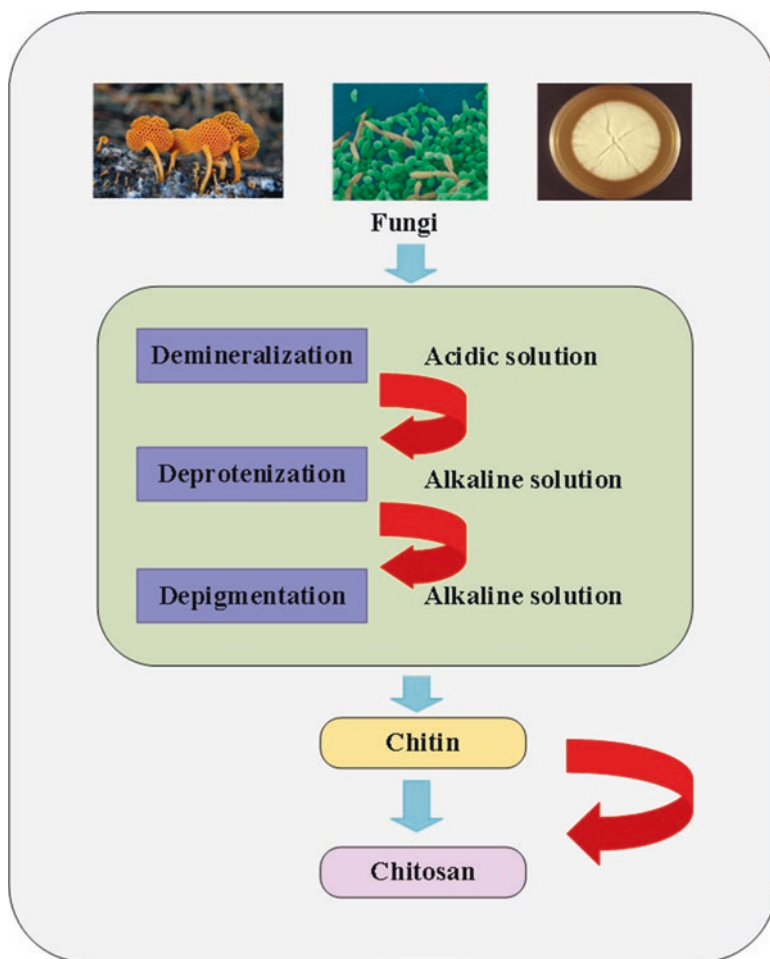


Fig. 12.3 Three major steps in the extraction of chitin and chitosan

mins) and acetic acid (2%, 95 °C, 8 h) (George et al. 2011). Table 12.4 discusses the various methods followed for the synthesis of chitin and chitosan.

Another significant type of production method to obtain chitin and chitosan from fungal species is the fermentation process. There are two types of fermentation methods- Solid-state fermentation (SSF) and submerged fermentation (SmF). In the case of SSF, microbial growth occurs on the moist solid substrates without free water, whereas in SmF, the microbial growth occurs in the liquid medium. Generally, the biomass concentration of fungi is associated with the production of chitin and chitosan from the fungal cell wall. In the act of biomass production, the efficiency of the SSF is higher in comparison to SmF (Abo Elsoud and El Kady 2019). The substrates utilized for SSF can be classified into two types- (i) homogenous, e.g., PUF (polyurethane foam) and (ii) heterogenous, e.g., by-products of agriculture,

Table 12.4 Various methods used for the synthesis of chitin, chitosan, and pullulan

Fungal species	Isolated polysaccharide	Isolation/extraction method	Reference
<i>Ganoderma lucidum</i>	Chitin	Alkaline treatment	(Ospina Álvarez et al. 2014)
<i>Absidia coerulea</i> ATCC 14076	Chitosan	Hot alkaline and acid treatment	(Bansal et al. 2011)
<i>Lentinus edodes</i>	Chitosan	Submerged and solid-state fermentation	(Crestini et al. 1996)
<i>Aspergillus flavus</i>	Chitosan	Alkaline treatment	(George et al. 2011)
<i>Botrydipodia theobromae</i>	Chitosan	Alkaline treatment	(George et al. 2011)
<i>Cladosporium cladosporioides</i>	Chitosan	Alkaline treatment	(George et al. 2011)
<i>Fusarium Sp.</i>	Chitosan	Alkaline treatment	(George et al. 2011)
<i>Phoma Sp.</i>	Chitosan	Alkaline treatment	(George et al. 2011)
<i>Aureobasidium spp.</i>	Pullulan	Submerged fermentation, supernatant is precipitated using organic solvents followed by ultra-filtration and ion exchange resins	(Cheng et al. 2011a)
<i>Tremella mesenterica</i>	Pullulan	Continuous fermentation (for 17 days), supernatant was dialyzed against tap-water, ethanol or 5% cetyltrimethylammonium bromide precipitation followed by ultra-filtration	(Fraser and Jennings 1971)
<i>Cryphonectria parasitica</i>	Pullulan	Mycelia mat was filtered and the filtrate was treated with absolute ethanol (cold).	(Forabosco et al. 2006)
<i>Teloschistes flavicans</i>	Pullulan	The culture medium was treated with chloroform-methanol and 100% ethanol solution to get a residue which was then treated with Aq. KOH (10%). The extract is centrifuged to give β -glucans and supernatant. The supernatant is again centrifuged and pullulan was obtained.	(Reis et al. 2002)

(continued)

Table 12.4 (continued)

Fungal species	Isolated polysaccharide	Isolation/extraction method	Reference
<i>Rhodotorulabacarum</i>	Pullulan	The culture was heated at 100 °C in water bath for 15 min, cooled to room temperature. The heated culture was centrifuged and cold ethanol was added to the supernatant which is held at 4 °C for 12 h to precipitate the extracellular polysaccharide. The precipitate was dissolved in deionized water at 80 °C and the solution was dialyzed against deionized water. After the precipitation the precipitate was dried using cold ethanol at 80 °C to obtain a constant weight.	(Badr-Eldin et al. 1994)

wheat straw, soybean, and rice bran. Comparatively, the homogenous type of substrate is beneficial as the oxygen transfer rate, composition of the medium, the feed rate can be controlled together with supporting the fungal biomass quantification whereas, the use of a heterogeneous substrate can hinder the quantification of fungal biomass, which affects the extraction of chitin and chitosan (Sparringa and Owens 1999).

On the contrary, few cases reported higher biomass production by SmF. (Mazumder et al. 2009) studied the biomass production by *Pleurotus ostreatus* to compare the efficiency of SSF and SmF and reported that during the initial growth SSF and SmF did not show a great difference in biomass production. However, a significant difference was noted at second day of growth due to higher biomass production through SmF. The simplicity in the quantification of fungal biomass production makes SmF as a feasible method for the extraction of chitin and chitosan. The major disadvantage of SmF over SSF is the higher risk of contamination, the higher energy requirement for the agitation process, and the subsequent production of wastewater (Sitanggang et al. 2012). However, it is essential to note that the appropriate fermentation method for the extraction of chitin and chitosan from fungi is highly species-specific (Bhargav et al. 2008; Abo Elsoud and El Kady 2019).

Owing to the intrinsic characteristics of fungal species, environmental and nutritional requirements, the quality and quantity of the cell wall, yield of chitin and chitosan can differ from species to species. To decrease the cost of production and increase the production yield, many researchers investigated the effect of traditional fermentation incorporated with additional nutrient supplements. Chatterjee et al. (2008) and Chatterjee et al. (2009) investigated the effect of gibberellic acid, kinetin, and auxins on the yield of chitosan by *M. rouxii* in whey medium and *R.oryzea* in a medium containing molasses salt. As a result, an increase in the molecular weight of the obtained chitosan was noted. Lower concentration of gibberellic acid, kinetin, and auxins enhanced the growth of fungal mycelia and chitosan yield. However, the growth of *M. rouxii* and *R.oryzea* was suppressed as the concentration

of plant hormones gradually increased in the medium. Nwe and Stevens (2004) studied the effectiveness of the urea on the production of chitosan by *Gongronella butleri* in the solid substrate medium and reported a notable increase in the chitosan yield and difference in the molecular weight of the obtained chitosan. The effect of various nitrogen sources such as ammonium sulfate, dried cyanobacterial biomass obtained from *Anacystis nidulans*, urea and yeast extract on *Pleurotus ostreatus* was studied and indicated a significant increase in the chitosan production due to the higher nutritional value of cyanobacterial biomass. It is important to note that the fungi with the capacity to produce chitin and chitosan should be harvested at the late exponential growth phase to obtain the maximum yield (Akila 2014).

12.2.1.3 Applications of Chitin and Chitosan in Food and Allied Sector

Chitin and chitosan upon complete hydrolysis or partial hydrolysis have long been used in various industrial sectors, including agriculture, textile, biomedical, photography, electronics, chromatography, wastewater treatment, and food industry. Chitin and chitosan are relatively strong candidates for application in the food industry, especially in the packaging sector due to their film-forming properties (Priyadarshi and Rhim 2020). The use of chitosan in the food packaging sector is validated due to their antimicrobial and antifungal activities. Chitosan-based active packaging film reduces the requirement of chemical preservatives.

Chitosan can be used to form a thin film around the surface of the food (e.g., fruits and vegetables) as a protective layer, which is termed as an edible coating (Duan et al. 2019) and has been widely studied for its application as a food preservative. Owing to the antibacterial property, biodegradability, and nontoxicity, chitosan has been extensively used as an antimicrobial agent, which can be benefitted to extend the shelf life and enhance the quality of the food. Chitosan and its modified form can be used in combination with other ingredients to optimize the antimicrobial effects (Muzzarelli et al. 2012). Chitosan in the form of powders, microspheres, coatings, suspensions, is highly effective in comparison to chitosan solution. Their antimicrobial property has been effective in dairy and dairy products, fruits and fruit juices, meat and meat-based products, cereals, and seafood. Chitosan has been recognized for its utility as a flocculant and has higher adsorption capacity due to its cationic and molecular structure. Generally, the enzyme pectinol has been utilized for the elimination of pectin and carbohydrates from the juice to obtain clear beverages. Chatterjee et al. (2004) studied the effect of chitosan in fruit juice clarification and reported that it has a strong potential to remove the suspended particles present in the beverages. Chitosan, as a flocculant for fruit juice clarification, has been extensively studied for pineapple, apple, orange, carrot, lemon, and grape (Rungsardthong et al. 2006). Also, chitosan can be widely used to remove the polyphenolic compounds to obtain transparency in the beverages and changes the initial color of the juice (Spagna et al. 1996). Additionally, chitosan is used as an acidity controller in fruit beverages. Among the hydrocolloids approved for food applica-

tions, chitosan has been considered as a significant source of emulsifier (Gutiérrez 2017).

It can be used to stabilize the oil in water emulsion without the requirement of any additional surfactant. As an example, mineral oil-chitosan (Torrico et al. 2011) and soybean oil-chitosan (Wardy et al. 2011) emulsions have been studied on chicken eggs to enhance the shelf life and maintain the quality. The study reported that the shelf life was continued for 3 weeks without affecting the sensorial property. Perdones et al. (2012) studied the effect of lemon oil-chitosan emulsion on strawberries and reported that the coating maintained and preserved the quality when stored at 5 °C. Further chitosan is one of the significant candidates studied for the encapsulation and delivery of bioactive compounds and nutrients in food (Gutiérrez 2017). Chitosan also has the potential to act as the dehazing agent and in comparison to conventional fining methods; it is feasible, cost-effective, and time-saving. It is also used in bioconversion for the production of value-added food products (Products and Discards 1991), purification of water, and recovery of waste material from food processing discards (Hwang and Damodaran 1995). Fig. 12.4 illustrates the applications of chitosan in food industry.

12.2.2 Pullulan

Pullulan is a commercially used microbial exopolysaccharide that is produced from *Aureobasidium pullulans* with ecological versatility by submerged fermentation on a medium supplemented with carbon, nitrogen, and other essential nutrients. It is a linear, unbranched exopolysaccharide consisting of maltotriose units linked with α (1 $\rightarrow$ 6) glycosidic bonds, and each maltotriose containing glucose subunits are further connected with α (1 $\rightarrow$ 4) glycosidic bonds (Satyanarayana et al. 2013). Fig. 12.5 represents the chemical structure of the pullulan. It is soluble in water and insoluble in organic solvents (Nishinari et al. 1991). Parameters such as tempera-

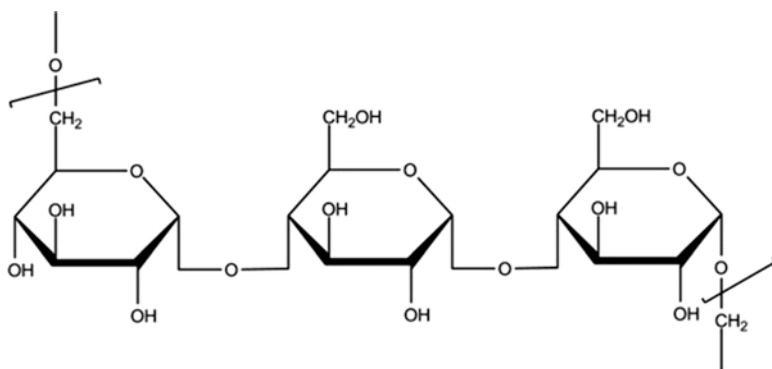


Fig. 12.5 Chemical structure of pullulan

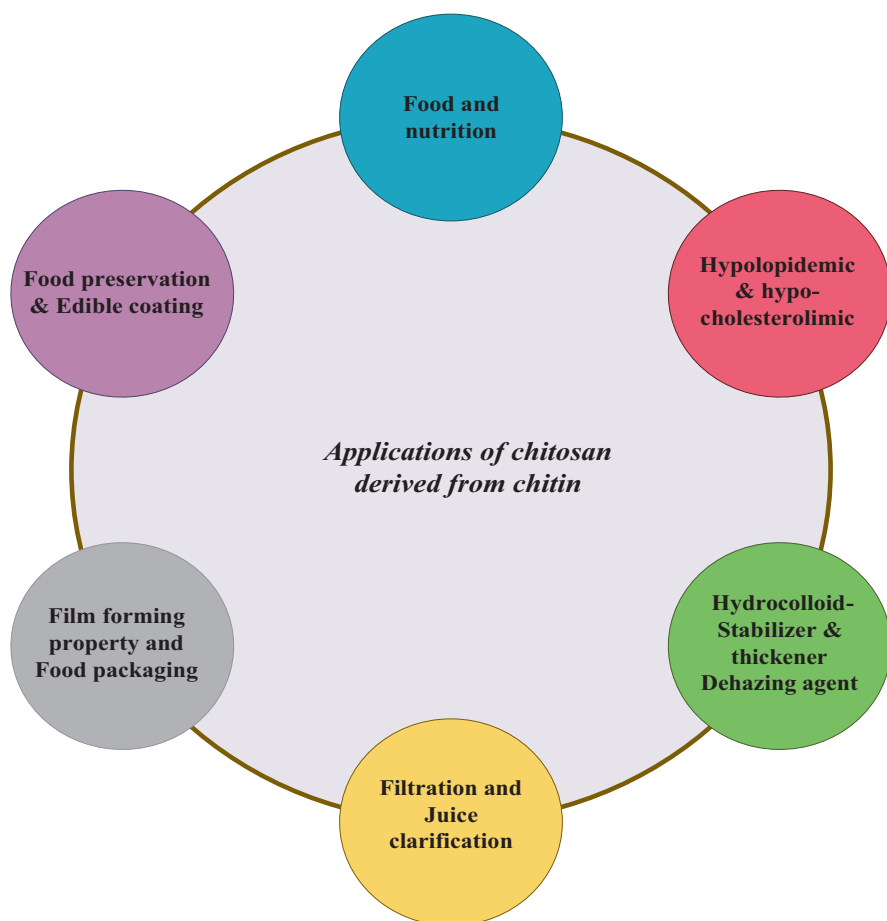


Fig. 12.4 Applications of chitin and chitosan in food industry

ture, pH, and salt concentration do not influence the viscosity of pullulan. The nutrients that are essential for the production of pullulan are expensive, which increases the production cost.

However, the wastes recovered from agro-based industries are highly rich in organic and inorganic compounds that have been used as a significant alternative precursor substrate for the isolation of pullulans by submerged or solid-state fermentation. Pullulan is validated as nontoxic, notmutagenic, noncarcinogenic, non-ionic, and nonhygroscopic exopolysaccharide. Table 12.5 describes the properties of pullulan. Due to these desired properties, it has a wide range of applications (Tabasum et al. 2018) in the food industry, pharmaceutical industry (Satyanarayana et al. 2013), biomedical sector, drug delivery, gene delivery (Singh et al. 2015), tissue engineering, and wound healing (Singh et al. 2008; Singh et al. 2018).

Table 12.5 Properties of Pullulan

Properties	Specifications
Nature	Powder
Molecular weight	45–600 kDa
pH (1% (w/v) solution)	5–7
Color	White or off-white
Melting point	250 °C
Solubility	Easily soluble in cold water, hot water, and dilute alkali
Insolubility	Insoluble in organic solvents except formamide and dimethylsulfoxide
Viscosity (10% (w/v) solution, 30 °C)	100–180 cP
Moisture content (without tackiness or hygroscopy)	10–15%
Specific optical activity [α]D ₂₀ (1% (w/v) in water)	$\geq + 160^\circ$
Mineral residue-ash (sulphated) Polypeptides	$\leq 3\%$

12.2.2.1 Fungal Strains Producing Pullulans

For the large-scale and commercial purpose, pullulans are produced from *Aureobasidium pullulans*, which is a ubiquitous species mainly found in the soil, including antarctic soils, freshwater, ocean water, rock, wood, plant, and animal tissue. Few strains of *A. pullulans* are also found in the coastal region and deep sea (Li et al. 2007). Due to its ability to secrete melanin pigment, it is also termed as “Black yeast fungus.” *A. pullulans* is a polymorphic fungus and has different cell morphologies- (i) yeast-like cells, (ii) blastospores, (iii) swollen blastospores, (iv) chlamydospores, and (v) mycelium (Ronen et al. 2002). Seviour et al. (1984) reported that the pullulan production is independent of the morphological forms of *A. pullulans*. Later (Yurlova and De Hoog 1997; Simsn and Bouchep 1988; Heald and Kristiansen 1985) stated that all five morphological forms of *A. pullulans* are essential for the pullulan production. In addition to *Aureobasidium* spp., microorganisms such as *Cryphonectriaparasitica*, *Rhodotorulabaccharum*, *Teloschistesflavicans*, and *Cytaria* spp. are also used for the production of pullulan at an industrial scale (Sugumaran and Ponnusami 2017). Table 12.6 enlists few fungi that produce pullulans. Production of pollutants by *Aureobasidium* spp. is highly studied because of their easy adaptability to the nutrient source and also gives a higher yield in comparison to other pullulan-producing fungal species. The biosynthesis pathway of pullulan production is illustrated in Fig. 12.6.

12.2.2.2 Pullulan Production by Microbial Fermentation

The microbial production of pullulan is influenced by fermentation type, design of the bioreactor, and its operating conditions. It also depends on medium composition, fermentation conditions, and inoculum (Cheng et al. 2011b). Various methods involved in pullulan production include batch fermentation, fed-batch fermentation, chemostat, immobilized cell fermentation, and airlift fermentation. Among them, batch fermentation is the most commonly employed. Several reports in the literature indicated two-step temperature, pH, and agitation control in the batch mode of fermentation to enhance the microbial growth and pullulan production (Wang et al. 2013; Xia et al. 2011; Wu et al. 2010). The conditions, for the production of pullulan, such as carbon source (starch, beet molasses, cornmeal hydrolysates, olive oil, sucrose, spent sulfite liquor, hydrolyzed potato starch, peat hydrolysate, corn syrup) (Wu et al. 2009), nitrogen source (yeast extract, ammonium ions, soybean pomace) (Seo et al. 2004), pH (5.5–7.5), temperature (around 30 °C) should be optimized based on the type of strain cultured (Li et al. 2009). The upstream production can be done either in batch, fed-batch, or continuous fermentation. Downstream processing of pullulan requires additional care because of the co-production of melanin, which decolorizes the pullulan with its black color (Sugumaran and Ponnusami 2017). Steps involved in the fermentation type of pullulan production are depicted in Fig. 12.7. Few commonly used methods for the synthesis of pullulan are shown in Fig. 12.7.

12.2.2.3 Applications of Pullulan

Owing to its unique linkage pattern, pullulan possesses distinguishing physical properties, such as adherent nature, nonhygroscopic, the ability to form thin biodegradable films and fibers which are resistant to oxygen permeation (Pinar et al. 2013). Therefore, pullulan has been extensively used in various industries, including cosmetics, food, and flocculants. Among, all the industrially significant exopolysaccharide, pullulan is an interesting candidate which has potential applications in food industries. It is a nontoxic, noncarcinogenic, and edible compound. Pullulan is declared safe by the Food and Drug Administration within the US to be used in food applications and has GRAS (Generally considered as Safe) status. It is jointly listed within the Japanese Standards for Ingredients for drugs and extensively utilized in Japan as an additive and glazing agent with oxygen-barrier properties. Fig. 12.8 illustrates the applications of pullulan in the food industry. It is used to increase the shelf life of foods and shield them from oxidation. It is used as a low-calorie sugar substitute to produce low-calorie food products such as doughnuts, biscuits, cookies, and supplementation of the dietary fiber (Pinar et al. 2013).

Pullulan is also used as a prebiotic as it promotes the growth of bifidobacteria in the gut and helps improve the health of the consumer. Pullulan has antifungal activity and prevents the fungal spoilage of the foods and beverages. It is used for the preparation of the maltotriose syrup. Also, it is used to enhance the viscosity and

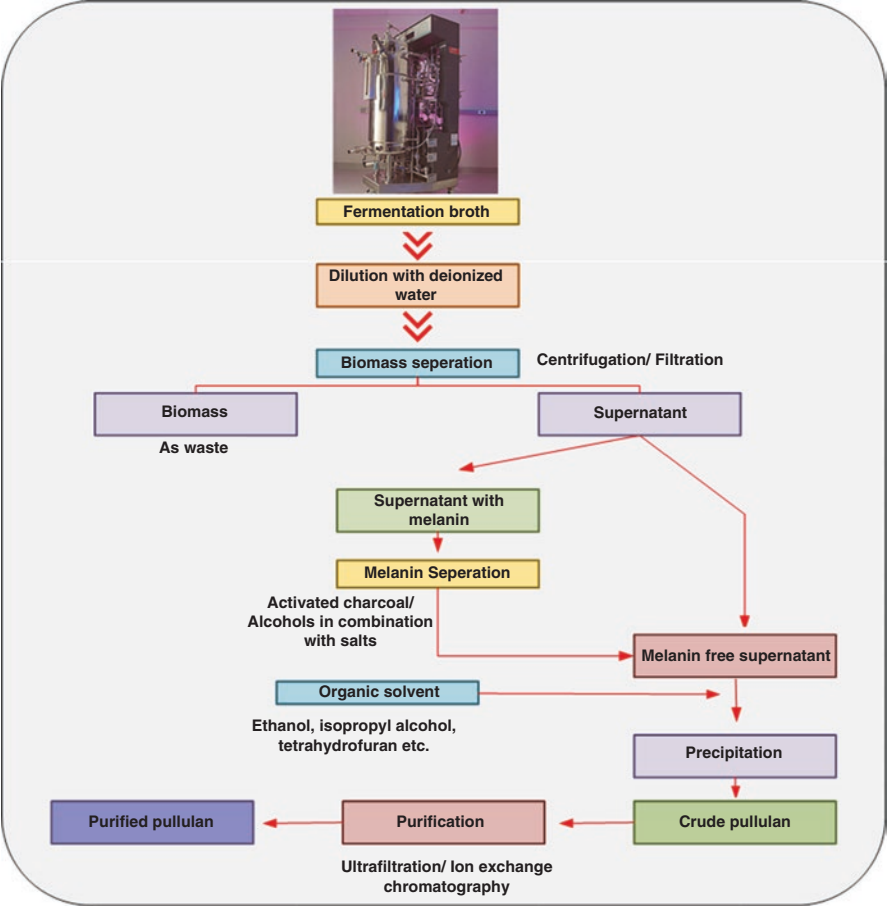


Fig. 12.7 Steps involved in the production of pullulan through fermentation



Fig. 12.8 Applications of pullulan in food industry

gloss of the food products containing a higher concentration of the salt, e.g., pickled fruits and vegetables, soya sauce, etc. It can also be used as a binding agent in food products as they have excellent adhesive properties. As a binding agent, it helps to attach nuts on cookies, etc., and used for the preparation of snacks such as fish sticks, beef, and pork sheets. Owing to the low viscosity, it can be applied to lyophilized or spray-dried food products to increase the solubility of the product and for easier granulation. It can be used as a stabilizer and protective agent for the mayonnaise. It can be used as texture improver and filler in various beverages and juices. The colorless nature of pullulan can be further molded into films and used as novel packaging materials (Pinar et al. 2013) (Fig. 12.8).

12.2.3 *Elsinan*

Elsinoe species, *E. leucospila*, *E. ampelina*, and *E. fawcetti* produce an extracellular novel polysaccharide elsinan. It is an odorless, nontoxic, tasteless, biodegradable, consumable exopolysaccharide that can be hydrolyzed by α -amylase and hydrophilic in nature. It has a broad range of applications including pharmaceuticals, food, and allied sectors. Elsinan is a linear polymer containing (1 $\rightarrow$ 4) and (1 $\rightarrow$ 3)- α -D-glucan glycosidic linkages. It is comprised of maltotetraose and maltotriose units, five-fold helical structure, and has molecular weight ranging between 5000 and 10,000 kDa (Synytsya and Novak 2014). Owing to its unique structure, it has an interesting rheological property and exhibits higher viscosity. It is stable over a broad range of temperature (30 ± 70 °C), salt concentrations, and pH (3–11). It is effectively soluble in water, formic acid (90%), NaOH (0.1 N), dimethyl sulfoxide or formamide, whereas, it is insoluble in chloroform, ethyl acetate, methanol, ethanol, and acetone.

The color of elsinan changes to green upon anthrone-sulfuric reaction, yellow upon reaction with cysteine-sulfuric acid, colorless by Morgan-Elson reaction, and is negative to iodine stain. It has the ability to form a film which is easily soluble in hot water at temperature ≥ 80 °C. In contrast, at a temperature below 40 °C, the film swells while maintaining its initial structure and shape. Room temperature with high humidity or moisture does not influence the blocking of the film, whereas the quantity of plasticizer used such as water and glycerin can change and modify the elongation properties of the film. The commonly used method for the preparation of film is casting, molding, and winding. Due to its irreversible nature, the initial properties, including folding nature, transparency, and flexibility, can be maintained for a long duration. The elsinan film is highly impermeable to gas, oils, fats, fat-soluble vitamins, and oily foods. The film is nonconductive and electrically chargeable. The film is ecologically safe and biodegradable. Elsinan can be effortlessly framed into several formed bodies, for example, fiber, string, wipe, pellet, granule, rope, stick, pole, fabric, bandage, film, net, paper, sheet, tube, tablet, case, cover, and coating (Synytsya and Novak 2014).

12.2.3.1 Fungal Production of Elsinan

Elsinan is formed extracellularly by *Elsinoe leucospila* and other *Elsinoe* Species. The fungi are cultured in a suitable culture medium (solid or fluid form) that contains essential sources such as carbon, nitrogen, minerals, and various additional supplements. Elsinan can be isolated from submerged cultures or static cultivation with the former resulting in higher yields. The carbon source for the production of elsinan includes glucose, sucrose, maltose, starch hydrolysates or fructose, and the nitrogen source can be either natural organic or synthetic compounds along with essential minerals such as potassium salts, magnesium salts, sulfates, and phosphates. The fermentation process should be carried out at the appropriate initial conditions such as temperature (20–30 °C), pH (5–8), and duration (3–7 days). The mycelia and the cell walls are eliminated from the culture medium through filtration or centrifugation, whereas the elsinan is isolated through precipitation with methanol, acetone, or ethanol. Further, the elsinan is recovered by filtration or centrifugation and subjected to purification by re-dissolution in water, re-precipitation with a suitable solvent, and drying of the purified elsinan (Synytsya and Novak 2014).

12.2.3.2 Applications of Elsinan

Elsinan has a variety of industrial applications (Fig. 12.9), including those of film-forming property and pharmaceutical formulations. It shows a huge dietary fiber impact and subsequently diminishes the cholesterol level in the serum of hypercholesterolemic individuals (Giavasis 2013). As mentioned earlier, this polysaccharide can be formed into different shapes, and these formed bodies are edible, nontoxic, transparent, hot water soluble, moisture, water-resistant, and extended timeframe without losing their attractive properties. Such formed bodies can be utilized for a variety of purposes, including nourishment, agriculture, textile, fisheries, forestry, cosmetics, chemicals, and pharmaceutical products. However, the application of elsinan as food additives is still limited.

12.2.4 Galactomannan

Galactomannan is the product of hazardous fungal species *Aspergillus* and *penicillium*. Its chemical structure consists of a single D-galactose branch-linked α -(1–6) in the backbone of β -(1–4)-D-mannan. The chemical structure of galactomannan is shown in Fig. 12.10. It can also be extracted from seed sources such as locust bean, guar, tara, and fenugreek. Galactomannan is one kind of an exopolysaccharide that usually has a mannan core and lactofuransyl side-chains. It is mostly found in the cell wall of fungi, especially in *Aspergillus* sp., *Penicillium* sp., and is also found in other species of fungi.

Fig. 12.9 Applications of elsinan in various sectors

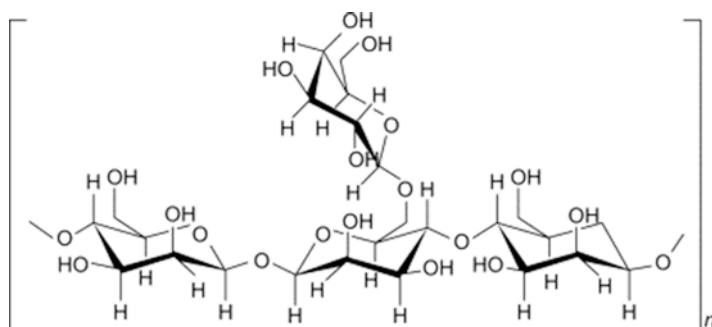


Fig. 12.10 Chemical structure of galactomannan

Owing to its unique properties such as high molecular weight, hydrophilic nature, and nonionic character (Pollard et al. 2010), they are used as film-forming agent, coating agents, gelling agents, stabilizers, thickeners, and cometic materials (Albuquerque et al. 2016). In food industries, they are used for a wide range of

applications in the dairy, bakery, confectionery, meat, canned and frozen products, spices, sauces, and beverage industries (Bourbon et al. 2010). They increase the texture and nature of the food substances and are also used as fat substitutes. As they are not digested by the human digestive system, they are used as dietetic foods. In the case of the nonfood sector, they are used as stabilizers and thickeners in pharmaceutical and cosmetic industries as a formulation in creams and lotions, respectively. They are also used to control the release of drugs in the small intestine. In combination with proteins, it has been used to coat tablets and capsules. In paper industries, it is used to enhance the mechanical properties of the paper. In the form of films, membranes, and hydrogel, they are used as supporting material for immobilization (Albuquerque et al. 2016). The list of fungi that can produce various exopolysaccharides are listed in the Table 12.7.

12.2.4.1 β -Glucans

β -glucan is a water-soluble exopolysaccharide that is abundant in the cell wall of baker's yeast, *saccharomyces cerevisiae*. β -glucans produced by yeast and mushrooms have a branched structure in which the monomers of glucose are linked with β -(1 $\rightarrow$ 3) and β -(1 $\rightarrow$ 6) glycosidic bonds, whereas the glucose monomers obtained from oats and barley are linked with β -(1 $\rightarrow$ 4) and β -(1 $\rightarrow$ 3) glycosidic bonds (Bin-fen et al. 2008). The chemical structure of β -glucan is shown in Fig. 12.11. The macrostructure and description of β -glucan vary depending on the source from which it was obtained. The synthesis of β -glucans from the cell wall of fungus is a complex process due to the presence of different classes of β -glucans. The β -glucan is present either in water-soluble or insoluble form in the fungal species.

The extraction conditions that are employed in the isolation of β -glucans and its structure strongly determine the soluble and insoluble fractions. The water-soluble

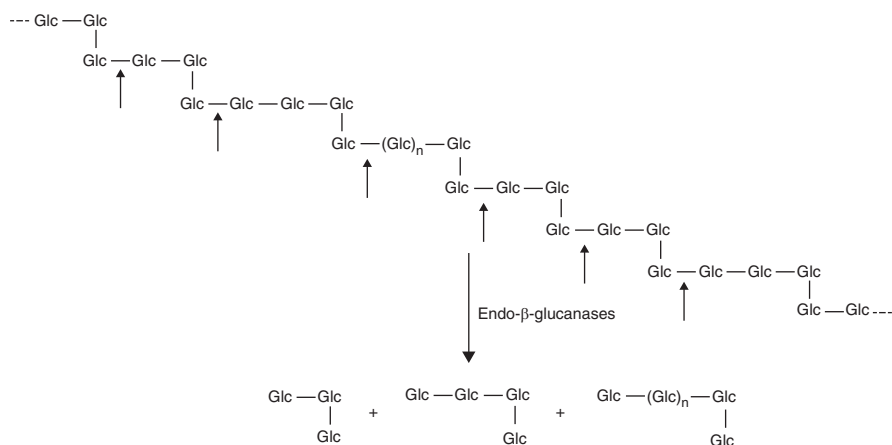


Fig. 12.11 Chemical structure of β -glucan

Table 12.7 List of different fungal strains that can produce exopolysaccharides

Fungi	Reference
<i>Absidia corymbifera</i>	(De Ruiter et al. 1994)
<i>Absidia cylindrospora</i>	(Misaki 1968)
<i>Acremonium charticela</i>	(Nehad and El-Shamy 2010)
<i>Acremonium diospyri</i>	(Stasinopoulos and Seviour 1989)
<i>Acremonium persicinum</i>	(Maziero et al. 1999)
<i>Agaricus nevoi</i> HAI610	(Elisashvili et al. 2009)
<i>Agaricus xanthodermus</i>	(Maziero et al. 1999)
<i>Agrocybe cylindracea</i>	(Kim et al. 2005)
<i>Agrocybe platensis</i>	(Maziero et al. 1999)
<i>Akanthomyces pistillariiformis</i>	(Madla et al. 2005)
<i>Alternaria alternata</i>	(Nehad and El-Shamy 2010)
<i>Alternaria mellea</i>	(Misaki 1968)
<i>Alternaria solani</i>	(Misaki 1968)
<i>Antrodia camphorata</i>	(Shu and Lung 2004)
<i>Antrodia cinnamomea</i>	(Lin and Sung 2006)
<i>Antrodiella ginestae</i>	(Maziero et al. 1999)
<i>Armillaria mellea</i>	(Sandford 1979)
<i>Aschersonia samoensis</i>	(Madla et al. 2005)
<i>Aspergillus alliaceus</i>	(Gomez-Miranda and Leal 1981)
<i>Aspergillus fumigatus</i>	(Yin et al. 2011)
<i>Aspergillus niger</i>	(Kumaran et al. 2014)
<i>Aspergillus parasiticus</i>	(Ruperez and Leal 1981)
<i>Aureobasidium pullulans</i>	(Wu et al. 2012)
<i>Auricularia fuscossuccinea</i>	(Maziero et al. 1999)
<i>Beauveria bassiana</i>	(Madla et al. 2005)
<i>Botryosphaeria rhodina</i>	(Miranda et al. 2008)
<i>Botrytis cinerea</i>	(Misaki 1968)
<i>Byssosclamyces nivea</i>	(Gomoiu and Catley 1996)
<i>Calvatia cyathiformis</i>	(Maziero et al. 1999)
<i>Candida bogoriensis</i>	(Misaki 1968)
<i>Candida boidinii</i>	(Petersen et al. 1990)
<i>Cephalosporium serraie</i>	(Stasinopoulos and Seviour 1989)
<i>Cephalosporium stiehmeri</i>	(Stasinopoulos and Seviour 1989)
<i>Cephalosporium subverticillatum</i>	(Stasinopoulos and Seviour 1989)
<i>Ceratocystis stenoceras</i>	(Misaki 1968)
<i>Cladosporium tricarum</i>	(Nehad and El-Shamy 2010)
<i>Cladosporium tricoidea</i>	(Misaki 1968)
<i>Claviceps purpurea</i>	(Sandford 1979)
<i>Climacodon pulcherrimus</i>	(Maziero et al. 1999)
<i>Collybia maculata</i>	(Lim et al. 2004)
<i>Cookeina tricholoma</i>	(Madla et al. 2005)
<i>Cordyceps dipterigena</i>	(Madla et al. 2005)

(continued)

Table 12.7 (continued)

Fungi	Reference
<i>Cordyceps militaris</i>	(Kim et al. 2005)
<i>Cordyceps nipponica</i>	(Madla et al. 2005)
<i>Cordyceps sinensis</i>	(Kim and Yun 2005)
<i>Cordyceps sphecocephala</i>	(Oh et al. 2007)
<i>Cordyceps taii</i>	(Xiao et al. 2010)
<i>Coriolus (Trametes) versicolor</i>	(Misaki 1968)
<i>Coriolus hirsutus</i>	(Misaki 1968)
<i>Cryphonectria parasitica</i>	(Forabosco et al. 2006)
<i>Cryptococcus elinorii</i>	(Misaki 1968)
<i>Cryptococcus laurentii</i>	(Petersen et al. 1990)
<i>Drechslera spicifera</i>	(Aouadi et al. 1992)
<i>Epicoccum nigrum</i>	(Schmid et al. 2011)
<i>Flammulina velutipes</i>	(Misaki 1968)
<i>Fomes fomentarius</i>	(Chen et al. 2008)
<i>Fomitopsis pinicola</i>	(Xiao et al. 2004)
<i>Fusarium coccophilum</i>	(Madla et al. 2005)
<i>Fusarium solani</i>	(Nehad and El-Shamy 2010)
<i>Ganoderma applanatum</i>	(Lee et al. 2007)
<i>Ganoderma lucidum</i>	(Papinutti 2010)
<i>Gibellula pulchra</i>	(Madla et al. 2005)
<i>Glomastix gueg</i>	(Nehad and El-Shamy 2010)
<i>Gomphidius rutilus</i>	(Gao et al. 2012)
<i>Grifola frondosa</i>	(Bae et al. 2005)
<i>Hansenula capsulate</i>	(Petersen et al. 1990)
<i>Hansenula holstii</i>	(Sandford 1979)
<i>Hansenula minuta</i>	(Sandford 1979)
<i>Helotium</i> sp.	(Sandford 1979)
<i>Hirsutella</i> sp.	(Madla et al. 2005)
<i>Hymenostilbe</i> sp.	(Madla et al. 2005)
<i>Hypocrella tamurai</i>	(Madla et al. 2005)
<i>Hypsizigus marmoreus</i>	(Zhang et al. 2012)
<i>Inonotus levis</i> HAI796	(Elisashvili et al. 2009)
<i>Isaria farinosa</i> BO5	(Jiang et al. 2008)
<i>Lachnum</i> sp. YM261	(Ye et al. 2012)
<i>Lentinus edodes</i>	(Feng et al. 2010)
<i>Lipomyces starkeyi</i>	(Petersen et al. 1990)
<i>Lyophyllum decastes</i>	(Pokhrel and Ohga 2007)
<i>Melanoporia nigra</i>	(Maziero et al. 1999)
<i>Moniliella pollinis</i>	(Mózo 2017)
<i>Morchella crassipes</i>	(He et al. 2012)
<i>Mucor circinelloides</i>	(De Ruiter et al. 1994)
<i>Mucor hiemalis</i>	(De Ruiter et al. 1994)

(continued)

Table 12.7 (continued)

Fungi	Reference
<i>Mucor mucedo</i>	(Misaki 1968)
<i>Mucor racemosus</i>	(De Ruiter et al. 1994)
<i>Mucor rouxii</i>	(Abdel-aziz et al. 2012)
<i>Nothopanus hygrophanus</i>	(Maziero et al. 1999)
<i>Oligoporus</i> sp.	(Maziero et al. 1999)
<i>Oudemansiella canarii</i>	(Maziero et al. 1999)
<i>Oudemansiella radicata</i>	(Zou 2005)
<i>Paecilomyces japonica</i>	(Sinha et al. 2001)
<i>Paecilomyces sinclairii</i>	(Kim et al. 2002)
<i>Paecilomyces tenuipes</i>	(Madla et al. 2005)
<i>Panaeolus papilionaceus</i>	(Maziero et al. 1999)
<i>Penicillium charlesii</i>	(Sandford 1979)
<i>Penicillium luteum</i>	(John et al. 1933)
<i>Penicillium paraphergal</i>	(Nehad and El-Shamy 2010)
<i>Penicillium varians</i>	(Jansson and Lindberg 1980)
<i>Penicillium vermiculatum</i>	(Kogan et al. 2002)
<i>Peniophora cinerea</i>	(Maziero et al. 1999)
<i>Perenniporia piperis</i>	(Maziero et al. 1999)
<i>Phellinus baumii</i>	(Hwang et al. 2005)
<i>Phellinus gilvus</i>	(Maziero et al. 1999)
<i>Pholiota nameko</i>	(Maziero et al. 1999)
<i>Phomopsis foeniculi</i>	(Corsaro et al. 1998)
<i>Phytocordyceps</i> sp.	(Madla et al. 2005)
<i>Pichia mucosa</i>	(Sandford 1979)
<i>Platymonas</i> sp.	(Sandford 1979)
<i>Plectania occidentalis</i>	(Sandford 1979)
<i>Pleurotus cornucopiae</i>	(Gutiérrez et al. 1996)
<i>Pleurotus eryngii</i>	(Gutiérrez et al. 1996)
<i>Pleurotus flabellatus</i>	(Maziero et al. 1999)
<i>Pleurotus floridanus</i>	(Gutiérrez et al. 1996)
<i>Pleurotus ostreatoroseus</i>	(Maziero et al. 1999)
<i>Pleurotus ostreatus</i>	(Maziero et al. 1999)
<i>Pleurotus pulmonarius</i>	(Gutiérrez et al. 1996)
<i>Pleurotus sajor-caju</i>	(Maziero et al. 1999)
<i>Pleurotus tuber-regium</i>	(Zhang and Cheung 2011)
<i>Polyporus fomentarius</i>	(Misaki 1968)
<i>Polyporus ignarius</i>	(Misaki 1968)
<i>Polyporus tuberaster</i>	(Misaki 1968)
<i>Psilocybe castanella</i>	(Maziero et al. 1999)
<i>Psilocybe subcubensis</i>	(Maziero et al. 1999)
<i>Pullularia pullulans</i>	(Catley 1971)
<i>Pycnoporus sanguineus</i>	(Maziero et al. 1999)

(continued)

Table 12.7 (continued)

Fungi	Reference
<i>Rhinocladellia elatior</i>	(Petersen et al. 1990)
<i>Rhizomucor pusillus</i>	(De Ruiter et al. 1994)
<i>Rhizopus nigricans</i>	(Misaki 1968)
<i>Rhizopus stolonifer</i>	(Misaki 1968)
<i>Rhodotorula glutinis</i>	(Petersen et al. 1990)
<i>Rigidoporus microporus</i>	(Maziero et al. 1999)
<i>Ripartitella</i> cf. <i>brasiliensis</i>	(Maziero et al. 1999)
<i>Schizophyllum commune</i>	(Maziero et al. 1999)
<i>Sclerotium glucanicum</i>	(Wang and Mcneil 1995)
<i>Sclerotium rolfsii</i>	(Schmid et al. 2011)
<i>Shiraia bambusicola</i>	(Yang and He 2008)
<i>Sorangium cellulosum</i>	(Zhang et al. 2002a)
<i>Sporothrix schenckii</i>	(Misaki 1968)
<i>Stemphylium</i> sp.	(Banerjee and Jana 2009)
<i>Stereum sanguinolentum</i>	(Sandford 1979)
<i>Stropharia rugosoannulata</i>	(Zhai et al. 2013)
<i>Syncephalastrum racemosum</i>	(De Ruiter et al. 1994)
<i>Thamnidium elegans</i>	(De Ruiter et al. 1994)
<i>Torrubiella tenuis</i>	(Madla et al. 2005)
<i>Torulopsis melibiosum</i>	(Sandford 1979)
<i>Torulopsis pinus</i>	(Sandford 1979)
<i>Trametes versicolor</i>	(Maziero et al. 1999)
<i>Trametes villosa</i>	(Maziero et al. 1999)
<i>Tremella brasiliensis</i>	(Sandford 1979)
<i>Tremella encephala</i>	(Sandford 1979)
<i>Tremella foliacea</i>	(Sandford 1979)
<i>Tremella fuciformis</i>	(Zhu et al. 2011)
<i>Tremella mesenterica</i>	(Polysaccharides and Sandford 1979)
<i>Tremella subanomala</i>	(Polysaccharides and Sandford 1979)
<i>Trichaptum byssogenum</i>	(Maziero et al. 1999)
<i>Tricholoma crassum</i>	(Maziero et al. 1999)
<i>Trichosporon asahii</i>	(Fonseca et al. 2009)
<i>Trogia buccinalis</i>	(Maziero et al. 1999)
<i>Tuber sinense</i>	(Tang et al. 2008)
<i>Tyromyces pseudolacteus</i>	(Maziero et al. 1999)
<i>Ustilago maydis</i>	(Cornejo-Mazón Maribel 2012)
<i>Zygosporium masonii</i>	(Madla et al. 2005)

form has shown more effective biological activity in the animals and humans, and above all, it has a much more significant effect on the immune systems. The cell walls of fungal species usually contain two types of biopolymers, β -glucan and chitin. In both these molecules, every separate chain is linked with the hydrogen bonds, and covalent bonds are formed between both the biopolymers. These bonds

lead to the formation of a stronger cell wall, where the chitin fibers are linked with one another, resulting in a network within the glucan matrix. Today, the most important sources of beta-glucans are found to be the members of the genus Oyster (*Pleurotus spp.*) and Shiitake (*Lentinus edodes*). In *Lentinus edodes*, the capable substance is found to be the glucan lentinan (Manzi and Pizzoferrato 2000), whereas the pleuran is the effective substance present in Oyster mushrooms (InternationalMB 1996).

12.2.4.2 Lentinan

Lentinan is made up of β -(1 $\rightarrow$ 3)-glucose with two (1 $\rightarrow$ 6)- β -glucose branches in each five units of glucose (Fig. 12.12) and is derived from an edible mushroom, *Lentinus edodes*. This polysaccharide has been proven to be active against several different syngeneic and allogeneic tumors through the activation of the human immune system. Lentinan being an anticancer carbohydrate also acts as a potential immunomodulator, which stimulates the function and generation of many effector cells (Mostecka et al. 1991). In vivo studies conducted earlier, described lentinan as the T-cell-oriented adjuvants. Also, it improves the cellular activity of the natural killer cells and functions of many monocyte/macrophage (Mostecka et al. 1991; Bel et al. 1989; Fruehauf et al. 1982). The structural parameters and the molecular weight of the lentinan have been determined using various instrumental analysis such as LLS (laser light scattering), viscometry, size exclusion chromatography combined with LLS and ^{13}C NMR, which indicated that the polysaccharide lentinan has as triple-helical chains when present in aqueous solution (Wang et al. 2008; Zhang et al. 2002b).

This fungal polysaccharide can be converted from its triple helix structure to a single random coil in the presence of aqueous NaOH solution, water/dimethyl sulfide (DMSO) mixtures solution or this condition can also occur at 140 °C. The

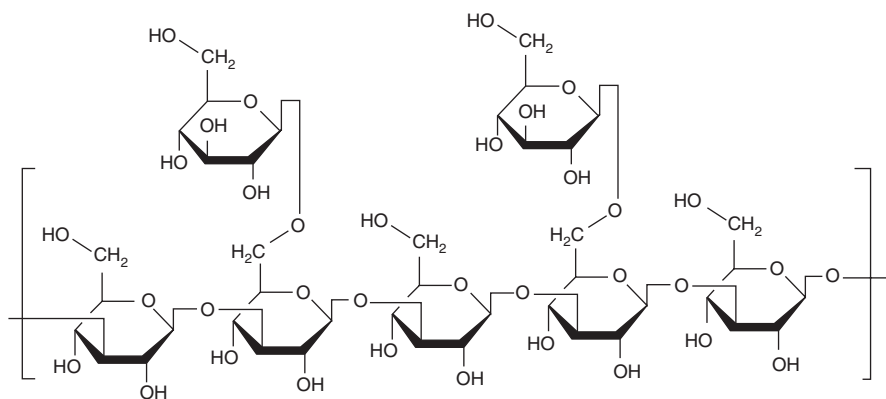


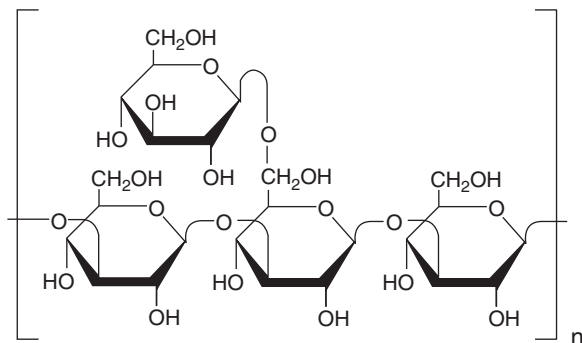
Fig. 12.12 Chemical structure of lentinan

triple-helical structure of lentinan contributes to the distinct antitumor activities and incase of any damage or break to this triple-helical structure, its antitumor activity is significantly reduced and in few cases it may even disappear. So, interestingly the anticancer activity of lentinan is dependent on its triple-helical structure, its combined proteins, and its moderate molecular weight (Zhang et al. 2005). It is widely used as hypocholesterolemic agent for humans as it is found to reduce the levels of lipoproteins (both LDL and HDL) in the blood (Khatun 2012). Lentinan is also used as polysaccharide drugs in both domestic and foreign market. The drugs which have been made from lentinan and are commercially available are Lentinan capsules, Lentinan injection, *Lentinus edodes* mycelia polysaccharide tablets (Zhang et al. 2018; Chen et al. 2013).

12.2.4.3 Schizophyllan

Schizophyllan is an exopolysaccharide that is majorly found on the surface of hyphae, sclerotia of ascomycete, and basidiomycete fungi. Among this, Schizophyllan is mainly isolated from strains of filamentous basidiomycete *Schizophyllum commune*. It is a nonionic, hydrophilic homoglucon which consists of β -(1 $\rightarrow$ 6)-linked glycopyranosyl residues in the β -(1 $\rightarrow$ 3)- glucan backbone (Fig. 12.13) and has a molecular weight around 20,000 Da (Zhang et al. 2013). Schizophyllan is a class of carbohydrates which when dissolved in water, forms triple-helix and a single chain in alkaline medium or DMSO. The structure of this polysaccharide is similar to another β -glucan called scleroglucan, which is mainly produced by fungus *Sclerotium rolfii* (Koenig 2019). For the fungal synthesis of schizophyllan, it is essential to optimize reaction parameters for an effective fermentation process to occur. To get an acceptable product purification, all the parameters need to be supplied in a set specification (such as pH of the medium, solubility of the media components, their gel strength, and purity) (Kumari et al. 2008). *S. commune* utilizes carbon sources like sugars and agricultural residues for polysaccharide production. Sutivisedsak et al. 2013 studied around 11 strains of *Schizophyllum commune*, which can produce schizophyllan. Those strains include

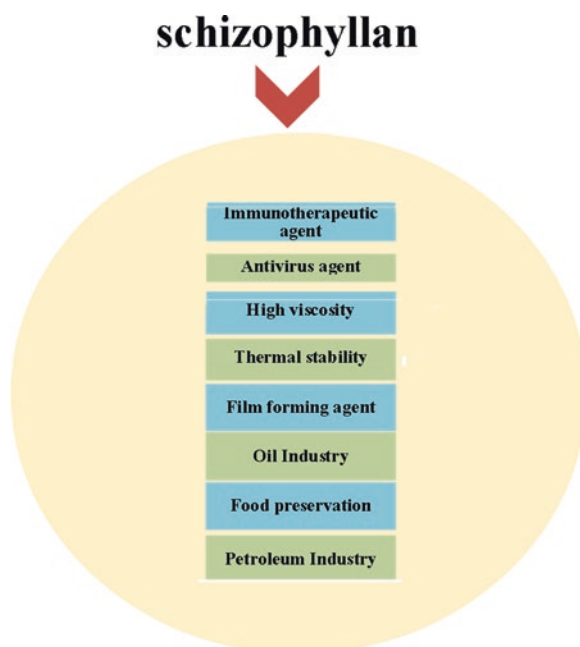
Fig. 12.13 Chemical structure of schizophyllan



ATCC- 20165, 26,262, 26,890, 28,095, 44,200, 38,548; CBS – 199.27, 249.69, 266.60, 579.83, 109,645.

The schizophyllan obtained from these strains showed similar physical properties to that of marketable schizophyllan, signifying that they would be applicable for use in the field of biomaterials. Sutivisedsak et al. 2013 investigated the isolation of schizophyllan and reported that *Schizophyllum commune* strain was grown on a solid medium containing malt extract for about 8 days at 30 °C. The whole culture was homogenized to obtain the supernatant and the insoluble pellet. The obtained pellet was resuspended on the deionized water, homogenized, and centrifuged. The supernatant was mixed with 95% ethanol, and the precipitate was collected using centrifugation, which upon air drying and lyophilization yielded schizophyllan. Industrially schizophyllan is synthesized for its application in cancer treatment and as active cosmetics ingredients (Fig. 12.14). On the other hand, Schizophyllan also has the distinctive physical property of thermal stability, high viscosity, and capacity to form a film with high oxygen resistance, which makes it useful in food preservation and packaging. It can also act as a response modifier in a biological system and nonspecific stimulator of the immune system. It plays a vital role in pharmaceutical industries for the clinical treatment of patients undergoing antitumor therapy (Mansour et al. 2012; Schulz et al. 1992; Daba and Ezeronye 2003).

Fig. 12.14 Various applications of schizophyllan



12.2.4.4 Scleroglucan

Scleroglucan is one of the versatile polysaccharides produced by the fungi *Sclerotium rolfssii*. The chain length of scleroglucan varies from organism to organism. The average molecular weight of the polysaccharide is approximately 5.3×10^6 Da (Brigand 2012). This basidiomycete hosts in plants found in the tropical and temperate regions. Scleroglucan upon hydrolysis gives D-glucose since it is a homopolymer, and its chemical structure comprises of (1 → 3)-linked β -D-glucopyranosyl which at every third unit consists of β -D-glucopyranosyl unit linked with (1 → 6) (Fig. 12.15). The structure of scleroglucans is indicated as a rod-like triple-helical structure, which is similar to the curdlan (Brigand 2012).

Oxalic acid formation is one of the major characteristic features of *Sclerotium rolfssii*. These fungi synthesize enzymes such as cellulases, phosphatidase, arabinanase, exomannanase, α -1,6- galactosidase, endogalactanase, exogalactanase, endocellular, and exocellular polygalacturonase for invading the plant cells (Lachke and Deshpande 1988; Haltrich et al. 1994). The selected stain of *S. rolfssii* is used for the commercial production of scleroglucans by aerobic submerged culture method. The culture should contain around 3% glucose or sucrose as carbon source, nitrates, salts, and corn-steep liquor (Punja 1986). Other carbohydrates can also be used as an energy source. Due to its stable nature, this organism can also be cultivated in the seawater. The temperature required for the fermentation is 28–30 °C and approximately 3 days. The initial pH of 4.2 falls to 2, which helps in reducing the chances of contamination. This acidification enhances the co-synthesis of oxalic acid. The usage of ammonium salts instead of corn-steep liquor helps to suppress the formation of oxalic acid (Punja 1986). The biosynthetic pathway of scleroglucan is illustrated in Fig. 12.16, and the isolation through fermentation is represented in Fig. 12.17.

Due to its favoring rheological properties, this particular polysaccharide is broadly used in various fields of industries. One significant application of scleroglucan is utilization in the oil industry for the enhancement of oil recovery. Scleroglucan is also resistant to temperature, electrolytes, and also exhibits antitumor activities (Coviello et al. 2005). Scleroglucan can be used as a formulation in hair control

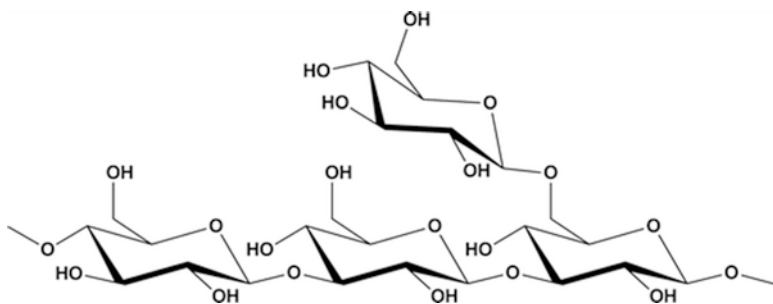


Fig. 12.15 Chemical structure of scleroglucan

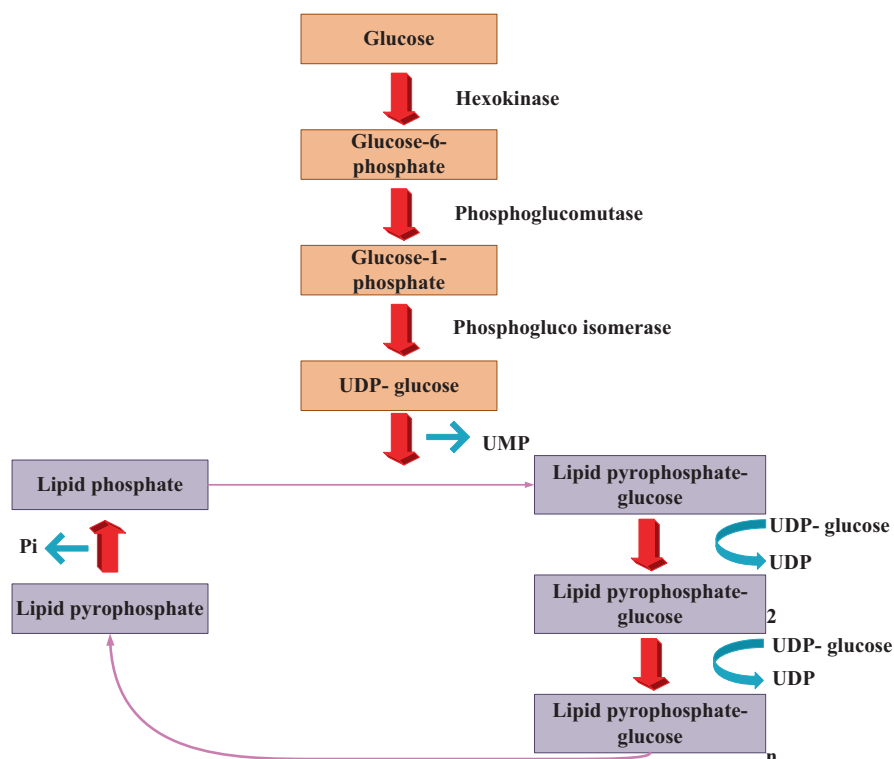


Fig. 12.16 Biosynthesis pathway of scleroglucan

preparations, skincare preparations, creams and emollients, protective skin lotions, antisoilants, and demulcents in the cosmetic industry. Scleroglucan is also used as a coating agent, laxatives, and also as stabilizers in the pharmaceutical industry (Brigand 2012). In terms of the food industry, they can be used as stabilizers for aerated and frozen foods, creams, and sauces by the process of semigelation at 7 °C. However, this might be disadvantageous in certain cases. Other industrial applications of scleroglucan include printing inks, binders for ceramics, adhesives, refractories, fire extinguishing foams, watercolors, etc. It is also used to thicken nitrate solutions that are used in explosives. Scleroglucan, upon specific enzyme-catalyzed process, can yield gentiobiose for industrial applications (Brigand 2012) (Fig. 12.18).

12.2.4.5 Pleuran

Pleuran, an exopolysaccharide that belongs to the β -glucans is isolated from the fruit bodies of the fungal species, *Pleuran ostreatus*, which is most commonly known as the pearl oyster mushroom or the tree oyster mushroom. The molecular

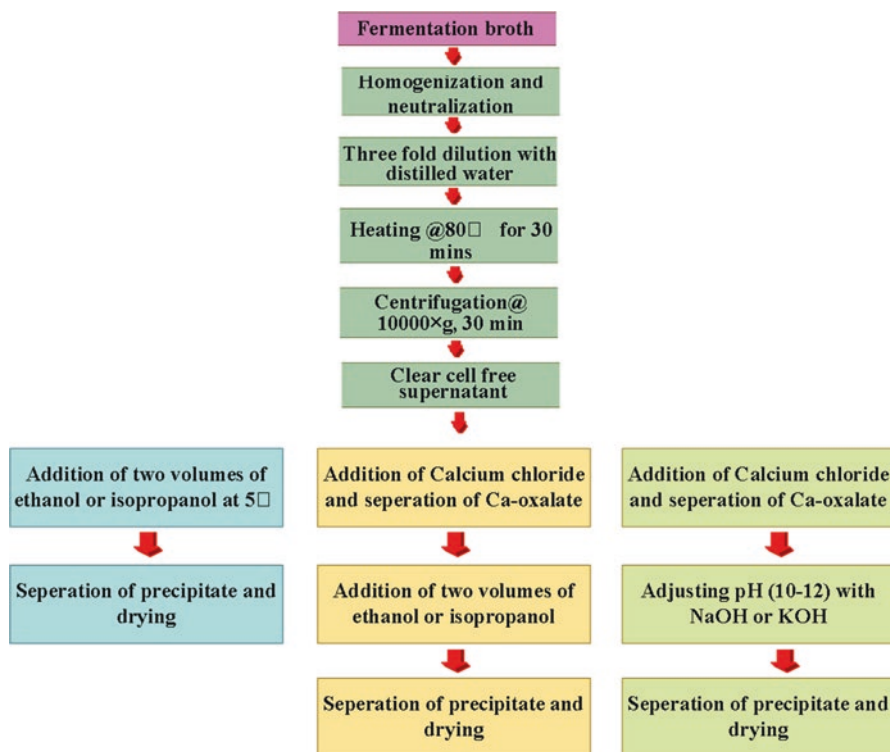


Fig. 12.17 Steps involved in the production of scleroglucan through fermentation

weight of pleuran is 600–700 kDa and is alkali insoluble. Pleuran chain has β -1,3 and β -1,6 with branches bound to β -1,6 and has a single molecule of glucose (Fig. 12.19). It is generally not digestible by the humans as the enzymes produced by the epithelial cells are not capable of hydrolyzing the pleuran. Various species that belong to the genus *Pleurotus* are a huge source of pleuran. A few strains that produce pleuran are *P. djamon* (pink oyster), *P. tuber-regium* (king tuber oyster), *P. nebrodensis* (white ferula mushroom), *P. citrinopileatus* (golden oyster), *P. cystidiosus* (abalone mushroom), *P. pulmonarius* (phoenix oyster), *P. cornucopiae* (branched oyster mushroom), *P. sajorcaju* (gray abalone oyster), and *P. eryngii* (king oyster) (Corrêa et al. 2016).

12.2.4.6 Grifolan

Grifola frondosa (maitake mushroom) is a saprophytic species from the family Meripilaceae (Poryporales), and the production of *G. frondosa* has been tested in three different forms: (i) bag culture, (ii) outdoor bed culture, and (iii) bottle culture (Mayuzumi and Mizuno 1997). Grifolan (Maitake glucan) is a β -glucan which is

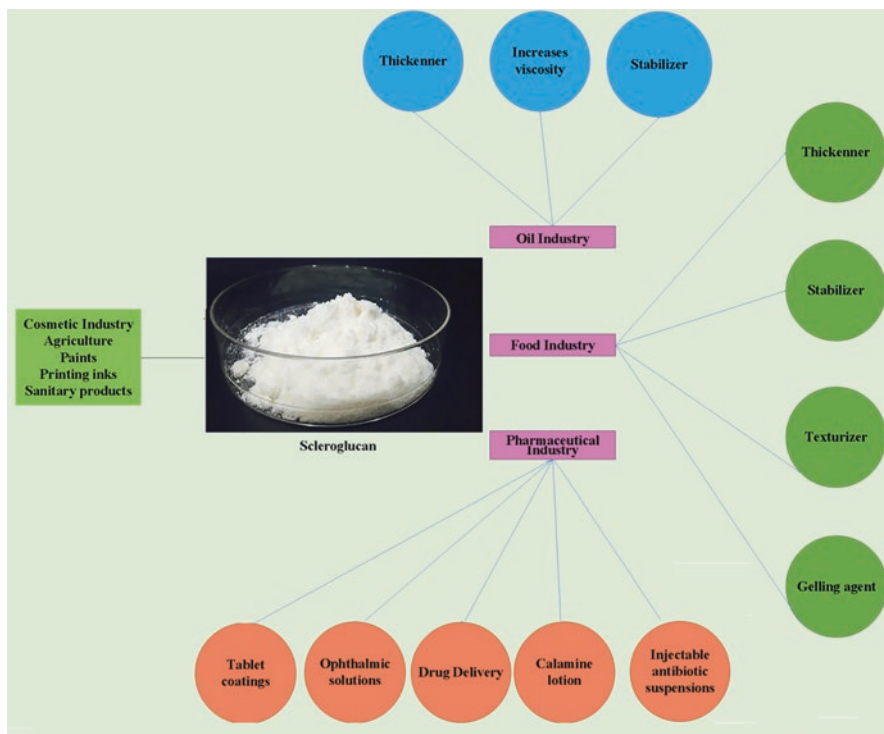


Fig. 12.18 Applications of scleroglucan

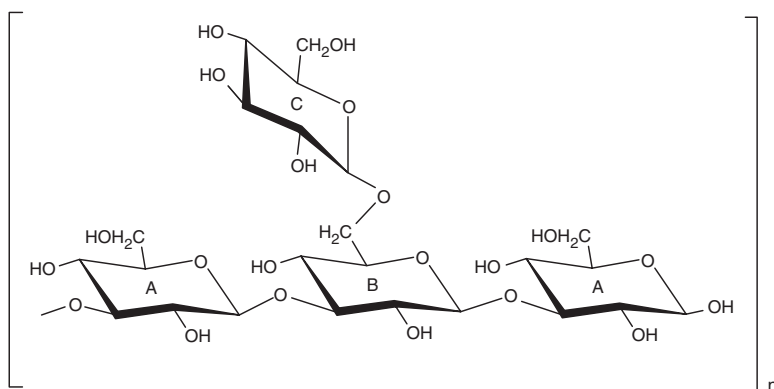
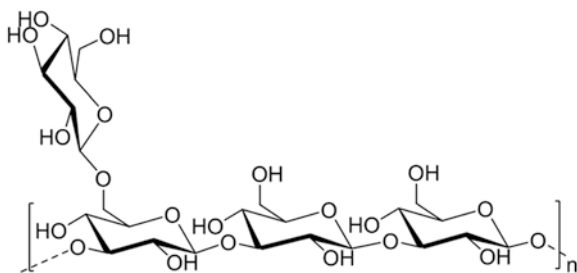


Fig. 12.19 Chemical structure of pleuran

extracted from mycelium or fruiting bodies of fungus *Grifola frondosa* (Minato 2010). The active component in grifolan is a branched polymer of glucose molecules linked by (1 → 3), (1 → 6) β -glucan (Fig. 12.20), which is analogous to former β -glucans (schizophyllan and scleroglucan) (Bohn and BeMiller 1995). The molec-

Fig. 12.20 Chemical structure of grifolan



ular weight of grifolan ranges between 450–500 kDa and has a triple-helix structure during the purification step with the help of NaOH and urea, which is similar to curdlan. The structural features of grifolan result in various physicochemical properties. This grifolan also adopts random-coil confirmation similar to laminarin, which has a lower molecular weight in liquid culture (Mao et al. 2007).

There are two methods for the extraction of grifolan from the mycelium. They are a hot-water extraction method and cold-alkali extraction method. There is evidence that the grifolan extracted from these two methods is quite similar (Nonami 2002). After the cultivation of this fungus, the fermentation broth is usually subjected to centrifugation (8000 rpm for 20 mins) in the presence of boiling water to extract mycelium. After the separation of mycelium from the filtrate, it is heated with 80% ethanol at 60 °C for 4 hours to eliminate fatty substances. This mycelium is then mixed with distilled water and subjected to autoclave. This process is repeated thrice to remove all other substances except polysaccharide. The extract is intensified, and dialyzation is carried out with the help of deionized water. The obtained polymer portion is subjected to precipitation by the addition of four volumes of ethanol (95%) for 24 hours at 4 °C. The precipitate is then lyophilized and stored. This extract is further treated with a solution of sodium hydroxide containing 4% urea for 24 hours at a temperature of 4 °C. The procedure is repeated thrice and then neutralized with acetic acid. The neutralized sample is precipitated to get the supernatant. The latter is dialyzed with deionized water and precipitated with 95% ethanol. Finally, the sample is lyophilized and stored for their future use (He et al. 2017) (Nonami 2002). Maitake glycans show potent medicinal properties (Yang et al. 2019). Grifolan is used in the treatment of hypertension, hyperlipidemia, HIV, and viral hepatitis, as a gelling agent in the food industry as it is similar to curdlan (Mayell 2001).

12.2.4.7 Applications of β -Glucan in the Food Industry

The fungal exopolysaccharide, β - glucan, has a wide range of applications in the food industry (Fig. 12.21). They have been incorporated in many food items such as dairy, cereals, and meat-based products and investigated by many researchers (Murtaza et al. 2016) (Eduardo et al. 2016; Kinner et al. 2011). In spite of its incorporation into various food products, further investigation is required for a better

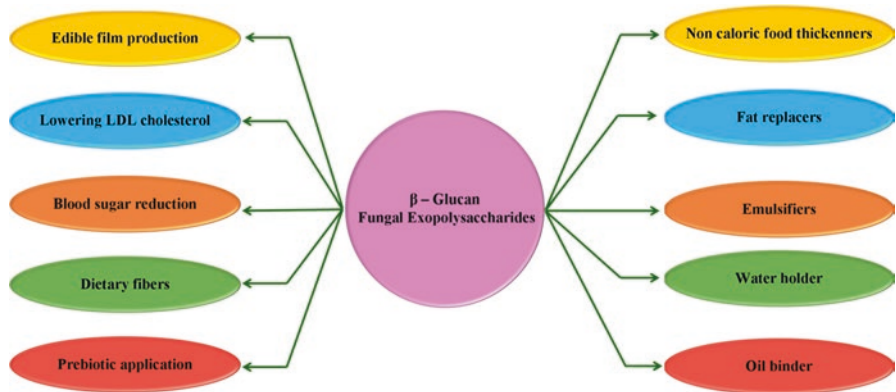


Fig. 12.21 Potential and current application of β -glucans in food production



Fig. 12.22 β -glucan and its applications in various food products

understanding of the β -glucan in food products. Fig. 12.22 shows the application of β -glucan in various food products. A new functional bioactive dairy-based food containing β -glucan has been eventually developed by Bekers et al. The idea of the product developed was that during the fermentation process, a symbiotic combination of prebiotics, dietary fibers, and probiotics was released (Bekers et al. 2001). Similarly, promising results were shown when a functional yogurt was developed after the incorporation of β -glucan. In such a product, the texture of the unsweetened yogurts and appearance were enhanced after the addition of β -glucan. Apparently, the viscosity of the product was also improved along with the sweeteners combined (Fernández-García et al. 1998). Also, in many dairy products, the incorporation of β -glucan ultimately triggers the fermentative bacteria and produces a functional bioactive substance (Kontula et al. 1998). The β -glucan has a multifunctional role in various food sectors which facilitates the processors to incorporate this as a formulation in wide varieties of products such as low-fat-containing sausages (Morin et al. 2004), low-fat-containing ice-cream (Abdel-Haleem and Awad 2015), brined and soft cheese varieties, and low-fat cheese (Ningtyas et al. 2018). They are also used as an ingredient in the preparation of soups, sauces, and beverages for the past two decades (Ahmad et al. 2009). Their incorporation in such products helped replace the conventional thickening agents. Elsinan is utilized as an oxidation barrier for food products due to its incredibly low air or oxygen permeability. The products may include processed foods, transient food sources, and pharmaceuticals. Elsinan film is favorable in retaining the original flavors of the substances. Due to its biodegradable nature of elsinan, it is used as a packaging material in the food industry (Giavasiz 2013). Pleuran possesses various properties such as immunomodulatory properties, lipid-lowering effect, antidiabetic effects, cardio-protective, antioxidative properties, hepato-protective, and antimicrobial and antitumor properties. They are being used widely as supplements in the food and the pharmaceutical industries considering the fact that they have high protein content, low fat, high dietary fiber content, and also immunomodulatory properties. Pleuran is stable at high temperatures; hence they are used in bakery industries. In addition, pleuran improves the nutritional, nutraceutical properties upon its incorporation in foods and is used as probiotics (Pelaes Vital et al. 2015; Aida et al. 2009; Synytsya et al. 2009).

12.3 Conclusion

Exopolysaccharides are natural carbohydrates that can be isolated from the cell wall of fungi and purified through the upstream and downstream processing. Investigations on the exopolysaccharides from the fungal source are increasing considerably on their physicochemical properties. Owing to the unique properties of the fungal exopolysaccharides, they are used as film-forming agents, stabilizers, coating agents, functional compounds, fat and sugar substitutes, preservatives, texturizer, prebiot-

ics, etc. They are also used in other industries, including pharmaceutical, cosmetics, paper, textiles, and agriculture, etc. However, the literature available on the fungal exopolysaccharides and its application in the food industry is limited. Considering the fact that they have antimicrobial, antitumor, antioxidant, and other immunomodulatory properties, further investigations and researches on fungal exopolysaccharide should be encouraged. Also, the knowledge on the interaction of exopolysaccharides in the food matrix and changes in the physicochemical and sensory properties should be studied in detail. The outlook for the novel food products containing fungal exopolysaccharides is considered major.

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Chapter 13

Neoteric Trends in Medicinal Plant-AMF Association and Elicited Accumulation of Phytochemicals



Banadipa Nanda, Samapika Nandy, Anuradha Mukherjee,
Devendra Kumar Pandey, and Abhijit Dey

13.1 Introduction

Since the beginning of life on the Earth, a diverse array of living beings have evolved under the mother nature's meticulous nurture and care—be it the lush green trees and myriad of plants; large animals like mammals, birds, or reptiles; or smaller ones like in the form of sponges, Annelids, insects, and other invertebrates. Alongside them also evolved the thousands of kinds of microscopic unicellular or multicellular organisms like bacteria, protozoa, archaea, and fungi; living deads like viruses; infectious molecules like prions; and so on, bringing with them a myriad of diseases and pathological maladies. As the research fields of life science and medical science grew, the researchers continued to seek more and more accurate, target-specific and efficient cues for these diseases; the organisms responsible for them also evolved and continued to advance in order to survive, thus making it hard for the scientists to rest even for a moment. Furthermore, our contemporary way of life, trying to keep up with the fast-forward lifestyle of twenty-first century, has steered us into consuming high quantity of synthetic allopathic compounds and anxiolytics, which has been making our body considerably drug resistant and prone to a myriad of very harmful side effects. To keep track with this endless cycle and desperate for new weapon to fight against these minuscule but seemingly invincible enemies, the mankind has now turned to mother nature to look for new answers.

B. Nanda · S. Nandy · A. Dey
Department of Life Sciences, Presidency University, Kolkata, India
e-mail: abhijit.dbs@presiuniv.ac.in

A. Mukherjee
MMHS, South 24Pgs, West Bengal, India

D. K. Pandey (✉)
Department of Biotechnology, Lovely Faculty of Technology and Sciences,
Lovely Professional University, Phagwara, Punjab, India

Nature, being the grand master and unrivaled chemist, has created almost an inexhaustible and never ending array of molecular entities, thus opening up an entirely new world, full of chemical structures, derived from a number of biochemical processes that have been exposed to a variety of changes and modulations over the millennia, and has successfully established itself to be an infinite resource of pharmacophores, novel chemotypes, and many other valuable bioactive agents, taking drug development to a whole new level (Veeresham 2012). Since time immemorial, our ancestors have relied upon this vast natural resource and delved into their depths to establish the natural products as the backbone of traditional system of medical treatment throughout the globe, thus rendering them as an integral part of history and culture.

The wisdom of traditional medicine has attained considerable appreciation and experimental research-based exposure in recent era. The emergence of the concept and practice of “complementary and alternative medicine” throughout the globe has considerably heightened the demand of medicinal plants and plant-based pharmaceuticals. It has been estimated by WHO that almost 80% populations throughout the world utilize different plants and products obtained from them, as the primary source of medicinal agents. Currently, more or less 119 compounds acquired from at least 90 different plant species are in use as important drugs worldwide. Among them, almost 77% have been extracted and derived from the traditionally used medicinal plants (Pandey et al. 2018). In 4000 BCE, ‘hul gil’ (joy plant) was very popular among the Sumerians. It is none other than *Papaver somniferum*, the original source of two of the most important drugs of modern world, the morphine and codeine.

Scientists hypothesized that these two alkaloids, popular in human civilization for thousands of years, could have originated initially as a part of the plant’s innate defense mechanism against herbivores. *P. somniferum* has served as a testament to the significance and importance of secondary metabolites, being one of the first few plants to be cultivated by humans for a purpose other than being served as a food (Theis and Lerda 2003). Some of the most important and still gloriously functional traditional medicine systems in modern world are the Indian System of Medicines (ISM), the Japanese Kampo, the Traditional Chinese Medicines (TCM), and African folklore. Being heavily dependent on plant derived remedies, the traditional Chinese medicines constitute almost 50% of total drugs used in China. More than 500 medicinal plants among almost 5500, used in TCM, currently occupy the front row seats in regular prescription.

One of the most ancient, unconventional but comprehensive medicinal systems, containing a thorough summarization of unorthodox remedies, is the traditional Indian System of Medicine, mainly constituting Ayurveda, Unani, and Siddha, with reserpine being one of the best examples. It is an indole alkaloid, developed from *Rauwolfia serpentina*, a very important and effective Indian medicinal plant that exhibits potent antihypertensive and tranquilizing properties. Among others, the Japanese Kampo medicine is very popular and its formulations are prescribed by at least 70% physicians. Baicalein (from *Scutellaria baicalensis*), glycyrrhizin (from

Glycyrrhiza uralensis), and saikosaponin I (from *Bupleurum falcatum*) are some of the plant derivatives commonly used in Kampo system of medicine.

Also, more than 80% population in some African countries exclusively depends on phytomedicines as a valuable medicinal resource. Caffeine is a purine alkaloid, developed from the coffee tree, *Coffea arabica*, originated from the high lands of south-west Ethiopia, exhibits potent central nervous system (CNS) activity and positive inotropic activity, and activates lipolysis. Another example is ouabain (γ -strophanthin), a cardiac glycoside isolated from *Strophanthus gratus* found in tropical West Africa (Hostettmann et al. 2000), and is useful in treating heart problems in acquires cardiac insufficiency. Therefore, the first question that arises in our minds is what exactly are ‘secondary metabolites’ and how do they function against pathogens. Let’s take a look at the following passage to be acquainted with the nature and function of secondary metabolites and more specifically ‘plant secondary metabolites’.

13.2 Secondary Metabolites

The plant kingdom manufactures over 200,000 distinct chemical compounds, most of which arise from specialized metabolism (Pyne et al. 2019). A. Kossel in 1981 coined the term “secondary,” which unlike the primary metabolites that reside in every living cell with dividing capacity, are present only incidentally, and thus are not of cardinal significance in the organism’s living. Despite being derived from primary metabolism, secondary metabolites do not contribute to building the fundamental molecular skeleton of the said organism, and while its deprivation does not necessarily retrench the life of an organism, a trait exclusively belonging to primary metabolites, it can severely affect the survival of the said organism to a significant extent (Thirumurugan et al. 2018; Devi et al. 2020; Rastegari et al. 2019; Yadav et al. 2019). Therefore, the definition of secondary metabolites can be given as follows:

“Secondary metabolites or natural products can be defined as a heterogeneous group of natural metabolic products that are not essential for vegetative growth of the producing organisms, but they are considered differentiation compounds conferring adaptive roles, for example, by functioning as defense compounds or signaling molecules in ecological interactions, symbiosis, metal transport, competition, and so on” (Demain and Fang 2000).

Among various functions of secondary metabolites, the most notable ones are as follows:

- (i) To serve as “competitive weapons” against other living beings.
- (ii) Act as transporting agents of metal ions essential for the organism’s survival.
- (iii) Play a crucial role in the establishment as well as maintenance of symbiotic relationships with other organisms.

- (iv) Act as “differentiation effectors” and reproductive agents.
- (v) Operate as the communicating agents between organisms alongside interfering in the formation of spores and germination.

Most importantly, besides having antimicrobial and antiparasitic activities, secondary metabolites also perform as enzyme inhibitors and antitumor and immunosuppressive agents, for which they are a very effective and important natural resource in the process of drug development.

13.2.1 Plant Secondary Metabolites

Enormous amount of enemies surrounds the plants in all natural habitats along with different kinds of abiotic environmental stresses (Kumar et al. 2019; Thakur et al. 2020). While primary metabolites actively participate in nutrition and reproduction by performing essential metabolic roles, the secondary metabolites function as the plants’ defense strategist by protecting them against these biotic and abiotic stresses (Pagare et al. 2015; Kour et al. 2019a, b). Thus, having interesting biological properties and important medicinal values, secondary metabolites are a group of highly sought after natural products for the commercial production of valuable drugs like the anticancer drugs Vinblastine, paclitaxel, or camptothecin in a more economy-friendly biotechnological process (Schäfer and Wink 2009; Singh and Yadav 2020).

In accordance with their vast diversity in structure, function, and biosynthesis, almost 2,140,000 known plant secondary metabolites have been arranged in various categories. Based on their biosynthetic origin, they have been separated into three groups, namely, terpenoids, polyketides, and phenylpropanoids (Verpoorte 2000). Alkaloids are an auxiliary class of secondary metabolites, biosynthesized mainly from amino acids. Aside from these, there are also substances derived from fatty acids and alkaloids, polyketides, enzyme cofactors, and nonribosomal polypeptides.

13.2.1.1 Terpenoids

Terpenoids or Isoterpenoids, the largest class of plant secondary metabolites, are a major group of substances obtained from isopentenyl diphosphate biosynthetically. At present, almost 35,000 known terpenoids and steroids have been discovered (Thirumurugan et al. 2018). The number of isoprenoid units present in the structure is the determining factor in the classification of the terpenoids; for example, menthol from peppermint and citronella in lemon are monoterpenes consisting of two isoprenoid units, while sesquiterpenes, such as artemisinin and zingiberene, contain three isoprenoid units. Gibberellic acid with four isoprenoid unit is a diterpene. Triterpenes like ursolic acid, oleanolic acid, and betulinic acid are composed of six,

while tetraterpenes consist of eight isoprenoid units, for example, stigmasterols, lupeol, lanosterol, diosgenin, etc. (Gershenzon and Kreis 1999).

13.2.1.2 Alkaloids

Almost 12,000 alkaloid compounds containing nitrogen as a part of a cyclic system are present in numerous plants with a variety of pharmacological attributes, based on the particular alkaloid structure. The best examples of potent alkaloids are ajmaline, vinblastine, mescaline, reserpine, colchicines, morphine, nicotine, and cocaine. In plant bodies, these compounds are synthesized from aromatic compounds, amino acids, and terpenes (Herbert 2001).

13.2.1.3 Plant Phenolics

These compounds consist of substances that are made of an aromatic ring with a hydroxyl group. Among the plant phenolics, the largest group is composed of the flavonoids, which are followed by phenolic quinones, coumarins, ligans, and monocyclic phenols (Croteau et al. 2000).

13.2.2 *Plant Secondary Metabolites: Transport, Storage, and Turnover*

In plants, while the biosynthesis of secondary metabolites is confined to different compartments, their accumulation, storage, and release are carried out in immensely specialized organs like in glandular tissues, roots, stems, barks, and flowers (Croteau et al. 2000). It was reported by many researchers that high polar water soluble compounds generally accumulate in the vacuole (Wink 1999a, b; Boller and Wiemken 1986), whereas low polar compounds are preserved in resin ducts, thylakoid membranes, glandular hairs, lactifers, trichomes, or the cuticle (Wiermann et al. 1981). Monoterpene essential oils in peppermint are stored especially in glandular trichome during leaf development (McGarvey and Croteau 1995).

Being the unique sources for many pharmaceuticals, flavors, food additives, and other industrial products, production of plant secondary metabolites often takes place when plants are under stresses with diverse elicitors or signal chemicals. By understanding the signal transduction paths that serve the fundamental basis of the elicitor-induced biosynthesis of secondary metabolites, optimization of their commercial production is possible (Zhou and Fan 2007).

To keep up with the ever-changing, continually advancing pathogen population that is running almost neck in neck with the ever-growing human population density, the researchers have discovered and developed quite a large number of very

potent drugs along with many therapeutic leads and new pharmacologically active constituents. The following are some of the plant-derived drugs under commercialization.

However, in spite of putting in four decades of efforts, due to many biological and biotechnological limitations, synthesis of plant secondary metabolites by plant cell culture is still being hampered (Zhao et al. 2000). The low yield of plant secondary metabolites is one of the major obstacles in the aforementioned process. The primary roles of secondary metabolites are to protect their parent organisms from the attacks of herbivores, pathogens, and insects and against other biotic and abiotic stresses (Singh et al. 2020). Therefore, few of the advancements for in vitro production of the metabolites are based on this principle and have been developed to elicit the production including the treatment with diverse signal molecules, elicitors, and abiotic stresses (Zeng et al. 2007; Zhou and Fan 2007).

13.2.3 Biotic and Abiotic Factors and Accumulation of Secondary Metabolites

The accumulation of SM in a plant species relies mostly on (1) climate, (2) altitude, and (3) soil nature. It has been found that various microbes and insects also play a major role in building up the SM in medicinal plants. Some important microorganisms like rhizobacteria promote plant growth, and Mycorrhiza affects the secondary metabolite accumulation in medicinal plants (Tiwari et al. 2020). Detailed studies in this direction will not only help in the identification of the high yielding plant source but also be useful in the optimization of the parameters so that the medicinal plants can be cultivated under unfavorable and stringent conditions. Among these, arbuscular mycorrhizal fungi (AM fungi) exist in symbiotic (mutually beneficial) associations with the fine young roots of higher plants (Smith and Read 1997) and play a very crucial role in promoting the increased accumulation of secondary metabolites in its host plants, thus helping them to cope with environmental biotic and abiotic stresses and ward off the pathogenic microorganisms and harmful insects and pests. The rest of this review briefly discusses the miscellaneous beneficial actions of AMF and its role in the commercial production of plant-based natural medications.

13.3 Arbuscular Mycorrhizal Fungi (AMF)

Asexual, obligatory symbiotic fungi with unique morphological features and genomic structure, the arbuscular mycorrhizal fungi (AMF) occupy a dual niche consisting of soil and the host root and thus belong to the group of 'root obligate biotrophs' having close mutual relationship with over 80% of plants (Vályi et al.

2016). They serve the purpose of natural biofertilizers and provide the host with nutrients, water, and protection against pathogens in exchange for the resultant side products from photosynthesis (Yadav et al. 2020a, b, f). Thus, AMF plays the role of primary biotic soil components, which, when absent or deprived of, can become the reason for the loss of efficiency in the ecosystem functioning (Berruti et al. 2016). Belonging to the phylum “Glomeromycota”, these fungi are ubiquitous soil-borne microbial fungi, dwelling on this earth for more than 450 million years (Redecker et al. 2000). The fungal hyphae of arbuscular mycorrhiza establishes colony exclusively in the root cortex of the host plant, forming immensely branched meshwork of structures inside the cells, i.e., arbuscules, the functional site for the exchange of nutrients (Balestrini et al. 2015). Therefore, AMF is capable of alleviating the limitation in the growth and development of plants caused by an inadequacy in nutrient supply (Nouri et al. 2014). Thus, the role of arbuscular mycorrhizal fungi, as a biofertilizer, is becoming increasingly urgent in the light of sustainable agriculture (Berruti et al. 2016). They were also found to effectively function as elicitors of plant stress response system, root system development and in the field of nutrient uptake and production of secondary metabolites (Szczałba et al. 2019). It protects the host plants from abiotic and biotic stresses and modulates the antioxidant enzyme activity besides playing a crucial role in the biosynthesis of secondary metabolites or phytochemicals like anthocyanin, carotenoids, polyphenols, and phytoestrogens. These plays a fundamental role in promoting human health. Many studies are currently focusing on how the production of food and supplements with enhanced nutritional and nutraceuticals value can be increased efficiently by using AMF. Although several plant species were investigated, the number of AMF utilized is just a handful, and therefore, the full exploitation of their wide physiological and genetic diversity is limited (Avio et al. 2018; Yadav et al. 2020c, d).

13.4 Agronomic and Ecological Roles of Arbuscular Mycorrhizal Fungi

AMF belonging to the group of soil microorganisms acts as a significant and potent linker between the soil mineral nutrients and plants so much that even if there is a remarkable diversity among below ground AM fungal communities, they also depend on soil type, seasons, diversity of plant species, or a combination of all these factors (Smith and Smith 2012). Actually, in normal situation, a nonmycorrhizal condition should be abnormal for most of the species (Smith and Smith 2012). Among the other notable benefits provided to plants by the AMF interaction are improved drought and salinity tolerance (Augé et al. 2015) and disease resistance (Pozo and Azcón-Aguilar 2007). In spite of tremendous studies and research works regarding the effect of AM symbiosis on the response of plants against abiotic stresses (flooding, salinity, and draught) in the last few years, the mechanisms that are actually responsible for the plants’ enhanced tolerance to stress have not been

fully understood yet (Augé et al. 2015; Sánchez-Romera et al. 2016). AMF also enhances the root surface area and biomass and increases phosphate intake (Koide 1991). AMF produces organic acids that solubilize the insoluble mineral phosphates (Lapeyrie 1988) and improves the P uptake by host plants. Arbuscular mycorrhiza attenuates the toxicity resulted from the heavy metal ions like Fe, Cu, and Zn, which, although play a major role in several subcellular compartments, also constitute a very toxic and highly reactive group of elements. By these functions, AM fungi help the host plant to tolerate the high concentration of heavy metals in soil (Tamayo et al. 2014; Meier et al. 2015; Yadav 2020; Yadav et al. 2020e). They also improve the plant nutrition by performing the following functions:

- Increase in the root surface area via the extraradical hyphae, extending beyond the root depletion zone.
- Promoting degradation of organic material.
- Influencing variation of the microbial community in the rhizosphere/

In nutrient suppressive soil, to their hosts, the influence of AMF is very high, while it is low under P-sufficient condition (Koide and Schreiner 1992). On the other hand, AM fungi also affect ecosystem directly by improving the soil structure and aggregation, thus positively influencing the plant community structure and net productivity (Van Der Heijden et al. 1998). They enhance the nitrogen uptake by plants and their assimilation, which lessens the soluble N in the soil and consequently limits denitrification. Through this cycle, AM fungi can regulate N₂O emission, which is a major greenhouse gas. Therefore, it is thought that AMF is able to cause a significant change in climatic by lowering the level of pollution and can be recognized as primary biotic soil components; if absent or impoverished, like in the case of anthropic input, it can lead to the lessening of the functionality of ecosystem (Bender et al. 2015; Berruti et al. 2016; Rastegari et al. 2020a, b).

13.5 AMF Positively Interferes with the Secondary Metabolite Accumulation in Medicinal Plants

AMF, in a symbiotic relationship with the host plant roots, at times, boosts the secondary metabolite production such as vitamins, carotenoids, polyphenols, anthocyanins, lycopene, and flavonoids. When inoculated with the crop plants, AM fungi induced increase in growth, nutritional value, and production of secondary metabolites in various industrially and medicinally important plants. This is attributed to the increased uptake of supplements and nutrients, production of substances that promote growth and development, tolerance to biotic and abiotic stresses, and synergic association with plant growth promoting rhizobacteria (PGPR) like nitrogen fixers and phosphate solubilizing bacteria (PSB) (Bagyaraj and Varma 1995). Due to the AMF-host plant symbiosis establishment, quite a significant range of biological

and biochemical parameters are affected in the host plant, including the molecular and physiological pattern of secondary metabolite production and accumulation. It was observed that AMF enhances secondary metabolites, i.e., terpenoids, alkaloids, and phenolics in various economically and industrially important crop plants.

The accumulation of flavonoids (Morandi et al. 2009; Vierheilig et al. 2000; Wang et al. 1998), cyclohexanone derivatives and apocarotenoids (Maier et al. 1999; Vierheilig et al. 2000), phytoalexins (Yukimune et al. 1996), phenolic compounds (Devi and Reddy 2002), triterpenoids (Akiyama and Hayashi 2002; Akiyama et al. 2002), and glucosinolates in plants colonized by AM fungi (AMF) has also been reported. In field cultivation conditions, it was investigated that AMF inoculation in tuber flesh and peel of all the tested yam species significantly increased the secondary metabolite content in yam tubers (Lu et al. 2015). Mechanism of resistance during AMF interaction in *Piper aduncum* induces variation in the secondary metabolites, the said plant originally produced (de Oliveira et al. 2019a, b). Also, AM triggers the metabolomic response in wheat cultivars, which in turn enhances its tolerance to water stress (Bernardo et al. 2019). Again, *Mimosa tenuiflora* seedling in relationship with AM exhibits significantly larger growth rate, increased photosynthetic ability, and increased content of soluble carbohydrates and secondary metabolites when compared to plants without mycorrhizal association, the most benefited party being the soil with low to moderate phosphate content. AMF, thus, seems to remove the severe restriction imparted on the growth of plants, by soil with low phosphate content (Pedone-Bonfim et al. 2018). AM fungi also increase the flavonoid content and phenolic compound concentration in the bark of *Libidibia ferrea* in field condition (dos Santos et al. 2017). The AMF inoculation increased the concentration of a flavonoid named vitexin, having antidepressant and anxiolytic activities, obtained from *Passiflora* sp. in almost 63.64%.

The same occurs with *A. longula* where the total saponin and flavonoid content in the leaves increases with respect to the control (de Oliveira et al. 2019a, b). On the other hand, AM fungi modify the hyperforin, pseudohypericin, and hypericin content in the flowers of *Hypericum perforatum*, cultivated under contrasting phosphate availability in a highly organic substrate (Lazzara et al. 2017). As an elicitor, AMF affects the production of rosmarinic acid and antioxidants by transformed roots of *Ocimum basilicum* in in vitro culture (Srivastava et al. 2016). NaCl causes adverse effect on the seedlings of *Brassica juncea*; but AMF colonization is responsible for enhancing the tolerance to NaCl through the reformation of the physiobiochemical attributes, secondary metabolite and phytohormone production, and antioxidant enzyme activity (Sarwat et al. 2016). Colonization of olive trees with AM fungi significantly modifies the quality and quantity of the phenolic compounds stored in olive tree roots and their antioxidant capacity (Mechri et al. 2015), besides enhancing mineral nutrition, i.e., N, P, Fe, Cu, Zn, and B, which in turn helps in chlorophyll synthesis, thus enhancing the rate of photosynthesis (Bian et al. 2001; Feng et al. 2002).

Furthermore, AM fungi play a role in morphological modification of their hosts like causing the thickening of cell wall, strengthening the vascular bundles, and

increasing the concentration of phenolics, sulfur containing amino acids, phosphate content etc., which is responsible for the reduction in the severity of various disorders resulted from different pathogen infections (Singh and Kapoor 1999). Currently, all over the world, more and more attention is being subjected to the symbiosis between AMF and its host plant and how they facilitate nutrition uptake through their large network of extraradical fungal hyphae, spreading all over the colonized roots and surrounding soil alongside of being an additional, auxiliary absorbing system (Avio et al. 2018) (Tables 13.1 and 13.2).

13.6 Conclusion

Over the last century, advanced health care and improved medical infrastructure have considerably increased the life expectancy of human population while bringing up new difficult challenges, because, the more we live longer, the more the capacity of cognitive aging increases. Our dependency on the conventional allopathic medicines and treatment procedure to fight against a myriad of pathogenic conditions and diseases has rendered us susceptible to a number of harmful side effects and related health problems. Thus, plant-based alternative and complementary medicine brings us a handful of oxygen in the midst of smog-filled air. For its commercialization and increased production, AMF is one of the main protagonists on the floor.

The microbiome of medicinal plants especially AMF has now been proved to directly and indirectly impact the metabolome of the host and thus could influence the efficacy of herbal medicine by increasing the growth and concentration of primary and secondary metabolites in several plant species, which serves as a vast resource pool of natural drugs and medicines. Therefore, to commercialize and amplify the limited secondary metabolite production, scientists have already started to look for a few suitable, accurate, and cost friendly method. To meet these objectives, in future, the research should focus on: (1) selection and screening of potent and efficient AM fungal strains adapted to production conditions, (2) procedures for the production of seedlings of medicinal plants with mycorrhiza, (3) intensive cultivation of medicinal plants and the production of organic medicinal materials, and (4) mechanisms by which AM fungi change the contents of active ingredients in medicinal plants. But the mode of function and complete understanding of the physiological interaction between the host plant and AMF are still a little vague. In production of herbal materials, much research is required to elucidate the full scenario and their implications of the mode of function of AM fungi and the use of mycorrhizal technology in the cultivation of medicinal plants.

Table 13.1 Medicinal plants and their collaboration with AM

Medicinal plants	Family	Medicinal value (as/against)	Major marker compounds	References
<i>Aloe barbadensis</i>	Asphodelaceae	Antibacterial, anti-inflammatory, and immunity booster	Barbaloin	Cardarelli et al. (2013), Gong et al. (2002)
<i>Coleus forskohlii</i>	Lamiaceae	Anticancerous, cardiopathy, and glaucoma	Forskolin	Sailo and Bagyaraj (2005)
<i>Panax notoginseng</i>	Araliaceae	Pain, detumescence, passive congestion, and hemostasis maintainance	Ginsenosides	Ren et al. (2007), Wu et al. (2016), Wu et al. (2015)
<i>Schizonepeta tenuifolia</i>	Lamiaceae	Headache, flu, and vomiting	<i>Pulegone</i> , limonene, and menthofuran	Wei and Wang (1991), Raei and Weisany (2013)
<i>Bupleurum scorzonrifolium</i>	Apiaceae	Cold, chest pain, fever, irregular menstruation, uterine falloff, and rectoceles	Saikosaponin	Teng and He (2005), Zhang et al. (2011)
<i>Pogostemon cablin</i>	Lamiaceae	Cold and diarrhea	Pogostol	Arpana et al. (2008), Singh et al. (2013)
<i>Datura stramonium</i>	Solanaceae	Rheumatism, cough, and pain	Hyoscyamine	Wei and Wang (1989), Honggang (1989), Garrido et al. (2010), Muthukumar et al. (2006)
<i>Gentiana manshurica</i>	Gentianaceae	Acute icterohepatitis, convulsions, and fever	Amarogentin and swertiamarin	Huo et al. (2008)
<i>Panax ginseng</i>	Araliaceae	Vital energy reinforcement, and physiological weakness restorer	Ginsenosides	Zhang et al. (1990), Xing et al. (2003), Xing et al. (2000), Li (2003a) Cho et al. (2009), Kil et al. (2014)
<i>Ginkgo biloba</i>	Ginkgoaceae	Hypertension, cough and gasp, high urination frequency, and wet dream	Ginkgolic acid and bilobalide	Qi et al. (2002, 2003), Wu and Wei (2008)

(continued)

Table 13.1 (continued)

Medicinal plants	Family	Medicinal value (as/against)	Major marker compounds	References
<i>Paris polyphylla</i>	Melanthiaceae	Malignant boil and swell, sore throat, and trauma	Polyphyllin	Zhou et al. (2010, 2015), Zubek et al. (2010)
<i>Allium sativum</i>	Amaryllidaceae	Arteriosclerosis and high blood pressure, cardiovascular, and immune system stimulator	<i>Allicin</i> and ajoene	Borde et al. (2009)
<i>Salvia miltiorrhiza</i>	Lamiaceae	Cardiovascular diseases, irregular menstruation, hepatitis, and asthma	<i>Salvinorin</i>	Geneva et al. (2010), He et al. (2009a, b), Wang and He (2009), Ma et al. (2009), Meng and He (2011)
<i>Astragalus membranaceus</i>	Fabaceae	Immunity booster and diabetes mellitus	Astragaloside	Loomis and Correau (1972)
<i>Coix lacryma-jobi</i>	Poaceae	Pulmonary abscess, edema, diarrhea, and dermatophytosis	Palmitic acid, stearic acid, oleic acid, and linoleic acid	Li (2003b)
<i>Camptotheca acuminata</i>	Nyssaceae	Tumors	Camptothecin and piperine	Huang et al. (2003), Zhao et al. (2006, 2007), Yu et al. (2010)
<i>Inula ensifolia</i>	Asteraceae	Inflammation and antiviral	Borneol, β -caryophyllene, p-cymene, and bornyl acetate	Zubek et al. (2010)
<i>Atractylodes lancea</i>	Asteraceae	Diarrhea, abdominal fullness and distention, dermatophytosis edema, cold and nyctalopia, and rheumatism	Astragaloside	Guo et al. (2006), Zhang et al. (2010, 2011)
<i>Phellodendron amurense</i>	Rutaceae	Dysentery and furuncles	Nexrutine, quercetin, and berberine	Fan et al. (2006), Jie et al. (2007), Cai et al. (2008)

(continued)

Table 13.1 (continued)

Medicinal plants	Family	Medicinal value (as/against)	Major marker compounds	References
<i>Echinacea purpurea</i>	Asteraceae	Antibacterial and antiviral	Cichoric acid	Araim et al. (2009)
<i>Atractylodes macrocephala</i>	Asteraceae	Diarrhea, edema, abdominal distension, fetal irritability, and spontaneous perspiration	Astragaloside	Lu and He (2005, 2008), Lu et al. (2008a, b, 2011), Wang et al. (2010),
<i>Salvia officinalis</i>	Lamiaceae	Inflammations and mouth and throat infections	<i>Salvinorin</i>	Nell et al. (2009)
<i>Ophiopogon japonicus</i>	Asparagaceae	Cough, constipation, and insomnia	Ophiopojaponin	Pan et al. (2008)
<i>Artemisia umbelliformis</i>	Asteraceae	Antibacterial and antiviral	Artemisinin	Binet et al. (2011)
<i>Tagetes erecta</i>	Asteraceae	Inflammation, cold, and cough	Carotenoids, scopoletin, and ferulic acid	Asrar and Elhindi (2010)
<i>Arnica montana</i>	Asteraceae	Bruises, swellings, and mouth diseases	<i>Helenalin</i> and chamissonolid	Jurkiewicz et al. (2010)
<i>Phellodendron chinense</i>	Rutaceae	Dysentery and furuncles	Nexrutine, quercetin, and berberine	Zhou and Fan (2007)
<i>Artemisia annua</i>	Asteraceae	Malaria	Artemisinin	Kapoor et al. (2007), Rapparini et al. (2008), Chaudhary et al. (2008), Awasthi et al. (2011), Huang et al. (2011)
<i>Pueraria lobata</i>	Fabaceae	Diarrhea, fever, and headache	Quercetin, tryptamine, and apigenin 5-hydroxytryptamine	Wang et al. (2006)
<i>Glycyrrhiza inflata</i>	Fabaceae	Detoxicator, phlegm eliminator, and cough	Glycyrrhizin	Liu et al. (2007), Liu and He (2009)
<i>Citrus aurantium</i>	Rutaceae	Digestion promoter and appetitanregend	Limonin glucoside and phlorin	Wu et al. (2010)
<i>Origanum</i> sp.	Lamiaceae	Appetitanregend, digestion promoter, and antibacterial	Carvacrol, cosmocide, vicenin-2 thymol, and rosmarinic acid	Khaosaad et al. (2006)

(continued)

Table 13.1 (continued)

Medicinal plants	Family	Medicinal value (as/against)	Major marker compounds	References
<i>Mentha arvensis</i>	Lamiaceae	Headache and cough	Eucalyptol, menthol, linalool, pulegol, terpinolene, menthofuran, and menthyl acetate	Gupta et al. (2002), Karagiannidis et al. (2011)
<i>Ocimum basilicum</i>	Lamiaceae	Trauma and snake bite	Eugenol and linalool	Guo et al. (2006), Toussaint et al. (2007), Lee and Scagel (2009), Prasad et al. (2011), Rasouli-Sadaghiani et al. (2010)
<i>Origanum vulgare</i>	Lamiaceae	Appetitanregend, digestion promoter, and antibacterial	Carvacrol, cosmocide, thymol, vicenin-2, and rosmarinic acid	Morone Fortunato and Avato (2008)
<i>Prosopis laevigata</i>	Fabaceae	Immunity booster	Juliflorine and patulitrin	Rojas-Andrade et al. (2003)
<i>Taxus chinensis</i>	Taxaceae	Anticancer	Cephalomannine, 10-deacetylbaaccatin, baccatin III, and paclitaxel	Ren et al. (2008)
<i>Pinellia ternata</i>	Araceae	Cough and vomiting	Atractylon, β -cubebene, methyl eugenol, and δ -cadinene	Chen et al. (2009a, b, 2010), Guo et al. (2010), Shen et al. (2011)
<i>Angelica dahurica</i>	Apiaceae	Toothache, headache, and nasosinusitis	Ferulic acid and ligustilides	Cao and Zhao (2007), Zhao and He (2011)
<i>Trachyspermum ammi</i>	Apiaceae	Gaseous distention, diarrhea, and cholera	Thymol	Kapoor et al. (2002a)
<i>Anethum graveolens</i>	Apiaceae	Flatulence and antispasm	Alpha-phellandrene, apiole, limonene, and geraniol	Kapoor et al. (2002a)
<i>Foeniculum vulgare</i>	Apiaceae	Appetitanregend and digestion promoter	Anethole, fenchone, and methyl chavicol	Kapoor et al. (2004)
<i>Valeriana officinalis</i>	Caprifoliaceae	Sedatives, tranquilizers, and sleep inducer	Valerenic acid	Nell et al. (2010), Geneva et al. (2010)

(continued)

Table 13.1 (continued)

Medicinal plants	Family	Medicinal value (as/against)	Major marker compounds	References
<i>Hypericum perforatum</i>	Hypericaceae	Antibacterial, anti-inflammation, hemostasis maintenance, detumescence, and detoxicating	Hypericin	Zubek et al. (2012)
<i>Coriandrum sativum</i>	Apiaceae	Appetitanregend, analgesia, and detoxicating	Caffeic acid, chlorogenic acid, quercetin, keampferol, rhamnetin, and apigenin	Kapoor et al. (2002b), Farahani et al. (2008)
<i>Origanum onites</i>	Lamiaceae	Appetitanregend, digestion promoter, and antibacterial	Carvacrol, thymol, cosmocide, vicenin-2, and rosmarinic acid	Karagiannidis et al. (2011)
<i>Forsythia suspensa</i>	Oleaceae	Detoxicating, cold, fever, and sore throat	Phillyrin, pinoresinol, phillygenin, lariciresinol, and forsythiaside	Zhao et al. (2007)
<i>Castanospermum australe</i>	Fabaceae	AIDS, Pompe's disease, cancer, and diabetes	1-epilexine	Abu-Zeyad et al. (1999)
<i>Hypericum perforatum</i> , <i>Levisticum officinale</i> , <i>Mentha citrata</i> , and <i>Thymus vulgaris</i>	Hypericaceae, apiaceae, lamiaceae, and lamiaceae respectively	Antidepressant, anti-inflammatory, diaphoretic, antispasmodic, carminative, analgesic, antiseptic, cholagogic, and vasodilator	Hypericin, naphthodianthron, and hyperforin	Zubek et al. (2013)
<i>Aloe vera</i>	Asphodelaceae	Antioxidant and antimicrobial	Barbolin	Burni et al. (2013)
<i>Gloriosa superba</i>	Colchicaceae	Abortifacient, antigout, antipyretic, antileprotic, thermogenic, and anticancerous	Colchicine	Yadav et al. (2013)
<i>Catharanthus roseus</i>	Apocynaceae	Anticancer	Vinblastine, vincristine, vindoline, and catharanthine	Rosa-Mera et al. (2011)

Table 13.2 Effects of AM symbiosis on secondary metabolism of medicinal plants under the conditions of artificial inoculation

Secondary metabolite		Medicinal plant	AMF	Change in secondary metabolite	References
–	Terpene	<i>Schizo tenuifolia</i>	<i>Glomus mosseae</i> and <i>G. epigaea</i>	Increase	Wei and Wang (1991)
Attractol	Terpene	<i>Arctylodes macrocephala</i>	<i>Glomus mosseae</i>	Increase	Pandey et al. (2018)
Menthol, menthone, carvone, and pulegone	Terpene	<i>Mentha arvensis</i>	<i>Glomus etunicatum</i> , <i>G. fasciculatum</i> , and <i>G. lamellosum</i>	Increase	Gupta et al. (2002), Karagianmidis et al. (2011)
Carvacrol and thymol	Terpene	<i>Origanum</i> sp.	<i>G. mosseae</i>	Increase	Khaosaad et al. (2006)
Thymol	Terpene	<i>Origanum vulgare</i>	<i>G. viscosum</i> , strain A6	No change	Morone Fortunato and Avato (2008)
Linalool and geraniol	Terpene	<i>Ocimum basilicum</i>	<i>G. rosea</i> and <i>G. margarita</i>	Increase	Copetta et al. (2006)
			<i>G. fasciculatum</i>	Increase	Rasouli-Sadaghiani et al. (2010)
Anethole	Terpene	<i>Anethum graveolens</i>	<i>G. fasciculatum</i> and <i>G. macrocarpum</i>	90% of the control	Kapoor et al. (2002a)
Thymol	Terpene	<i>Trachyspermum ammi</i>	<i>G. fasciculatum</i> and <i>G. macrocarpum</i>	72% of the control	Kapoor et al. (2002a)
β -caryophyllene, p-cymene, and geraniol	Terpene	<i>Coriandrum sativum</i>	<i>G. hoi</i>	Increase	Farahani et al. (2008)
Anethol	Terpene	<i>Foeniculum vulgare</i>	<i>G. fasciculatum</i> , <i>G. macrocarpum</i> , and <i>G. fasciculatum</i>	78% of the control	Kapoor et al. (2004)

Sesquiterpenes and artemisinin	Terpene	<i>Artemisia Annua</i>	<i>G. fasciculatum</i> and <i>G. macrocarpum</i>	Increase	Welling et al. (2016), Domokos et al. (2018)
Patchoulol	Terpene	<i>Pogostemon cablin</i>	<i>Gigaspora margarita</i> , <i>Acaulospora laevis</i> , <i>G. bagyarajii</i> , <i>G. etunicatum</i> , <i>G. fasciculatum</i> , <i>G. intraradices</i> , <i>G. leptotichum</i> , <i>G. mosseae</i> , <i>G. macrocarpum</i> , <i>G. monosporum</i> , and <i>Scutellospora calaspora</i>	Increase	Arpana et al. (2008)
Atractylol	Terpene	<i>Atractylodes lancea</i>	<i>G. mosseae</i> , <i>G. aggregatum</i> , <i>G. versiforme</i> , and <i>G. intraradices</i>	No change	Guo et al. (2006), Zhang et al. (2010, 2011)
Menthol, menthone, carvone, and pulegone	Terpene	<i>Mentha spicata</i>	<i>G. etunicatum</i> and <i>G. mosseae</i>	No change	Bagheri et al. (2014)
Thymol	Terpene	<i>Trachyspermum ammi</i>	<i>G. fasciculatum</i>	51.21%	Kapoor et al. (2002a)
Geraniol and linalool	Terpene	<i>Coriandrum sativum</i>	<i>G. macrospermum</i> and <i>G. fasciculatum</i>	28% and 43% over control	Kapoor et al. (2002b)
Anethol	Terpene	<i>Foeniculum vulgare</i>	<i>G. macrospermum</i> and <i>G. fasciculatum</i>	Increase	Kapoor et al. (2004)
Artemisinin	Terpene	<i>Artemisia annua</i>	<i>G. macrospermum</i> , <i>G. fasciculatum</i> , <i>Glomus mosseae</i> , and <i>G. versiforme</i>	15.2–32.8% over the control	Kapoor et al. (2007), Domokos et al. (2018)
Sesquiterpene lactones	Terpene	<i>Arnica montana</i>	<i>G. geosporum</i> , <i>G. constrictum</i> , <i>G. intraradices</i> , and <i>G. mosseae</i>	Either increase or decrease	Jurkiewicz et al. (2010)
Glycyrrhizic acid, bornylacetate, 1,8-Cineole, α -Thujones, and β -Thujones	Terpene	<i>Glycyrrhiza inflata</i>	<i>G. mosseae</i> and <i>G. versiforme</i>	Increase	Liu et al. (2007)

(continued)

Table 13.2 (continued)

Secondary metabolite		Medicinal plant	AMF	Change in secondary metabolite	References
Valerenic acid	Terpene	<i>Valeriana officinalis</i>	<i>G. intraradices</i>	Increase	Geneva et al. (2010)
Stevioside and rebaudioside-A	Steviol glycosides	<i>Stevia rebaudiana</i>	<i>Rhizophagus fasciculatus</i>	Increase	Mandal et al. (2013)
Stevioside and rebaudioside-A	Steviol glycosides	<i>Stevia rebaudiana</i>	<i>G. intraradices</i>	Upregulates genes for biosynthesis	Mandal et al. (2015)
Rosmarinic acid and caffeic acid	Phenols	<i>Ocimum basilicum</i>	<i>G. caledonium</i> and <i>G. mosseae</i>	Increase	Toussaint et al. (2007), Lee and Scagel (2009)
Rosmarinic acid and caffeic acid	Phenols	<i>Ocimum basilicum</i>	<i>G. intraradices</i>	No change	Lee and Scagel (2009)
Diobulbinone	Phenols	<i>Dioscorea</i> sp.	<i>G. clarum</i> , <i>G. fasciculatum</i> , <i>G. etunicatum</i> , <i>G. mosseae</i> , and <i>Gigaspora</i> sp.	Increase	Singh et al. (2013)
Phenolics	Phenols	<i>Echinacea purpurea</i>	<i>G. intraradices</i>	Increase in roots	Araim et al. (2009)
Caffeic acid and chlorogenic acid	Phenolic acids	<i>Arnica Montana</i>	<i>G. geosporum</i> , <i>G. constrictum</i> , <i>G. intraradices</i> , and <i>G. mosseae</i>	Increase in roots	Jurkiewicz et al. (2010)
Caffeic acid	Phenolic acids	<i>Viola tricolor</i>	<i>Rhizophagus irregularis</i>	Significant influence	Zubek et al. (2015)
Caffeic acid	Phenolic acids	<i>Viola tricolor</i>	<i>Funneliformis mosseae</i>	No response	Zubek et al. (2015)
Salvianolic acid B	Phenolic acids	<i>Salvia miltiorrhiza</i>	<i>G. mosseae</i> , <i>G. aggregatum</i> , <i>G. versiforme</i> , and <i>G. intraradices</i>	Increase	Yang et al. (2017)
Gallic acid	Phenolic acids	<i>Libidibia ferrea</i>	<i>Claroideoglossum etunicatum</i>	21% over the control	Silva et al. (2014)
Gallic acid	Phenolic acids	<i>Libidibia ferrea</i>	<i>Acaulospora longula</i>	No response	Silva et al. (2014)

Gallic acid	Phenolic acids	<i>Libidibia ferrea</i>	<i>Gigaspora albida</i>	No response	Silva et al. (2014)
Rutin, quercetin, and kaempferol	Phenolic acids	<i>Catharanthus roseus</i>	<i>Glomus</i> sp.	Increase	Rosa-Mera et al. (2011)
Caffeic acid, rosmarinic acid, and luteolin	Phenolic acids	<i>Mentha spicata</i>	<i>G. etunicatum</i> and <i>G. mosseae</i>	Increase	Bagheri et al. (2014)
Coumaric acid and ferulic acid	Phenolic acids	<i>Myracrodruon urundeuva</i>	<i>Acaulospora longula</i>	81.03%	de Sousa et al. (2013)
Thymol derivative	Phenolic acids	<i>Inula ensifolia</i>	<i>G. intraradices</i> and <i>G. clarum</i>	Either increase or decrease	Zubek et al. (2010)
Gallic acid, chlorogenic acid, catechin, and hydroxyl benzoic acid	Phenolic acids	<i>Valeriana jatamansi</i>	<i>G. intraradices</i>	Increase	Jugran et al. (2015)
–	Flavonoid	<i>Salvia miltiorrhiza</i>	<i>G. mosseae</i>	Increase	He et al. (2009c), Meng and He (2011)
–	Flavonoid	<i>Astragalus membranaceus</i>	<i>G. mosseae</i>	Increase	He et al. (2009b)
–	Flavonoid	<i>Myracrodruon urundeuva</i>	<i>Acaulospora longula</i>	57.5% over control	He et al. (2009c)
–	Flavonoid	<i>Dioscorea</i> spp	<i>G. clarum</i> , <i>G. etunicatum</i> , <i>G. fasciculatum</i> , <i>G. mosseae</i> , and <i>Gigaspora</i> sp.	42% over the control	Lu et al. (2015)
–	Flavonoid	<i>Viola tricolor</i>	<i>Rhizophagus irregularis</i>	Increase	Zubek et al. (2015)
Imperatorin and total coumains		<i>Angelica dahurica</i>	<i>Glomus</i> sp.	Increase	Zhao and He (2011)
Total Phenolics and rosmarinic acid		<i>Salvia officinalis</i>	<i>G. mosseae</i> and <i>G. intraradices</i>	No change in leaves and roots	Nell et al. (2009)
Valeric acid		<i>Valeriana officinalis</i>	<i>G. mosseae</i> and <i>G. intraradices</i>	Increase	Nell et al. (2010)

(continued)

Table 13.2 (continued)

Secondary metabolite		Medicinal plant	AMF	Change in secondary metabolite	References
–	Tannins	<i>Valeriana jatamansi</i>	<i>G. intraradices</i>	Increase	Jugran et al. (2015)
Castanospermine	Alkaloid	<i>Castanospermum australe</i>	<i>G. intraradices</i> and <i>Gigaspora margarita</i>	Significant improvement with <i>G. intraradices</i>	Abu-Zeyad et al. (1999)
Trigonelline	Alkaloid	<i>Prosopis laevigata</i>	<i>Gigaspora rosea</i>	1.8 fold increase in roots	Rojas-Andrade et al. (2003)
Vinblastine	Alkaloid	<i>Catharanthus roseus</i>	<i>Glomus</i> sp.	Increase	Rosa-Mera et al. (2011)
Hyosine and hyoscyamine	Alkaloid	<i>Datura stramonium</i>	<i>G. mosseae</i> and <i>G. epigaeum</i>	Increase	Wei and Wang (1989)
Berberine, jatrorrhizine, and palmatine	Alkaloid	<i>Phellodendron amurense</i>	<i>G. mosseae</i> , <i>G. etunicatum</i> , <i>G. versiforme</i> , and <i>G. diaphanum</i>	Increase	Fan et al. (2006)
Berberine	Alkaloid	<i>Phellodendron Chinese</i>	<i>Acaulospora laevis</i> , <i>Acaulospora mellea</i> , <i>G. ditum</i> , <i>G. intraradices</i> , <i>G. mosseae</i> , and <i>G. versiforme</i>	Increase in the tree bark	Zhou and Fan (2007)
Vindoline, vinblastine, vincristine, catharanthine, ajmalicine, and serpentine	Alkaloid	<i>Catharanthus roseus</i>	<i>G. etunicatum</i> and <i>G. intraradices</i>	Increase	Zhao et al. (2000)
Nicotine, anabasine, and nomicotine	Alkaloid	<i>Nicotiana tabacum</i>	<i>G. etunicatum</i> and <i>G. intraradices</i>	Increase	Gupta et al. (2002)
L-ephedrine and guanosine	Alkaloid	<i>Pinellia ternate</i>	<i>G. intraradices</i> and <i>G. mosseae</i>	Increase in the tuber	Guo et al. (2010)
Camptothecin	Alkaloid	<i>Camptotheca acuminata</i>	<i>Acaulospora mellea</i> and <i>G. intraradices</i>	Increase	Zhao et al. (2006), Yukimune et al. (1996)

Forskolin	Alkaloid	<i>Coleus forskohlii</i>	<i>Acaulospora laevis</i> , <i>G. monosporum</i> , <i>G. bagyarajii</i> , <i>G. margarita</i> , <i>G. fasciculatum</i> , <i>G. mosseae</i> , <i>G. leptotichum</i> , <i>G. macrocarpum</i> , <i>G. intraradices</i> , <i>G. etunicatum</i> , and <i>Scutellospora calospora</i>	Increase	Sailo and Bagyaraj (2005)
Alliin	Alkaloid	<i>Allium sativum</i>	<i>G. fasciculatum</i>	Increase	Borde et al. (2009)
Hypericin and pseudohypericin	Others	<i>Hypericum perforatum</i>	<i>G. intraradices</i> , <i>G. mosseae</i> , <i>G. constrictum</i> , and <i>G. geosporum</i>	Increase	Zubek et al. (2012)
Oleoresin	Others	<i>Zingiber officinale</i>	<i>Scutellospora heterogama</i> , <i>Gigaspora decipiens</i> , <i>Acaulospora koskei</i> , and <i>Entrophospora colombiana</i>	Increase	Silva et al. (2008)

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Chapter 14

Fungal Endophytes from Orchidaceae: Diversity and Applications



Ru Wei Chua and Adeline Su Yien Ting

14.1 Introduction

Endophytic fungi are microorganisms that colonize and inhabit the inner tissues of living plants without causing any disease, harm or infection to their host (Dhayanithy et al. 2019). They are considered to have a mutual symbiotic relationship with their host, although in some cases, saprophytic and pathogenic relationships may be demonstrated (Arora and Ramawat 2017; Ting 2020; Rana et al. 2019c; Suman et al. 2016). Endophytic fungi receive nutrition and protection from their host plants, which in return, produce beneficial compounds (secondary metabolites, bioactive compounds) that enhance plant growth, nutrient uptake and tolerance towards biotic and abiotic stresses (Arora and Ramawat 2017; Hamzah et al. 2018; Velma et al. 2018; Devi et al. 2020b; Rana et al. 2019a). Dreyfuss and Chapela (1994) estimated that there are approximately one million species of fungal endophytes existing worldwide, indicating the possibility of their presence in almost all plant species. And of these, grasses, ferns and flowering plants are well-known hosts of a great diversity of fungal endophytes (Sudheep et al. 2017).

Orchidaceae (or commonly known as orchids) is one of the largest families of flowering plants that can be found in various habitats, ranging from damp forest floors to tree barks to sandy dunes (Ma et al. 2015). Orchids form an interesting association with endophytes as the lack of endosperm in orchid seeds suggests their absolute dependence on endophytes for seed germination and growth (Shah et al. 2019). Aside from their ornamental uses, orchids are also valued for their medicinal properties especially in Ayurvedic and Chinese medicine to cure various diseases (Pant et al. 2017). The leaves, roots and pseudo-bulbs of the orchid are potential remedies for bronchitis, inflammation, rheumatism, cancer and microbial infection

R. W. Chua · A. S. Y. Ting (✉)
School of Science, Monash University Malaysia, Jalan Lagoan Selatan,
Selangor Darul Ehsan, Malaysia
e-mail: adeline.ting@monash.edu

(Pant et al. 2017). This propels the interest in exploring endophytes from Orchidaceae as possible sources of potent compounds for applications.

The interest in sourcing endophytes from Orchidaceae for valuable bioactive compounds, were attributed to the discovery of endophytes from medicinal plants, which reportedly produced similar bioactive compounds as their host plants (Yap et al. 2017; Bungtongdee et al. 2018). These natural compounds of endophytic origin have shown anti-microbial, anti-oxidant, anti-cancer, immunosuppressive, anti-diabetic and cytotoxic properties (Cui et al. 2012; Jin et al. 2017; Rana et al. 2019). Evidently, endophyte-derived bioactive compounds are important for medical and pharmaceutical use. They present possible new chemotherapeutic agents and agrochemicals, to mitigate anti-biotic resistance or pesticide resistance in pathogens and pests, respectively (Cui et al. 2012). Endophytes can be upscaled and cultured to harness the natural products for medicinal, industrial, pharmaceutical and agricultural applications (Bungtongdee et al. 2018).

Early works by Bernard (1900) discovered fungal endophytes from *Neottia nidus-avis* (bird's nest orchid). The fungal endophytes were found in the imbibed seeds, later identified as *Fusarium* sp., which were associated with orchid germination. Bernard (1908) further isolated *Rhizoctonia* sp. from roots of *Odontoglossum* sp. and *Vanda tricolor*. Therefore, early discoveries of endophytes in orchids were mostly as symbionts for orchid germination. Gradually, more fungal endophytes were discovered and they were found to confer different benefits to the orchids. For example, the presence of *Rhizoctonia repens* in tubers of *Himantoglossum hircinum* rendered the tubers with fungicidal properties and resistance to further fungal infections (Bernard 1911). In recent years, studies on fungal endophytes from Orchidaceae are slowly gaining momentum, with initial studies focused on their diversity and distribution (Bayman et al. 1998) and gradually to include revelations of their beneficial applications. To date, several endophyte species from Orchidaceae have been discovered to demonstrate anti-microbial, anti-viral, anti-inflammatory and anti-tumor properties (Singh et al. 2012a). This chapter reviews the diversity of endophytic fungi from the Orchidaceae family and various applications.

14.2 Fungal Endophytes

Fungal endophytes are microorganisms that reside internally in living plants. They can be found in the stems, roots, leaves, flowers, as well as in the seeds, of various plant species (i.e. algae, lichens, grasses, ferns, shrubs, forest trees, medicinal plants and vegetables) growing in terrestrial, freshwater and marine ecosystems (Sridhar 2019; Zhang et al. 2019; Rana et al. 2020a). Most plants harbour one or more endophyte species (Strobel 2018; Yadav et al. 2020c). Endophytes form mutualistic relationships with their host plant (Saikkonen et al. 1998), benefiting from protection and nutrition from the host plant while providing host plants with better tolerance to biotic and abiotic stresses (Khare et al. 2018; Zhang et al. 2019; Rana et al.

2020b). Endophytes have also been known to produce beneficial secondary metabolites, which render protective effects to the host from pests and pathogenic fungi (Duan et al. 2019; Devi et al. 2020a; Yadav et al. 2020a, 2020b). Among the metabolites produced include alkaloids, polyketides, terpenoids and glycosides, which have various capacities as anti-bacterial, anti-fungal, anti-cancer and anti-oxidant agents (Duan et al. 2019; Rana et al. 2019b).

This chapter discusses fungal endophytes from Orchidaceae. The fundamental relationship between endophytes and orchids is the dependency of orchids on fungal endophytes for germination and growth (Shah et al. 2019). The germination process in orchid is initiated when the fungal endophyte penetrates the seed (Pant et al. 2017). The endophyte-seed association supplements the seed with nutrients from the endophyte, resulting in the formation of a protocorm, which is absent in other flowering plants (Pant et al. 2017). The orchid protocorm is a distinct structure that will only be formed when symbiosis is established with a compatible fungus, leading to the formation of a shoot apical meristem prior to plantlet formation (Yeung 2017). Unlike other flowering plants, the reproductive system of orchids strongly depends on protocorm development for the initiation of organogenesis, which is absent during orchid embryogenesis (Fang et al. 2016).

Yeung (2017) reported that apical cells of the protocorm body form the meristematic zones, whereas larger parenchymatous basal cells house the symbiont, suggesting preprogrammed cell fates. Once the protocorm is successfully infected with a compatible fungal isolate, peloton digestion by protocorm cells occurs at regular intervals, which is a crucial process for plantlet formation (Yeung 2017). With the absence of endosperm, this organ acts as a source of nutrients for germination. Pereira et al. (2015) reported *Epulorhiza* isolates demonstrated compatibility with *Cyrtopodium glutiniferum*, hence the development of protocorm was successful which leads to seed germination. Digested pelotons were also observed in the protocorm structures. In addition, Wright and Guest (2005) discovered that incompatible isolates could lead to parasitization of protocorm and the undesirable degradation of nutrient reserves, causing soft rot of protocorm and seedling death. In recent years, endophytes from orchids are not only recognized for their role in seed germination, but have also been explored for their diversity and other valuable attributes such as growth-promoting properties and their anti-microbial, anti-oxidant and anti-cancer properties (Bungtongdee et al. 2018; Salazar-Cerezo et al. 2018; Shrestha et al. 2018; Shah et al. 2019; Salazar et al. 2020).

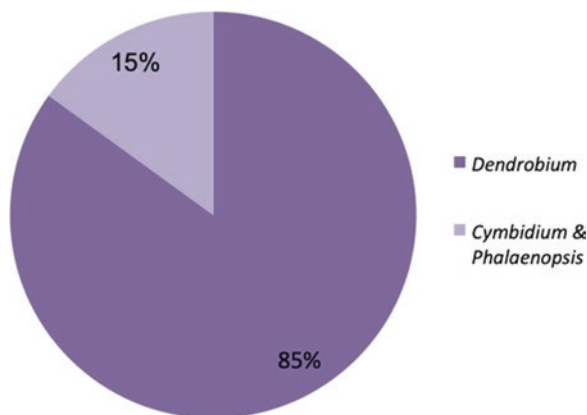
14.3 Orchidaceae

Orchidaceae is the second largest family of flowering plants, comprising of 25,000 to 35,000 species of orchids, existing as terrestrial, saprophytic, lithophytic or epiphytic orchids in various habitats (Lam et al. 2015; Zhang et al. 2018; Sarsaiya et al. 2020). Asia is one of the primary regions with diverse orchid species (De et al. 2014a). Orchids are cultivated mostly for their high aesthetic ornamental value (cut

flower, potted plant), and also for medicinal and food applications. Orchids contribute greatly to the cut flower industry, with 85% of *Dendrobium*, and 15% of *Cymbidium* and *Phalaenopsis* dominating the market (Cheamuangphan et al. 2013) (Fig. 14.1). Other important orchids in the cut flower industry include *Cattleya*, *Oncidium*, *Paphiopedilum* and *Vanda* (De et al. 2014a). Although *Dendrobium* orchids are common, *Phalaenopsis*, *Cymbidium* and *Paphiopedilum* have higher commercial success owing to their beautiful blooms (Zhang et al. 2018). For the orchid *Vanilla planifolia*, they are not cultivated for their blooms, rather for their seed pods produced. The dried seedpods of this orchid are sold commercially as vanilla flavouring used in the many different food industries (Zhang et al. 2018).

Interestingly, there are also orchid species appreciated for their roles in traditional medicine. Ethnobotanical knowledge would reveal orchids having therapeutic properties to treat diseases such as inflammations, arthritis, rheumatism, muscular pain, tuberculosis, asthma and wounds (Hossain 2011). Among the many genera of orchids, *Anoectochilus*, *Calanthe*, *Cypripedium*, *Dendrobium*, *Eria*, *Gastrodia* and *Luisia*, are well known for their medicinal properties (Pant 2013). *Anoectochilus roxburghii* ('King Medicine') is traditionally used in China (Pant 2013), whereas *Cypripedium acaule* Aiton is an orchid species popular in North American folk medicine (Hinsley et al. 2018). *Dendrobium* species are also most prominently used in traditional Chinese medicine, specifically *Dendrobium loddigesii* Rolfe, *D. officinale*, *D. monoliforme* Sw. and *D. nobile* Lindl (Hinsley et al. 2018). *Dendrobium* species are valued for their anti-oxidative, anti-tumor, anti-inflammatory, analgesic and anti-pyretic properties (Gutiérrez 2010; Singh et al. 2012b). *D. officinale* is also used extensively in traditional medicine to treat fever and hypoglycaemia (Sarsaiya et al. 2020). There are also some lesser-known orchids such as *Vanilla madagascariensis* Rolfe, which have been used in African traditional medicine. *Cymbidium goeringii* is another example, which has hypotensive and diuretic activities as well (Singh et al. 2012a). The orchid *Vanda roxburghii* has also shown anti-inflammatory capacity and is used in Indian traditional medicine (Singh

Fig.14.1 Major orchid genera in the cut flower industry. (Data summarized from Cheamuangphan et al. (2013) on global orchid trade from 2007–2012)



et al. 2012b). It is evident that medicinal orchids remain appreciated, as they have been utilized as remedies for many diseases.

The medicinal values of Orchidaceae are attributed to their high phytochemical content, which includes an array of alkaloids, carotenoids and flavonoids (Hossain 2011). In some orchids such as *Dendrobium*, 32 various types of alkaloids, 22 phenanthrenes, 15 bibenzyls, 7 sesquiterpenoids, 6 coumarins and 4 fluorenones have been discovered from 42 *Dendrobium* species (Lam et al. 2015). As such, a single *Dendrobium* plant (i.e. *D. nobile*), can be used to treat fever, gastric, pains, inflammation and to regulate the immune system (Yuan et al. 2009; Alurappa et al. 2018; Sarsaiya et al. 2020).

In recent years, endophytic fungi discovered from orchids demonstrated stronger antibacterial activities compared to existing antibiotics such as ampicillin (Xing et al. 2010). The endophytic fungi were from *Dendrobium devonianum*. Another *Dendrobium* species, *D. monoliforme*, reportedly host endophytes with anti-microbial and anti-oxidant activities, and with the potential to cure osteoporosis (Shah et al. 2019). These discoveries propel the interest in further exploring fungal endophytes from orchids for beneficial applications.

14.4 Diversity of Fungal Endophytes from Orchidaceae

Of the 763 genera of Orchidaceae, only 200 genera (<30%) have been explored for fungal endophytes (Sarsaiya et al. 2019). To date, fungal endophytes from Orchidaceae have been reported to produce anti-microbial compounds (Xing et al. 2010; Shah et al. 2019), and enhance growth and tolerance of host plants towards various biotic and abiotic stresses (Sarsaiya et al. 2019). Common fungal endophytes found predominantly in most Orchidaceae include *Colletotrichum*, *Fusarium*, *Trichoderma*, *Phomopsis*, *Aspergillus* and *Verticillium* species (Chen et al. 2013a; Ma et al. 2015). In recent years, *Xylaria* species are also increasingly discovered and are concluded to be abundant and dominant in tropical Orchidaceae (Chen et al. 2013b). The following sections will discuss the main orchid genera and the diversity of fungal endophytes they harbour.

14.4.1 Fungal Endophytes from *Bulbophyllum*

Bulbophyllum is pantropical, found in Asia, Australia, Madagascar, Continental Africa, East Indian Islands, the tropical Pacific islands to the Neotropics, with New Guinea and Madagascar as the centres of diversity (Kowalkowska et al. 2017). This is the largest genus in the Orchidaceae family, consisting of approximately 2200 species worldwide (Li et al. 2018). They are mostly epiphytic (sometimes lithophytic), and can be found in tropical forests to wet montane cloud forests (Hosseini and Dadkhah 2016) as most *Bulbophyllum* species grow well in the warm

climate, favouring moderate to bright light. *Bulbophyllum* flowers have fleshy lips that are trilobed with a large callus (Teixeira et al. 2004). Their blooms are of hues of dull cream, purple-brown or yellow-green colours, with spots or are hirsute, with fruity or carrion-like scents (Kowalkowska et al. 2017) (Fig.14.2).

Fungal endophytes are highly diverse in the leaves of *Bulbophyllum* (Sudheep and Sridhar 2012; Sawmya et al. 2013). To date, *Aspergillus*, *Penicillium* and *Fusarium* sp. have been identified as common endophytes discovered from *Bulbophyllum*, whereas species of *Gliocladium*, *Guignardia* and *Nigrospora* are relatively rare. A summary of the endophytic fungal species from various *Bulbophyllum* orchids is tabulated in Table 14.1.

The dominant fungal endophytes in *Bulbophyllum* orchids (e.g. *Aspergillus*, *Penicillium* sp.) are not exclusive to *Bulbophyllum*, as they are commonly recovered from other crops as well, such as maize (*Zea mays* L.), rice (*Oryza sativa* L.) and banana (*Musa acuminata*) (Cao et al. 2002; Tian et al. 2004; Zakaria et al. 2016; Potshangbam et al. 2017). The occurrence of these genera in multiple host-plants and their widespread nature suggested that they are neither host-specific nor restricted to a single geographical region. *Aspergillus* and *Penicillium* endophytes are known to produce gibberellin, which promotes plant growth (Potshangbam et al. 2017), and are able to suppress disease from pathogens as well (Waqas et al. 2015). *Fusarium* is another endophyte commonly found in *Bulbophyllum* orchids. As with other endophytes, *Fusarium* is also non-specific to *Bulbophyllum* orchids, as they are found in *Axonopus compressus*, *Holcus lanatus*, *Oryza granulata*, various vegetables (cucumber, tomato, red pepper, pumpkin and Chinese cabbage), Rhizophoraceae mangrove trees, members of Leguminosae family and *Musa acuminata* (Zakaria and Chua 2013; Zakaria et al. 2016). *Fusarium* endophytes are well represented in various plants from Malaysia, India, Spain and Korea, establishing their prevalence and non-host specific nature across geographical regions (Kim et al. 2007; Macia-Vicente et al. 2008; Naik et al. 2009; Zakaria et al. 2016).

Interestingly, endophytes that are less commonly found in *Bulbophyllum* such as *Gliocladium*, *Guignardia* and *Nigrospora*, are relatively common in other plant species. An extensive range of host plants reportedly host *Guignardia* species,



Fig.14.2 *Bulbophyllum* orchids. (Source: www.pixabay.com)

Table 14.1 Diversity of endophytic fungi isolated from various *Bulbophyllum* species

Host plants	Fungal endophytes	Country/Region	References
<i>Bulbophyllum kaitiense</i>	<i>Aspergillus</i> , <i>Penicillium</i>	Eastern Ghats, India	Kasmir et al. (2011)
<i>Bulbophyllum neilgherrense</i>	<i>Xylariaceae</i> sp., <i>Pestalotiopsis</i> , <i>Trichoderma</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Colletotrichum</i>	Western Ghats of Karnataka, India	Sawmya et al. (2013)
<i>Bulbophyllum neilgherrense</i>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Gliocladium</i> , <i>Fusarium</i> , <i>Nigrospora</i>	Western Ghats, India	Sudheep and Sridhar (2012)

which includes *Taxus baccata*, *Tsuga sieboldii*, *Podocarpus macrophyllus*, *Taxus cuspidata*, *Lonicera morrowii*, *Euonymus alatus*, *Cassia fistula*, *Centella asiatica*, *Ocimum sanctum* and *Azadirachta indica* (Okane et al. 2003; Sreekanth et al. 2011; Balakumaran et al. 2015). Additionally, *Guignardia* species have been reported as causal agents of leaf spots and they also inflict fruit diseases on various hosts, with orchids being prone to this fungus (Wikee et al. 2011). For *Nigrospora* sp., they have been isolated as endophytes in medicinal plants such as *Stephania kwangsiensis* (in traditional Chinese medicine) and *Loranthus micranthus* (a Nigerian mistletoe used in African traditional medicine) (Ebada et al. 2016; Luo et al. 2017), and in some cases, found to be a common plant pathogen (Wang et al. 2017). Nevertheless, *Nigrospora* sp. reportedly produces bioactive compounds with anti-fungal properties as well (Luo et al. 2017).

14.4.2 Fungal Endophytes from *Dendrobium*

Dendrobium is the second largest genus of the Orchidaceae family, with many of the species predominantly found in the tropics (Lam et al. 2015). Dendrobiums are mainly epiphytic orchids, with some species thriving well in intermediate conditions (14–26 °C), some preferring cooler climates (10–24 °C), as long as there is exposure to warm bright light for 12–14 hours each day all year round (De et al. 2014b). *Dendrobium* has long pseudobulbs and canes with soft leaves, but certain species have short or swollen pseudobulbs instead (De et al. 2015). Their leaves are thick, deciduous or evergreen, and the blooms produced are of various sizes and colours (pink, purple, white, yellow, brown, greenish-yellow) (Fig. 14.3). *Dendrobium* orchids are typically valued as cut flowers or as potted plants (De et al. 2015). In some countries, they are also appreciated for their medicinal values (Yuan et al. 2009).

Dendrobium orchids harbour endophytic fungi in various parts of the plant, but most abundantly and diverse in the roots (Chen et al. 2013a; Jin et al. 2017). Studies have revealed that common fungal endophytes isolated from *Dendrobium* include species of *Alternaria*, *Aspergillus*, *Acremonium*, *Colletotrichum*, *Fusarium*, *Phomopsis*, *Rhizoctonia*, *Trichoderma* and *Xylaria* (Yuan et al. 2009; Ma et al. 2015; Parthibhan et al. 2017; Shrestha et al. 2018). There were also several less



Fig.14.3 *Dendrobium* orchids. (Source: www.pixabay.com)

common species discovered from *Dendrobium*, such as *Curvularia*, *Scolecobasidium*, *Westerdykella*, *Chaetomella*, *Thielavia*, *Aureobasidium*, *Chaetomium* and *Nigrospora*. A summary of the endophytic fungal species from various *Dendrobium* orchids is provided in Table 14.2.

Species of *Fusarium*, *Xylaria*, *Colletotrichum*, *Phomopsis*, *Trichoderma* and *Aspergillus* are predominantly found in *Dendrobium* species in countries such as China, Thailand, Nepal, Indonesia and Australia. The cosmopolitan nature of *Fusarium* is evident as they are also found in various plants such as *Holcus lanatus*, *Axonopus compressus*, mangrove trees, banana trees and vegetable crops (Kim et al. 2007; Macia-Vicente et al. 2008; Naik et al. 2009; Zakaria et al. 2016). While some *Fusarium* species are crop pathogens, there are also non-pathogenic isolates, which confer benefits to host plants by producing bioactive secondary metabolites (Demers et al. 2014). For endophytic *Xylaria*, they are common endophytes found in tropical plants (Bayman et al. 1998; Davis et al. 2003; Rajulu et al. 2013), which have been reported capable of switching from endophytic to saprophytic nature once the host tissues begin to senesce (Rajulu et al. 2013). *Xylaria* species are valued for the vast enzymes produced for industrial use (Rajulu et al. 2013). Similarly, endophytic *Colletotrichum* sp. have also been isolated from other non-Orchidaceae host species such as *Pennisetum purpureum*, *Cymbopogon citratus*, *Mangifera indica* and *Camellia sinensis* (Manamgoda et al. 2013; Vieira et al. 2014; Rabha et al. 2016). Some *Colletotrichum* (e.g. *C. gloeosporioides*) species are pathogenic, inflicting diseases on crops, but some produce valuable bioactive compounds with anti-oxidant, anti-tumor and anti-microbial properties with pharmaceutical importance (Gonzaga et al. 2015).

Several lesser-known endophyte species have also been found in *Dendrobium*, which includes *Curvularia*, *Scolecobasidium*, *Westerdykella*, *Thielavia*, *Aureobasidium* and *Chaetomium*. They have been found in the roots and leaves of *Dendrobium* from China, Indonesia and Thailand. Some of these isolates, though not often found in *Dendrobium*, are common endophytes of other plants. *Curvularia* is present in *Eugenia jambolana*, *Parthenium hysterophorus* and *Cymbopogon*

Table 14.2 Diversity of endophytic fungi isolated from various *Dendrobium* species

Host plant	Fungal endophytes	Country/Region	References
<i>Dendrobium aphyllum</i>	<i>Xylariaceae</i> sp., <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Phomopsis</i>	Yunnan Province, Southwestern China	Chen et al. (2013a, b)
<i>Dendrobium chrysanthum</i>	<i>Xylariaceae</i> sp., <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Phomopsis</i>	Yunnan Province, Southwestern China	Chen et al. (2013a, b)
<i>Dendrobium chrysotoxum</i>	<i>Xylariaceae</i> sp., <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Phomopsis</i>	Yunnan Province, Southwestern China	Chen et al. (2013a, b)
<i>Dendrobium crystallinum</i>	<i>Xylariaceae</i> sp., <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Phomopsis</i>	Yunnan Province, Southwestern China	Chen et al. (2013a, b)
<i>Dendrobium crumenatum</i>	<i>Xylaria</i> sp., <i>Colletotrichum</i> , <i>Cladosporium</i> , <i>Curvularia</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Pestalotiopsis</i> , <i>Scolecobasidium</i> , <i>Westerdykella</i> , <i>Xylohypha</i>	Indonesia	Mangunwardoyo et al. (2012)
<i>Dendrobium crumenatum</i>	<i>Colletotrichum</i> , <i>Xylaria</i> , <i>Cladosporium</i> -like fungi	Chantaburi Province, Thailand	Sour et al. (2015)
<i>Dendrobium falconeri</i>	<i>Xylariaceae</i> sp., <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Phomopsis</i>	Yunnan Province, Southwestern China	Chen et al. (2013a, b)
<i>Dendrobium fimbriatum</i>	<i>Xylariaceae</i> sp., <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Phomopsis</i>	Yunnan Province, Southwestern China	Chen et al. (2013a, b)
<i>Dendrobium friedricksianum</i>	<i>Xylaria</i> , <i>Fusarium</i> , <i>Pestalotiopsis</i>	Chantaburi Province, Thailand	Sour et al. (2015)
<i>Dendrobium hercoglossum</i>	<i>Chaetomium cochliodes</i> , <i>Xylaria</i> , <i>Colletotrichum</i> , <i>Nigrospora</i> sp.	Chantaburi Province, Thailand	Sour et al. (2015)
<i>Dendrobium loddigesii</i>	<i>Acremonium</i> , <i>Fusarium</i> , <i>Colletotrichum</i> , <i>Chaetomella</i> , <i>Cladosporium</i> , <i>Nigrospora</i> , <i>Pyrenochaeta</i> , <i>Sirodesmium</i> , <i>Thielavia</i>	Guizhou Province, China	Chen et al. (2010)
<i>Dendrobium moniliforme</i>	<i>Aspergillus</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Fusarium</i> , <i>Helminthosporium</i> , <i>Hypoxylon</i> , <i>Leptosphaerulina</i> , <i>Trichoderma</i>	Central hills of Nepal	Shrestha et al. (2018)
<i>Dendrobium nobile</i>	<i>Pestalotiopsis</i> , <i>Colletotrichum</i> , <i>Phomopsis</i> , <i>Clonostachys</i> , <i>Penicillium</i> , <i>Trichoderma</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Xylaria</i> , <i>Hypoxylon</i> , <i>Rhizoctonia</i>	Yunnan Province, China	Yuan et al. (2009)

(continued)

Table 14.2 (continued)

Host plant	Fungal endophytes	Country/Region	References
<i>Dendrobium officinale</i>	<i>Fusarium</i> , <i>Xylaria</i> , <i>Colletotrichum</i> , <i>Pestalotiopsis</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Trichoderma</i> , <i>Cochliobolus</i> , <i>Cystobasidium</i> , <i>Aureobasidium</i> , <i>Epicoccum</i> .	Tropics/ subtropics of South China	Jin et al. (2017)
<i>Dendrobium palpebrae</i>	<i>Fusarium</i> , <i>Cladosporium</i> -like fungi	Chantaburi Province, Thailand	Sour et al. (2015)
<i>Dendrobium speciosum</i>	<i>Fusarium</i> , <i>Nigrospora</i> , <i>Phialophora</i> , <i>Epicoccum nigrum</i> , <i>Trichoderma</i> , <i>Tulasnella</i>	Rainforests of South East Queensland, Australia	Boddington and Dearnaley (2008)
<i>Dendrobium transparens</i>	<i>Aspergillus</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Fusarium</i> , <i>Helminthosporium</i> , <i>Hypoxylon</i> , <i>Leptosphaerulina</i> , <i>Trichoderma</i>	Central hills of Nepal	Shrestha et al. (2018)

caesius (Avinash et al. 2015; Yadav et al. 2016; Priyadharsini and Muthukumar 2017). *Curvularia* spp. are known for their pathogenicity but some species such as *C. lunata* are known to be highly active against *Staphylococcus aureus* and *Candida albicans* (Avinash et al. 2015; Yadav et al. 2016). On the contrary, species of *Scolecobasidium* and *Westerdykella* are also considered rare in other host plants. *Scolecobasidium* has been recovered from tomatoes and Chinese cabbages in Japan, albeit in low abundance (Mahmoud and Narisawa 2013). The *Westerdykella* isolates however, could be exclusive to *Dendrobium* as the only other report on their endophyte nature was from pigeon orchids (*D. crumenatum*) in Indonesia (Mangunwardoyo et al. 2012).

14.4.3 Fungal Endophytes from Cymbidium

Cymbidiums are semi-terrestrial, epiphytic orchids, found in areas with higher elevations (De et al. 2014c). As *Cymbidium* orchids favour cooler climates, they tolerate temperatures as low as 7°C with 10–15 °C being ideal for growth initiation of flower spikes (De et al. 2014c). Therefore, *Cymbidium* orchids grow best below 30 °C, with good lighting (avoiding direct exposure to scorching sunlight). *Cymbidium* orchids originate from tropical and subtropical Asia, found in China, North Eastern India, Malaysia, Japan, Philippines, North Australia and the Borneo islands (De et al. 2014b). *Cymbidium* has long, ribbon-shaped leaves with short pseudobulbs that encircle the base of the leaves. Their flower spikes develop from pseudobulb, and their blooms are typically white, pink, yellow, green or red in colour (De et al. 2014b) (Fig. 14.4).



Fig.14.4 *Cymbidium* orchids. (Source: www.pixabay.com)

Table 14.3 Diversity of endophytic fungi isolated from *Cymbidium* species

Host plant	Fungal endophytes	Country/Region	References
<i>Cymbidium aloifolium</i>	<i>Aspergillus</i> , <i>Colletotrichum</i> , <i>Penicillium</i> , <i>Alternaria</i> , <i>Trichoderma</i> , <i>Fusarium</i> , <i>Cyperus</i>	Western Ghats of Karnataka, India	Shubha and Srinivas (2017)
<i>Cymbidium bicolor</i>	<i>Armillaria</i> , <i>Ceratobasidium</i> , <i>Erythromyces</i> , <i>Moniliopsis</i> , <i>Mycena</i> , <i>Russulaceae</i> , <i>Serendipita</i> , <i>Thanatephorus</i> , <i>Tulasnella</i>	Eastern Ghats, India	Senthilkumar et al. (2018)
<i>Cymbidium dayanum</i>	<i>Chaetomium</i> , <i>Xylaria</i> , <i>Colletotrichum</i> , <i>Phoma</i> , <i>Fusarium</i> , <i>Pestalotiopsis</i> , <i>Corynascus</i> , <i>Cladosporium</i> -like fungi	Chanthaburi Province, Thailand	Sour et al. (2015)
<i>Cymbidium faberi</i>	<i>Trichoderma</i> , <i>Fusarium</i> , <i>Tulasnella</i> , <i>Umbelopsis</i>	Hubei Province, China	Yu et al. (2015)
<i>Cymbidium goeringii</i>	<i>Tulasnella</i> , <i>Exophiala</i> , <i>Fusarium</i> , <i>Nigrospora</i> , <i>Cryptosporiopsis</i> , <i>Cylindrocarpon</i>	Hubei Province, China	Yu et al. (2015)
<i>Cymbidium sinense</i>	<i>Tulasnella</i> , <i>Epulorhiza</i>	Chiang Rai and Chiang Mai Provinces, Thailand	Nontachaiyapoom et al. (2010)

Cymbidium orchids host various endophytes as well, with the highest diversity and colonization rate in the roots (Yu et al. 2015; Shubha and Srinivas 2017). *Tulasnella* and *Fusarium* are commonly isolated found in *Cymbidium* orchids (Nontachaiyapoom et al. 2010; Yu et al. 2015). In contrast, *Cyperus*, *Cryptosporiopsis* and *Cylindrocarpon* were less frequently discovered from *Cymbidium* orchids. The common endophytic fungal species from *Cymbidium* orchids are summarized in Table 14.3.

Tulasnella is a relatively common fungal endophyte found recurrently in most *Cymbidium* species from Thailand, China and India. Species of *Tulasnella* are predominantly isolated from the root tissues of *Cymbidium*, as they are known for

their role as mycorrhizal fungi (Senthilkumar et al. 2018). *Tulasnella* also appears to be closely associated with other orchid species as well, such as *Anacamptis laxiflora*, *Ophrys fuciflora*, *Orchis purpurea*, *Serapias vomeracea* and various *Holcoglossum* orchids (Girlanda et al. 2011; Tan et al. 2012). *Fusarium* spp. are also found in *Cymbidium* orchids, although reports have suggested their prevalence in *Dendrobium* orchids, and other host species such as grasses, trees, vegetable and rice crops (Kim et al. 2007; Macia-Vicente et al. 2008; Naik et al. 2009; Zakaria et al. 2016).

The less common endophytes found in *Cymbidiums* are *Cryptosporiopsis*, *Cylindrocarpon*, *Erythromyces*, *Cyperus* and *Umbelopsis*. *Cryptosporiopsis* is rare in *Cymbidium* orchids but they are abundantly recovered from *Eremanthus erythropappus* trees in Brazil (Godinho et al. 2019). *Cryptosporiopsis* spp. have also been found in *Zanthoxylum lepreurii*, *Alnus incana* and *Alnus alnobetula* (Talontsi et al. 2012; Lalancette et al. 2019), with some species known as stem pathogens to woody host plants. Nevertheless, certain species of *Cryptosporiopsis* are known to confer growth-promoting properties and inhibitory activities against pathogens (Talontsi et al. 2012; Lalancette et al. 2019). Other less common endophytes of *Cymbidium* orchids are *Cylindrocarpon* and *Erythromyces*, with the former detected in low-frequency isolations from other plants such as *Dendrobium moniliforme*, *Parthenium hysterophorus* and *Sapium ellipticum* (Romero et al. 2001; Kamdem et al. 2018; Shah et al. 2019); whereas the latter is found mainly in other orchids such as *Erythrorchis altissima* Blume and *Galeola altissima* (Umata 1995; Dearnaley 2006).

14.4.4 Fungal Endophytes from *Phalaenopsis*

Phalaenopsis orchids, also commonly known as moth orchids, are popular in the floral market with high values as cut flowers or ornamental plants. They are mostly epiphytic and monopodial orchids, native to southeastern Asia (Wang and Gregg 1994). The plants have short stems, with no pseudobulb formation, and are covered by clasping leaves (De et al. 2014b). Their leaves are thick and leathery and the blooms formed are white, yellow, pink in colour, or with slightly mottled flowers (De et al. 2014b) (Fig. 14.5).

Phalaenopsis are tropical plants, favouring temperatures around 19–27 °C for appropriate growth, and tend to stop growing at temperatures above 30 °C (De et al. 2014b). The roots of *Phalaenopsis* harbour various fungal endophytes (Saha and Rao 2006; Rachanarin et al. 2018), with *Tulasnella* and *Rhizoctonia* more commonly discovered compared to species of *Trichoderma* and *Cochliobolus*. A summary of the endophytic fungal species from various *Phalaenopsis* orchids is tabulated in Table 14.4.

Tulasnella and *Rhizoctonia* species are common endophytes of orchids, with high frequencies of isolation from *Cephalanthera longibracteata*, *Anacamptis laxiflora*, *Ophrys fuciflora*, *Orchis purpurea*, *Serapias vomeracea* and various



Fig.14.5 *Phalaenopsis* orchids. (Source: www.pixabay.com)

Table 14.4 Diversity of endophytic fungi isolated from various *Phalaenopsis* species

Host plants	Fungal endophytes	Country/Region	References
<i>Phalaenopsis manni</i>	<i>Rhizoctonia</i> , <i>Trichoderma</i> , <i>Cochliobolus</i>	Kalimpong, India	Saha and Rao (2006)
<i>Phalaenopsis pulcherrima</i>	<i>Tulasnella</i> , <i>Epulorhiza</i> , <i>Rhizoctonia</i>	Phitsanulok Province, Thailand	Rachanarin et al. (2018)

Holcoglossum orchids (Girlanda et al. 2011; Tan et al. 2012); and *Orchis purpurella*, *Listera ovata*, *Coeloglossum viride*, *Platanthera bifolia*, *Platanthera chlorantha* and *Liparis japonica* (Harvais and Hadley 1967; Ding et al. 2014), respectively. Both *Tulasnella* and *Rhizoctonia* form symbiotic association with orchids as important mycorrhizal fungi (Linde et al. 2017). *Trichoderma* and *Cochliobolus* have also been found as endophytes of *Phalaenopsis*, although they are less frequently isolated. Nevertheless, both these endophytes are valued for the anti-microbial, anti-oxidant and cytotoxic compounds produced (Alurappa et al. 2014; Park et al. 2018).

14.4.5 Fungal Endophytes from *Cattleya*

Cattleya orchids, also known as ‘Queen of Orchids’, are epiphytic or lithophytic species native to tropical America (De et al. 2014b). They are cultivated for the cut flower industry. They have elongated pseudobulbs, thick and leathery leaves as well as large fleshy roots (De et al. 2014b). Their flowers are fragrant and long-lasting, available in a wide range of colours (De et al. 2014b) (Fig.14.6).

Cattleya orchids achieve optimum growth when cultivated at 15–30 °C, with exposure to medium and bright light (De et al. 2014b). The roots of *Cattleya* harbour a large number of fungal endophytes with high species diversity (Ovando et al. 2005). *Colletotrichum* and *Epulorhiza* species are typically isolated with higher frequencies, whereas *Botrytis*, *Monilliopsis* and *Tetracladium* are less commonly



Fig.14.6 *Cattleya* orchids. (Source: www.pixabay.com)

Table 14.5 Diversity of endophytic fungi isolated from various *Cattleya* species

Host plants	Fungal endophytes	Country/Region	References
<i>Cattleya jongheana</i>	<i>Colletotrichum</i>	Minas Gerais, Brazil	da Silva et al. (2018)
<i>Cattleya skinneri</i>	<i>Botrytis</i> , <i>Monilliopsis</i> , <i>Pestalotiopsis</i> , <i>Verticillium</i> , <i>Tetracladium</i> , <i>Aspergillus</i> , <i>Fusarium</i> , <i>Trichoderma</i> , <i>Penicillifer</i> , <i>Epulorhiza</i>	Chiapas, Southern Mexico	Ovando et al. (2005)

reported in *Cattleya* (Ovando et al. 2005; da Silva et al. 2018). A summary of the endophytic fungal species from various *Cattleya* orchids is tabulated in Table 14.5.

Colletotrichum and *Epulorhiza* are major endophytes in *Cattleya*, but they are not exclusive to *Cattleya*. *Colletotrichum* has also been found in many *Dendrobium* species, as well as *Pennisetum purpureum*, *Cymbopogon citratus*, *Mangifera indica* and *Camellia sinensis* (Manamgoda et al. 2013; Vieira et al. 2014; Rabha et al. 2016). For *Epulorhiza* species, they are endophytes of *Holcoglossum* orchids, *Epidendrum rigidum*, *Polystachia concreta* and *Phalaenopsis pulcherrima* (Pereira et al. 2003; Tan et al. 2012; Rachanarin et al. 2018). Accordingly, *Epulorhiza* is commonly isolated from terrestrial orchid roots due to their mycorrhizal associations with the host, and their importance in orchid germination (Pereira et al. 2003; Tan et al. 2012).

The lesser-known endophytes from *Cattleya* include *Botrytis*, *Moniliopsis*, *Tetracladium* and *Verticillium*, among others. Although these endophytes are less frequently isolated from *Cattleya*, they are relatively common in other orchid or plants. For example, *Moniliopsis* is found in several other orchids such as *Dipodium variegatum*, *Coeloglossum viride*, *Amerorchis rotundifolia*, *Corallorhiza maculata*, *Cypripedium passerinum*, *Goodyera oblongifolia*, *Platanthera hyperborea* and *Amerorchis rotundifolia* (Currah and Sherburne 1992; Zelmer et al. 1996; Bougoure and Dearnaley 2005). *Moniliopsis* species are *Rhizoctonia*-like fungi, and form mycorrhizal associations with orchids (Zelmer et al. 1996). For *Tetracladium* sp., they are also found in *Cassiope tetragona*, *Saxifraga cespitosa*, *Saxifraga oppositifolia*, *Silene acaulis* and *Orchis militaris* (Elena et al. 2010; Zhang and Yao 2015). The endophyte *Botrytis*, though not as frequently isolated from *Cattleya*, is

rather prevalent in non-orchid plants, such as lettuce, *Centaurea stoebe* and *Hemerocallis* sp. (Shipunov et al. 2008; Sowley et al. 2010; van Kan et al. 2014).

14.4.6 Fungal Endophytes from *Oncidium*

Oncidium species are mostly sympodial epiphytes, found in the tropics and subtropics of America, Argentina and Mexico (De et al. 2014b). *Oncidium* orchids generally grow well with near direct sunlight, and at temperatures 25–30 °C (De et al. 2014b). They have pseudobulbs, small and thin leaves and flowers in yellow or brown shades (occasionally, pink, red, green, white or magenta shades are also found) (Fig. 14.7). Due to their appearance, they are also called ‘Golden Showers’ or ‘Dancing Ladies’ (De et al. 2014b). *Oncidium* blooms are favoured as cut flowers or potted plants due to their beautiful appearance.

Oncidium orchids host various fungal endophytes, with higher species diversity in the leaves (Otero et al. 2002; Pereira et al. 2005; Mohamed and Joseph 2016). *Rhizoctonia* is most frequently isolated, whereas *Aspergillus* and *Pestalotia* have only been isolated from one of the *Oncidium* species. A summary of the endophytic fungal species from various *Oncidium* orchids is tabulated in Table 14.6.

As a common resident in orchids, *Rhizoctonia* is known to form mycorrhizal associations with the host, and is often isolated from the roots (Ding et al. 2014). *Rhizoctonia* is also a common endophyte found in other orchids distributed across Thailand, India, China and Scotland. They include *Phalaenopsis* orchids, *Liparis japonica*, *Platanthera chlorantha*, *Orchis purpurella*, *Listera ovata*, *Coeloglossum viride* and *Platanthera bifolia* (Harvais and Hadley 1967; Ding et al. 2014). Apart from their symbiotic relationship with orchids, *Rhizoctonia* spp. may also produce beneficial anti-microbial compounds (Tan et al. 2018). *Oncidium* orchids also harbour *Pestalotia*, *Aspergillus* and *Cercospora*. *Pestalotia* is predominant in the roots, whereas *Aspergillus* and *Cercospora* are abundant in the leaves. *Pestalotia* sp. are commonly isolated from other plants such as *Camellia oleifera*, *Heritiera fomes*,



Fig.14.7 *Oncidium* orchids. (Source: www.pixabay.com)

Table 14.6 Diversity of endophytic fungi isolated from various *Oncidium* species

Host plants	Fungal endophytes	Country/Region	References
<i>Oncidium altissimum</i>	<i>Xylaria</i> , <i>Pestalotia</i> , <i>Colletotrichum</i> , <i>Rhizoctonia</i>	Puerto Rico	Otero et al. (2002)
<i>Oncidium flexuosum</i>	<i>Rhizoctonia</i> -like fungi, <i>Ceratorhiza</i>	Minas Gerais, Brazil	Pereira et al. (2005)
<i>Oncidium</i> sp.	<i>Aspergillus</i> , <i>Cercospora</i> , <i>Rhizoctonia</i>	Changanacherry, India	Mohamed and Joseph (2016)



Fig.14.8 *Paphiopedilum* orchids. (Source: www.pixabay.com)

Pinus strobus and *Pinus sylvestris* (Hu et al. 2007; Nurunnabi et al. 2017; Yu et al. 2018). This endophyte produces the important anti-cancer drug taxol (Strobel et al. 1996). Although *Aspergillus* sp. is less common in *Oncidium* orchids, they are present in other orchids (*Bulbophyllum*, *Dendrobium*) (Kasmir et al. 2011; Sudheep and Sridhar 2012; Jin et al. 2017; Shrestha et al. 2018) and in other plants (*Euphorbia geniculata*, *Cannabis sativa*, *Pryza sativa*) (Zakaria et al. 2016; Lubna et al. 2018; Kamel et al. 2020). *Aspergillus* endophytes can confer growth-promoting benefits to their host plants through production of gibberellins and indole acetic acid (Lubna et al. 2018).

14.4.7 Fungal Endophytes from Paphiopedilum

Paphiopedilum or ‘Slipper Orchids’ are lithophytic or terrestrial orchids, found in China, Indonesia, South East Asia, Himalayas and Papua New Guinea (De et al. 2014d). *Paphiopedilums* mostly require shading from direct sunlight as they favour cooler conditions (15–26 °C) and low light intensity (De et al. 2014d). Species of *Paphiopedilum* are popular flowering plants in horticulture due to their distinctive colours and shapes. They have no pseudobulb, no stems and their leaves are leathery,

lanceolate and elliptic in shape (De et al. 2014d). Flowers are typically a mixture of white, yellow, green, pink or red in colour (Fig. 14.8).

A higher diversity of endophytes is found in the leaf tissues of *Paphiopedilum* (Nontachaiyapoom et al. 2010; Govinda Rajulu et al. 2016). *Tulasnella* is the most common genus recovered from various species of *Paphiopedilum*, which also includes other endophytes such as *Lasiodiplodia*, *Penicillifer*, *Rigidoporus*, *Flavodon* and *Valsa* spp. A summary of the endophytic fungal species from various *Paphiopedilum* orchids is tabulated in Table 14.7.

The prevalence of *Tulasnella* sp. in the roots of *Paphiopedilum* was attributed to their mycorrhizal association with orchids. As such, *Tulasnella* sp. is also found in several other orchid species such as *Holcoglossum* sp., *Cephalanthera longibracteata*, *Anacamptis laxiflora*, *Ophrys fuciflora*, *Orchis purpurea* and *Serapias vomeracea* (Girlanda et al. 2011; Tan et al. 2012; Lee et al. 2017). *Lasiodiplodia*, *Rigidoporus*, *Penicillifer* and *Flavodon* are also endophytic species found in *Paphiopedilum*, although literature would reveal that their association with orchids is rare. *Lasiodiplodia* species are common tree endophytes and some species such as *L. theobromae* are pathogenic (Ismail et al. 2012). For *Flavodon* sp., they are discovered in *Distylium chinense*, *Catharanthus roseus* and *Rhizophora apiculata* (Klaiklay et al. 2013; Palem et al. 2015; Duan et al. 2019). *Rigidoporus* sp., on the other hand, are found in *Hevea* trees from Peru, Mexico, Brazil and Cameroon as pathogens of the rubber trees (Hamidson and Naito 2004; Martin et al. 2015). Nevertheless, *Lasiodiplodia* was isolated with higher frequencies from the leaves (Govinda Rajulu et al. 2016), whereas *Rigidoporus*, *Flavodon* and *Penicillifer* colonized the roots of *Paphiopedilum* (Khamchatra et al. 2016; Parthibhan and Ramasubbu 2020).

Table 14.7 Diversity of endophytic fungi isolated from various *Paphiopedilum* species

Host plants	Fungal endophytes	Country/ Region	References
<i>Paphiopedilum druryi</i>	<i>Tulasnella</i> , <i>Penicillifer</i> , <i>Colletotrichum</i>	Western Ghats, India	Parthibhan and Ramasubbu (2020)
<i>Paphiopedilum fairrieianum</i>	<i>Cladosporium</i> , <i>Fusarium</i> , <i>Lasiodiplodia</i> , <i>Penicillium</i> , <i>Xylaria</i>	Arunachal Pradesh, India	Govinda Rajulu et al. (2016)
<i>Paphiopedilum sukhakulii</i>	<i>Tulasnella</i> , <i>Epulorhiza</i> ,	Chiang Rai, Thailand	Nontachaiyapoom et al. (2010)
<i>Paphiopedilum villosum</i>	<i>Tulasnella</i> , <i>Rigidoporus</i> , <i>Ceratobasidium</i> , <i>Flavodon</i> , <i>Nigroporus</i> , <i>Corioloropsis</i> , <i>Valsa</i>	Chiang Mai, Thailand	Khamchatra et al. (2016)



Fig.14.9 *Vanda* orchids. (Source: www.pixabay.com)

Table 14.8 Diversity of endophytic fungi isolated from various *Vanda* species

Host plants	Fungal endophytes	Country/ Region	References
<i>Vanda cristata</i>	<i>Alternaria</i> , <i>Fusarium</i> , <i>Pseudochaetosphaeronema</i> , <i>Paraconiothyrium</i> , <i>Agaricus</i> , <i>Mycoleptodiscus</i>	Central hills of Nepal	Chand et al. (2020)
<i>Vanda testacea</i>	<i>Aspergillus</i> , <i>Gliocladium</i> , <i>Penicillium</i> , <i>Nigrospora</i> , <i>Alternaria</i> , <i>Fusarium</i> , <i>Paecilomyces</i> , <i>Phialophora</i>	Western Ghats of India	Sudheep and Sridhar (2012)
<i>Vanda testacea</i>	<i>Armillaria</i> , <i>Ceratobasidium</i> , <i>Erythromyces</i> , <i>Moniliopsis</i> , <i>Mycena</i> , <i>Russulaceae</i> , <i>Serendipita</i> , <i>Thanatephorus</i> , <i>Tulasnella</i>	India	Gomathi et al. (2018)
<i>Vanda testacea</i>	<i>Rhizoctonia</i> , <i>Xylaria</i> , <i>Fusarium</i> , <i>Ceratobasidium</i>	India	Behera et al. (2013)

14.4.8 Fungal Endophytes from Vanda

Vanda is a type of monopodial orchid, inhabiting vast tropical regions (South East Asia, China, India, Sri Lanka, Himalayas and Philippines) as epiphytes (De et al. 2014b). They are cultivated for their ornamental value. They also have medicinal properties used to treat fever, diarrhea, dysentery and skin diseases (De et al. 2014b). The leaves of the *Vanda* orchids are mostly strap shaped, and their blooms are heavy textured and in various colours (yellow, purple, blue, brown, magenta and lavender) (De et al. 2014b) (Fig.14.9).

Vanda orchids grow well in conditions with high intensity of light, and a temperature of 16–32 °C (De et al. 2014b). Most endophytes are found in the roots of the *Vanda* orchids compared to the leaves (Sudheep and Sridhar 2012; Chand et al. 2020). Some of the common endophytes recovered from *Vanda* orchids include *Fusarium*, *Alternaria* and *Ceratobasidium*, as well as some lesser-known species such as *Armillaria*, *Moniliopsis*, *Mycena*, *Russulaceae* and *Agaricus*. A summary of the endophytic fungal species from various *Vanda* orchids is tabulated in Table 14.8.

The endophytic *Ceratobasidium* typically colonizes the roots (Behera et al. 2013; Gomathi et al. 2018), *Alternaria* commonly inhabits the leaves and *Fusarium* is dominant in both leaf and root tissues (Sudheep and Sridhar 2012; Behera et al. 2013; Chand et al. 2020). The widespread nature of these fungal endophytes can be seen in other hosts as well, including other orchid species such as *Dendrobium* and *Cymbidium* orchids (Boddington and Dearnaley 2008; Yuan et al. 2009; Chen et al. 2010; Mangunwardoyo et al. 2012; Chen et al. 2013b; Sour et al. 2015; Yu et al. 2015; Jin et al. 2017; Shubha and Srinivas 2017; Shrestha et al. 2018), and other non-orchid species such as trees, rice, vegetable crops, *Salvadora persica*, *Azadirachta indica*, soybean, *Securinega suffruticosa*, *Epidendrum melinanthum*, *Rhynchostylis retusa*, *Anacamptis laxiflora*, *Ophrys fuciflora*, *Orchis purpurea* and *Serapias vomeracea* (Kim et al. 2007; Macia-Vicente et al. 2008; Naik et al. 2009; Girlanda et al. 2011; Mosquero-Espinosa et al. 2013; Zakaria and Chua 2013; Zakaria et al. 2016; Chatterjee et al. 2019; Elgorban et al. 2019; Batzer and Mueller 2020; Du et al. 2020).

On the contrary, lesser-known endophytes of *Vanda*, i.e. *Armillaria*, *Moniliopsis*, *Mycena*, *Agaricus*, *Serendipita* and *Russulaceae*, are typically found in the root tissues with the exception of *Agaricus*, which is from the stem tissues (Gomathi et al. 2018; Chand et al. 2020). *Armillaria* as an endophyte is relatively rare, as they are more well-known as honey fungus (Guo et al. 2016). Nevertheless, *Armillaria* has been discovered in *Gastrodia elata*, a tuberous orchid used for epilepsy, convulsions, headaches and stroke treatments in China (Gao et al. 2009). For *Moniliopsis* sp., they are common endophytes of a variety of orchids such as *Dipodium variegatum*, *Coeloglossum viride*, *Amerorchis rotundifolia*, *Corallorhiza maculata*, *Cypripedium passerinum*, *Goodyera oblongifolia*, *Platanthera hyperborea* and *Amerorchis rotundifolia* (Currah and Sherburne 1992; Zelmer et al. 1996; Bougoure and Dearnaley 2005). The genus *Agaricus*, however, was only found in *Vanda* and there is insufficient information to further establish the endophytic nature of *Agaricus* sp. (Chand et al. 2020).

14.5 Applications of Fungal Endophytes

Fungal endophytes from Orchidaceae are excellent sources of bioactive metabolites. The compounds produced include alkaloids, flavonoids, peptides, quinones, terpenoids, semiquinones, phenolic compounds, as well as compounds that are growth-promoting, anti-microbial, anti-oxidant and anti-cancer in nature (Jin et al. 2017; Pant et al. 2017; Bungtongdee et al. 2018; Shah et al. 2019). Endophytes from Orchidaceae also produce extracellular enzymes (e.g. amylases, pectinase, lipase, cellulase and laccases), which have potential use in various industries (Paramanantham et al. 2019).

14.5.1 Medicinal Uses

The medicinal use of endophytic fungi became popular with the discovery of Taxol, an anti-tumour agent produced by the endophytic fungi *Taxomyces andreanae* found from the yew tree (*Taxus brevifolia*) (Stierle et al. 1993). In recent years, fungal endophytes from different host plants are also investigated for medicinal use, primarily as anti-microbial, anti-oxidant and anti-tumour agents (Rastegari et al. 2020a, 2020b; Singh and Yadav 2020; Yadav et al. 2020d).

Endophytes from orchids have been discovered to produce effective anti-microbial compounds. To date, species of *Fusarium*, *Xylaria* and *Phoma*, isolated from orchids such as *Acianthera teres*, *Anoectochilus setaceus*, *Dendrobium*

Table 14.9 Fungal endophytes from Orchidaceae with anti-microbial activities

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Acianthera teres</i>	<i>Fusarium oxysporum</i>	Anti-microbial against <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Candida krusei</i> and <i>Candida albicans</i>	Vaz et al. (2009)
<i>Anoectochilus setaceus</i>	<i>Xylaria</i>	Helvolic acid extracts are anti-bacterial against <i>Bacillus subtilis</i> and methicillin-resistant <i>Staphylococcus aureus</i>	Ratnaweera et al. (2014)
<i>Dendrobium crumenatum</i>	<i>Fusarium nivale</i>	Anti-microbial against <i>Candida albicans</i> and <i>Candida tropicalis</i>	Mangunwardoyo et al. (2012)
<i>Dendrobium devonianum</i>	<i>Fusarium</i> , <i>Phoma</i>	Anti-bacterial against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> and <i>Bacillus subtilis</i>	Xing et al. (2010)
<i>Dendrobium lindleyi</i>	<i>Fusarium oxysporum</i>	Anti-fungal against <i>Fusarium</i> , <i>Colletotrichum</i> and <i>Curvularia</i> pathogens	Bungtongdee et al. (2018)
<i>Dendrobium loddigesii</i>	<i>Pyrenochaeta</i>	Anti-microbial against <i>Bacillus subtilis</i> and <i>Aspergillus fumigatus</i>	Chen et al. (2010)
<i>Dendrobium officinale</i>	<i>Fusarium oxysporum</i> , <i>Aureobasidium pullulan</i> , <i>Aspergillus niger</i>	Anti-microbial against <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Sarcina lutea</i> , <i>Saccharomyces cerevisiae</i> and <i>Candida albicans</i>	Jin et al. (2017)
<i>Dendrobium officinale</i>	<i>Pestalotiopsis</i>	Anti-microbial against <i>Candida albicans</i> , <i>Aspergillus fumigatus</i> , <i>Cryptococcus neoformans</i> and <i>Trichophyton rubrum</i> .	Wu et al. (2016)
<i>Dendrobium thyrsiflorum</i>	<i>Fusarium</i> , <i>Phoma</i> , <i>Epicoccum</i>	Anti-bacterial against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> and <i>Bacillus subtilis</i> .	Xing et al. (2010)
<i>Oncidium warmingii</i>	<i>Alternaria</i>	Anti-bacterial against <i>Bacillus cereus</i> and <i>Staphylococcus aureus</i>	Vaz et al. (2009)

crumenatum, *Dendrobium devonianum*, *Dendrobium lindleyi*, *Dendrobium officinale* and *Dendrobium thyrsiflorum*, have all been studied for their anti-microbial properties (Table 14.9). Among the endophyte species, *Fusarium oxysporum* is the most extensively studied, with anti-microbial activities established against fungal pathogens (e.g. *Fusarium*, *Colletotrichum*, *Curvularia*, *Saccharomyces cerevisiae*, *Candida krusei* and *Candida albicans*) and bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Sarcina lutea*) (Vaz et al. 2009; Jin et al. 2017; Bungtongdee et al. 2018). Another valuable endophyte is *Phoma* sp., with potent anti-bacterial effects against *S. aureus*, *E. coli* and *Bacillus subtilis* (Xing et al. 2010).

Endophytic *Xylaria* species also produce anti-microbial compounds, i.e. helvolic acid, which is a potent anti-bacterial compound against *S. aureus* and *B. subtilis* (Ratnaweera et al. 2014). Interestingly, some lesser-known endophytes have also demonstrated anti-microbial activities. For example, endophytic *Pyrenochaeta* sp. from *Dendrobium loddigesii* produces anti-microbial compounds against *B. subtilis* and *Aspergillus fumigatus* (Chen et al. 2010). Literature would reveal that most of the endophytes with anti-microbial activities are from the *Dendrobium* species, and this may be associated with the nature of *Dendrobium* itself, in which extracts of *Dendrobium* are also anti-microbial in nature (Sandrasagaran et al. 2014; Paudel et al. 2018b). As such, the endophytes that they harbour are likely to produce anti-microbial compounds as well.

Anti-oxidant compounds have also been discovered from endophytes from Orchidaceae. Nevertheless, this beneficial property has not been fully explored with only endophytes from *Dendrobium lindleyi* and *D. monoliforme* that were studied (Table 14.10). Among the endophyte species, *Fusarium oxysporum* has been found to produce phenolic compounds with high anti-oxidant activities (Bungtongdee et al. 2018). The free radical scavenging activity by the ethyl acetate extracts of *F. oxysporum* was higher than the positive control (Butyl-1-hydroxytoluene) (Bungtongdee et al. 2018). In addition, *Colletotrichum* sp. also produces a variety of anti-oxidant compounds including pentadecanoic acid, methyl ester and diethyl phthalate (Shrestha et al. 2018). Phenolic and eicosene compounds were recovered from endophytic *Hypoxydon* sp. as well, which demonstrated high anti-oxidant activities (Shrestha et al. 2018).

Table 14.10 Fungal endophytes from Orchidaceae with anti-oxidant activities

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Dendrobium lindleyi</i>	<i>Fusarium oxysporum</i>	Possess high anti-oxidant activity	Bungtongdee et al. (2018)
<i>Dendrobium monoliforme</i>	<i>Colletotrichum</i> sp.	Pentadecanoic acid, methyl ester and diethyl phthalate have anti-oxidant properties	Shrestha et al. (2018)
<i>Dendrobium monoliforme</i>	<i>Hypoxydon</i> sp.	Phenolic and eicosene compounds acquire anti-oxidant properties	Shrestha et al. (2018)

Table 14.11 Fungal endophytes from Orchidaceae with anti-tumour activities

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Cymbidium goeringii</i>	<i>Chaetomium cochliodes</i>	Diketopiperazines exhibit anti-tumor properties against MCF-7 breast cancer cells	Wang et al. (2015)
<i>Dendrobium lindleyi</i>	<i>Fusarium oxysporum</i>	Mutagenic against Trp-P-1, TA98 and TA100 cells.	Bungtongdee et al. (2018)
<i>Dendrobium officinale</i>	<i>Pestalotiopsis</i> sp.	Monoterpenoids, pestalotin and ergosterol derivative exhibit cytotoxic activities against MKN45, LOVO, A549 and HL-60 cancer cell lines	Wu et al. (2016)

Anti-tumour compounds with cytotoxic activities have been discovered from endophytes colonizing *Cymbidium goeringii*, *Dendrobium lindleyi* and *D. officinale* (Table 14.11). *Chaetomium cochliodes* is one such endophyte, which is known to produce diketopiperazines (Wang et al. 2015). These compounds have potent anti-tumour activities against MCF-7 breast cancer cells (Wang et al. 2015). Another valuable endophyte is *Fusarium oxysporum*, where fungal extracts have shown cytotoxic activities against mutagenic Trp-P-1, TA98 and TA100 cells (Bungtongdee et al. 2018). *Pestalotiopsis* sp. also produces anti-tumour compounds, in the form of monoterpenoids, pestalotin and ergosterol derivatives (Wu et al. 2016). These compounds exhibit cytotoxic activities against various cancer cell lines (MKN45, LOVO, A549 and HL-60) (Wu et al. 2016). From the literatures, most endophytic species with anti-tumour capabilities were isolated from *Dendrobium* orchids, coinciding with the nature of *Dendrobium* orchids that has anti-tumour extracts (Paudel et al. 2018a). As such, the endophytes that they host appear to be able to produce similar compounds.

14.5.2 Agricultural Uses

Fungal endophytes from Orchidaceae have been discovered to confer many beneficial associations to their host plants; from plant-growth promotion, and biocontrol activities, to enhancing tolerance to stressful environmental conditions (Rana et al. 2019). These endophytes are therefore potential agents that could be applied for agricultural use, to stimulate plant growth, enhance plant defense mechanisms against pathogenic infections and mitigate effects of abiotic stresses (Paramanantham et al. 2019).

Fusarium sp. is prevalent in *Acampe papillosa*, *Dendrobium desiflorum* and *D. moscatum* (Wu et al. 2002; Tsavkelova et al. 2008). The ability of these endophytes to promote plant growth is attributed to the production of growth-promoting compounds such as gibberellic acid, vitamin B2, vitamin B6 and folic acid (Table 14.12). Their symbiotic relationship with host plants also enhances growth, as better nutrient uptake and assimilation can occur, improving

Table 14.12 Fungal endophytes from Orchidaceae with growth-promoting properties

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Acampe papillosa</i>	<i>Fusarium proliferatum</i>	Produces gibberellic acid with plant growth-promoting properties	Tsavkelova et al. (2008)
<i>Dendrobium desiflorum</i>	<i>Fusarium</i> sp.	Secretes vitamin B2, B6, folic acid and gibberellin which promotes plant growth and development	Wu et al. (2002)
<i>Dendrobium moniliforme</i>	<i>Colletotrichum alatae</i>	Promotes plant growth and development	Shah et al. (2019)
<i>Dendrobium moschatum</i>	<i>Fusarium proliferatum</i>	Produces gibberellic acid with plant growth-promoting properties	Tsavkelova et al. (2008)
<i>Dendrobium officinale</i>	<i>Sebacina</i> , <i>Tulasella</i>	Stimulant for seed germination and growth	Zhang et al. (2020)
<i>Vanda coerulea</i>	<i>Rhizoctonia zeae</i>	Promotes growth and development of orchid seedlings	Aggarwal et al. (2012)
<i>Vanilla planifolia</i>	<i>Ceratobasidium</i>	Stimulant for seed germination	Bayman et al. (2011)
<i>Vanilla poiteaei</i>	<i>Ceratobasidium</i>	Stimulant for seed germination	Bayman et al. (2011)

Table 14.13 Fungal endophytes from Orchidaceae with biocontrol properties

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Epidendrum melinanthum</i>	<i>Ceratobasidium</i>	Reduces expression of disease on rice crops from <i>Rhizoctonia solani</i>	Mosquera-Espinosa et al. (2013)
<i>Odontoglossum</i> sp.	<i>Phomopsis</i> sp.	Produces volatile organic compounds with anti-fungal properties against several plant pathogens	Singh et al. 2011
<i>Oncidium</i> sp.	<i>Piriformospora indica</i>	Defends the plant against pathogenic infection by <i>Erwinia chrysanthemi</i>	Ye et al. (2019)
<i>Vanilla planifolia</i>	<i>Ceratobasidium</i>	Reduces disease severity from pathogenic <i>Fusarium</i> sp.	Bayman et al. (2011)

photosynthetic ability, growth and productivity (Behie and Bidochka 2014). *Ceratobasidium* is another example of common endophyte from Orchidaceae with growth-promoting abilities. Their symbiotic association with host plants stimulates germination and growth, especially in *Vanilla* orchids (Bayman et al. 2011). This clearly shows the potential of endophytes for plant growth enhancement and their prospect for development as biofertilizer for plants.

Some endophytic species have potential as biocontrol agents (Table 14.13). *Ceratobasidium* and *Phomopsis* species, found inhabiting *Epidendrum melinanthum* and *Vanilla planifolia* and *Odontoglossum* sp., respectively, have showed biocontrol activities against common pathogens. *Ceratobasidium* sp. reduces disease incidence caused by pathogenic *Fusarium* sp. and *Rhizoctonia solani* (Bayman et al. 2011;

Table 14.14 Fungal endophytes from Orchidaceae with tolerance against environmental stress

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Anoectochilus</i> sp.	<i>Colletotrichum globosum</i>	Enhances seedling tolerance to stress conditions	Ye et al. (2020)
<i>Anoectochilus</i> sp.	<i>Colletotrichum gloeosporioides</i>	Enhances seedling tolerance to stress conditions	Ye et al. (2020)
<i>Cattleya skinneri</i>	<i>Trichoderma</i> sp., <i>Epulorhiza</i> sp., <i>Botrytis</i> sp.	Supports plant acclimatization in nutrient-poor environments	Ovando et al. (2005)
<i>Dactylorhiza maculata</i>	<i>Piriformospora indica</i>	Confers plant with stress tolerance	Desisa (2017)

Table 14.15 Fungal endophytes from Orchidaceae as enzyme producers

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Cymbidium aloifolium</i>	<i>Colleotrichum truncatum</i> , <i>Fusarium oxysporum</i> , <i>Penicillium chrysogenum</i> , <i>Penicillium purpurogenum</i> , <i>Colletotrichum gloeosporioides</i> , <i>Nigrospora</i> sp.	Produces valuable enzymes (phosphatase, amylase, lipase, laccase, protease, pectinase, cellulase)	Shubha and Srinivas (2017)
<i>Cyrtostylis reniformis</i>	<i>Sebacina vermifera</i>	Produces valuable enzymes (carbohydrate-active enzymes, peptidase, lectins)	Lahrman et al. (2015)
<i>Dendrobium aphyllum</i>	<i>Pseudopestalotiopsis theae</i>	Secretes high amount of pectinase enzyme	Sopalun and Iamtham (2020)
<i>Vanda spathulata</i>	<i>Streptomyces</i>	Produces high amount of cellulase enzymes	Senthilmurugan et al. (2013)

Mosquera-Espinosa et al. 2013), whereas *Phomopsis* sp. suppresses disease incidence caused by pathogenic *Fusarium*, *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Phytophthora*, *Botrytis*, *Colletotrichum* and *Verticillium* (Singh et al. 2011).

Endophyte association with host plants is also able to improve their tolerance to environmental stress. Species of *Colletotrichum*, *Piriformospora*, *Trichoderma*, *Epulorhiza* and *Botrytis* tolerates abiotic stresses, with some species encouraging host acclimatization as well (Table 14.14). These species are from *Anoectochilus* sp., *Cattleya skinneri* and *Dactylorhiza maculata*, are also resistant against nutrient starvation and drought (Ovando et al. 2005; Desisa 2017; Ye et al. 2020). The fungal endophytes adapt to stressful environments by expressing stress-related enzymes (peroxidases and superoxide dismutases), whereas others intensify nutrient and water access, to improve plant survival under harsh conditions (Ovando et al. 2005; Ye et al. 2020). The potential of endophytes in tolerating biotic and abiotic stresses is clearly demonstrated, which allows their prospect for utilization as bioprotectors for plants (Singh et al. 2020).

14.5.3 Industrial Uses

Some of the endophytes from Orchidaceae have been explored for the production of compounds that may be beneficial for industrial use. This typically includes endophytes with the ability to produce enzymes, such as phosphatase, amylase, lipase, laccase, protease, pectinase, cellulase, lectin, carbohydrate-active enzymes, peptidase and lectin (Table 14.15).

Most endophytes are able to produce hydrolytic enzymes such as phosphatase. This enzyme is secreted by numerous endophytes including *Colleotrichum truncatum*, *C. gloeosporioides*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *P. purpurogenum* and *Nigrospora* sp. (Shubha and Srinivas 2017). This is followed by pectinase, which is another common enzyme reportedly produced by *C. gloeosporioides*, *P. chrysogenum*, *C. truncatum*, *F. oxysporum* and *Pseudopestalotiopsis theae* (Shubha and Srinivas 2017; Sopalan and Iamtham 2020). By contrast, only a few endophytes are capable of producing laccase, which include *P. purpurogenum* and *Nigrospora* sp. (Shubha and Srinivas 2017). In addition, endophytes from Orchidaceae (e.g. *Cymbidium aloifolium*) are also capable of producing proteolytic enzymes such as protease, as observed in *C. gloeosporioides*, *F. oxysporum*, *P. chrysogenum* and *C. truncatum* (Shubha and Srinivas 2017). Some enzymes are less commonly produced by fungal endophytes, such as the production of peptidase by *Sebacina vermifera* (Lahrman et al. 2015). Evidently, this demonstrates the potential of endophytes in enzyme production and their prospect for development for industrial uses (Yadav 2020).

Table 14.16 Fungal endophytes from Orchidaceae with metal tolerance

Orchidaceae	Fungal endophytes	Potential applications	References
<i>Bipinnula fimbriata</i>	<i>Tulasnella</i> sp.	Tolerates heavy metal (copper, zinc, arsenic, iron, aluminium) contamination in soil	Herrera et al. (2018)
<i>Bromheadia finlaysoniana</i>	<i>Penicillium chrysogenum</i> , <i>Rhizoctonia</i> sp.	Tolerates lead metal stress	Idris et al. (2019)
<i>Dendrobium crumenatum</i>	<i>Penicillium chrysogenum</i> , <i>Rhizoctonia</i> sp.	Tolerates lead metal stress	Idris et al. (2019)
<i>Epipactis helleborine</i>	<i>Ilyonectria</i> sp.	Tolerates heavy metal (iron, copper, zinc, lead, cadmium) contamination in soil	De Agostini et al. (2020)
<i>Phalaenopsis pulcherrima</i>	<i>Penicillium chrysogenum</i> , <i>Rhizoctonia</i> sp.	Tolerates lead metal stress	Idris et al. (2019)

14.5.4 Bioremediation

The use of fungal endophytes from Orchidaceae for bioremediation has not been studied extensively. Nevertheless, the few existing reports suggested that these endophytes do have tolerance to metals, which may present potential use in metal removal (Table 14.16). *Penicillium* sp. present in orchids such as *Bromheadia finlaysoniana*, *Dendrobium crumenatum* and *Phalaenopsis pulcherrima*, have been found to tolerate high levels of lead (Idris et al. 2019). Another *Penicillium* isolate, *P. funiculosum*, is tolerant to copper and their association with host plants (soybean) enables the plants to mitigate copper-induced stress (Khan and Lee 2013). Therefore, there is potential to explore endophytes from orchids for bioremediation of metals.

14.6 Conclusion

A plethora of fungal endophytes are harboured by several common orchid genera such as *Dendrobium*, *Bulbophyllum*, *Cymbidium*, *Phalaenopsis*, *Cattleya*, *Oncidium*, *Paphiopedilum* and *Vanda*. Prominent endophyte species include *Colletotrichum*, *Fusarium*, *Trichoderma*, *Phomopsis*, *Aspergillus*, *Tulasnella* and *Xylaria*. These endophytes produce valuable bioactive compounds and have potential for various applications. It is evident that fungal endophytes from Orchidaceae are not just useful for medicinal applications, but for agricultural and industrial usage as well. With the discovery of more useful fungal endophytes from Orchidaceae, it is expected that this may facilitate the discovery of novel drugs and bioactive compounds. With better understanding and improved techniques, the exploration into potential applications presented by diverse endophytic fungi from Orchidaceae is infinite.

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Chapter 15

Fungal Mycotoxins: Occurrence and Detection



Anju Kumari, Rehema Joshua, Rakesh Kumar, Partibha Ahlawat,
and Sangeeta C. Sindhu

15.1 Introduction

Fungi are members of eukaryotic organisms that include yeasts, molds, mushrooms, and toadstools; differentiated from the algae and higher plants by the lack of chlorophyll. They are among the most widely distributed organisms on earth, free-living in soil, water, and decaying organic matter; others form parasitic or symbiotic relationships with plants or animals. Molds are part of a larger group of fungi; more apt to cause spoilage in foods, feedstuff, spices, and herbal leading to reduction of yield, safety, and quality with significance economic losses. Changes due to spoilage can be sensorial, nutritional, and qualitative nature like pigmentation, discoloration, rotting development of off-odors and off-flavors (Samuel and Adeyeye 2016; Devi et al. 2020b; Singh et al. 2020). The extent of contamination is influenced by prevailing environment conditions. They are widely distributed and found wherever moisture is present (Adejumo and Adejoro 2014); producing toxic substance named mycotoxin. The term mycotoxin was first used in the 1960s to describe the toxin associated with contaminated peanuts in animal feed and the loss of turkeys in England.

Mycotoxins are low-molecular weight molecules formed by fungi (molds) at the end of the exponential growth process as toxic secondary metabolites and do not adversely affect the producing organism but cause harm to animals, domestic

A. Kumari · R. Joshua · P. Ahlawat (✉)

Centre of Food Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

R. Kumar

Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

S. C. Sindhu

Department of Food and Nutrition, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

animals, and humans (Devi et al. 2020a; Singh and Yadav 2020; Yadav et al. 2020a, b). Mushroom-produced poisonous compounds are almost never called mycotoxins, but are called “mushroom poisons” or “mushroom toxins” (Bennett and Klich 2003). Mycotoxin accumulates in food crops in the field, during transportation and storage (Ahmad and Jae-Hyuk 2017). Its stability against heat, physical, and chemical treatments makes difficult to eliminate during food processing (Marin et al. 2014). The acute, chronic, mutagenic, and teratogenic are four mycotoxins toxicity highly reported (Pitt 2000). The consequence of acute toxicity is the reduction in liver and kidney function, which in extreme cases leads to death. Higher neurotoxin levels cause damage to the brain. Some mycotoxins chronic effect causes liver cancer and inhibits the replication of DNA that may contain mutagenic or teratogenic properties (Smith and Moss 1985). Mycotoxicosis’ multiple effects include necrosis of the skin, leucopenia, and immunosuppression (Pitt 2000).

15.2 Types of Mycotoxins

Mycotoxin contamination in animal feed and human food is a global problem. According to Miller (1995), there are four types of toxic fungi that can be classified as *Fusarium graminearum*, *Alternaria alternata*, *Aspergillus flavus*, and *Penicillium verrucosum* (Fig. 15.1).

Over 300 mycotoxins have currently been identified, few of which have been reported to cause regular contamination of food, feed, spices, and herbs. Mycotoxin toxicity has a greater impact in reducing animal productivity, raising disease incidence due to immune suppression, damage to vital organs, and reproductive capacity disruption, eventually death. Mycotoxins reported on a regular basis are summarized in Fig. 15.2. Among these, Aflatoxins are the most toxic compound,

Fig. 15.2 Regularly reported mycotoxins to contaminate food, feed, spice, and herbs

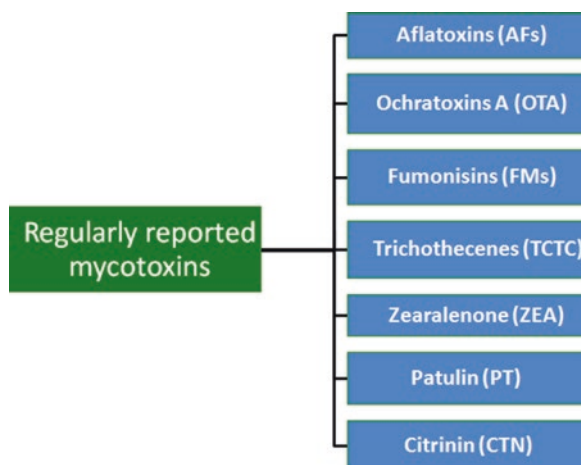




Fig. 15.1 Major mycotoxins producing species

with significant economic pressure on agriculture (Mitchell et al. 2016; Ostry et al. 2017).

15.2.1 Aflatoxins (AFs)

Aflatoxin compounds are produced primarily by *Aspergillus* spp. and classified into four groups as Aflatoxins B1, B2, G1, and G2, where the most potent toxin is Aflatoxins B1. It has been documented that *Aspergillus parasiticus* produces all four of these AFs while *Aspergillus flavus* produces only Aflatoxins B. After an outbreak of Turkey-X-disease in England, the discovery of AFs happened in 1960. Bennett and Klich (2003) identified AFs as an acute and chronic potent toxic to animals and humans; cause liver and kidney dysfunction, induce mutagenic, carcinogenic, and immunosuppressive effects (Bennett and Klich 2003; Aiko and Mehta 2015). Aflatoxins have been recorded for thousands of human deaths each year in combination with hepatitis B virus, which occurs mostly in nonindustrialized

tropical countries due to poor infrastructure and lack of sophisticated equipment to lower food AFs levels.

In 1975, Krishnamachan reported a hepatitis outbreak occurred 1974 that affected 400 Indians, 100 of whom died. With regard to this outbreak, maize was tracked and found to be heavily infected with *A. flavus* up to 15 mg/kg AF; equated to 2–6 mg daily intake by some of the adults affected. People are much more likely to develop liver cancer in areas where AFs and hepatitis B are prevalent (Campbell 1983). The number of deaths from liver cancer due to AFs in Indonesia have been estimated to reach 20,000 per year, according to Lubulwa and Davis (1994). The signs of human acute aflatoxicosis include nausea, stomach pain, pulmonary, and cerebral edema, coma, seizures, and even death (Mwanda et al. 2005). Symptoms of gastrointestinal dysfunction have been reported in animals, reduced reproduction, reduced conversion and efficiency of feed, reduced milk and egg production, and anemia (Pier 1992). Increasing proinflammatory cytokine IFN- γ and reducing anti-inflammatory cytokine IL-4 are the mechanisms of AF toxicity described by Qian et al. (2014). Mehrzad et al. (2014) documented interference with nucleotide excision repair, gene methylation modification, and DNA mutations.

15.2.2 *Ochratoxins A (OTA)*

Ochratoxins are originally produced by *A. ochraceus*; however, *Penicillium viridicatum* has also been reported to produce with three OTA groups namely ochratoxins A, B, and C. The most prevalent toxin is ochratoxin. Heenan et al. (1998) and Magnoli et al. (2007) described *A. carbonarius* as a common producer of OTA widespread in tropical foods, particularly in cereals, coffee, bean feed, wine, grape juice, and sun-drying survival, especially in dried fruit. Ostry et al. (2017) identified coffees and wines among all potential sources of OTA as major contributors to OTA intake. OTA may also contaminate items derived from animals, such as eggs, poultry, animal milk, and human milk, according to Stoev (2013). Due to its tolerance in thermal processing and high stability in acidic environments, OTA can be found under normal cooking conditions in cereal products and roasted coffee (Bennett and Klich 2003; Hussein and Brasel 2001).

Mantle (2002) reported that OTA caused immunotoxicity, genotoxicity, neurotoxicity, teratogenicity, and embryo toxicity; impacts animal productivity by reducing the conversion of feed and body weight gain and reducing the production of eggs in laying hens (Heussner and Bingle 2015). OTA also inhibits the synthesis of RNA and DNA; it accumulates in animal tissue, particularly pigs because of its fat solubility (Stoev et al. 2002). The Food and Drug Administration of the United States has not yet defined controlling limit for OTA. Nevertheless, the European Union has set limits of 5–50 ppb in numerous products (EC.No.1881/2006). OTA's reported toxicity mechanism (Walker et al. 2005) is production of genotoxin by reactive oxygen species. Wangikar et al. (2005) documented teratogenic activity that induced congenital fetal deficiencies and histopathological changes. Jennings

et al. (2012) identified the mechanism for inhibiting the erythroid 2-like nuclear factor, which is the main response to oxidative stress.

15.2.3 *Fumonisin*s (FMs)

Fumonisin)s are mainly developed by *Fusarium moniliforme*, *F. Proliferatum*, *F. verticillioides*, and other closely related species, widespread in maize, make it a major source of FMs compounds. In the United States, approximately 80% of all harvested maize was infected by *F. verticillioides*. Other potential substrates of FMs are barley, sorghum, black tea, rice, soybean, figs, and herbal plants (Bennett and Klich 2003). There are four classes of FMs, namely A, B, C, and P (Rheeder et al. 2002). FMs B have Fumonisin B1 (FB1), Fumonisin B2 (FB2), and Fumonisin B3 (FB3) subgroups. FB1 predominates in maize kernels and accounts for 70–80% of the total FMs family. In China, 98.1% of the maize product samples collected from the province of Shandong in 2014 were reported to contain FB1, FB2, and FB3 (Li et al. 2015). According to Turner et al. (1999), FB1 exposure varies broadly depending on climate, processing technologies, and frequent of consuming of maize-based diet. Müller et al. (2012) reported low concentrations of FB1 in maize-based food in the western countries like Germany due to industrialized food production, which appears to constitute an effective means for preventing and mitigating its contamination.

In contrast, maize grown in South America, the Far East, and Africa is more frequently infected by fumonisin-producing fungi due to unfavorable climate as well as inappropriate cultivation and storage conditions, resulting in high levels of FB1 in food. Esophageal cancer, infant growth retardation, neural tube deficits in fetal development, and cardiovascular effects are correlated with FMs use. In southern Africa, an outbreak of esophageal cancer has been identified with area with high incidence of FMs infected maize, which is regularly consumed in that locality as the primary staple food. For parts of China, the same claim was made (Chu and Li 1994). There has not yet been a fundamental relationship; however, there are other factors that play a role in the disease etiology. When infected corn was fed to pigs, FMs caused pulmonary edema. The toxicity mechanism of FBs is inhibition of the enzyme ceramide synthase modulating sphinganine acylation and sphingosine recycling (Solfrizzo et al. 2004).

15.2.4 *Trichothecenes* (TCTC)

Trichothecenes are metabolites that are mostly formed by species of *Fusarium*; where *F. graminearum* is an important source that is endemic in cereal grains including oats, wheat, barley, maize, rye, and rice (Yazar and Omurtag 2008). In addition, sunflower seeds, soybeans, peanuts, bananas, and potatoes have been

stated to be the source of TCTC (Bennett and Klich 2003). Deoxynivalenol (DON) is widespread and well-studied toxin among TCTCs; cereals have been documented to be mostly contaminated in many countries (Yoshizawa 1991). TCTC is quite toxic quickly embrace lipid bilayers of cell membrane to interact with nucleic acids and cell organelles (Wannemacher et al. 1991). The effects posed in humans and animals include retarded growth, reduced cattle milk production, refusal of feed, gastrointestinal bleeding, immunocompromise, and decrease egg production in hen (Pestka 2007). DON-contaminated grains in humans have reported fever, nausea, abdominal pain, dizziness, vomiting, and diarrhea (Wan et al. 2020). The main mechanism of TCTC toxicity, according to Richard (2007), is inhibition of ribosomal protein synthesis, followed by DNA destruction and embarrassment of RNA synthesis. In 1988, TCTC was reported to be responsible for a large-scale human toxicosis in India; China, Japan, and Korea also reported the same effects. The advisory standard of the US FDA has defined the DON level of 1 ppm for wheat product such as bran and flour that can be used up by humans. Feinberg and McLaughlin (1989) identified the toxicity mechanism of DON by inhibiting RNA and DNA synthesis. It was identified by Girgis et al. (2010) as a reduction of TNF- α with immune implications in plasma. Other studies explained protein biosynthesis inhibition and alteration of the proinflammatory cytokine production as potential DON toxicity mechanism.

15.2.5 Zearalenone (ZEA)

Zearalenone (ZEA) is mainly produced by *F. graminearum* and *F. semitectum* in high humidity and low-temperature conditions (Bhatnagar et al. 2002). *F. graminearum* contaminated maize, barley, and wheat grains cause genital problems in domestic animals, particularly pigs; reproductive disorders have also been reported in sows, including testing infertility, abortion and atrophy, reduced libido, and mammary gland hypertrophy in male pigs (Marasas et al. 1984). In the United States and Canada, maize and wheat are more frequently contaminated with ZEA, while in European countries, wheat, rye, and oats are reported as major sources of ZEA (Hussein and Brasel 2001). ZEA concentrations reported low in field-contaminated grain, but increased with moisture above 30–40% under storage conditions (Gupta 2007). Bennett and Klich (2003) pinpointed ZEA as estrogenic mycotoxins due to its structural resemblance to natural estrogen-causing apparent estrogenic effects in humans and animals. The interference occurs concurrently with DON (Yazar and Omurtag 2008) and less often with AFs. It is stable and normal temperatures of cooking and partly eliminated at high temperatures (Castelo et al. 1998). The maximum levels of ZEA in different food commodities have been regulated by the European Committee (EC No 1126/2007); (Richard 2007). However, till to date, there have been no ZEA advisory levels set by the US FDA.

15.2.6 *Patulin (PT)*

Patulin was originally isolated from *Penicillium claviforme*. Bennett and Klich (2003); Drusch and Ragab (2003), reported that *P. expansum* is a major producer of PT; primarily contaminating apples and apple products, pears, peaches, and grapes (Moake et al. 2005; Yang et al. 2014). Subsequent research has shown human toxicity to PT, including nausea, vomiting, ulceration, and hemorrhage (Puel et al. 2010); seizures, lung inflammation, degeneration of epithelial cells, carcinogenic, genotoxic, immunosuppressive, and teratogenic (Sant'Ana et al. 2008). The toxicity mechanism of PAT as identified by Glaser and Stopper (2012) is due to cross-linking structural DNA damage and consequent formation of micronucleus. Wu et al. (2008) identified the toxicity mechanism through the interaction of macromolecular sulfhydryl groups, and the production of reactive oxygen species triggering the apoptotic process. Liu et al. (2006) documented activation of the p38 kinase signaling pathway leading to cell death; and enterocyte cytotoxicity and increased permeability and ion transport in intact intestinal mucosae as explained by Mohan et al. (2012). The patulin exposure cause death of rats with 55 mg /kg body weight (LD50) (Saleh et al. 2019). The U.S. FDA Committee set the limit total of PT to be 50 ppb for food meant for human.

15.2.7 *Citrinin*

Citrinin is one of the mycotoxins firstly isolated from a *Penicillium citrinum* culture. Nevertheless, this mycotoxin has also been produced by several other fungal species within the three genera, *Penicillium* (*P. expansum*, *P. verrucosum*), *Aspergillus* (*A. terreus*), and *Monascus* (*M. ruber*). It typically co-occurs with OTA, contaminating cereal grains, including wheat, barley, oats, maize, and rice. Peanuts and fruits are also contaminated with Citrinin. The neck is the main target organ of citrinin toxicity, although other target organs such as the liver and bone marrow have been reported as well (Tola and Kebede 2016).

15.3 Occurrence of Mycotoxin

The pervasive presence of fungi makes food crops vulnerable to fungal contamination during preharvest and postharvest conditions (Aiko and Mehta 2015). Mycotoxins can be produced during the growth and processing of crops and are chemically and thermally stable, thus they are carried over into processed foods. *Fusarium species* producing fumonisins are dominant in the field where water activity (aw value) is not a limiting factor for the majority of the growing period. *Aspergillus species*, on the other hand, which produce aflatoxins, are more xerophilic

and are usually “storage fungi” due to the low-moisture content of grains during processing (Gnonlonfin et al. 2013). Preharvest contamination of crops with mycotoxin may be caused by environmental factors such as high temperature, humidity, drought, and heavy rainfall. Such conditions usually occur in different African countries. Delayed harvesting and intercropping are agricultural practices linked to the contamination of mycotoxins (Gnonlonfin et al. 2013); crops which grow year after year in the same field increase the risk of contamination with toxins. Harvested maize grains in tropics may already contain spores of fungi such as *Fusarium*, *Aspergillus*, and *Penicillium*, which may grow and compete for food under ideal environmental conditions (Gnonlonfin et al. 2013). *Fusarium* head blight is a significant fungal infection of several crops of cereals, including wheat, barley, maize, oat, rye, and triticale. *Fusarium* head blight causes significant loss of yield, decreases grain value, and contaminates the grain with fungal mycotoxins that are detrimental to the health of humans, animals, and ecosystems. One of the major sources of mycotoxin infection in malting barley is *Fusarium* head blight (Spanic et al. 2018). An emerging method to control *Fusarium* head blight disease and mycotoxin contamination in cereals is RNA interference (RNAi) (Machado et al. 2017). In growing-stressed plants, fungal invasion and mycotoxin development are highly marked for seeds. Drought, insect disruption, and irrigation timing are the most commonly recognized crop stressors. The field-infected crops increase the possibility of post-harvest and storage fungal growth. Poor hygienic conditions tend to stimulate fungal growth and infection during storage, transportation, and processing facilities.

The aflatoxin is the most well-known active mycotoxin. It has been documented that the toxin can be transported from contaminated soil to the fruit in apparently healthy, intact seeds (Alegbeleye et al. 2018). Aflatoxins may be present in all cereal crops (Tola and Kebede 2016). Aflatoxin B1 can be transferred to the stems and leaves from the roots. If the soil microorganisms do not rapidly kill the aflatoxin found in the plowed under stove and grains, there is a risk that the seedlings’ roots of the plant of the following year will both consume and translocate the aflatoxins to the stems and leaves (Mertz et al. 1980). This could be hazardous to the plant’s growth and development as well as to the consumer’s health. It is produced by *A. flavus* and *A. parasiticus*. Aflatoxin-producing fungus grows widely in cereal crops, and oil seeds such as ground nuts, peanuts, pistachio nuts, walnuts, almonds, and cottons (Bennett and Klich 2003; Boevre et al. 2012). Such crops and many other products that are processed under inadequate temperature and humidity conditions are also affected (Jeswal and Kumar 2015). In addition, contamination may also pose an additional safety threat due to the potential transfer of mycotoxins to animal products such as meat, milk, and egg, contributing to consumption of mycotoxins (Richard 2007). Sometimes mycotoxins are found in animal feeds with ingredients such as oilseed cakes, peanut, cotton seeds, coconut cake, and maize grits. Many toxins can be metabolized if animals consume polluted feed and stay in milk, food, and eggs (Samuel and Adeyeye 2016). In AFB1-contaminated animals, milk may be infected with aflatoxin M1 (AFM1), a major biotransformed hydroxylated metabolite of AFB1 by hepatic microsomal cytochrome P450 (Bennett and Klich 2003). AFM1 in milk can be observed up to 24 hours after eating cow feed

contaminated with AFB1, and its concentration depends on the levels of AFB1 contamination in feed (Prandini et al. 2009). AFM1 can also be present in dairy products, including cheese with a higher concentration than raw milk, as AFM1 binds well with casein, heat stable, and is not influenced by the cheese production method (Govaris et al. 2002; Cavallarin et al. 2014). OTA was found in a number of foods/feeds, with levels in products used as feed ranging up to 27 ppm and levels in foods for human consumption ranging from approximately 100 ppb (Gupta 2007). OTA and citrinin levels in human food have been found to be much lower than in raw animal feed because citrinin is almost removed during processing and baking of human food and OTA is significantly reduced.

15.4 Phytotoxicity

Based on the remarkable inhibitory effect on chlorophyll and carotenoid synthesis, reduction of seed germination, seedling growth, and yield, the phytotoxic effects of mycotoxins in plants were observed. Inhibition of phases of plant elongation due to PAT effect followed by reduction of seed size, seed weight, number of flowers, and biomass gain was observed when PAT was applied to these cultivated plants in aqueous solution (Ismaiel and Papenbrock 2014). Aflatoxin has been suggested to prevent chlorophyll synthesis resulting in the infected plants becoming virescent or albinized. Citrinin reduced the bean plants' radical production. Some studies have suggested that cell death in plants is caused by OTA (Wang et al. 2011).

15.5 Mycotoxin Substrates

Plant products and animal products are substrates that allow the development of mycotoxins (Fig. 15.3). After the fungi are created, climate is the most important factor in determining contamination. Storage at high temperatures and humidity leads to significant contamination of mycotoxins.

15.5.1 Mycotoxin in Food

Mycotoxin-contaminated cereals, pulses, fruits, and oil seeds are common source of mycotoxicosis. OTA's major source is nuts and dried foods of various varieties. Grapes are highly affected by *Aspergillus carbonarius* producing OTA. Through using them for the manufacture of alcohol, high starch products contaminated with AFs can be saved. Beer production implies a variety of operations which might impact the initial level of mycotoxins in a positive or negative way. The complexity of these operations do not give to the brewer a complete control on chemical and

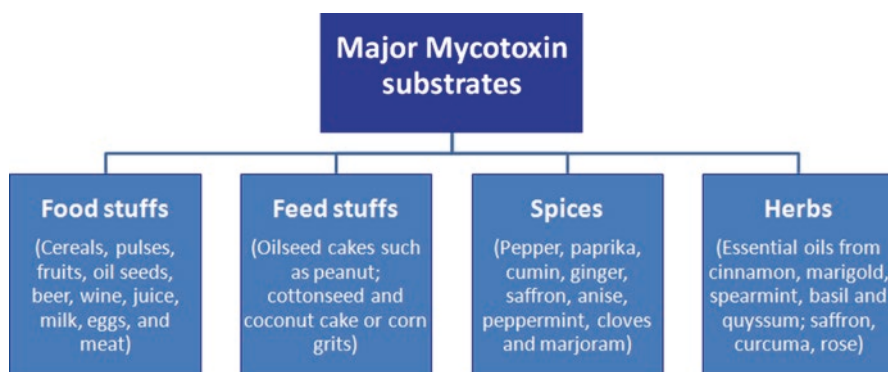


Fig. 15.3 Major mycotoxin substrates

biochemical reactions that take place in the batch, but the knowledge about mycotoxin properties can help in identifying the operations decreasing their level in food-stuffs and in the development of mitigation strategies. The operations that may lead to a decrease in mycotoxin load are found to be steeping, kilning, roasting, fermentation, and stabilization operations applied over the process (e.g., clarification). Also, other general decontamination strategies usually employed in food industry, such as hot water treatment of barley, ozonation, or even the use of lactic acid bacteria starter cultures during malting or fermentation are considered. Around 40% of AFs stated to be in the fraction of syrup (soluble for distillers) and 60% in the fraction of solids (Tola and Kebede 2016). Green coffee beans are prone to attack of *Aspergillus* species such as *Aspergillus carbonarius*, *A. niger*, *A. ochraceus*, and *A. westerdijkiae*, which are widely OTA producers in tropical and semitropical coffee plantations. Nowadays, it is estimated that coffee accounts for approximately 12% of the total intake of OTA, being considered the third source of OTA exposure. The inclusion of gamma radiation in the postharvest processing chain of green coffee beans could be a promising approach in order to not only eliminate OTA producer fungi but also the toxicity of performed OTA. Ozone and ultrasound could be applied as pretreatments before coffee fermentation, while high-pressure processing could be introduced in steps presenting high risk of microbiological hazards in the coffee industry without altering the manufacturing process (Leitão 2019). Bread made from barley or wheat may be a source of OTA.

Long-term exposure to mycotoxin significantly increases health risk, particularly in children and women, and this is thought to be related to nutritional deficiencies in minerals and vitamins resulting from diets predominantly based on contaminated cereal in their areas. High incidence of human liver cancer has been related to exposure to AFs in central Africa and parts of Southeast Asia. High intakes of AFs are causally associated with high incidence of cancer of the human liver (Groopman et al. 1988). Because maize-based food represents a significant part of the typical African diet (Turner et al. 1999), FB1 dietary intakes in rural areas can be as high as 354.9 lg/kg body weight per day due to consumption of moldy unprocessed maize;

compared to FB1 intakes of 1.2 lg/kg body weight per day in urban areas of South Africa, which is still considerably above estimates of exposure in Europe and North Africa. High levels of FB1 maize contamination were also found in rural parts of China (up to 41 mg/kg) where maize is commonly consumed and represents a significant portion of the staple diet, resulting in mean FB1 intakes of up to 740 lg/kg bw per day (Qiu and Liu 2001).

15.5.2 *Mycotoxin in Feed*

Animal feeds such as oilseed cakes, peanut, cotton seeds, coconut cake, and maize grits produce mycotoxins on a regular basis. Highest level of mycotoxins reported in concentrated animal feedstuffs. Cottonseed lipids and proteins promote the development of aflatoxins (Mellon et al. 2000). Many contaminants can be metabolized if animals consume infected feed and linger in milk, food, and eggs (Samuel and Adeyeye 2016). The ingestion of mycotoxin-contaminated feed increases exposure to infectious diseases, chronic infection reactivation, and diminished effectiveness of the vaccine. High mycotoxin concentrations may lead to acute symptoms, but low mycotoxin levels may have a long-term effect on animal performance. This affects the internal organs of animals such as liver, kidney, and intestinal tract, neurotransmitter and endocrine systems, and immune functions. In addition, animal consumption of mycotoxin-contaminated feed can cause reproductive failure, loss of appetite, decrease in egg and milk production, refusal of feed, decrease in feed quality, poor weight gain, immunosuppression, and mortality. According to Richard (2007), products derived from animals like milk, meat, and egg contribute to human consumption of mycotoxin as carry-over effects from contaminated food. A proportion (about 1.5%) of aflatoxins B1 and B2 are hydroxylated and excreted in the milk as aflatoxins M1 and M2. OTA is not readily excreted because of its fat solubility, which makes it accumulate in the fat depot of infected animals, and from there OTA is consumed by people eating pork (Krogh et al. 1974).

In England in the early months of 1960, the outbreak of Turkey-X-disease was attributed to the impact of aflatoxin traced to the feed, and more precisely to one ingredient in the feed, a peanut meal imported from Brazil. Ducklings in Kenya had the same problem. The resistance to the toxicity of mycotoxins varies among species of animals (Denli 2015). Swine is the most susceptible to mycotoxins among animal species. Certain species susceptible to the toxicity of mycotoxin include broilers, laying hens, chicken, and quails. Ruminants are more resistant to mycotoxins than other species due to their rumen flora being shielded from exposure to mycotoxins. Pigs are particularly susceptible to mycotoxins because of their high cereal intake (Pierron et al. 2016). TCTC toxin causes pigs to vomit and refuse to eat at levels close to 8 mg/kg of infected feed (Williams et al. 1989). Regulations and/or warnings in pig feed for AFs, OTA, FMs, ZEA, TCTC, DON, and toxin T-2 exist in the European Union. Contaminated feed management procedures and

approaches used to avoid the development of mycotoxins are not economically viable; binding agents (adsorbents) could be used to mitigate the deleterious effects of contaminated feed on animals (Denli 2015).

15.5.3 *Mycotoxin in Herbs and Spices*

Mycotoxin is a major herbal and spicy contaminant. Severe human health complications can result from the use of contaminated medicinal herbs (Kosalec et al. 2009; Posadzki et al. 2013). Mycotoxins cause a variety of kidney, liver, digestive tract, skin, respiratory organs, genital organs, and nervous system disorders. AFs, OTA, FMs, ZEA, and DON are the most frequently found mycotoxins in spices and medicinal herbs. Comparison research on the incidence of mycotoxin in medicinal herbs and spices from various areas of India indicated high black pepper and long pepper contamination with AFB1. Of the 150 specimens, 43% were infected with AFB1, 6% OTA, 6% citrinin, and 4% ZEA (Do et al. 2015). Certain studies showed that 14% of specimens of Indian medicinal herbs and spices were found to be infected with AFB1 and OTA. Nevertheless, herbal specimens of cinnamon, saffron, curcuma, rose, and lesser galangal have been found free of mycotoxin contamination; and all herbs collected from India have been found free of penicillic acid, ZEA, and toxinT-2 (Aziz et al. 1998). The incidence rate of aflatoxins contained in Chinese herbs is 15.5% in China (Liu et al. 2012). Highest levels of contamination shown in Guangxi province goods with aflatoxins in 27 infected samples were up to 290.8 µg/kg. In South Africa, up to 139 µg/kg of FB1 was found in 81% of traditional medicinal herb samples (Katerere et al. 2008). Twenty-five percent of red paprika samples in Morocco had higher aflatoxin levels (9.68 µg/kg) (Zinedine et al. 2006).

In Saudi Arabia, 20% of spices, such as anise, black cumin, black pepper, peppermint, and marjoram, were found to be up to 40 µg/kg contaminated with aflatoxins; while some red pepper, cumin, and marjoram samples were contaminated with sterigmatocystin up to 25 µg/kg. In Turkey, up to 3649 µg/kg (Karbancioglu-Guler and Heperkan 2009), 75% of dried fig samples were contaminated with extremely high FB1 levels. In Spain, 99% of samples were found to be infected with T2, 98% with ZEA, 96% with AF, 63% with OTA, 62% with DON, 61% with citrinin, and 13% with FB1. Up to 31 µg/kg aflatoxin levels have been found in ginger products in the United States, which are above national regulatory limits (Trucksess et al. 2007). White pepper, powdered black, and cardamom have been found to inhibit the development of *A. flavus* and *A. parasiticus* in several reports. Furthermore, essential oil extracted from medicinal herbs, including cinnamon, marigold, spearmint, basil, and quyssum, sufficiently inhibits fungal growth, such as *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, and *F. moniliforme* (Soliman and Badeaa 2002).

15.6 Exposure to Mycotoxin

Before birth, mycotoxin exposure begins. Blood testing has shown that 98% of Western Africans were susceptible to AFs. In Benin, for example, 99% of children had traces of AFs in their blood, with some of the highest levels of aflatoxin ever observed in humans. Mycotoxins designated to endanger the economy, human health, plant, and animal production of countries (World Health Organization 2006). It also hampers international trade, food and feed waste, resource diversion to science, compliance, control, and applications for reducing mycotoxin problems (Hussein and Brasel 2001; Stoev 2013). The consumption of mycotoxin-contaminated commodities is related to several acute and chronic diseases in humans as well as in animals. The acute diseases for which there is some evidence of an association with mycotoxins include: Aflatoxic hepatitis in India and Kenya; enteric ergotism in India; vascular ergotism in Ethiopia; and deoxynivalenol mycotoxicosis in India and China. A common feature in all these outbreaks has been the involvement of staple foods such as corn, wheat, or pearl millet, following unseasonable rains or drought during either the growing season or harvest (Jelinek et al. 1989). The severity of a mycotoxicosis can be increased by stresses like starvation and alcoholism. In turn, mycotoxicoses can heighten vulnerability to microbial diseases and worsen the effects of malnutrition (Bennett and Klich 2003). The major mycotoxins associated with human and veterinary diseases include AFs, citrinum, ergot alkaloids, FMs, OTA, PT, and the TCTC (Zain et al. 2011).

Mycotoxins use different mechanisms to exert their toxicity. For example, aflatoxins intercalate with DNA, fumonisins are a sphingolipid biosynthesis inhibitor, and trichothecenes interfere with protein synthesis (De Vries et al. 2002). Zearalenone has main effects of hyperestrogenism on reproduction. Clinical signs of hyperestrogenism include vulva swelling, increased uterine volume and secretion, hyperplasia and secretion of the mammary gland, excessive estrus, anestrus, increased incidence of pseudo-pregnancy, miscarriage, decreased libido, and secondary complications of rectal and vaginal prolapse, stillbirth, and small litters (Gupta 2007).

Figure 15.4 shows mycotoxin exposure effects in human and animal. Experimentally, fumonisins have been found to cause liver damage in multiple species including pigs, goats, cows, rabbits, and primates (Gumprecht et al. 1995; Osweiler et al. 1993) and species-specific target organ toxicity such as pig lung (Haschek et al. 1992), horse brain (Ross et al. 1993), rat kidney, rabbit and sheep, and esophagus in rats (Gumprecht et al. 1995). Epidemiological data have linked *F. verticillioides*-contaminated maize intake to human esophageal cancer (Rheeder et al. 1992). Oxidative stress is well known to lead to renal toxicity and carcinogenicity, resulting from increased production of reactive oxygen species (ROS) or compromised antioxidant defense mechanisms (Lock and Hard 2004). ROS can damage proteins, lipids, and DNA and has therefore been hypothesized to play a causative role in damage to DNA caused by FB1.

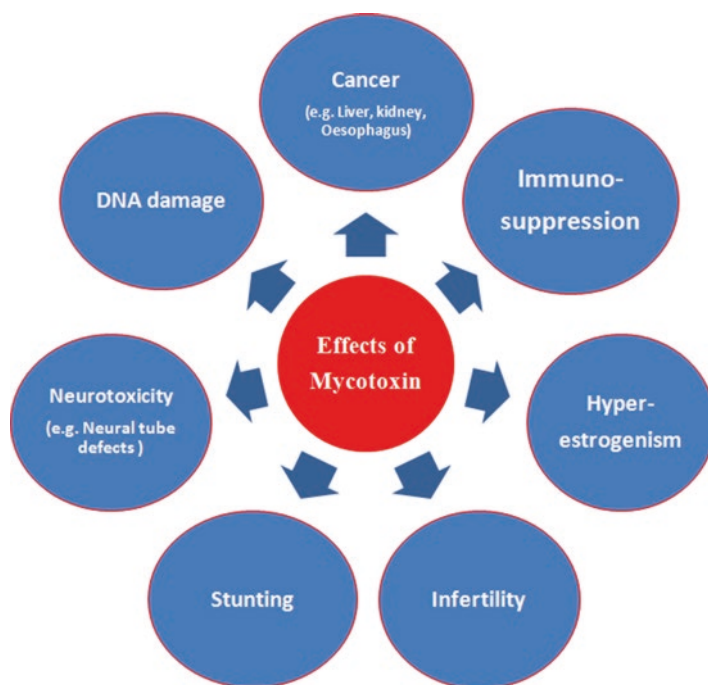


Fig. 15.4 Major mycotoxicoses in human and animal

Consumption of aflatoxin-contaminated diets is associated with high incidence of African liver cancer. In sub-Saharan Africa, up to 1 in 10 people are often infected with hepatitis B and C and, in combination with exposure to AFs, the risk of liver cancer is much more than ten-fold compared to exposure to both hepatitis alone (Gnonlonfin et al. 2013; Wagacha and Muthomi 2008). AFs are primarily an economic problem in the United States and the member States of the European Union, contributing to hundreds of cases of malignant hepatoma in the Asian and African developing countries (Mitchell et al. 2016; Liu and Wu 2010). Daily intake of AFB1 infected foods can lead to chronic aflatoxicosis with impaired growth and kwashi-orkor in infants, immune suppression, cancer, and reduced life expectancy (Gnonlonfin et al. 2013). Maize, together with other cereals such as sorghum, rice, wheat, and finger millet, is the major part of cereals used in complementary foods in Africa. Sadly, maize is at high risk of mycotoxin infection, such as AFs and FMs (Miller 2008). Kids in Northern Tanzania consuming maize-based supplemental foods are at risk of exposure to multiple mycotoxins (Kimanya et al. 2014). Contamination of fumonisin and aflatoxin can lead to childhood growth retardation and neural tube defects in the development of the fetus. In Burkina Faso, infant formulas from a mixture of several cereals (maize, millet, rice, or sorghum), leguminous (bean), oleaginous (peanut, soya, or sesame), sugar or salt, and sometimes milk powder, are consumed as food supplements after the weaning age with high

mycotoxin levels. The highest values for AFB1 (EU threshold: 0.1 mg/kg), OTA (EU threshold: 0.5 mg/kg), and FMs (EU threshold: 200 mg/kg) were 900, 6, and 3 times higher than the EU regulatory threshold (1881/2006); specimens from semi-industrial units and small craft industries tended to be the most affected by AFB1 (Ware et al. 2017). In terms of intake of these pollutants, children up to 12 years of age are known as a potentially vulnerable subgroup. Children have a different physiology than adults, apart from having a higher dose per kg body weight. They may therefore be more sensitive to neurotoxic, endocrine, and immune effects (Raiola et al. 2015). Research conducted to provide an overview of the prevalence of mycotoxin in Italian consumer commercial infant formulas and baby foods found that 31% of cereal-based baby foods had mycotoxin rates. Co-occurrence of various mycotoxins was also found in the same study. The number of positive samples indicating co-occurrence of multiple mycotoxins was 92%. They primarily contained around three and four mycotoxins (Juan et al. 2014). For oat-based infant cereals, co-occurrence of multiple mycotoxins was found most frequently followed by mixed grain cereals. While none of the cereals contain mycotoxin levels that met the U.S. regulatory limits, 21 samples exceeded EU mycotoxin limits including OTA, aflatoxin B1, and ZEN (Al-Taher et al. 2017).

The major contaminants contained in baby foods are *Fusarium* mycotoxins (mainly DON) in Tunisia; high levels of contamination have been reported, reaching ML in several instances. The results obtained showed that the 67% of the infant foods tested were infected and the highest Estimated Daily Intakes (EDI) values for DON were found (690 ng /g) (Oueslati et al. 2018). The study to assess the frequency of multimycotoxin in cereal and oilseed products most consumed in C^{ote} d'Ivoire showed that all peanut paste samples were infected with aflatoxin B1 with 99% above the EU limits of 2 mg/kg for AFB1 and 4 mg/kg for total aflatoxins (AFs: B1+B2+G1+G2) and concentrations of up to 4535 mg/kg (AFB1) and 8094 mg/kg (AFs). Samples of maize (96%) and rice (57%) were also infected with AFB1 at 58% and 24%, respectively EU limits and 80 mg/kg total for maize and 14 mg/kg for rice. Samples of peanut paste were the highest consumer health risk accompanied by samples of maize and rice (Manizan et al. 2018).

15.7 Mycotoxin Bioaccessibility

Bioaccessibility of mycotoxin is based on compound form, food product type, mode of contamination, and rate of contamination (Kabak et al. 2009). Thirty-two percent of ZEA intake in children reported release as a bioaccessible fraction from the food matrix and absorption in the intestinal tract (Avantaggiato et al. 2003). Bioaccessibility of peanut slurry AFB1 and buckwheat OTA was found to be around 94% and 100%, respectively (Carolien et al. 2005; Raiola et al. 2012). In children over 3 years of age, the bioaccessibility of PAT in apple products artificially contaminated at the EU regulatory level is the same as the limit set in adults at 50 mg/l in juices and 25 mg/kg in purees. Mean values of pulp juice bioaccessibility were

shown between 67.30% and 70.89%, purees showed mean values of 58.15% and 55.69%, while nectar and two clarified juice showed values of 38.88%, 28.59%, and 25.28%, respectively.

15.8 Detection of Mycotoxin

Because of their toxicity, total allowable levels of mycotoxins are restricted world-wide and tracking their presence in multiple products is mandatory to ensure food safety and health security for consumers. Sampling, homogenization, collection, cleanup, and finally identification and quantification are standard steps (Fig. 15.5) taken to assess levels of mycotoxin in food samples that may be carried out using instrumental or noninstrumental techniques (Whitaker 2003; Pereira et al. 2014; Shephard 2016). Chromatography, immunoassay, and rapid-on-site test are major techniques used in analysis of mycotoxin. There are also several other research methods with possible utility for studying mycotoxin. Such methods, however, were not widely used outside the context of the study. Recognized bodies such as AOAC, ISO, etc. need further confirmation and validation.

15.8.1 Sampling for Mycotoxin Detection

Sampling is a key step in the study, which contributes significantly to the accuracy of the tests and the final decision of either compliance or noncompliance for a whole food batch (Yao et al. 2015). Since mycotoxins are not distributed homogeneously in food, conventional food sampling methods are typically not appropriate for testing mycotoxins with exceptional liquid food samples such as milk or some highly processed foods such as peanut butter (Pereira et al. 2014; Ridgway and Scientific 2012). This uneven distribution of mycotoxins in food poses the difficulty of

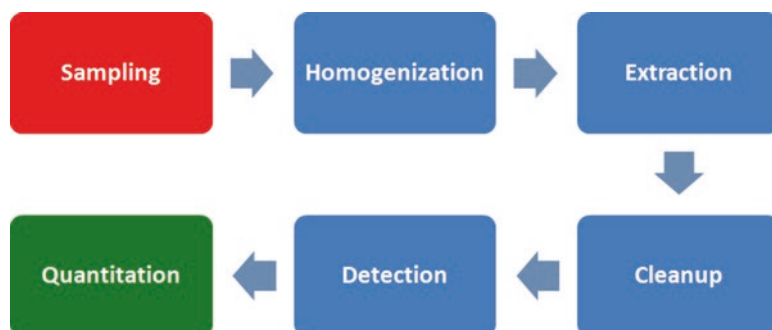


Fig. 15.5 Basic steps in analysis of mycotoxin

collecting true representative bulk specimens (Ridgway and Scientific 2012). Therefore, the sampling method must be carefully applied to ensure that the sample examined is a true representative of the entire bulk and to ensure that the findings are accurate (Pereira et al. 2014).

15.8.2 Extraction of Mycotoxin from Food Sample

During the study of mycotoxins (Krska et al. 2008; Shephard 2008), extensive sample preparation is required to extract the toxins from the food components. It is important to remove mycotoxins from solid food samples in a liquid phase before cleaning up processes to improve specificity and sensitivity of detection technique (Krska 1998). Selection of food sample extraction and cleanup methods for testing mycotoxins, typically controlled by mycotoxin chemical properties, the essence of the food components, and technique used for detection (Ridgway and Scientific 2012). Extraction of liquid-liquid is mostly used in samples of liquid foods; however, extraction of solid-liquid can also be used, particularly for cereal grains and other solids (Pereira et al. 2014). Many mycotoxins are hardly water-soluble but greatly resolvable in organic solvents including chloroform, ethyl acetate, dichloromethane, methanol, and acetone (Ridgway and Scientific 2012). Nevertheless, fumonisins are water-soluble and FB1 in a mixture of acetonitrile and water is greatly stable (Bennett and Klich 2003). Therefore, a mixture of organic solvents with water improves organic solvents penetration in the food components to increased efficiency of extraction (Krska 1998; Rahmani et al. 2009). Solvents like cyclohexane and hexane are used in food samples with high lipid content (Turner et al. 2009). Rapid solvent extraction, Supercritical fluid extraction, and microwave-assisted extraction are recent automated instrumental solvent extraction methods used in the study of mycotoxins (Pereira et al. 2014; Maragos and Busman 2010). These methods improve mycotoxin extraction, allow smaller, environmentally friendly amounts of chemical solvent, and provide better extraction efficiencies compared to conventional methods; however, they are very expensive (Kralj Cigic and Prosen 2009; Yadav 2020; Yadav et al. 2020d).

15.8.3 Cleanup of Mycotoxin Sample

Extract cleaning is an important process for eliminating interfering substances when mycotoxins are detected successively. Through cleaning the extract, the specificity, tolerance, reliability, and precision are improved (Turner et al. 2009). Cleanup methods used in mycotoxin analysis are multifunctional cleanup columns, immune affinity columns, ion-exchange columns, liquid-liquid separating, solid-phase extraction, and column chromatography (Pereira et al. 2014; Rahmani et al. 2009). Extraction by solid phase and immune affinity columns are the most widely used

techniques for the cleanup of mycotoxins, because they are quick, effective, reproducible, and secure and have a wide range of selectivity (Spanjer et al. 2008; Hu et al. 2016). In the technique of solid phase extraction, the specimen is run in the mobile phase solution and the stationary phase containing a solid adsorbent in which the mycotoxins are bound and then eluted on the basis of the unique partitioning (Rahmani et al. 2009). Column packing types are available commercially with various functional groups such as octyl, ethyl, octadecyl, phenyl, cyclohexyl, aminopropyl, and cyanopropyl. The use of these sorbents is based on the food components, solvent used, and mycotoxin's chemical composition (Pereira et al. 2014; Saez et al. 2004). For a particular mycotoxin, columns of immune affinity are filled by stimulated solid phase bound to a common antibody. When the analyte goes through the column, mycotoxin selectively binds to the antibodies, while other components of the matrix are extracted through the washing step; hence mycotoxin is elucidated with solvent (Pereira et al. 2014).

15.8.4 QuEChERS Technique in Mycotoxin Detection

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique was used to extract and cleaning up mycotoxins from different food components (Koesukwiwat et al. 2014). The technique was developed primarily in 2003 to analyze pesticides, adapt to obtain a varied range of analytes such as mycotoxins, acrylamide, polycyclic aromatic hydrocarbons, and aromatic amines (Anastassiades et al. 2003). Dispersive solid-phase extraction and acetonitrile in the existence of salts such as sodium chloride and magnesium sulfate are the two solvent-based extraction steps taken by QuEChERS (Yogendrarajah et al. 2013). Magnesium sulfate is typically used in the sample to remove water from the organic process, while sodium chloride is used to decrease the amount of polar interference. C18 and primary secondary amine such as silica and alumina are commonly used to extract lipids and sugar, organic acids, and certain colorants during the cleanup process (Ridgway and Scientific 2012). QuEChERS techniques have been revealed to be fast, cheap, and simple. It also uses less solvent than other methods (Pereira et al. 2014; Shephard 2016).

The determination of multimycotoxin is possible through the QuEChERS technique which significantly alleviated the errors associated with different matrix materials and resulted in different physicochemical properties due to the co-occurrence of several analyte mycotoxins. Furthermore, more than 30 mycotoxins can be studied simultaneously. Numerous mycotoxins have recently been identified in various food components, including cereal foodstuffs, milk, coffee, beer, and spices (Desmarchelier et al. 2014; Frenich et al. 2011; Zhou et al. 2016; Sun et al. 2016). This method has also been refined, validated, and implemented to analyze contamination from the local market in vegetable oils such as corn oil, palm oil, and sunflower oil using LC-MS/MS. Palm oil and corn oil found to be contaminated

with some mycotoxins. The highest levels of contamination are observed in palm oil (100%), followed by maize oil (43%), and sunflower oil (40%) (Sharmili et al. 2016).

15.8.5 Quantification Techniques of Mycotoxin

There are many methods that are used to detect and measure mycotoxin (Fig. 15.6). Some of the techniques are widely used due to their simplicity, flexibility, selectivity, precision, and reliability.

15.8.5.1 Chromatographic Techniques

Chromatographic methods are widely used for analysis of mycotoxin (Shephard 2016); aimed at the quantitative detection of mycotoxins by liquid chromatography (LC) or gas chromatography (GC) attached with fluorescence (FLD), ultraviolet (UV), or mass spectrometric (MS) detection. Thin layer chromatography (TLC) was the earliest approach usually used by visual assessment and instrumental densitometry as a fast screening tool for particular mycotoxins (Shephard 2008). Nevertheless, current trends in the study of mycotoxins in food, concentrate on the implementation of robust, simple, easy to use, and low-cost technologies that can detect and quantify several mycotoxins with great selectivity and sensitivity in one run (Rahmani et al. 2009). High-performance liquid chromatography (HPLC)

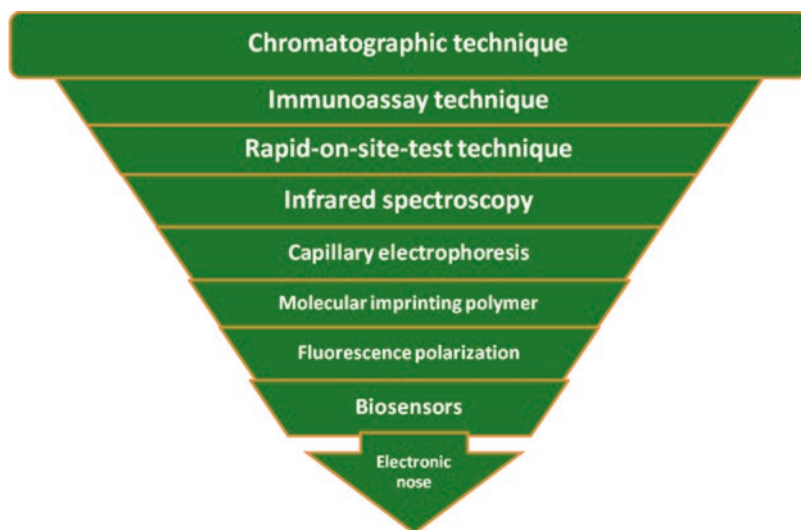


Fig. 15.6 Detection and quantification techniques with commonly used on the top narrowing down

(Krska et al. 2001; Gilbert and Anklam 2002); combined with diode array (DAD), ultraviolet (UV), mass spectrometry (MS), or fluorescence (FLD) detectors (Pereira et al. 2014); other chromatographic techniques are commonly used because of their low sensitivity and specificity (Pereira et al. 2014; Laura et al. 2016). In GC (Pereira et al. 2014), unstable mycotoxins such as patulin and TCTC are quantitated. However, the phase of derivatization in GC is often required because of the insignificant volatility and pronounced polarity of most mycotoxins, thus, makes GC techniques to be rarely used in mycotoxins analysis (Orata 2012).

HPLC-FLD technique for the quantification of mycotoxins in cereals (Pascale 2016) was adopted by the AOAC and the Standardization Committee of Europe. Sensitivity equivalent to others produced by LC coupled with MS can be obtained through this technique; however, HPLC coupled with FLD is usually best suited to single mycotoxin or multimycotoxins (Kong et al. 2013; Rahmani et al. 2013). A HPLC-FLD system has recently been used to simultaneously detect several OTA and AF mycotoxins in cereal products, ginger, ginseng, and peanut butter (Chan et al. 2004; Trucksess et al. 2008); OTA, ZEA, and AFs in cereals such as rice, rye (Gobel and Lusky 2004; Rahmani et al. 2010); and AFs, ZEA, OTA, and DON in maize (Ofitserova et al. 2009). Over recent years, LC-MS/MS has emerged as the preferred method for multiresidue analysis of mycotoxins (Schenzel et al. 2010) or pesticides (Zhao et al. 2014) since the adoption of the QuEChERS sample preparation process (Anastassiades et al. 2003). Determination of multiclass mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, ZEN, DON, FB1, FB2, FB3, and T2) was developed in a variety of herbal samples using LC-MS/MS methods; different sample preparation methods were compared and optimized to obtain satisfactory mycotoxin extraction due to the complexity of herbal matrices. The analytes were successfully determined in all herbal species using the immune-affinity column method with additional QuEChERS extraction.

Reasonable recovery and sensitivity was achieved under optimized conditions and no matrix effect was observed on the analytes (Cho et al. 2019). Despite the pronounced benefits of conventional HPLC techniques, MS technique provides numerous distinctive benefits above entire LC techniques in food study (Ahmad and Jae-Hyuk 2017). As an ideal confirmatory procedure, it offers information of chemical structure, greater selectivity, and sensitivity through the molecular distinctiveness of the extract centered on mass/charge (Liao et al. 2011; Li et al. 2013). MS detection reduces the time necessary for fluorescence enhancement by removing the necessity for inaccuracy of analyte derivation and cleanup phases (Shephard 2016). Various MS interfaces and analyzers were used, such as chemical photo-ionization of atmospheric pressure, electrospray ionization, and ionization of atmospheric pressure (Pereira et al. 2014). In addition, there are various categories of mass analyzers, including time-of-flight, quadrupole, ion-trap, Fourier transform-ion, and cyclotron resonance. In fact, MS technique can quantify more than 100 mycotoxins simultaneously in one sprint, makes it remarkable techniques for the detection of multimycotoxins in various foodstuffs, but the primary uses of MS were for the study of singular mycotoxins (Spanjer et al. 2008).

15.8.5.2 Immunoassay Technique

Due to simplicity and cheapness coupled to sensitivity and selectivity, immunoassays are preferably employed for the first level screening and survey studies on mycotoxin contamination. Immunoassays like ELISA (enzyme-linked immunosorbent assay) and fluorescence polarization immunoassay are among methods employed in this technique. ELISA's concept is built on associations between mycotoxins that deed as an antigen and a toxin-enzyme conjugate antibody apporioned to binding sites (Turner et al. 2009; Pittet 2005). The amount of antibody-bound toxin-enzyme conjugate in the analyzed sample (Yao et al. 2015) depends on the level of color production during research. ELISA is a quick, precise, and easy to use technique to analyze mycotoxins in food. It was used to conduct the study within a relatively short period of time. ELISA-based kits are available commercially for all regulated mycotoxins; and provide the most widely used analytical tool for food safety in the food chain (Pereira et al. 2014); by detecting and quantifying main mycotoxins comprising AFM1, AFs, OTA, DON, ZEA, T-2, and FM's toxins. ELISA's foremost shortcoming is that the package detects just individual specific mycotoxin and is intended for one-time usage, making it expensive to test multiple mycotoxins contaminated samples; practicable cross-reactivity and dependence on a specific material does not make the package ideal for wholly food materials and contamination levels (Yao et al. 2015). If ELISA kit is used in analyte that is not stated by the manufacturer, a suitable chromatographic technique will confirm the positive results (Shephard 2016). A comparative study of ELISA and HPLC found that ELISA appeared to underestimate OTA content compared to HPLC content (Matrella et al. 2006). Nonetheless, ELISA continues to be an invaluable tool to identify OTA on-site and rapidly. In addition, in the wine and wheat samples, underestimation and overestimation of OTA content were observed and attributed to the pH dependence of OTA's chemical stability in the cleanup phase and citrinin intervention in the immunoaffinity column cleanup process (Marquardt and Madhyastha 2018).

15.8.5.3 Rapid On-site Test Techniques

Rapid test strips are aimed for on-site detection and give results within a very short time. Price, tolerance, and reliability, however, tend to limit their applications in various matrices (Goryacheva et al. 2007). Such techniques can identify significant pollutants in food such as microbial contaminant, residues of veterinary medicines, allergens, pesticides, and mycotoxins (Krska and Molinelli 2009). The commercially available quick visual immunoassay strips for on-site mycotoxin screening comprise lateral flow devices, dipsticks, and flow-through devices (Pereira et al. 2014; Maragos and Busman 2010). Lateral flow is formed as a single phase experiment that comprises a sample line together with the same strip's negative control line. Lateral flows are commercially obtainable for the identification of DON, AFs, T-2 toxins, ZEA, and OTA and can produce semiquantitative results in less than

10 minutes (Pereira et al. 2014). It comprises plate, porous membrane, and absorbent pad (Maragos and Busman 2010). The experiment is based on a competitive immunoassay which uses a labeled antibody as a signal reagent (Krska and Molinelli 2009). To run quantitative tests, the lateral flow system was coupled with spectrometric readers (Zheng et al. 2006; Krska and Molinelli 2009). Dipstick analysis involves steps of preparation and incubation to get results similar to how ELISA operates, and usually takes more than 30 minutes to get results (Maragos and Busman 2010). Dipsticks are commercially available with a visual limit of 40–60 ng/g, primarily designed for the analysis of FB1 in maize-based foodstuffs (Schneider et al. 1995). Afterward, multianalyte dipstick immunoassay for the analysis of multiple mycotoxins was introduced; however, it has limited sensitivity. The available multiplex dipstick immunoassay is used for instantaneous detection ZEA, DON, T-2 toxins, and FMs in oats, wheat, and maize DON and ZEA in wheat (Molinelli et al. 2009), and ZEA and OTA in maize (Zheng et al. 2006). Flow-through membranes use similar approach as lateral flow devices; however, reliable results may not be obtained near the detection limit (Zheng et al. 2006; Krska and Molinelli 2009). Flow-through immunoassays technique (Sibanda et al. 2001; Sibanda et al. 2002) is used to test OTA in roasted and green coffee. Similar methods are also tested for AFB1 in nuts (Trullols et al. 2004) and ZEA in cereals and feedstuff samples (Urraca et al. 2005).

15.8.5.4 Infrared Spectroscopy

Incorporate infrared analyzers are paired with main component analysis to monitor and measure mycotoxins in the food sample without sample preparation. This technique detects cereal mycotoxins easily and nondestructively (Pettersson and Aberg 2003). IR spectroscopy was reported to detect DON contamination in maize and wheat (De Girolamo et al. 2009). For on-site analysis of DON and AFB1 in maize, wheat, and peanuts, portable incorporate infrared laser spectroscopy has been recently developed (Sieger et al. 2017). Incorporate infrared spectroscopy involves no additives, no sample preparation or processing, ease of operation, and very short-term results (Pettersson and Aberg 2003); however, more research still needs to be done to detect multiple mycotoxins. Both portable incorporate infrared laser spectroscopy and incorporate infrared spectroscopy are facing challenges of mycotoxin nonhomogeneity within the food materials, ground grain particle size dispersal, and detection limits (Shephard 2008).

15.8.5.5 Capillary Electrophoresis

Capillary electrophoresis (CE) is a method that uses fluorescence or UV absorbance to isolate various electrochemical potential components. This technique requires minor quantity of solvents and buffers and therefore, merely lesser quantities of waste are generated (Shephard 2008). Separate AFs, DON, FMs, OTA, and ZEA are

confirmed to be CE (Maragos and Appell 2007). Sensitivity is low, however, so only small quantities of specimens can be checked. CE, combined with laser-based fluorescence, has improved sensitivity to analyze AFs, OTA, and FB1 in coffee, sorghum, and maize at efficiencies similar to those obtained by certain chromatographic techniques (Corneli and Maragos 1998). Recently, ZEA research in maize with a detection limit of 5 ng/g (Maragos and Appell 2007) was reported in combination with cyclodextrin-enhanced fluorescence.

15.8.5.6 Molecular Imprinting Polymers

A molecular imprinting polymer (MIPs) is aimed to imitate natural recognition units like antibodies and biological receptors with specificities equivalent to those of antibody-antigen interactions (Giuseppe et al. 2011). It uses cross-linked polymers in the presence of an analyte, such as mycotoxins (Appell and Mueller 2016) that are electrochemically produced by the response of monomer and cross-linker. MIPs' main advantages are their high selectivity and sensitivity to the target molecule used in the printing process, their chemical strength, ease of preparation, and low cost (Haupt 2003). MIPs were planned for AFs (Appell and Mueller 2016), OTA (Jodlbauer et al. 2002; Zhou et al. 2004), DON, and ZEA (Weiss et al. 2003; Gambacorta et al. 2018) research. It has yet to be demonstrated and confirmed its applicability to food matrices, and also provides potential for further growth (Maier et al. 2004).

15.8.5.7 Fluorescence Polarization

Fluorescence polarization (FP) is a method commonly used for mycotoxin detection. It is a simple method that tests the interaction between fluorescently marked antigen and a particular antibody (Maragos 2006). This method basically calculates indirectly the frequency of spin in solution of a fluorophore that is straight correlated to the size of a particle. It thus enables the identification in solution of low-molecular weight constituents without needing a step to isolate the toxin (Maragos 2006). AFs (Sheng et al. 2014), OTA (Shim et al. 2004), DON (Galkina et al. 2019), FMs (Hendrickson et al. 2020), and ZEA (Liu et al. 2021) used this technique. Nevertheless, compared with HPLC (Maragos and Plattner 2002), it has less sensitivity and accuracy.

15.8.5.8 Biosensors

Biosensor is a measuring device which adds in a particular biological component (e.g., antibody) which produces an activity of biorecognition. It also has a physico-chemical component that transforms the recognition incident into an electro-chemical, optical, piezoelectric, or thermal signal (van der Bram et al. 2003). It is a

simple, accurate, and low-cost method to quantify mycotoxins in foods (Logrieco et al. 2005). Surface plasmon resonance, biosensor array, and fiber-optic probes are biosensor verities built and used for mycotoxin detection (van der Bram et al. 2003). Competitive surface biosensors identified ZEA, AFB1, OTA, DON, and FB1 in naturally contaminated matrices (van der Bram et al. 2003; Tüdös et al. 2003). Biosensor recycled use differentiates it from ELISA single-use kits and other rapid strip screening tests. Without significant loss of activity (Tüdös et al. 2003), DON immobilized surface biosensor chips can be reused more than 500 times. Biosensor procedures also require sample cleaning; and cannot perform multiple mycotoxin simultaneous analyzes (Logrieco et al. 2005; Yadav et al. 2020c).

15.8.5.9 Electronic Nose

An electronic nose (EN) is a modified GC that imitates the sensory olfactory system of humans and offers a nondestructive, quick and low-cost detection of mycotoxins in foodstuff (Keshri and Magan 2000). It involves number of chemical sensors with various specificities communicating with various volatile compounds. Such interactions produce signals that can be successfully used as a fingerprint of the unstable molecules from the samples analyzed (Feast 2001). Using pattern recognition method, odors can be detected and quantified after obtaining a fingerprint (Falasconi et al. 2005). Current EN methods for the detection of mycotoxins rely on detecting toxigenic fungi rather than detecting the mycotoxin itself. This method is basically suitable in distinguishing among toxigenic and nontoxigenic fungi (Keshri and Magan 2000; Olsson et al. 2002) and moldy and nonmoldy grains (Magan and Evans 2000). Few studies have documented its use in maize AF detection and grain DON (Olsson et al. 2002; Rastegari et al. 2020a, b).

15.9 Conclusion

Mycotoxin exposure is widespread worldwide due to trade globalization and starts before birth; endangers human health, development of plants and animals, and the economy of countries. Mycotoxin's bioaccessibility depends on the compound, the food product, the level of contamination, and the contamination method. Ubiquitous nature of the fungi makes food crops vulnerable during preharvest and postharvest conditions to fungal contamination. Thus, the problem of mycotoxin cannot be dealt with by regulation alone, but requires a comprehensive approach towards its identification and quantification. Combined and endless efforts by government, academia, farmers, food handlers, and the wider community are needed to counteract mycotoxin targeting its source, occurrence, exposure, impact, and sensitive detection techniques, in order to improve food safety.

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Chapter 16

Preservative Efficacy of Essential Oils Against Postharvested Fungi and Insects of Food Commodities – A Prospect to Go Green



Manindra Mohan, Shiv Shanker Gautam, S. Zafar Haider, Neha Sen, Sanjay Gupta, and Prashant Singh

16.1 Introduction

Essential oil (EO) is the concentrated hydrophobic volatile liquid which can be extracted from various parts of plants such as roots, shoots, barks, flowers, leaves, seeds, peel, fruits, and whole plant (Hammer et al. 1999; Sanchez et al. 2010; Baser and Demirci 2011). The major constituents of EOs are secondary metabolites that play an essential role in plant defense, as antimicrobial agents (Fraenkel 1959; Tajkarimi et al. 2010; Muthaiyan et al. 2012), antiparasitic (George et al. 2009; Albanese et al. 2009; Maggiore et al. 2012; Moazeni et al. 2012), insecticidal (Essam 2001; Kim et al. 2003; Ketoh et al. 2006; Singh et al. 2012), antiviral (Schnitzler et al. 2011), and antifungal properties (Haider et al. 2011; Kalemba and Kunicka 2003; Mohan et al. 2011; Silva et al. 2011; Tserennadmid et al. 2011; Vitoratos et al. 2013). EOs are a good source of different bioactive compounds like antioxidative, insecticidal and antimicrobial, and antiphlogistic and spasmolytic

M. Mohan

Uttarakhand Council for Biotechnology, Haldi, U.S. Nagar, Uttarakhand, India

S. S. Gautam (✉)

Department of Microbiology, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

S. Z. Haider

Centre for Aromatic Plants (CAP), Selaqui, Dehradun, Uttarakhand, India

N. Sen

Pal College of Nursing and Medical Sciences, Haldwani, Uttarakhand, India

S. Gupta

Department of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, India

P. Singh

Department of Chemistry, DAV (PG) College, Dehradun, Uttarakhand, India

properties. It has received attention as natural additives for the food products in place of synthetic preservatives (Tongnuanchan and Benjakul 2014). Consequently, EOs can serve as the alternative additives or processing aids as green technology (Singh and Yadav 2020).

Insect and fungal infections are the main cause of postharvest disease of fresh fruits, vegetables, and cereals during storage and transportation which causes significant economic losses in the commercialization phase (Gatto et al. 2011; Thakur et al. 2020). Infections caused during postharvest conditions lower the shelf life and adversely affect the market value of fruits (Tripathi and Kumar 2007). However, mycotoxins produced by molds have adverse effect on human and animals also (Zain 2011). The contamination of fruits and vegetables with mycotoxins not only caused health hazards but also resulted in economic losses, especially for exporting countries (Fernandez-Cruz et al. 2010). EOs obtained from plants have the potential to replace synthetic fungicides and insecticides in management of such postharvest diseases of fruits, vegetables, and cereals (Hyldgaard et al. 2012).

Biodeterioration of harvested and stored products is another chronic problem in tropical and subtropical countries which get aggravated due to hot and humid climate. The dominating genera of fungi proliferating on agricultural commodities are *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Rhizopus*, *Mucor*, *Armillaria*, *Phoma*, *Phialophora*, etc. that easily grow and produce toxic metabolites called as mycotoxins (Devi et al. 2020; Rastegari et al. 2020a, b; Singh et al. 2020). These fungi pose serious phytopathological and mycotoxicological risks at preharvest and postharvest stages (Logrieco et al. 2003). The purpose of this chapter is to provide an overview of current scenario of EOs as antifungal and insecticidal agent, its constituents, sources, and chemical nature, and its implementation as natural food preservatives in food commodities.

16.2 Sources and Chemical Nature of EOs

EOs are volatile in nature, carry a distinctive scent or essence, hydrophobic liquid and containing volatile aroma compounds from plants. They have the tendency to evaporate on exposure at ambient temperature. EOs are either secreted by plant protoplasm or by hydrolysis of some glycosides and other associated organs like oil tubes, parenchymal cells, and schizogenous passages. A variety of antimicrobial compounds are produced by plants. Some compounds are already present in plants while other produced in response to microbial invasion or physical injury of the plant (Roller 2003). The identification of most active antimicrobial compounds is difficult because EOs have complex mixture of constituents (Delaquis et al. 2002; Djenane et al. 2011; Espina et al. 2010). The composition of a particular EOs may vary depending on the season of harvest and the methods used to extract the oil from plant sources (Djenane et al. 2011; Nannapaneni et al. 2009; Combrinck et al. 2011; Demuner et al. 2011; Paibon et al. 2011). The constituent of EOs is a diverse family of low-molecular weight organic compounds with large differences in antimicrobial

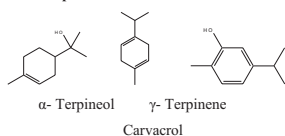
and insecticidal activity. The active compounds can be divided into four groups according to their chemical nature including terpenes, terpenoids, phenylpropanes, and others.

Several isoprene units (C_5H_8) combined to form terpenes. Terpenes are hydrocarbons, synthesized in the cytoplasm of plant cells via either the methylerythritol 4-phosphate (MEP) pathway or the mevalonate-dependent (MVA) pathway (Campolo et al. 2018). Terpenes have a hydrocarbon backbone which can be rearranged into cyclic structures through enzyme cyclases, forming monocyclic or bicyclic structures (Caballero et al. 2003). The main terpenes are monoterpenes ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$), but longer chains such as diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$) etc., also exist. Examples of terpenes include p-cymene, limonene, terpinene, sabinene, and pinene (Fig. 16.1).

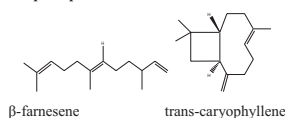
Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and remove methyl groups from terpenes (Caballero et al. 2003). Terpenoids can be subdivided on the basis of functional groups attached to benzene ring such as alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Examples of terpenoids are thymol, borneol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol. The antimicrobial activity of most terpenoids is based on their functional groups. Phenolic terpenoids have been shown the maximum antimicrobial activity due to hydroxyl group which has delocalized electrons. Phenylpropanoids are synthesized from the precursor of

1. Terpenoids

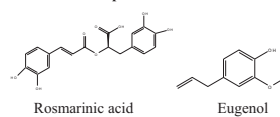
Monoterpenes



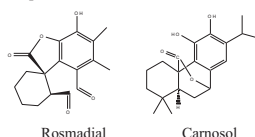
Sesquiterpenes



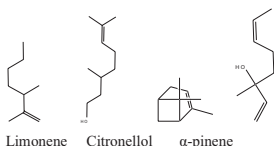
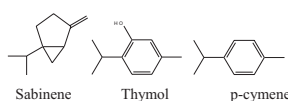
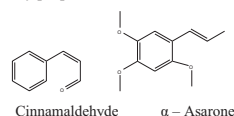
4. Phenolic compounds



Diterpenes

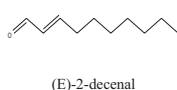


5. Phenylpropanoid

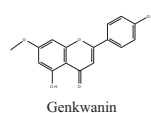


Linalool

2. Oxygenated derivatives



3. Flavonoids



7. Sulfur and nitrogen derivatives

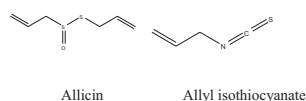


Fig.16.1 Major bioactive compounds of essential oils

phenylalanine in plants such as phenol and cinnamic acid. Examples of phenylpropanoids are eugenol, vanillin, safrole, and cinnamaldehyde.

EOs have a number of different degradation products which originates from unsaturated fatty acids, lactones, terpenes, glycosides, and sulfur-nitrogen-containing compounds (e.g., allicin and allyl isothiocyanate) (Caballero et al. 2003). Lemongrass oil was found to contain the highest concentration of citral with geraniol (42.5%) and neral (31.7%) in comparison to the other citral-rich oils derived from various oil-bearing plants (Combrinck et al. 2011).

16.3 EOs against Postharvested Fungi of Food Commodities

Fungi are the most prevalent microorganism of food commodities. Long-term storage and transportation provide enough duration for fungal spore germination and making its fruiting body. The major factor of food spoilage includes the occurrence of mycotoxins produced by certain fungi. EOs can be a useful alternative on behalf of synthetic fungicides for the management of such fungi during postharvest handling of fruit and vegetables (Gatto et al. 2011). The EO activities may vary with fungi due to variation in chemical constituents, its mode and site of action, and activity with mycotoxins. The development of natural crop protective products as alternatives to synthetic antifungal agent is currently in the spotlight (Combrinck et al. 2011) and with major problems related to fresh fruit vegetables and crops (Antunes and Cavaco 2010). The application of EOs in this context can turn to fulfill consumer requirements for more natural and healthy foods (Gachango et al. 2012; Martinez-Romero et al. 2008).

16.3.1 Mechanism of Antifungal Activity of EOs

EOs act on fungal cells with multiple means of mechanisms. The constituents of EOs may responsible for such antimicrobial or antifungal activity. Terpenes/terpenoids are lipophilic in nature which may cause disruption of cell membrane, inhibition of sporulation, and germination of food spoilage fungi (Nazzaro et al. 2017; Yadav et al. 2020a, b). On hyphal wall, EO causes thinning and disruption and finally flattens and empties hyphal tips divided into bud-like structure (Jing et al. 2014). In addition, cytoplasm leakage, inhibition of DNA, RNA, and protein synthesis, dysfunction of fungal mitochondria by inhibiting mitochondrial dehydrogenases, interaction with electron transport system (ETS), inhibiting its ATP synthesis, altered level of reaction oxygen species (ROS), etc. are key inhibitory role of EOs (Nazzaro et al. 2017). H⁺-ATPase plays an essential function of electrochemical proton gradient across cell membrane. Monoterpenes interact with such efflux pump by 70–90% and block the transportation (Ahmad et al. 2013). EOs exert a metabolic interference with biofilm formation and damage to its constituents

(Fig. 16.2). Therefore, the antifungal properties of EOs are the synergistic effects of its components and active substances can serve as additives in food commodities (Yadav 2020; Yadav et al. 2020c).

EOs can play significant role in day to day life and industrial fields. It is used in different sectors like personal care, flavor enhancer in foods and beverage, preservatives, antimicrobial agents, antioxidant, and others. Annually food commodities like fruits and vegetables loss cause major decay by fungi (Du Plooy et al. 2009). During pos-harvest periods, fruits deteriorate rapidly which result in the loss of weight, color change, and softening of fruits which are accompanied by occurrence of decay due to species of genera *Penicillium*, *Botrytis*, *Monilia* among other fungi. Currently, the control of fungal decay of fruits is prevented by use of pesticides and fungicides, i.e., prochloraz, guazatine, imazalil, thiabendazole, and pyrimethanil for treating fruit crops such as mango, avocado, and citrus. These are applied by passing freshly harvested fruit through large volume dip solutions. The chemical pesticides and fungicides can easily contaminate the soil, water, and vegetation and may cause of toxic to other animals like birds, fishes, insects, higher animals, and human. The use of EOs as natural antimicrobial agents can able to reduce or eliminate toxic pesticides and fungicides and to control bacterial and fungal pathogens also. EOs and their application as antifungal agents are represented in Table 16.1.

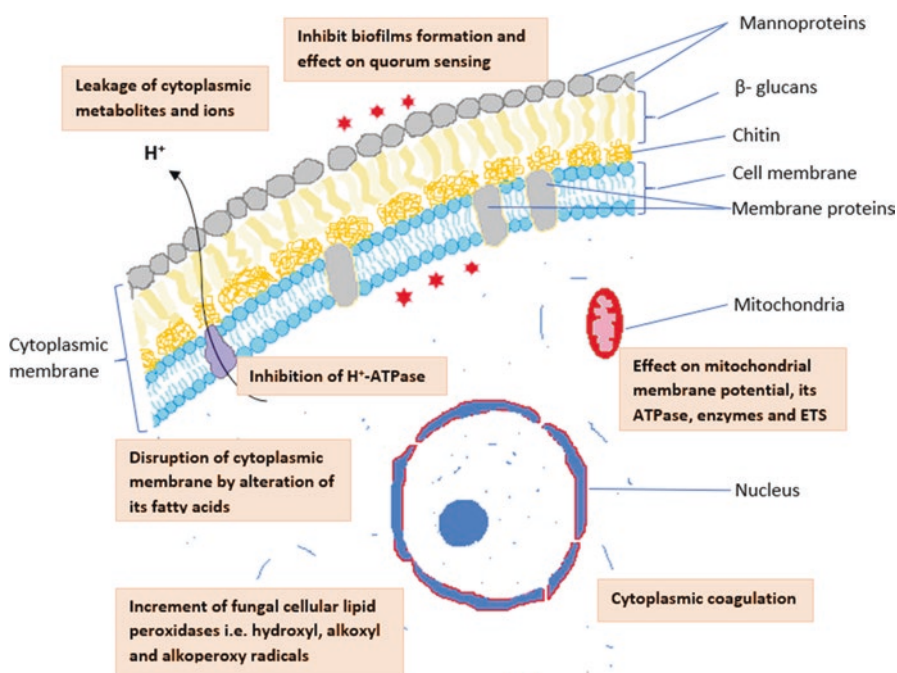


Fig.16.2 Fungal cell and site of action of essential oils

Table 16.1 Essential oils and their application as antifungal agents

Common name	Scientific name	Major constituent (%)	Model organisms	References
Basil	<i>Ocimum basilum</i>	Eugenol	<i>Alternaria alternata</i> , <i>Aspergillus fumigatus</i> , <i>A. niger</i> , <i>A. flavus</i> , <i>Curvularia lunata</i> , <i>Fusarium nivale</i> , <i>Fusarium oxysporum</i> , and <i>Penicillium italicum</i>	Du Plooy et al. (2009)
Camphor	<i>Cinnamomum camphora</i>	Terpenoids	<i>A. niger</i> , <i>A. flavus</i> , <i>Penicillium sp.</i>	Mahilraj et al. (2014)
Citronella	<i>Cymbopogon sp.</i>	–	<i>A. niger</i> , <i>A. flavus</i> , <i>Penicillium sp.</i>	Mahilraj et al. (2014)
Coriander	<i>Coriandrum sativum</i>	Linalool, (E)-2-decenal, decanol, (E)-2-decen-1-ol	<i>Candida sp.</i>	Silva et al. (2011); De et al. (1999)
Curry leaf	<i>Murraya koenigii</i>	α -pinene, sabinene, transcaryophyllene	<i>Candida albicans</i> , <i>C. parapsilosis</i> , <i>Aspergillus fumigatus</i> , and <i>Aspergillus niger</i>	Mohan et al. (2012)
Garlic	<i>Allium sativum</i>	Allicin	<i>Candida albicans</i>	Ankri and Mirelman (1999)
Ginger	<i>Zingiber officinale</i>	Camphene, Zingiberene, 1,8 cineole	<i>Aspergillus flavus</i> , <i>Penicillium expansum</i> , <i>Alternaria alternata</i> , <i>Fusarium oxysporum</i> , <i>Epidermophytonfloccosum</i> , <i>Microsporium gypseum</i>	Sharma et al. (2013); Tiwari et al. (2003)
Haines	<i>Nepeta hindostana</i>	β -farnesene, ageratochromene	<i>Aspergillus flavus</i> , <i>A. niger</i> , <i>A. ochraceus</i> , <i>A. terreus</i> , <i>Fusarium nivale</i> , <i>F. oxysporum</i>	Pandey et al. (2015)
Kaffir lime	<i>Citrus hystrix</i>	Citronellol, Limonene, Linalool	<i>A. flavus</i> , <i>A. parasiticus</i>	Rammanee and Hongpattarakere (2011)
Tansy	<i>Tanacetum dolichophyllum</i>	–	<i>C. albicans</i>	Häider et al. (2011)
Lime	<i>Citrus aurantifolia</i>	Limonene, p-cymene	<i>A. flavus</i>	Rammanee and Hongpattarakere (2011)
Rosemary	<i>Rosmarinus officinalis</i>	Carnosic acid, Carnosol, Rosmadial, Genkwanin, Rosmarinic acid	<i>C. albicans</i> , <i>Saccharomyces cerevisiae</i>	Panizzi et al. (1993)

Common name	Scientific name	Major constituent (%)	Model organisms	References
Savory	<i>Satureja Montana</i>	Thymol, p-cymene, linalool, carvacrol	<i>C. albicans, S. cerevisiae</i>	Panizzi et al. (1993); De Oliveira et al. (2011)
Tejpat	<i>Cinnamomum tamala</i>	Cinnamaldehyde	<i>Candida albicans, C. parapsilosis, Aspergillus fumigatus</i> , and <i>A. niger</i>	Mohan et al. (2012)
Thyme	<i>Thymus vulgaris</i>	Thymol, p-cymene, carvacrol, γ -terpinene	<i>A. flavus, C. albicans, S. cerevisiae</i>	Panizzi et al. (1993); Nguefack et al. (2004); Kumar et al. (2008)
Cotton Weed	<i>Gnaphalium affine</i>	Eugenol, linalool, trans caryophyllene, a-terpineol, p-cymene	<i>Saccharomyces cerevisiae, Aspergillus niger, A. flavus, Penicillium citrinum, Rhizopus oryzae</i>	Zeng et al. (2011)
Thin Sphallerocarpus	<i>Sphallerocarpus gracilis</i>	a-Asarone, γ -terpinene, p-cymene	<i>Aspergillus niger</i>	Gao et al. (2011)
Marshmallow	<i>Althaea officinalis</i>	Palmitic acid, heptacosane, nonacosane, linoleic acid, naphthalene decahydro 2,6-dimethyl	<i>Aspergillus niger</i>	Gautam et al. (2015)

16.4 EOs and Its Constituents against Harmful Insects of Food Commodities

EOs and its constituents are promising alternatives to chemical insecticides as natural insect pest control agents. They are low-toxic, eco-friendly alternative to synthetic repellents, and insecticidal against harmful insects of food commodities. They play an important role in protecting stored cereals and pulses from insects (Kumar et al. 2008; Raja et al. 2001; Ko et al. 2009). Above 2000 plant species have been studied for exhibiting the insecticidal activity with antifeedant, repellent, and insecticidal compounds (El-Sabrout et al. 2018). In Mexico, 114 species of 36 families had been identified between 1996 and 2018 with biological effects on 20 insect species and 7 nematode species (Hernandez-Carlos and Gamboa-Angulo 2019). The major bioactive compounds with insecticidal and nematocidal activity included alkaloids, terpenes, flavonoids, stilbenes, coumarin, ketone, and fatty acids (Hernandez-Carlos and Gamboa-Angulo 2019). The best-known neurotoxins among monoterpenes are the pyrethroids obtained from the leaves and flowers of certain *Chrysanthemum* spp. (Harborne 1993). EOs obtained from *Acorus*, *Artemisia*, *Curcuma*, *Cinnamon*, *Evodia*, *Foeniculum*, *Hyptis*, *Ocimum*, *Putranjiva*, *Syzygium*, and *Vitex* are known for their insect controlling properties. The EOs of *Ammomum villosum* containing four major compounds, i.e., bornyl acetate, camphor, camphene, and limonene have contact toxicity and high repellent activity against *Tribolium castaneum* and *Lasioderma serricorne*. The high concentration for repellent activity was 78.63 nL/cm² (Chen et al. 2018). The toxic properties of EOs against stored product insects are summarized in Table 16.2.

The mechanism behind insect control may be included with respect to oviposition, testes growth, and development, induces changes in genetic material (DNA and RNA), repellent, insecticidal, pupicidal, larvicidal, ovicidal, and insecticidal properties, etc. (El-Sabrout et al. 2018). The EOs enter the insect body by inhalation, ingestion, or skin absorption and interact with its physiology and nervous system (Mikhael 2011; Mann and Kaufman 2012). The nanoformulation of EOs is a new approach for insect pest control as biopesticides (Ibrahim 2019). The nanoencapsulated oils have higher penetration to insect tissues and insecticidal activity. The chief target cells of the pests include epithelial and endothelial cells which further can act on nervous and blood vascular system. Certainly, Eos-based insecticides are eco-friendly and natural with favorable mammalian toxicity.

16.5 Conclusions and Future Directives

No doubt EOs have enormous bioactive properties. They have a versatile preventive repellent, ovicidal, pupicidal, and larvicidal agent claiming its sustainability for control of insect pests. An attractive application of EOs and their constituents are in food products, i.e., to prolong the shelf life of foods by limiting growth or survival

Table 16.2 Essential oils and their applications as insecticidal agents

Properties	Plants sources/EO compounds	Organisms (Insect)	References
Repellent	<i>Cinnamomum zeylanicum</i>	<i>Callosobruchus chinensis</i>	Behal (1991); Tiwarei et al. (1998)
	<i>Citrus sinensis</i>	<i>Zabrotes subfasciatus</i>	Dawit and Bekelle (2010)
	<i>Cupressus sempervirens</i> , <i>Eucalyptus saligna</i>	<i>S. zeamais</i> , <i>Tribolium castaneum</i>	Tapondjou et al. (2005)
	<i>Eucalyptus nicholii</i> , <i>E. codonocarpa</i> , <i>E. blakelyi</i> , <i>Callistemon sieberi</i> , <i>Melaleuca fulgens</i> , <i>M. armillaris</i>	<i>Sitophilus oryzae</i> , <i>Tribolium castaneum</i> , <i>Rhizopertha dominica</i>	Lee et al. (2004)
	<i>Evodia rutaecarpa</i>	<i>T. castaneum</i> , <i>S. zeamais</i>	Liu and Ho (1999)
	<i>Foeniculum vulgare</i>	<i>T. castaneum</i>	Shukla et al. (1989)
	<i>Lippia cubeba</i>	<i>S. zeamais</i> and <i>T. castaneum</i>	Ko et al. (2009)
	<i>Ocimum kilimandscharicum</i>	<i>Sitophilus zeamias</i>	Bekele et al. (1995)
	<i>O. basilicum</i> , <i>O. gratissimum</i>	<i>Callosobruchus maculatus</i>	Keita et al. (2001)
	<i>Plectranthus grandifolius</i>	<i>C. maculatus</i>	Mikolo et al. (2009)
	<i>Putranjiva roxburghii</i>	<i>Trogoderma granarium</i>	Tripathi and Kumar, (2007)
	<i>Tagetes erecta</i>	<i>T. castaneum</i>	Ray et al. (2000)
Insecticidal	<i>Allium sativum</i>	<i>T. castaneum</i> , <i>S. zeamais</i>	Ho et al. (1996)
	<i>Cymbopogon schoenanthus</i> , <i>O. shave</i> , <i>Piper guinuse</i> , <i>Syzygium aromaticum</i> , <i>Xylopia aethiopica</i>	<i>C. maculatus</i>	Ketoh et al. (2006); Olonisakin et al. (2007)
	<i>Elletaria cardamomum</i>	<i>S. zeamais</i> , <i>Tribolium castaneum</i>	Huang et al. (2000)
	<i>S. aromaticum</i>	<i>Sitophilus oryzae</i> , <i>Rhizopertha dominica</i>	Sighamony et al. (1986)

(continued)

Table 16.2 (continued)

Properties	Plants sources/EO compounds	Organisms (Insect)	References
Fumigant	l-carvone	<i>Rhizopertha dominica</i>	Tripathi et al. (2003)
	Terpinen-4-ol, carvacrol, γ -terpinene, thymol, linalool, <i>R</i> -(+)-limonene, and geraniol	<i>Acanthoscelides obtectus</i> , <i>Tribolium confusum</i> , <i>Ephestia kuehniella</i>	Papachristos et al. (2004); Erler (2005); Stamopoulos et al. (2007)
	<i>Artemisia annua</i>	<i>Tribolium castaneum</i>	Tripathi et al. (2001)
	<i>A. sieberi</i>	<i>Tribolium castaneum</i>	Negahban et al. (2007)
	<i>Acorus calamus</i> , <i>A. sativum</i> , <i>Artemisia</i> spp., <i>Chenopodium ambrosioides</i> , <i>Curcuma longa</i> , <i>Mentha</i> spp., <i>Ocimum</i> spp., <i>Rosmarinus officinalis</i> , <i>Foeniculum vulgare</i> , <i>Citrus</i> spp., <i>Azadirachta indica</i> , <i>Cuminum cyminum</i> , <i>Elettaria cardamomum</i> , <i>Eruca sativa</i> , <i>Pimpinella anisum</i> , <i>Piper chaba</i> , <i>Evodia rutaecarpa</i> , <i>C. aromaticum</i> , <i>Lavandula hybrida</i> , <i>E. globulus</i> , <i>Thujopsis dolabrata</i>	<i>Sitophilus zeamais</i> , <i>Tribolium castaneum</i> , <i>Rhizopertha dominica</i> , <i>Acanthoscelides obtectus</i> , <i>Callosobruchus maculatus</i> , <i>Callosobruchus chinensis</i>	Rajendran and Sriranjini (2008)
	<i>Asiasarum sieboldi</i>	<i>Sitophilus oryzae</i>	Kumar et al. (2008)
	<i>Litsea cubeba</i>	<i>S. zeamais</i> , <i>T. castaneum</i>	Ko et al. (2009)
	<i>Melaleuca quinquenervia</i>	<i>C. Maculatus</i> , <i>Citrus aurantifolia</i> , <i>Ageratum conyzoides</i>	Aboua et al. (2010)
	<i>Thymus persicus</i>	<i>Tribolium castaneum</i> , <i>Sitophilus oryzae</i>	Taghizadeh-Saroukolai et al. (2010)
Ovicidal, Larvicidal, and Pupicidal	<i>A. calamus</i>	<i>S. granaries</i> , <i>S. oryzae</i> , <i>T. confusum</i> , <i>C. chinensis</i>	Risha et al. (1990)
	<i>A. calamus</i>	<i>C. phaseoli</i> , <i>C. maculatus</i>	Rahman and Schmidt (1999); Dimetry et al. (2004)
	<i>Aegle marmelos</i>	<i>S. oblique</i> , <i>C. chinensis</i>	Tripathi et al. (2003)
	<i>Calotropis procera</i>	<i>C. chinensis</i>	Kumar et al. (2008); Salunke et al. (2005)
Antifeedant Agent	<i>Annona sengalensis</i> , <i>Hyptis spicigera</i> , <i>Lippia rugosa</i>	<i>S. zeamais</i>	Nagmo et al. (2007)
	<i>Zanthoxylum alatum</i>	<i>Spodoptera litura</i>	Kumar et al. (2014)

of fungi and insects. Many plants-derived natural products that have insecticidal and fungicidal activity could be produced from locally available raw materials which make them relatively inexpensive. Further, it can be justified that EOs are helpful for the protection of stored products and to reduce the use of synthetic chemical insecticides. Recent studies in pest control have contributed the search for active natural products from plant EOs as an alternate to conventional pesticides. Thus, strong recommendation is needed for working with support from industries to shift towards green technology.

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Chapter 17

Fungal Biorefineries for Biofuel Production for Sustainable Future Energy Systems



Meena Kapahi, Roopa Rani, and Kashish Kohli

17.1 Introduction

Microorganisms form an integral part of all ecosystems and help in various ecological functions like decomposition and biogeochemical cycles. These versatile bioagents find various applications in industries like food processing, beverages, pharmaceutical and biorefineries (Singh et al. 2020; Singh and Yadav 2020). In a biorefinery, biomass is processed and converted to useful materials or products like biofuel, chemicals or energy through different physio-chemical and/or biological methods or their combination (Naik et al. 2010; Subramaniam et al. 2010). The bioconversion of lignocellulosic biomass encompasses initial treatment, hydrolysis, fermentation and separation to convert into useful products (Gupta et al. 2013). The physical and chemical pretreatment methods to delignify lignocellulosic compounds are cost- and energy-intensive processes and lead to the environmental impacts generating toxic chemicals and wastewater (Isroi et al. 2011). The biological processes depend on enzymes secreted by microbes leading to comparatively lower costs, less waste generation and minimal impacts on environment (Dashtban et al. 2009; Isroi et al. 2011). The world's energy needs are generally met by the ever-limited fossil fuel supplies. The associated environmental implications as well as the scarcity of conventional resources generate the requirement of non-conventional sources of energy as an alternative. Biomass is one of the sustainable resources which could be employed to generate various biofuels and chemicals in a biorefinery with the aid of microbes.

M. Kapahi (✉) · R. Rani · K. Kohli
Department of Chemistry, Manav Rachna University, Faridabad, Haryana, India
e-mail: meenakapahi@mru.edu.in

17.2 Biofuels

There are several types of biofuels (Fig. 17.1), each one has its own special properties, advantages and limitations. Traditionally, raw and unrefined biomass like animal dung, wood and charcoal (primary fuels) have been employed by people as the major sources of energy for heating or cooking in developing countries. With the advent of conversion technologies, biofuels can now be extracted from crops (food crops or energy crops) and other organic waste materials. The processed biofuels (secondary biofuels) are also classified as first-, second- or third-generation biofuels depending upon their sources. First-generation biofuels are obtained from edible crops through fermentation, distillation and/or transesterification. Ethanol production results from starchy crops such as sugarcane, wheat or other aged cereals. Biodiesel is extracted from animal fats or vegetable oils (sunflower, rapeseed oil) which can be utilised in pure as well as blend forms (along with conventional diesel) in engines.

The biofuel production worldwide was recorded to be approx. 135–140 billion litres during 2016 having biodiesel production as 23% of the total. However, countries like the USA, Brazil, Argentina, Germany and India lead in the production of such biofuels. In India, animal oil and used edible oils are being utilised to produce biodiesel (GAIN report 2016). During 2016, India, with six plants, produced 130–140 million litres of biodiesel through different varieties of vegetable or edible oils and animal fats which was supplied to the consumers including transport corporations, automobiles, institutions and brick kilns (GAIN Report 2016; Kour et al. 2019a; Yadav et al. 2020d). Although biodiesel can be used in conventional diesel

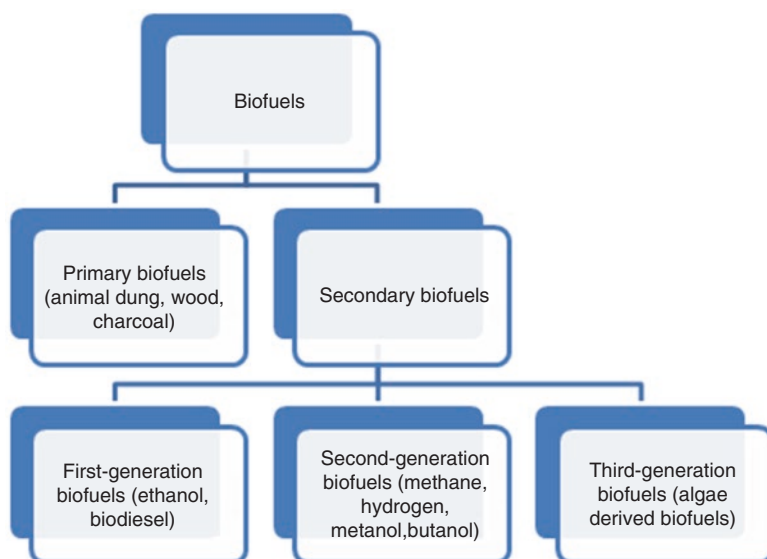


Fig.17.1 Types of biofuels

engines with the same fuel infrastructure, it is chemically distinct from conventional diesel.

It consists of a mixture of methyl esters synthesised after transesterification of various edible plant oils such as palm and soy oil giving fatty acid methyl ester (FAME) as the ultimate product that functions as a biofuel. However, different plant oils produce biodiesel with different chemical compositions; and thus, blends of various oils are required to produce desirable products. The process of transesterification produces monoglycerides (usually glycerine) as a by-product which largely functions as a feedstock for cosmetics (AMEC 2007; Kleinová et al., 2007). The production of bioethanol is carried out through some edible plant material such as sugarcane, sugar beet and corn kernels, since they can be easily decomposed to glucose which further can be fermented to ethanol through the microbes like yeasts (e.g. *Saccharomyces cerevisiae*). Another biofuel 'Biogas' can be produced through the process of methanation, anaerobic digestion of biodegradable waste (mostly organic waste, agricultural waste, manure, etc.) by microbes especially bacteria. The yield of biogas production increases with respect to the dry matter and the fatty acid content of the substrate (Moras 2007).

The use of edible oil for the production of biofuel is not much beneficial because of their low sustainability, use of water and fertilisers to produce different crops. Studies have also reported that the production of biofuel from these feedstocks is not fruitful due to its low economic viability (Dorado et al. 2006; Krawczyk 1996; Zhang et al. 2003). Therefore, research is being persuaded for utilising non-edible feedstock to synthesise biofuels in a cost-effective and eco-friendly manner (Pinzi et al. 2013). Biofuel such as biodiesel can be produced from different algal species like *Chlorella protothecoides* (Miao and Wu 2006), *Chlorella pyrenoidosa* (Li et al. 2011), *Dictyochloropsis splendida* (Afify et al. 2010), *Dunaliella tertiolecta* (Tang et al. 2011), *Fucus spiralis* (Maceiras et al. 2011), *Nannochloropsis oculata* (Carvalho Júnior et al. 2011), *Schizochytrium limacinum* (Johnson and Wen 2009) and *Stichococcus bacillaris* (Olivieri et al. 2011; Kour et al. 2019a, b). Similarly, the bioethanol production can be carried out through bacteria such as *Arthrospira platensis* (Markou et al. 2013), *Bacillus coagulans*, *Butyribacterium methylotrophicum*, *Clostridium autoethanogenum*, etc. (Ou et al. 2009; Genthner and Bryant 1982; Shen et al. 1999; Abrini et al. 1994), fungi as *Coriolus versicolor* (Zhang et al. 2007a, b), *Cyathus stercoreus* (Keller et al. 2003), *Irpex lacteus* (Xu et al. 2010), *Phanerochaete chrysosporium* (Sawada et al. 1995; Keller et al. 2003; Shi et al. 2009; Shrestha et al. 2008; Bak et al. 2009), *Pheblia tremellosus* (Mes-Hartree et al. 1987), *Pleurotus ostreatus* (Taniguchi et al. 2005; Hatakka 1983), *Polyporus giganteus* (Kirk and Moore 2007).

However, the quality of biofuel depends on the quality of feedstock oil and its fatty acid composition (Ramos et al. 2009). A group of scientists from Michigan State University have recently found an optimised method to produce oil and fatty acids with the help of algae and fungi. As per the research, one species from marine algae and one from soil fungi can be cultivated to produce biofuel and fatty acid for human benefit (Rastegari et al. 2019; Yadav et al. 2020c). The two species *Nannochloropsis oceanica* (algae) and *Mortierella elongata* (fungi) when kept in the

same chemical and physical environment can get attached and form agglomerate visible with naked eyes, known as bioflocculation. The synergy between the species produces oil and Omega-3 fatty acids. The former can be utilised to generate biofuels while latter component benefits human heart health (Igor 2018). *Phanerochaete chrysosporium*, *Mortierella isabellina* ATCC42613, *Cunninghamella echinulata*, *Cunninghamella japonica*, *Mortierella alpine* and *Mucor circinelloides* are some more species of oleaginous fungi that produce a promising amount of lipid and were found suitable to produce biodiesel (Lunin et al. 2013; Reddy and D'Souza 1994; Ruan et al. 2012; Sergeeva et al. 2008; Wynn et al. 2001). However, the total lipid content and nature of fatty acid are also an important measure for biodiesel synthesis from oleaginous fungus.

The sources for the first-generation biofuels are food sources that are 'already in demand' and scarce and can escalate the food prices leading to 'food or fuel' controversy. They pose a minimal impact on greenhouse gases (GHGs) emissions. Hence, these fuels get a lot of criticism (Michel 2012; Searchinger et al. 2008) and their use does not pose a sustainable solution and may threaten our present agricultural ecosystems.

On the contrary, second-generation biofuels (for example, methane, methanol, butanol, hydrogen, dimethyl ether, cellulosic ethanol) are obtained from non-food sources like wood and other organic wastes which are inexpensive and richly available yet underutilised biomass sources (Huang and Zhang 2011; Douskova et al. 2009). Third-generation biofuels are algal-based biofuels. The microalgae can be grown on wasteland and therefore have an advantage over plants/food crops requiring fertile lands. These photosynthetic microorganisms can be cultivated in wastewater, saltwater and sewage and can help in greenhouse gas mitigation. However, there is a need to use inexpensive but effective and high lipid-producing varieties to have commercial success. Cultivating algal biomass in wastewater is often associated with its remediation proving to be an added advantage.

17.3 Biorefinery: The Microbial Machineries

Extensive use of natural resources over a long time has led to the depletion of non-renewable sources that result in their limited use being expensive. Thus, some alternative energy sources are required with suitable methodologies for their generation which could be environment-friendly, cost-effective and of high quality. To obtain maximum atom economy or produce valuable by-products, the biorefinery approach is suggested. The biorefinery is an instrumental facility that can produce biofuel from biomass through various technologies. In infrastructure, it is similar to a petroleum refinery, except that it possesses renewable biomass feedstock, whereas in oil refinery non-renewable feedstock is being used. Three steps of processing raw material in a biorefinery can be described as given in Figs. 17.2 and 17.3.

Biorefining is a process that is based on the principles of Chemistry, Biology, Mathematics, Engineering as well as Biotechnology. It depends upon fermentation

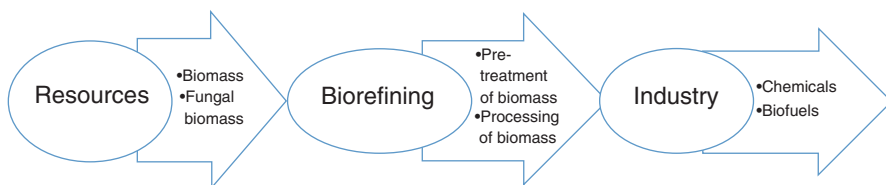


Fig.17.2 Biomass conversions in a biorefinery

Bioethanol	Biodiesel	Biogas
• <i>Aspergillus</i>, <i>Fusarium</i>, <i>Rhizopus</i>, <i>Trichoderma</i> (Anasontzis et al. 2011) • <i>Phanerochaete sordida</i> (Moriet al. 2016) • <i>Picher pastors</i> (Shin et al. 2015)	• <i>Ascocoryne sarcoides</i> (Gianoulis et al. 2012) • <i>Rhizopus oryzae</i> (Vodnar et al. 2013) • <i>Yarrowia lipolytica</i> (Rymowicz et al. 2009) • <i>Aspergillus niger</i> (Tudorache et al. 2012)	• <i>Piromyces rhizinflat</i> (Nkemka et al. 2015) • <i>Mucoromycotina</i>, <i>Agaricomycotina</i>, <i>Pucciniomycotina</i>, <i>Pezizomycotina</i> (Kazda et al. 2014)

Fig.17.3 Fungal species involved in biofuels production

technology and enzyme degradation. The biorefinery design should include a blend of chemicals and biological substances transforming into the desired product.

Biorefining of biomass can produce various useful products like biodiesel, bioethanol, biofibres, pulp and other bioactive compounds that can function as a feedstock for the manufacturing of many other useful products. A biorefinery has numerous advantages over the conventional petrochemical refinery such as being environmental-friendly and utilising biomass feedstock, i.e. it utilises waste biomass (especially kitchen waste) (Cherubini 2010; Kaur et al. 2020; Kumar et al. 2019; Prasad et al. 2020). The final products of a biorefinery are much demanded since it has renewable feedstock of plant-based biomass. Therefore, energy production through biorefinery has more energy surety generating less pollution and reducing the waste produced through fossil fuel combustion (Cherubini 2010). The concept of a biorefinery has gained importance in the present era since it provides the opportunities of employment/new businesses, development of rural areas and enhancing the economy of the country. The only demerit with the biorefinery is that most of the technologies utilised in this process are at their development stage, and thus the need for advanced processing technologies is much anticipated.

The developing techniques of biorefining have produced ample opportunities for further research and its commercialisation. Biofuel feedstock produces the product with different C:H:N:O ratios. The choice of biomass is adopted in such a way that the desired target products are formed. Plant biomass has various components like protein, carbohydrates, fats, lignin, vitamins and other aromatic compounds. The biorefineries can unite the techniques to convert these raw materials into an

intermediate which shall decompose into a final desirable product. This process requires separation techniques that practically extract the bioactive compounds of the biomass at the initial steps for further modification.

The biomass containing carbohydrate, lignin, proteins and fats is the main focus to function as a source for biorefinery processes. The major components of carbohydrates are glucose and starch which can act as effective chemicals for the hydrolysis process. The pre-treatment step involves proteins that hydrolyse cellulose is important to separate lignin-cellulose-hemicellulose composite. This pre-treatment step is temperature-dependent and consumes energy too. The treatment step of degradation of compounds through microbes is much expensive giving final vigorous products (Kamm et al. 2006a; Rastegari et al. 2020a, b). Based on the feedstock available, biorefineries are of following types as given in Table 17.1.

17.4 Fungi in Biomass Utilisation

Fungi are the types of saprophytic/parasitic/symbiotic eukaryotes that rely on environmental conditions to meet their nutritional requirements (Osiewacz 2002; Devi et al. 2020b; Yadav et al. 2020a, b). Free and complex enzymatic systems are produced by fungi for disintegrating the lignocellulosic substances. These free enzymes are produced commercially by aerobic fungi at about 30–35 °C temperature; *Aspergillus niger* and *Trichoderma reesei* are the highly active species to produce enzymes (Zhang and Lynd 2004; Devi et al. 2020a; Sharma et al. 2019). Single-celled fungi possess an enormous amount of lipid content that can be turned to biofuel.

Single-Cells Oils (SCOs) are the triglycerides generated through oleaginous microbes like yeasts, fungi, bacteria and algae. SCOs are important for giving solutions to ever-depleting fossil fuels (Beopoulos et al. 2009). The variable composition of fatty acids and lipid content in SCOs are dependent upon environmental factors such as temperature, pH, type of species and carbon source (Subramaniam et al. 2010; Venkata and Venkata 2011). For example, when the *Mortierella* species were grown in glucose, *M. alpina* produced ~40% SCOs, while *M. isabellina*

Table 17.1 Types of biorefineries

S. No.	Type of biorefinery	Nature of feedstock biomass	Example	Reference
1	Green biorefinery	Nature-wet	Clover, horse feed, green grass	Kromus et al. (2006)
2	Lignocellulosic feedstock biorefinery	Nature-dry	Cellulose, waste biomass	Koutinas et al. (2006)
3	Entire-crop biorefinery	Crude material	Maize, oats	Kamm et al. (2006b)
4	Biorefinery two platforms	Sugar-syngas	Sugar	Werpy and Petersen (2004)

ATHUM 2935 produced 50.4% oil (Papanikolaou et al. 2004; Wynn et al. 2001), whereas lipid content was 4.43% and 0.62%, respectively (Kumar et al. 2010). However, all oleaginous fungal cells cannot be utilised as a precursor to produce biodiesel. Most of the properties of biodiesel and biofuel depend on the feedstock profile. SCOs contain triacylglycerols (TAGs), sterol esters, fatty acids, monoacylglycerols, sterols and lipids. The limiting condition of the nutrients such as N, K, Na, P, S, Mg and Mn for cell creation results into the formation of lipids. Under such conditions, carbon assimilation gets accelerated and thus lipids are synthesised within cells. The lipid content of any microorganisms like microalgae, bacteria and fungi comprises its oil content which can be utilised for biodiesel (Li et al. 2008; Subramaniam et al. 2010).

The conversion of SCOs to biofuels can be done in two ways – direct and indirect transesterification. Direct transesterification occurs inside cell without SCO extraction from biomass, while indirect transesterification occurs after extraction of SCO from biomass. However, the extraction process is carried out through solvent extraction which involves high capital investment, thus, enhancing the demand of the direct transesterification method. This process involves the centrifugation of biomass obtained after fermentation and then washing with water followed by its drying and mixing with an acid and methanol (Liu and Zhao 2007). Liu and Zhao (2007) also reported straight transesterification of SCOs from yeasts like *L. starkei* and *R. toruloides* and fungus like *M. isabellina* which produces FAME. Single-cell oils of oleaginous fungi are known to be a large source of polyunsaturated fatty acids (PUFAs) which can also be used as food and medicinal purpose. (Certik and Shimizu 1999; Ratledge and Wynn 2002). *M. circinelloides* was the first commercially viable SCO which produces oil that is rich in γ -linolenic acid (fatty acid) (Cohen and Ratledge 2005). With different acid solution concentration and biomass: methanol ratio, different amount of FAME was produced. Lipid extraction from SCO through *M. circinelloides* was carried out with chloroform-methanol mixture in different proportion (Vicente et al. 2009). These two methods (direct and indirect) of transesterification were compared for biodiesel production. However, direct transesterification resulted in the production of a good yield of FAME and thus significant production of biodiesel (Vicente et al. 2009). The fatty acid profile of the feedstock is used to judge the quality of biodiesel such as oxidative stability, iodine value and ignition quality. Thus, it becomes important to examine the composition of fungal-derived fatty acids for their carbon content and unsaturation degree. Higher saturation in fatty acid results in desirable properties in biodiesel, i.e. better oxidative stability, flash point, iodine value and high cetane number.

These methods using oleaginous microbes to synthesise biodiesel have numerous merits such as being simple and rapid and is independent of climatic condition or season. The process requires less labour and time and is easy to handle. The lipid metabolism has been studied in heterotrophic microbes where the optimisation and standardisation of the process were carried out through agro-industrial residues producing a higher yield of SCOs (Kosa and Ragauskas 2011).

Various studies have demonstrated that fungal species can act as a crucial part of the production of biofuels from agricultural or other feedstocks. Out of several

oleaginous microbes, fungi hold various advantages because of their ability (Zheng et al. 2012). They concentrate up to 80% of lipid and yield fatty acids (Subramaniam et al. 2010); to degrade lignocellulosic materials and utilise a variety of biomass sources as corncob, wheat straw and oil (Andre et al. 2010; Conti et al. 2001; Economou et al. 2010; Gema et al. 2002; Peng and Chen 2008; Stredansky et al. 2000; Venkata and Venkata 2011; Weber and Tribe 2003) to produce biofuels with low-cost and lesser energy requirements (Conti et al. 2001); exhibit good lipid profiles (free fatty acids and saponifiable lipids) essential for good quality biodiesel (Vicente et al. 2009); ease of harvesting after the process by simple filtration (Xia et al. 2011); and their thermostability (Coyne et al. 2013). However, the yield depends upon the fungal species. Basidiomycetes and ascomycetes are fungi which can degrade lignin due to the production of extracellular enzymes (lignases, cellulases and hemicellulases) which have the potential to degrade the components of lignocelluloses (Coyne et al. 2013).

White-rot fungi can produce enzymes that can degrade all the components of the lignocellulose; whereas brown-rot fungi and ascomycetes produce specific enzymes degrading the components separately (Coyne et al. 2013). Ascomycetes fungi like *Aspergillus* spp. and *Neurospora* spp. can consume and grow on different types of lignocellulosic substrates (even non-pretreated substrates like wheat bran or wheat straw) (Panagiotou et al. 2011) and consume the monomeric units discharged from the hydrolysis of lignocellulosic materials.

SCO production involves the major issue of processing cost. The first step in the extraction process is cell disruption which ultimately influences the overall efficiency of the process (Ratledge et al. 1984). However, extraction of lipids from microorganisms has disadvantages that it involves much higher cost, fewer amounts but the main advantage with the microbes is that they can be genetically modified to generate the desired product (Pometto et al. 2005). The lignocelluloses hydrolysates can be converted to SCOs which can be further converted to biofuel. These microorganisms translate sugar into lipids which in turn are converted to biodiesel.

Bioethanol is obtained with the help of fermentation making use of glucose produced from carbohydrates in lignocellulosic materials (like agro-wastes and forest wastes) apart from crop sources. The production, initial treatment steps, saccharification and agitation processes (Alrumman 2016) are accomplished through enzymes (laccases, hemicellulases, cellulases, peroxidases) from various fungal species like *Saccharomyces cerevisiae*, *Picher pastoris*, *Neurospora intermedia* and *Aspergillus oryzae* (Bátori et al. 2015; Shin et al. 2015; Várnai et al. 2014). Various fungal species like *Rhizopus oryzae* and *Yarrowia lipolytica* have been recorded to produce valuable chemicals when grown on the carbon source as glycerol (Rymowicz et al. 2009; Vodnar et al. 2013). Several oleaginous fungal species including *Candida*, *Rhodospiridium*, *Mortierella* and *Cryptococcus* have been explored to obtain lipid content from them ~70–80% of the biomass weight (Rossi et al. 2011).

Biogas, another renewable energy source, is utilised majorly for cooking in rural regions. The by-product, spent slurry, is used as a fertiliser for plants. Anaerobic fungi have an important function as they contain cellulolytic and hemicellulolytic enzymes to hydrolyse plant polysaccharides. The role of anaerobic fungi like

Piromyces rhizinflata has been studied using substrates like cattail and corn silage (Nkemka et al. 2015).

17.5 Fungal Enzymes: The Forces behind

Fungi are storehouses of highly functional enzymes that are capable of hydrolysing recalcitrant substances like lignocellulose components (Rana et al. 2019b; Yadav 2020). These enzymes find an important role in different techniques in biorefineries. The hydrolysis of lignocellulosic materials to their respective monomers which are transformed into useful substances depends on the enzymatic machinery. Lignin-degrading enzymes can break down lignin components with the help of cellulases (cellulose-hydrolysis enzymes), cleaving the cellulose microfibrils into glucose monomers, hemicellulases acting on the hemicelluloses and lignases (delignifying enzymes) (Sreedharan et al., 2016). Some of the major enzymatic systems are discussed here.

Laccases, oxidoreductase enzymes, help in lignin breakdown by reducing oxygen to water molecules and oxidising aromatic compounds to their radicals (Lundell et al. 2010). For this property, laccases are extremely used in biotechnological and biodegradation applications (Martínez et al. 2005; Baldrian 2006; Lundell et al. 2010; Isroi et al. 2011). These enzymes are produced by white- and brown-rot fungi and certain ascomycetes (Martínez et al. 2005; Lundell et al. 2010). *Pleurotus ostreatus*, produces POXA1, containing one Cu, two Zn and one Fe atoms per molecule (Palmieri et al. 1997). Several isozymes have also been reported (Lundell et al. 2010) in several white-rot fungi and basidiomycetes like *Coprinopsis cinerea* (Lundell et al. 2010; Kour et al. 2019b).

The lignin-degrading peroxidase enzymatic consortia consist of manganese peroxidase (MnP), laccase, ligninperoxidase (LiP) and versatile peroxidase (VP). These heme-containing enzymes are extracellular and non-specific enzymes in nature and by various white-rot fungi. LiP enzymes delignify (Chen et al. 2011) in the presence of H_2O_2 producing LiP intermediates and free radicals cleaving various bonds and opening aromatic rings (Isroi et al. 2011). MnPs are another set of heme-containing and hydrogen peroxide-dependent enzymes that require Mn^{2+} as a cofactor to carry out oxidation function. The concentration of Mn^{2+} can affect the production of LiP and MnP; higher concentration leads to MnP production; while lower concentrations favour LiP production (Blanchette 1991). The presence of H_2O_2 gives rise to MnP intermediates oxidising amines and other lignin bonds (Dashtban et al. 2010; Isroi et al. 2011). VP enzymes contain heme group with Mn^{2+} and can oxidise phenols and non-phenol with Mn^{2+} oxidation. These enzymes have been recorded in various *Pleurotus* species.

The white-rot fungi produce hydrogen peroxide producing enzymes like glyoxal oxidase and aryl alcohol oxidase responsible for producing H_2O_2 by reducing oxygen to enable peroxidases to degrade lignin. Glyoxal oxidase contains Cu and is capable of oxidising aldehydes; while aryl alcohol oxidase (*Pleurotus eryngii*) can

oxidise chlorinated anisyl alcohols and aromatic alcohol (Martínez et al. 2005; Isroi et al. 2011).

Phenol oxidase enzymes oxidise phenols in presence of oxygen. This enzymatic set includes enzymes that modify lignin, catechol oxidases, tyrosinases and catalase phenol oxidases.

Hemicellulose degradation requires a variety of enzymes that are able to act synergistically such as xylanases and mannanases because of its heterogeneous and complex structure. Xylanases catalyse the hydrolysis of 1,4- β -D-xylosidic bonds in xylan that is an important structural polysaccharide in plant cells. Its complete hydrolysis requires different xylanolytic enzymes like 1,4- β -xylanases (to break the xylan backbone); β - (to breakdown xylose monomers); and enzymes like α -arabinofuranosidases and α -glucuronidases (to cleave side groups). These xylan-degrading enzymatic consortia have been observed in fungi (Belancic et al. 1995; Sunna and Antranikian 1997) like *Penicillium* and *Talaromyces* species (Saha 2003).

Cellulose is the most usual and an inexhaustible biopolymer and forms a chief component of the lignocellulosic agro-wastes. Its degradation by microbes is mediated through enzymatic systems like cellulases. They hydrolyse cellulose (b-1,4-D-glucan) bonds and generate products like glucose and cellobiose. The cellulase enzymatic machinery comprises three main enzymes viz. endo-b-1,4-glucanase, cellobiohydrolase and b-glucosidase acting together to hydrolyse cellulose to glucose monomers. Endoglucanases break glycosidic linkages of polymeric chains (Alvira et al. 2010) for which isozymes are produced in the fungi like *T. reesei* and *P. chrysosporium* (Dashtban et al. 2009). The b-glucosidases act on cellobiose and convert to glucose (Arantes and Saddler 2010). The extensively researched cellulolytic microbes encompass fungal species like *Aspergillus*, *Penicillium* and *Trichoderma* species can release extracellular enzymes (Schulein 1988). All the three fungal species were recombined to release a mutant strain to degrade the cellulose completely. With the help of *Saccharomyces* spp., the bagasse was processed to ethanol after pretreatment (El-Bondkly and El-Gendy 2012; Rana et al. 2019a).

17.5.1 Fungal Enzymes: Role in Biofuel Production

Biodiesel is the main biofuel component obtained by fermentation of agricultural wastes. Due to the availability of lignocellulosic materials worldwide, it is considered as one of the emerging renewable substrates for the bioenergy. The major challenges faced at the time of biofuel production are the usage of fast, cheap and effective methodologies. In biorefineries, the primary product produced is ethanol, which is generated from lignocellulosic precursor and hence called bioethanol. Due to the abundance of plant biomass, it becomes the most predicting substrate for the production of renewable energy. The major application of bioethanol is among flexible fuel vehicles (FFV) (Ibeto et al. 2011).

The intervention of fungal-based technology in the sugar-to-ethanol conversion process leads to the production of food-grade fungal proteins that are used to feed

animals (Nitayavardhana and Khanal 2010). It was further found that *Rhizopus microspores* (var. *oligosporus*) generate fungal biomass which thus found to possess 45.55% crude protein significantly rich in essential amino acids and poor in organic matters (Nitayavardhana and Khanal 2010; Rasmussen et al. 2007). Due to its five days achievement, such fungal processing is integrated in such a manner that they further supply protein to feed aquatic culture at very cheap prices as compared to commercial protein sources.

The production of biodiesel through lipids or oil content of SCOs can be feasible using various techniques like transesterification, pyrolysis, microemulsion and dilution. However, transesterification is the most recommended process for the same. Since the direct use of edible oils in diesel engines is not suggested because of the deposition of carbon in the inner walls of engine, and thus the processing of oil in biodiesel is needed (Lin et al. 2011). During the transesterification process, the triglyceride content of oil reacts with an alcohol (mainly methanol) giving rise to diglycerides which further decompose to monoglycerides, which in turn decompose to glycerol, however, every single step releases a fatty ester thereby producing three fatty ester molecules from a single molecule of triglyceride. In some cases, ethanol can also be used for transesterification, for example, *Mucor circinelloides* was esterified using ethanol and catalyst thereby producing fatty acids ethyl esters (FAEEs) (Carvalho et al. 2015). However, studies show that non-catalysed processes for transesterification have also been developed (Demirbas 2009; Leung et al. 2010). In some processes, a single-step transformation of triglycerides to monoglycerides was carried out which consumes less energy and time.

Some species of microorganisms are genetically engineered for increasing the production of SCOs, for example, *Ashbya gossypii*, a filamentous fungus, used for producing riboflavin, was engineered to enhance the production of lipid content which in turn used to produce biodiesel (Lozano-Martínez et al. 2016, Díaz-Fernández et al. 2017). The use of oleaginous fungi for the production of biodiesel has few major disadvantages, i.e. lack of high-throughput method, monitoring the capability of lipid accumulation, etc. However, some studies reveal that Duetz microtiter plate system can be utilised for monitoring the growth of oleaginous fungi and using FTIR spectroscopy for determining lipid content (Kosa et al. 2017).

17.5.2 Fungal Enzymes Substrate

Organic biomass consists of a large number of polysaccharide units which are formed by the combination of several thousand units of glucose (Mathews et al. 1999) and these polymers/polymeric chains are formed by three major biopolymers (Lee et al. 2014; Himmel et al. 2007) namely cellulose, hemicelluloses and lignin. The cellulose is a biopolymer present in each crystalline unit, thus helps in the formation of microfibrils in the higher plant. The enzyme can interact with the substrate and may become difficult due to the heterogeneous nature of lignocellulosic material. Innate structural characteristics and modifications during saccharification

are the two main reasons responsible for the efficacy of hydrolysis (Mansfield et al. 2008). Long spirals of glucose connected through β -acetal bonds are responsible for the structure of cellulose (Cooper 2015). Lignin and hemicelluloses contents, crystalline cellulose, degree of polymerisation and surface areas are some of the factors which affect the enzymatic hydrolysis of lignocellulosic biomass (Chang and Holtzapple 2000; Kim et al. 2003). Hence, lignin removal and development of eco-friendly pre-treatment contribute towards increased fungal biomass leading to hydrolysis through enzymes, cell wall porosity (Barakat et al. 2014; Wi et al. 2015).

To improve the yield of hydrolysis, cellulose-enriched fractions are digested with lesser processing time utilizing recalcitrance and low-costs chemicals in physical, chemical and biological processes (Jouzani and Taherzadeh 2015; Miranda et al. 2016; Saritha et al. 2012). To allow greater availability of enzymes to the fractions of cellulose, there is a greater need for successful alteration in lignocellulosic materials to rupture its biomass structure leading to separation.

17.6 Conclusion

Biofuels have the potential to substitute a considerable degree of conventional oil imported with locally produced fuels in the future. It would benefit the countries in being energy-secure and independent to some extent. The related positive-environmental impacts cannot be ruled out, for example, reduced global warming due to lower greenhouse-gas emissions and getting rid of massive amounts of solid wastes. This would indirectly benefit the local economy by providing employment opportunities and leading to the development of rural areas. Ever-increasing oil prices have led to an inclination towards biofuels with comparatively lesser costs. Biomass is one such sought after sustainable resource for producing various biofuels and useful products in biorefineries. Various fungal species have been researched for their use in biorefineries to produce biofuels like biodiesel, bioethanol and biogas. Fungi employ enzymatic machineries (lignases, cellulases and hemicellulases) to degrade lignocellulosic biomass for the production of biofuels. Oleaginous fungi and other such microbes that can be helpful in synthesising biodiesel or any other biofuel are an important aspect of the study. Genetic modification and mutation have made several species worth for the production of biodiesel in surplus amount.

17.7 A Long Way to Go

Under the current scenario, there is an urgent need to research to adopt newer technologies from non-edible plants to increase both the quality and quantity of biofuel. Due to the availability of agricultural residues, microbiological help and development in various biological treatments, the production of biofuel can meet 50% of biofuel demand by only 10% residue without even competing with the food chain.

And this development not only helps in the sustainability of bioenergy but also will help creating rural development and socio-economic impacts. Even multiple organisms consortia can be adopted (like fungal-algal) in order to maximise the benefits and fulfil multiple functions of bioremediation and the production of biofuels. And to make our production economically viable, one can produce biofuel in synthetic factories together with high-value products. The production of biofuels not only helps in creating the overall GHG balance but also helps in food security and protection of biodiversity and ecosystems. This can be achieved when the relevant policies are incorporated under government schemes in the presence of transparent sustainability criteria because otherwise, it could cause climate change impacts, natural habitat loss and disruption of staple food supplies.

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Chapter 18

Environmental and Industrial Perspective of Beneficial Fungal Communities: Current Research and Future Challenges



Ajar Nath Yadav, Tanvir Kaur, Rubee Devi, Divjot Kour,
Ashok Yadav, Praveen Kumar Yadav, Farhan Zameer, Murat Dikilitas,
Ahmed M. Abdel-Azeem, and Amrik Singh Ahluwalia

18.1 Introduction

Fungi are the one of the life forms that consist in the domain eukarya that have micro and macroscopic organism both. The organism belongs to this domain of life is known to exist in wide range of habitats like soil, water, air, animals and plants and also in the environments that have extreme conditions like low or high temperature, high concentration of metals and saline (Cox 2007; Devi et al. 2020a; Rana et al. 2019b). Fungi kingdom in nature plays various roles and one of the important roles is cleaning the ecosystem. Fungi use to survive on the dead bodies of animals, humans as well as of plants that help in the decomposition of the bodies and convert them into organic compounds (Rana et al. 2019a). Besides the natural cleaner of the

A. N. Yadav (✉) · T. Kaur · R. Devi · D. Kour
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture,
Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh, India

A. Yadav · P. K. Yadav
Department of Botany, Institute of Sciences, Banaras Hindu University,
Varanasi, Uttar Pradesh, India

F. Zameer
Department of Biological Sciences (Biochemistry), School of Basic and Applied Sciences,
Dayananda Sagar University, Shavige Malleshwara Hills, Kumaraswamy Layout,
Bengaluru, Karnataka, India

M. Dikilitas
Department of Plant Protection, Faculty of Agriculture, Harran University, S. Urfa, Turkey

A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, University of Suez Canal,
Ismailia, Egypt

A. S. Ahluwalia
Department of Botany, Akal College of Basic Sciences, Eternal University,
Baru Sahib, Sirmaur, Himachal Pradesh, India

ecosystem, some organism belonging to kingdom fungi also has negative effects on various organisms because of pathogenic nature and causes diseases to wide range of multi-cellular organism including plants and animals. Whereas, some fungi are beneficial and can be utilized in the various sectors and ease the development of human required products (Khan et al. 2014).

Beneficial fungi can be used in the sector like agriculture, industries as well as in the environment. In agriculture, fungi can be used as biofertilizers that help in the availing the various nutrients like phosphorus, potassium, zinc and iron by solubilizing or by chelating the nutrients from the soil (Kour et al. 2020; Yadav et al. 2020a). These organisms also known to protect the crops of various pathogens including fungi and one of the best known fungal biopesticides is *Trichoderma* (Schuster and Schmoll 2010; Sharma et al. 2019). Likewise fungi have various environmental applications such as bioremediation and cycling of nutrients. As it is known as a sweeper of the earth, so it can be used for the decomposition of several inorganic matters and hazardous chemicals like DDT, coloured dyes generated by industries that cannot or very slowly removes (Deshmukh et al. 2016).

Fungi also can be utilized in the various industries including food, pharmaceuticals, biorefineries and agricultural industries. Fungi have a capability to producing various types of enzymes like amylases, lipase, protease, cellulase, xylanase and many more which have been used in industries like food, detergents, dairy, textile, paper and pulp, rubber, oil and petroleum and plastic industries (Kour et al. 2019; Porras-Alfaro and Bayman 2011). Some fungi have also been used as medicines and can be used in the pharmaceutical industries for the production of wide range of medicines (Khan et al. 2014). In agricultural industries that produce agricultural-related products like fertilizers and pesticides also can use fungi in the production of biofertilizer and biopesticides that can be more beneficial for the environment as the product produced by the fungi is an eco-friendly approach (Thakur et al. 2020; Yadav et al. 2020b; Yadav et al. 2020c). Fungal communities and their enzyme products are exploited by a diverse array of major industries, including food and feed, detergent, pulp and paper, fuel, pharmaceutical and chemical (Fig. 18.1). In this chapter, all the perspectives related to the fungi in the field of environment and industries have been discussed in the following chapter.

18.2 Diversity and Distribution

18.2.1 Environmentally Important Fungal Communities

In microbial ecology, the diversity and distribution of fungi is one of the main discussed point in this topic. Fungi, the decomposers, pathogens and mutualism are highly diverse. The suggestion that fungi are an incredibly diverse and poorly known group of organism as well as these have found 1.5 million fungi around the

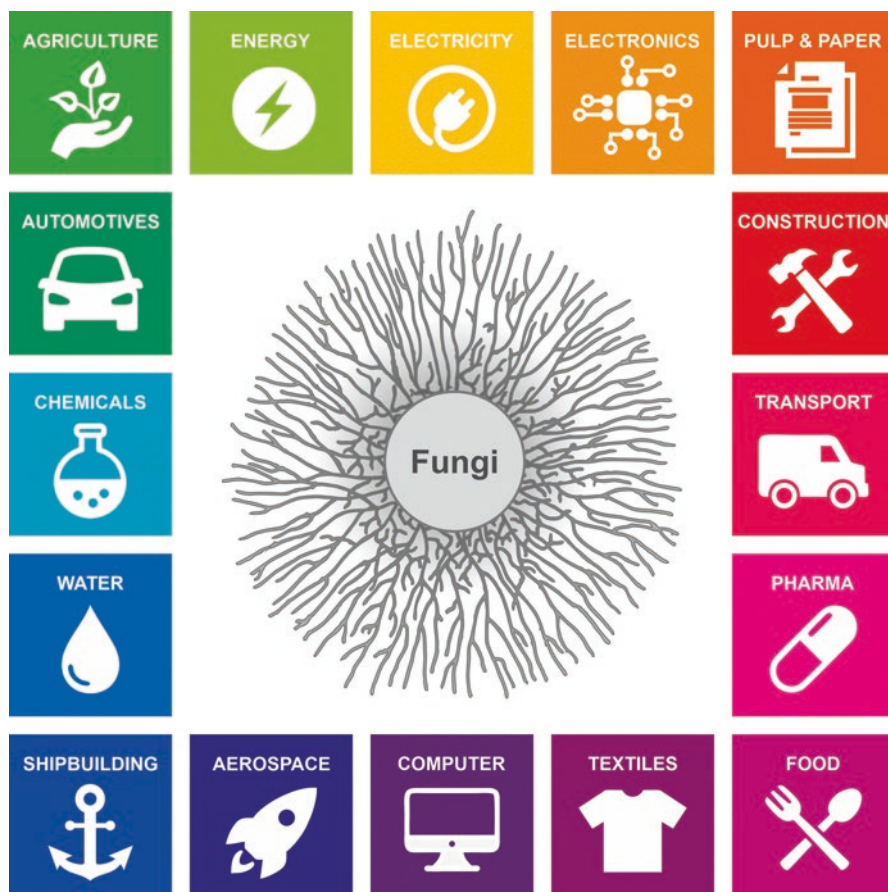


Fig. 18.1 Significant contributions of fungal biotechnology for sustainable development. (Adapted from Meyer et al. (2020))

whole world, in which 74,000 to 120,000 have been reported. These fungal species have found from diverse environmental regions such as air, soil, plant, heritages and also various extremes environments including mine, deep oceans, glaciers, deserts, hot springs and coastal region (saline areas) belonging to different phylum like Ascomycota, Basidiomycota, Mucoromycota and Zygomycota (Yadav 2020). Many of the reported fungi in the environment mostly from the sewage water, seawater, contaminated sites have been reported as environment important fungi as they help in the clean-up of environment pollutants like heavy metals, n-alkanes, dyes, tannery effluent and crude oil (Kour et al. 2021; Kumar et al. 2021). Fungi like *Phanerochaete chrysosporium*, *Aspergillus awamori*, *Trichoderma viride* (Joshi et al. 2011), *Aspergillus* sp. (Akhtar et al. 2013), *A. flavus* (Kurniati et al. 2014) and *A. fumigatus* (Talukdar et al. 2020b) have been reported for biodegradation of heavy

metals. Whereas, fungal species like *A. niger*, *Phanerochaete chrysosporium* (Rani et al. 2014) and *A. niger* (Mahmoud et al. 2017) have been reported for the degradation of coloured dyes released in the water by the industries. Fungi species including *Aspergillus* sp. (Silva et al. 2009), *Doratomyces nanus*, *D. purpureofuscus*, *Myceliophthora thermophila* and *Phoma eupyrena* (Mouhamadou et al. 2013) have been reported for the degrading of the n-alkanes. Some fungi have also been reported for the removal of crude oil like *Botrytis cinerea*, *Gonatobotryum apiculatum*, *Mycotypha microspore* (Ekundayo et al. 2012), *Cochliobolus lunatus*, *Fusarium solani*, *Penicillium documbens* (Al-Nasrawi 2012), *Alhagi persarum*, *Peganum harmala* and *Prosopis farcta* (Chehregani Rad et al. 2014) (Table 18.1).

18.2.2 Industrially Important Fungal Communities

The incredible group of organism's fungi is being distributed in the moderate as well as extreme habitats like soil (moderate, cold soil, soil with high concentration of salt, heavy metals, pollutant soil), water (oceans, hot springs, frozen water, waste water from industries), glaciers, plants, animals and food stuffs, where they accompanied by other organism and play different kind of roles in the environment. Fungi from different habitats can be utilized in the industries for several purposes like production of enzymes (amylase, pectinase, xylanase, laccase, protease, urease and cellulase), coloured dyes and secondary metabolites that have been used in the industries like food, biofineries and pharmaceutical (Rastegari et al. 2020a; Rastegari et al. 2020b; Yadav et al. 2020d) (Fig. 18.2).

Diverse range of fungi has been reported for the several industrial purposes like *Trichoderma atroviride* (Grujić et al. 2015), *Fusarium* sp. (Katoch et al. 2017), *Aspergillus niger* (Asrat and Girma 2018) (Gautam et al. 2011), *Trametes trogii*, *Trametes villosa*, *Coriolus versicolor* (Levin et al. 2010), *Aspergillus* sp. (Mukunda et al. 2012), *Penicillium granulatum*, *Aspergillus raperi*, *Aspergillus speluneus* (Khokhar et al. 2011), *Aureobasidium pullulans*, *Penicillium citrinum*, *Phialophora* sp., *Botrytis verrucosa*, *Cladosporium chlorocephalum* (Gawas-Sakhalkar and Singh 2011), *Aspergillus tubingensis*, *Aspergillus terreus*, (Bakri et al. 2010), *Talaromyces atroroseus*, *Talaromyces albobiverticillius* (Frisvad et al. 2013), *Acremonium strictum*, *Alternaria alternata*, *Aspergillus candidus*, *Chaetomium globosum*, *Cladosporium cladosporioides* (Saleem and Ebrahim 2014), *Pestalotiopsis* sp. (Wikee et al. 2019) has been reported for producing several types of enzymes. Fungi like *Talaromyces australis*, *Penicillium murcianum* (Hernández et al. 2019) have been reported for producing coloured dyes (Table 18.2).

Table 18.1 Biodiversity of environmentally important fungi

Fungi	Habitats	Applications	References
<i>Alhagi persarum</i>	Plants	Biodegradation of crude oil	Chehregani Rad et al. (2014)
<i>Aspegillus awamori</i>	Wastewater	Remediation of HM	Joshi et al. (2011)
<i>Aspergillus flavus</i>	Soil	Remediation of HM	Kurniati et al. (2014)
<i>Aspergillus flavus</i>	Soil	Degradation of HM, metabolic inhibitors and tannery effluent	Kumar and Dwivedi (2019)
<i>Aspergillus fumigatus</i>	Wastewater	Remediation of HM	Talukdar et al. (2020b)
<i>Aspergillus niger</i>	Soil	Biodegradation of dyes	Rani et al. (2014)
<i>Aspergillus niger</i>	Wastewater	Remediation of red azo dye	Mahmoud et al. (2017)
<i>Aspergillus</i> sp.	Soil	Remediation of PAH	Silva et al. (2009)
<i>Aspergillus</i> sp.	Soil	Remediation of HM	Akhtar et al. (2013)
<i>Aspergillus terreus</i>	Seawater	Remediation of hydrocarbons	Bovio et al. (2017)
<i>Aspergillus terreus</i>	Wastewater	Degradation of Pb	Sharma et al. (2020)
<i>Botryris cinerea</i>	Soil	Degradation of Bonnylight crude oil	Ekundayo et al. (2012)
<i>Cladosporeum perangustum</i>	Wastewater	Remediation of Cr	Sharma and Malaviya (2016)
<i>Cochliobolus lunatus</i>	Soil	Biodegradation of crude oil	Al-Nasrawi (2012)
<i>Coniothyrium</i> sp.	Soil	Remediation of PAH	Potin et al. (2004)
<i>Doratomyces nanus</i>	Soil	Remediation of PCB	Mouhamadou et al. (2013)
<i>Doratomyces purpureofuscus</i>	Soil	Remediation of PCB	Mouhamadou et al. (2013)
<i>Fusarium chlamydosporium</i>	Soil	Remediation of tannery effluent	Sharma and Malaviya (2014)
<i>Fusarium sambucinum</i>	Soil	Remediation of P&P mill effluent	Malaviya and Rathore (2007)
<i>Fusarium solani</i>	Soil	Biodegradation of crude oil	Al-Nasrawi (2012)
<i>Fusarium solani</i>	Soil	Biodegradation of DDT	Mitra et al. (2001)
<i>Fusarium</i> sp.	Soil	Remediation of PAH	Potin et al. (2004)
<i>Gliocladium deliquescence</i>	Soil	Degradation of Bonny light crude oil	Ekundayo et al. (2012)
<i>Gonatotobryum apiculatum</i>	Soil	Degradation of Bonny light crude oil	Ekundayo et al. (2012)
<i>Hordeum marinum</i>	Plants	Biodegradation of crude oil	Chehregani Rad et al. (2014)
<i>Merulius aureus</i>	Soil	Remediation of P&P mill effluent	Malaviya and Rathore (2007)
<i>Myceliophthora thermophila</i>	Soil	Remediation of PCB	Mouhamadou et al. (2013)
<i>Mycotypha microspora</i>	Soil	Degradation of Bonny light crude oil	Ekundayo et al. (2012)

(continued)

Table 18.1 (continued)

Fungi	Habitats	Applications	References
<i>Peganum harmala</i>	Plants	Biodegradation of crude oil	Chehregani Rad et al. (2014)
<i>Penicillium chrysogenum</i>	Soil	Remediation of PCB	Tigini et al. (2009)
<i>Penicillium citreonigrum</i>	Seawater	Remediation of hydrocarbons	Bovio et al. (2017)
<i>Penicillium citrinum</i>	Wastewater	Remediation of Cr	Zapana-Huarache et al. (2020)
<i>Penicillium commune</i>	Wastewater	Remediation of Cr	Sharma and Malaviya (2016)
<i>Penicillium digitatum</i>	Soil	Remediation of PCB	Tigini et al. (2009)
<i>Penicillium documbens</i>	Soil	Biodegradation of crude oil	Al-Nasrawi (2012)
<i>Penicillium</i> sp.	Soil	Remediation of alkanes	Husaini et al. (2008)
<i>Phanerochaete chrysosporium</i>	Soil	Biodegradation of dyes	Rani et al. (2014)
<i>Phanerochaete chrysosporium</i>	Wastewater	Remediation of HM	Joshi et al. (2011)
<i>Phlebia</i> sp.	Soil	Remediation of P&P mill effluent	Malaviya and Rathore (2007)
<i>Phoma eupyrena</i>	Soil	Remediation of PCB	Mouhamadou et al. (2013)
<i>Prosopis farcta</i>	Plants	Biodegradation of crude oil	Chehregani Rad et al. (2014)
<i>Scedosporium apiospermum</i>	Soil	Remediation of PCB	Tigini et al. (2009)
<i>Talaromyces islandicus</i>	Wastewater	Degradation of Pb	Sharma et al. (2020)
<i>Thermoascus crustaceus</i>	Soil	Remediation of PCB	Mouhamadou et al. (2013)
<i>Trichoderma viride</i>	Wastewater	Remediation of HM	Joshi et al. (2011)
<i>Trichoderma viride</i>	Wastewater	Remediation of Cr	Zapana-Huarache et al. (2020)

18.3 Biotechnological Applications

Fungi from different habitats can be used in the various fields like industries, agriculture and environment for the purposes like the production of enzymes, dyes, secondary metabolites, remediation of environmental pollutants like pesticides, n-alkanes, polyaromatic hydrocarbons, chemically synthesized dyes (Fig. 18.3).

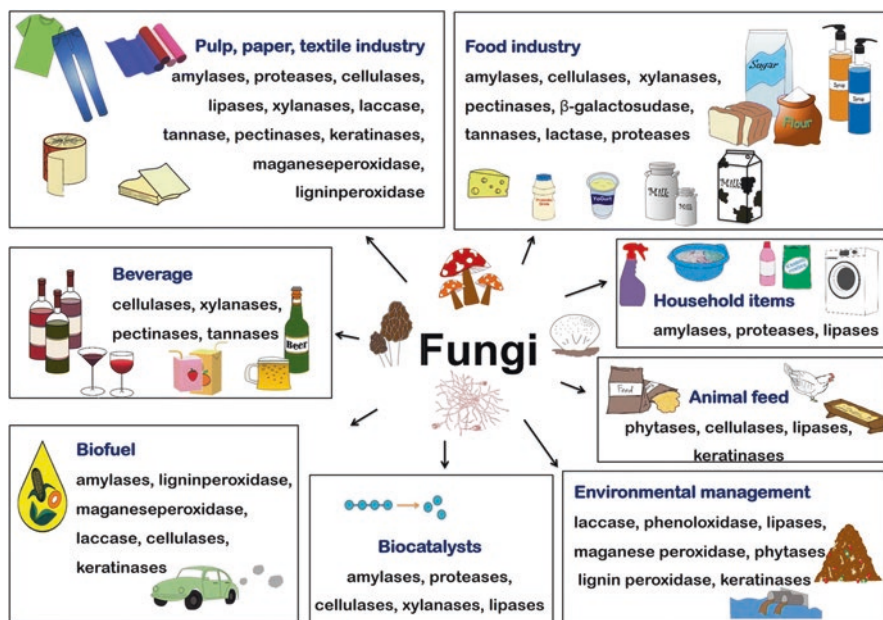


Fig. 18.2 Fungal enzymes and applications. (Adapted from Hyde et al. (2019))

18.3.1 Alleviation of Environmental Pollutants

In ecosystem, various types of pollutants like polyaromatic hydrocarbons, xenobiotics like DDT, colours dyes and heavy metals that have been released from the human activities like industrialization. Such pollutants have several types of hazardous effect, so, their removal is one important approach for scientist because naturally their degradation has a no or very less possibility. Therefore, mycoremediation is one strategy for the degradation of hazardous pollutants which are present in the nature from very long period of time (Ojuederie and Babalola 2017).

One of the pollutants that has been accumulated and possess serious threat to environment is heavy metals. Several types of heavy metals like cobalt, copper, iron, manganese molybdenum, mercury, chromium, cadmium, uranium, zinc, nickel, lead and arsenic present at high concentration in soil as well as in the water bodies (Ojuederie and Babalola 2017). These heavy metals have adversely effects in both soil and water quality which leads to reduction of yield of the crops and the marine animals (Chaturvedi et al. 2015; Sardar et al. 2018). To remove this contaminant from environment, mycoremediation is one of the best known strategy (Singh et al. 2020a). Many researches have been published in various journals in which different species of fungi have been reported for remediation of heavy metals like *Aspegillus awamori*, *Aspergillus flavus*, *Phanerochaete chrysosporium* and *Trichoderma viride* have been reported for the remediation of metals like Cd, Cr, Pb and Ni (Joshi et al. 2011). In another study, *Aspergillus fumigatus* has been reported

Table 18.2 Diversity of industrially important fungi

Fungi	Application	References
<i>Acremonium</i>	Huperzine A	Le et al. (2019)
<i>Acremonium strictum</i>	Amylase	Saleem and Ebrahim (2014)
<i>Alternaria alternata</i>	Amylase	Saleem and Ebrahim (2014)
<i>Aspergillus</i>	Huperzine A	Le et al. (2019)
<i>Aspergillus candidus</i>	Amylase	Saleem and Ebrahim (2014)
<i>Aspergillus foetidus</i>	Pectinase, Phytase	Pasha et al. (2013)
<i>Aspergillus fumigatus</i>	Pectinase	Banakar and Thippeswamy (2014b)
<i>Aspergillus fumigatus</i>	β -glucosidase, Xylanase	Saroj et al. (2018)
<i>Aspergillus niger</i>	β -glucosidase, Xylanase	Grujić et al. (2015)
<i>Aspergillus niger</i>	Amylase	Asrat and Girma (2018)
<i>Aspergillus niger</i>	Cellulase	Gautam et al. (2011)
<i>Aspergillus niger</i>	Tannin acyl hydrolase	Lal et al. (2012)
<i>Aspergillus raperi</i>	Amylase	Khokhar et al. (2011)
<i>Aspergillus</i> sp.	Amylase	Mukunda et al. (2012)
<i>Aspergillus</i> sp.	Amylase	Ominyi Matthias (2013)
<i>Aspergillus speluneus</i>	Amylase	Khokhar et al. (2011)
<i>Aspergillus terreus</i>	Xylanase	Bakri et al. (2010)
<i>Aspergillus terreus</i>	Amylase	Saleem and Ebrahim (2014)
<i>Aspergillus tubingensis</i>	Xylanase	Bakri et al. (2010)
<i>Aureobasidium pullulans</i>	Pectinase, Cellulase,	Gawas-Sakhalkar and Singh (2011)
<i>Aureobasidium pullulans</i>	Amylase, Xylanase	Singh et al. (2015)
<i>Botrytis verrucosa</i>	Cellulase, Protease,	Gawas-Sakhalkar and Singh (2011)
<i>Chaetomium globosum</i>	Amylase	Saleem and Ebrahim (2014)
<i>Cladosporium chlorocephalum</i>	Phosphatase, Esterase	Gawas-Sakhalkar and Singh (2011)
<i>Cladosporium cladosporioides</i>	Amylase	Saleem and Ebrahim (2014)
<i>Coniothyrium</i> sp.	Amylase, Pectinase	Sunitha et al. (2013)
<i>Coriolus versicolor</i>	Laccase	Levin et al. (2010)
<i>Discosia</i> sp.	Amylase, Cellulase	Sunitha et al. (2013)
<i>Emericella nidulans</i>	Amylase	Saleem and Ebrahim (2014)
<i>Fusarium</i> sp.	Hexanal	Katoch et al. (2017)
<i>Fusarium</i> sp.	Proteases, Amylases	Lanka et al. (2016)
<i>Fusarium</i> sp.	Huperzine A	Cruz-Miranda et al. (2020)
<i>Lasioidiplodia theobromae</i>	Lipases	Venkatesagowda et al. (2012)
<i>Lasioidiplodia theobromae</i>	Lipases	Venkatesagowda et al. (2018)
<i>Mortierella</i> sp.	Pectinase	Banakar and Thippeswamy (2014b)
<i>Mucor</i> sp.	Amylase	Ominyi Matthias (2013)
<i>Penicillium citrinum</i>	Amylase	Gawas-Sakhalkar and Singh (2011)
<i>Penicillium granulatum</i>	Amylase	Khokhar et al. (2011)
<i>Penicillium murcianum</i>	Yellow dye	Hernández et al. (2019)
<i>Pestalotiopsis</i> sp.	Amylase, Lipase, Laccase	Sunitha et al. (2013)
<i>Pestalotiopsis</i> sp.	Laccases	Wikee et al. (2019)
<i>Phialophora</i> sp.	Amylase	Gawas-Sakhalkar and Singh (2011)

(continued)

Table 18.2 (continued)

Fungi	Application	References
<i>Pleurotus</i> sp.	Laccase	Banakar and Thippeswamy (2014a)
<i>Podospora</i>	Huperzine A	Le et al. (2019)
<i>Rhizopus</i> sp.	Amylase	Ominyi Matthias (2013)
<i>Shiraia</i> sp.	Huperzine	Zhu et al. (2010)
<i>Syncephalastrum racemosum</i>	Pectinase	Banakar and Thippeswamy (2014b)
<i>Talaromyces albobiverticillius</i>	Red diffusible pigments	Frisvad et al. (2013)
<i>Talaromyces atroroseus</i>	Red diffusible pigments	Frisvad et al. (2013)
<i>Talaromyces australis</i>	Red dye	Hernández et al. (2019)
<i>Talaromyces purpurogenus</i>	Red diffusible pigments	Frisvad et al. (2013)
<i>Thermoascus aurantiacus</i>	Cellulases and Xylanase	Jain et al. (2015)
<i>Thermomyces lanuginosus</i>	Xylanase	Kumar et al. (2014)
<i>Trametes trogii</i>	Laccase	Levin et al. (2010)
<i>Trametes villosa</i>	Laccase	Levin et al. (2010)
<i>Trichoderma atroviride</i>	β -glucosidase	Grujić et al. (2015)
<i>Trichoderma reesei</i>	Ag Nano particles	Vahabi et al. (2011)
<i>Trichoderma reesei</i>	Xylanases, Exocellulases	Shrestha et al. (2015)
<i>Trichoderma</i> sp.	Cellulase	Gautam et al. (2011)
<i>Trichoderma virens</i>	Viridol, Virone	Kamala et al. (2015)
<i>Trichosporiella cerebriformis</i>	Polygalacturonase	Banakar and Thippeswamy (2014b)

for the alleviation of metals like Cd, Cr, Cu, Ni and Zn (Shazia et al. 2013). In a study, the alleviation of heavy metals like Pb, Cd and Cr has been remediated through the fungi like *Aspergillus nidulans*, *Rhizopus arrhizus* and *Trichoderma viride* (Shazia et al. 2013). In another study, *Beauveria bassiana*, which entomopathogenic fungi are reported for the alleviation of the heavy metals like Zn, Cu, Cd, Ni and Cr (Gola et al. 2016). Whereas, fungi like *Aspergillus fumigatus*, *Rhizopus* sp., *Penicillium radicum* have been reported for the remediation of metals Cr (bibi et al. 2018). In a study, even fungal consortium of *Perenniporia subtrophopora*, *Daldinia starbaeckii*, *Phanerochaete concrescens*, *Cerrena aurantiopora*, *Fusarium equiseti*, *Polyporales* sp., *Aspergillus niger*, *Aspergillus fumigatus* and *Trametes versicolor* has been used for remediation of As, Mn, Fe, Cu and Cr (Hassan et al. 2019). In another study, again different strains of *Aspergillus flavus* and *Aspergillus fumigatus* were reported for the removal of metals Cd (Talukdar et al. 2020a).

Another contaminant of environment, are xenobiotics which are chemical compounds produced by the humans synthetically for industrial use such as pesticides, fuels, alkanes, DDT, polycyclic hydrocarbons (PAH), synthetic azo dyes, chlorinated and nitro aromatic compounds (Hesham et al. 2021; Sharma et al. 2021; Yadav 2021). These manufactured compounds possess disastrous effects on the ecosystem and take very long period of time to disappear (Bharadwaj 2018). Therefore, their removal is an urgent need. Mycoremediation is one of the best techniques to alleviate it from the environment. Fungi like *Pleurotus ostreatus* (Purnomo et al. 2010), *Gloeophyllum trabeum*, *Fomitopsis pinicola*, *Daedalea dickinsii* (Purnomo et al. 2011), *Fomitopsis pinicola* (Sariwati et al. 2017) (Purnomo et al. 2020),

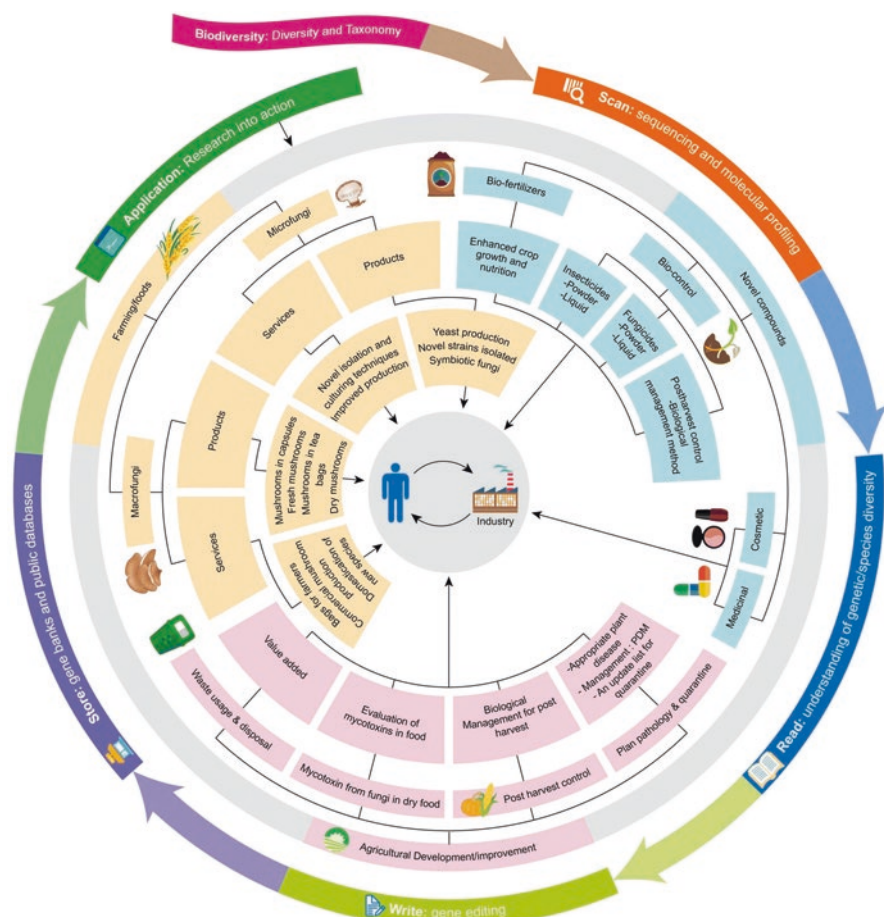


Fig. 18.3 Potential applications of fungi in biotechnology. (Adapted from Hyde et al. (2019))

Trichoderma hamatum, *Rhizopus arrhizus* (Russo et al. 2019), were reported for the degradation of DDT. *Fusarium* sp. (Sagar and Singh 2011), *Aspergillus terreus* (Silambarasan and Abraham 2013) and *Mortierella* sp. (Kataoka et al. 2010) were reported degrading pesticides. Whereas, fungi like *Curvularia* sp., *Lentinus* sp. and *Phanerochaete* sp. (Juckpech et al. 2012), *Penicillium chrysogenum*, *Mucor racemosus* and *Lasioidiplodia theobromae* (Balaji et al. 2014), *Agaricus bisporus* (García-Delgado et al. 2015) and *Scopulariopsis brevicaulis* (Mao and Guan 2016) have been reported for alleviating the polyaromatic hydrocarbons from the environment. Fungi like *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp. (Gopi et al. 2012), *Phanerochaete chrysosporium*, *Schizophyllum commune*, *Lenzites eximia* (Priya et al. 2013), *Penicillium oxalicum* (Saroj et al. 2014), *Aspergillus niger* and *Phanerochaete chrysosporium* (Rani et al. 2014), *Aspergillus niger* (Mahmoud et al. 2017) and *Lichtheimia* sp. (Abd El-Rahim et al. 2017) have been reported for the removal of synthetic azo dyes from the ecosystems.

There are some other pollutants which are affecting the environment is crude oil. This is caused by the shipping of oil from one place to another place. Oil contamination adversely affects diversity of soil as well as water. Various fungi species have been reported. *Polygonum aviculare* (Mohsenzadeh et al. 2010), *Agaricus* sp., *Coriolus* sp., *Lentinus edodes* and *Pleurotus ostreatus* (Pozdnyakova et al. 2011) have been reported for the removal of oil from the soil.

18.3.2 Industrial Applications

18.3.2.1 Food Industries

Fungi play an immense role in the food industries. Fungi act as the source of the food and a wide range of the food ingredients is obtained from fungi such as acidulants, carotenoids, colourants, enzymes, flavours, polyunsaturated fatty acids, proteins and vitamins (Copetti 2019; Singh et al. 2020b). The utilization of the fermentation-derived ingredients in food sector is increasing year after year such as gelling agents, flavour compounds and flavour enhancers. Fungi have a great potential in fermenting fruits and grains for cheese, bread and alcoholic beverages production (Singh et al. 2020b). Over past few years, commercial cultures of yeast have gained importance and are increasingly used for production of fermented cheese, ethanol and milk from lactose and whey solutions (Awasti and Anand 2020). Diverse species of molds find their application in ripening of Camembert and Roquefort cheese and enzyme production. Filamentous fungi have an amazing potential to produce pigments including flavins, indigo, monascins, phenazines and quinines (Dufosse et al. 2014). Pigments obtained from fungi have many advantages such as they are biodegradable, non-toxic, show various chemical profiles and could be produced from cheap raw materials (Heo et al. 2018).

Fungi are an excellent source of enzymes of industrial importance. *Aspergillus*, *Mucor* and *Rhizopus* are used for production of lipases. Lipases give stronger flavours in Italian cheeses by a modest lipolysis (Drożdżowska 2019). Lipase-modified butter fat has broad application in a variety of food processes (Aravindan et al. 2007). Xylanases have an important role in baking industry for improving bread quality and also in wine and juice clarification (Butt et al. 2008; Malathi and Devegowda 2001). Xylanases have been produced from *Paecilomyces thermophila* (Yang et al. 2006), *Aspergillus oryzae* (Pirota et al. 2013) and *Aspergillus niger* (Izidoro and Knob 2014). Glutaminases control the taste of fermented foods including soy sauce, especially in Asian countries (Nandakumar et al. 2003) and have been produced from *Candida scottii*, *Candida utilis*, *Hansenula* sp. and *Rhodotorula* sp. (Sarada 2013).

Emulsifiers are another important compounds derived from fungi. Emulsifiers have a major role in food industry as they increase the uniformity of nutrients, such as amino acids, fatty acids and fat-soluble vitamins. Other roles include generating interactions with proteins to produce a modifiable structure and adhesion correction

which affect the fluidity of molten chips and prevent fat appearance on the surface of chocolate (Alizadeh-Sani et al. 2018). Carbohydrate-protein complex from *Candida lipolytica* (Huang et al. 2011) and sophorous lipid and NDA from *Candida lipolytica* and *Candida utilis*, respectively (Shekhar et al. 2015) are fungal-derived emulsifiers.

18.3.2.2 Pharmaceuticals Industries

The pharmaceutical industry is a highly synchronized sector of industry and all manufacturing must be carried out in compliance with good production practices. Traditionally, considering cost disadvantages and the fact that continuous processing in certain cases might contribute to the production of purer products, virtually all production operations have been carried out batch wise. The industry of pharmaceuticals is by far the largest drugs producer, with an estimated worldwide annual budget for development of drug US\$6billion. In the company of pharmaceutical has manufacturing a wide range of organic compound of chemical structures. They are frequently classified according to their therapeutic function like anti-inflammatory/analgesic drugs, antibiotics, β -blockers, estrogens, lipid regulators, psychiatric drugs and iodinated contrast media (Cruz-Morató et al. 2012; Devi et al. 2020b).

Fungi have been found in all environment condition of old in the Pacific Ocean floor, where food availability is less and the sediments are more than 100 million years. This discovery contributes to the belief that life is everywhere and in unexpected locations and it also provides an incentive for pharmaceutical companies searching for new and more successful antibiotics to fix which drug-resistance bacteria crisis (Plumb 2005). In years 1940s, a new period has started in the field of biotechnology and medicine with the discovery of penicillin from the filamentous fungus *Penicillium notatum*, which can use as therapeutically. The *Penicillium* species are of great interest from a medicinal point of view. These species were found permanently in cold soils, including *Penicillium lanosum* and *Penicillium soppii*, they can efficiently produce useful secondary metabolites compounds cycloaspeptide A and Griseofulvin, both are antibiotic compound as well as showed antimicrobial activity (Frisvad et al. 2006).

Among fungi have the best tools for new bioactive agents that are commonly used to protect against many pathogenic fungi (Deshmukh et al. 2017). The great concern in pharmaceutical application is regarding antibiotic resistance, which in turn makes traditional medicine useless. Secondary metabolites compound were obtained from fungi for using in disease-causing agents to improve drug sensitivity. Fungi are potentially to produce secondary metabolites that include anti-oxidant, anti-tumor's activity (Mishra et al. 2017). It have anti-fungal compound like caspofungin-immunosuppressant drug like cyclosporine and lovastatin for lowering cholesterol (Devi et al. 2020b).

The physiological range of phenolic compounds of fungal species is wide such as anti-atherogenic, anti-thrombotic, anti-microbial, anti-inflammatory, anti-allergic, vasodilatory, anti-oxidant and cardio-protective activity (Pan et al. 2017).

Alkaloid is a category of β -carboline containing anti-microbial, anti-HIV and anti-parasitic secondary metabolites (Patel et al. 2012). *Aspergillus*, the genus of fungi from which alkaloid is secreted is *Penicillium*, *chromocleista* and *pestalotiopsis* (Ma et al. 2016). An anti-fungal protein, AfAFPR9, was isolated from the south Atlantic sediment sample isolated from *Aspergillus fumigatus* R9, which has an anti-fungal activity against pathogenic plants *Alternaria longipes*, *Trichoderma viride*, *Paecilomyces variotii*, *Colletotrichum gloeosporioides* and *Fusarium oxysporum* (Rao et al. 2015). A novel anti-microbial protein (Pc-Arctin) was purified from *Penicillium chrysogenum* A096 isolated from Arctic sediment in another study showing anti-microbial activity against *Penicillium variotii*, *Aspergillus Longipes* and *Tricoderma viride* (Chen et al. 2013). In this study, Pinheiro et al. (2017) have been reported the polyketide monocerin metabolites from *Exserohilum rostratum*, an endophytic fungus of the traditional Amazon plant (*Bauhinia guianensis*). This unique bioactive compound has the action of antagonist against *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923).

18.3.2.3 Biorefineries

The development and advancement of novel biotechnology methods can resolve the major concerns such as environmental pollution and global warming. It can be achieved through integrated biorefinery bioprocesses to introduce biomass as a best renewable source so that bio-based products could be developed on the large scale (Haddadi et al. 2018). Biorefineries actually take into account sustainable processing of biomass into a spectrum of marketable products and energy. The environment-threatening and non-renewable nature of fossil fuels and waste generation are actually leading to a paradigm shift from fossil fuel refineries to waste biorefineries. The successful examples include conversion of food waste into biogas, and compost and waste oil to biodiesel through microbial communities (Ferreira et al. 2019).

Filamentous ascomycetes including the species of *Aspergillus*, *Fusarium*, *Monascus* and *Neurospora* are known to be versatile fungi with immense potential to grow in an array of different substrates ranging from hexose and pentose monomers to disaccharides to carbohydrate polysaccharides (Angumeenal and Venkappayya 2005; Ferreira et al. 2015). This amazing versatility is perhaps related to diverse enzymes produced by these fungi while growing on a particular substrate. This capability makes fungi potent communities to play core roles within waste biorefineries through valorization of waste materials from different industrial sectors. Filamentous fungi are gaining greater interest as biocatalysts in biorefineries due to their capability of producing organic acids which could be used as building blocks in the chemical industry (Chowdhary et al. 2018). The species of *Aspergillus* are among the major fraction in commercially produced organic acids including itaconic acid, gluconic acid and citric acid (Pandey et al. 2015). Filamentous ascomycetes due to their metabolic versatility are capable of producing diverse value-added products from waste including enzymes, ethanol and biomass for feed

applications (Ferreira et al. 2016). Zygomycetes have been explored for ethanol production for a decade with promising results (Ferreira et al. 2013). The development of advanced biomass systems efficiently combining biomass production and its transformation, and the utilization of by-products would promote the implementation of biorefineries.

18.4 Conclusion

Fungi, the important group of organisms that naturally helps in the cleaning of the ecosystem, have several types of application in the field of environment and industries for the purpose of removal of pollutants and the production of various industrially important enzymes, dye and secondary metabolites. In the conclusion of fungi, is one of the important organisms for the mankind and in future, these organisms have a broad range of applications in both agriculture and industries.

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