Fungal Biology

Ajar Nath Yadav *Editor*

Recent Trends in Mycological Research

Volume 1: Agricultural and Medical Perspective

Springer

Fungal Biology

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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

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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 can be associated with plants epiphytically, endophytically and rhizospherically. Extreme environments represent unique ecosystems that harbour 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, ranging from nutrient cycling to environmental detoxification, which involves processes such as augmentation, supplementation and recycling of plant nutrients – particularly vital to sustainable agriculture. Fungal communities from natural and extreme habitats help promote plant growth, enhance crops yield and enhance soil fertility via direct or indirect plant growth promoting (PGP) mechanisms such as solubilization of phosphorus, potassium and zinc; and production of ammonia, hydrogen cyanides, phytohormones, Fe-chelating compounds, extracellular hydrolytic enzymes and bioactive secondary metabolites. These PGP fungal could be used as biofertilizers, bioinoculants and biocontrol agents in an ecofriendly manner for sustainable agriculture and environment instead of chemical fertilizers and pesticides. Besides agricultural applications, medically important fungi play a significant role in human health. For sustainable environment, fungal communities are used in bioremediation process that uses microorganism's metabolism to biologically degrade waste contaminants (sewage, domestic and industrial effluents) into nontoxic or less toxic substances. Fungi could be used as mycoremediation for the future of environmental sustainability. Fungi and fungal products have biochemical and ecological capability to degrade environmental organic chemicals and to decrease the risk associated with metals, semi-metals and noble metals either by modifying chemical structure or by manipulating chemical bioavailability.

The aim of book *Recent Trends in Mycological Research, Volume 1: Agricultural and Medical Perspective* is to provide an understanding of fungal communities from diverse environmental habitats and their potential applications in agriculture, medicine, environment and industry. The book will be useful to scientists, researchers and students involved in microbiology, biotechnology, agriculture, molecular biology, environmental biology and related subjects.

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Ajar Nath Yadav

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All authors are sincerely acknowledged for contributing up-to-date information on beneficial fungi, their biodiversity and biotechnological applications for sustainable agriculture and environment. The editor is thankful to all authors for their valuable contributions.

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

I am grateful to my Ph.D. research scholars Dr. Divjot Kour, Ms. Tanvir Kaur, Ms. Rubee Devi and colleagues for their support, love and motivation in all my efforts during this project.

I am very sure that this book will interest scientists, graduates, undergraduates and postdocs interested in fungal biology and biotechnology.

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About the Editor

Ajar Nath Yadav is an Assistant Professor (Senior Scale) in Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India. He has 5 years of teaching and 11 years of research experience in the field of microbial biotechnology, microbial diversity and plant–microbe interactions. He earned his Ph.D. in microbial biotechnology, jointly from ICAR-Indian Agricultural research Institute (New Delhi, India) and Birla Institute of Technology (Mesra, Ranchi, India); M.Sc. in Biotechnology from Bundelkhand University and B.Sc. in CBZ from the University of Allahabad, India. He has 211 publications, with *h*-index of 50, *i*10-index of 137 and 6821 citations (Google Scholar- on 01/01/2021) and 112 research communications in different national and international conferences.

Dr. Yadav is editor of 16 Springer-Nature, 7 Taylor & Francis, 2 Elsevier and 1 Wiley books. In his credit one granted patent "Insecticidal formulation of novel strain of *Bacillus thuringiensis* AK 47". He has got 12 Best Paper Presentation Awards and 1 Young Scientist Award (NASI-Swarna Jayanti Purskar). Dr. Yadav received "Outstanding Teacher Award" in 6th Annual Convocation 2018 by Eternal University, Baru Sahib, Himachal Pradesh, India. He has a long-standing interest in teaching at the UG, PG and Ph.D. level and is involved in taking courses in microbiology and microbial biotechnology. He is currently handling two projects: one funded by Department of Environments, Science & Technology (DEST), Shimla; and other by

HP Council for Science, Technology & Environment (HIMCOSTE). He has guided 1 Ph.D. and 1 M.Sc. Scholar; presently, he is guiding 5 scholars for Ph.D. degree. In his credit, ~6700 microbes (Archaea, bacteria and fungi) isolated from diverse sources and ~550 potential and efficient microbes deposited at culture collection ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh, India. He has deposited 2423 nucleotide sequences, 3 whole genome sequences (*Bacillus thuringiensis* AKS47, *Arthrobacter agilis* L77 and *Halolamina pelagica* CDK2) and 2 transcriptome to NCBI GenBank databases in public domain. The niche-specific microbes from extreme environments were reported as specific bioinoculants (biofertilizers) for crops growing in normal and diverse abiotic stress conditions. He and his group were the first to develop technology for screening archaea for phosphorus solubilisation. Dr. Yadav is editor-in-chief for "Journal of Applied Biology and Biotechnology". He has been serving as an editor/editorial board member and a reviewer for 25 different national and international peer-reviewed journals. He has lifetime membership of Association of Microbiologist in India and Indian Science Congress Council, India. Please visit https://sites.google.com/view/ajarnathyadav/ for more details.

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Chapter 1
Role of Useful Fungi in Agriculture Sustainability

Laith Khalil Tawfeeq Al-Ani, Surono, Liliana Aguilar-Marcelino, Viviana E. Salazar-Vidal, Alejandra G. Becerra, and Waqas Raza

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1.1 Introduction

Fungi are a group of eukaryotic organisms and source of food, organic acids, alcohol, antibiotics, growth-promoting substances, enzymes, and amino acids. They include microorganisms like molds, yeasts, and mushrooms. Fungi are tremendous decomposer of organic waste material and most readily attack cellulose, lignins, gums, and other organic complex substances. Fungi can act also under a wide range of soil reactions from acidic to alkaline soil reactions. Fungi can jointly play a basic role in different physiological processes as well as mineral and water uptake, chemical change, stomatal movement, and biosynthesis of compounds termed biostimulants, auxins, lignan, and ethylene to enhance the flexibility of plants to ascertain and cope with environmental stresses like drought, salinity, heat, cold, and significant metals. The primary functions of filamentous fungi in the soil are to degrade organic matter and help in soil aggregation. Besides this property, bound species of *Alternaria, Aspergillus, Cladosporium, Trichoderma*¸Dematium*, Gliocladium, Humicola,* and *Metarhizium* manufacture substance like organic compounds in soil and therefore could also be necessary for the maintenance of soil organic matter. Plant growth regulators and chemical fertilizers have been used to increase crop production (Islam 2008), as well as, a natural product used in the reduction of plant diseases (Al-Ani et al. 2012; Al-Ani 2017b; Mohammed et al. 2011, 2012) and control of pests (Adetunji et al. 2020) as well as, plant extract can use against plant pathogens (Sarker et al. 2020; Jatoi et al. 2020).

Although often inconspicuous, fungi occur in every environment on earth and play very important roles in most ecosystems. Along with bacteria, fungi are the major decomposers in most terrestrial and some aquatic ecosystems, and therefore play a critical role in biogeochemical cycles and in many food webs (Gadd 2007; Devi et al. 2020b). As decomposers, they play an essential role in nutrient cycling, especially as saprotrophs and symbionts, degrading organic matter to inorganic molecules, which can then re-enter anabolic metabolic pathways in plants or other organisms (Barea et al. 2005: Lindahl et al. 2007).

In agriculture sustainability, there are many useful resources possibly utilized in agriculture as biofertilizers and biopesticides, such as microbes comprising virus, bacteria, fungi, and nematode (Sharma et al. 2020; Kour et al. 2020a; Rastegari et al. 2020a, b). Biotechnological tools were utilized to determine the importance of useful microbes (Aguilar-Marcelino et al. 2020b). Useful virus as non-pathogenic or milder strain can be used to control high pathogen as a mechanism called “Cross-Protection” (Al-Ani and Furtado 2020). Bacteria interact with plants and affect the plant pathogens (Al-Ani 2017a; Singh et al. 2021) showing efficacy in the control of cucumber mosaic virus by induced systemic resistance (Al-Ani 2006; Al-Ani and Al-Ani 2011) and *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc*TR4) (Mohammed et al. 2013, 2014). Nematodes attacked insect and show efficiency as entomopathogenic such as *Steinernema carpocapsae* (Al-Jboory and Al-Zubai 2006; Katumanyane et al. 2020; Thakur et al. 2020). For fungi, it may be useful if they actively compete for nutrients and space with pathogenic microorganisms via

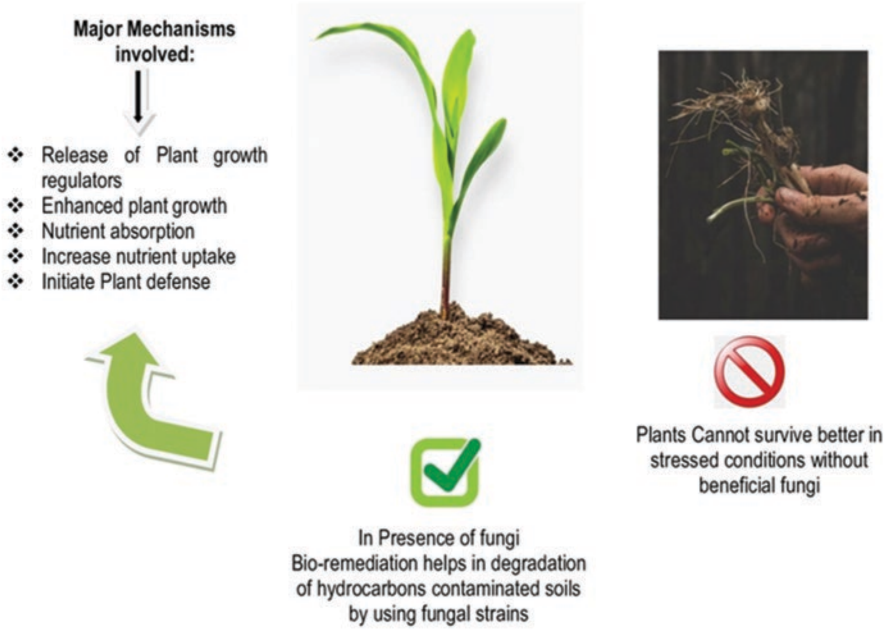

Fig. 1.1 Role of useful fungi in plant growth

the competitive exclusion principle (Fig. 1.1). Entomopathogenic fungi (i.e., a fungus that can act as a parasite of insects and kills or seriously disables them) can be used as biopesticides, as they actively kill insects like *Beauveria bassiana*, *Metarhizium* spp., *Hirsutella* spp., *Paecilomyces* (*Isaria*) spp., *Lecanicillium lecanii* (Keiller 2011), and *Fusarium proliferatum* (Al-Ani et al. 2018).

Endophytic fungi (living within the plant) of grasses of the genus *Neotyphodium*, such as *N. coenophialum*, produce alkaloids that are toxic to a range of invertebrate and vertebrate herbivores (Bouton et al. 2002). The use of fungi in the degradation contaminant in agricultural land is called mycoremediation which is a phrase coined by Paul Stamets, a form of bioremediation that uses fungi to degrade contaminants in the environment. The key to mycoremediation is determining the right fungal species to target a specific pollutant (Fomina et al. 2008). *Trichoderma* is a more beneficial fungus for the ability to control many plant pathogens, pests, and weeds by two main mechanisms, first: mycoparasitism, and second: non-mycoparasitism and active in different environment (Al-Ani 2018a, b).

The application of chemical fertilizers to crop plants negatively affects human health and environments. Recent studies have focused on the identification of alternative methods to enhance plant productivity and protect the soil. Soil-borne microbes can enter roots and establish their population in plants as endophytes, and many plant-associated fungi are well known for their capacity to promote plant growth; however, the relationship between these microbes and plants is still uncertain (Sylvia et al. 2005; Rana et al. 2020a). Microorganisms have the ability to produce phytohormones, solubilize insoluble phosphate, and convert complex organic substances to simple forms (Kour et al. 2020b; Singh et al. 2020a). Endophytic fungi have also been shown to impart plants with tolerance to salt, drought, heat, and diseases (Schulz et al. 2002; Jalgaonwala et al. 2017; Rana et al. 2019a).

Soil fungi can grow in a wide range of soil pH but the soil fungi is more acidic conditions tolerance, because is severe competition with bacteria at neutral pH. A majority of fungi are aerobic and prefer to grow at optimum soil moisture. The contribution of these organisms in biochemical transformation under excessive moisture is negligible (Smith and Read 2008). The fungi have ability to produce a wide variety of extracellular enzymes, they are able to break down all kinds of organic matter, decomposing soil components and thereby regulating the balance of carbon and nutrients for maintaining soil health (Kour et al. 2019b). This allows fungi to bridge gaps in the soil to transport nutrients relatively far distances back to the plants (Sturz et al. 1997). Soil is a primary source of fungal growth and is associated with the roots of all plant species. Fungi produce a wide range of bioactive metabolites, which can improve plant growth (Waqas et al. 2014; Devi et al. 2020a). In addition, fungi supply inorganic nutrients to plants, such as ammonium, nitrate, and phosphate (Seastedt et al. 2008), and they are used as biofertilizers. *Rhizosphere* microorganisms can overcome competition with other soil factors and survive under variable environmental conditions (Ferrara et al. 2012).

The fungi have been utilized for controlling insect pests. The microbial control of insect pests emerged 100 years ago. Insect is infected by fungi through the body surface and this property is different from the infection caused by bacteria, viruses, and protozoa. Fungi attacking insect are called entomogenous. The conidia of the insect attacking fungi are attached to the insect integument where they germinate and the germ tubes penetrate in insect body under optimum temperature and humidity. The fungus proliferates in the insect body and the insect body gets covered with mycelia and conidia.

1.2 Fungal Pathogens for Mite and Insect

Fungi caused diseases for mites and insects; many patents about entomopathogenic fungi were registered (Al-Ani 2019d). Fungi that affect mites are Zygomycetes such as *Erynia phalangicidae* that affect the mite *Pergamasus* sp. (Bałazy and Wisniewski 1984), other fungus is *Neozygites acaridis* that attacks *Halotydeus destructor* (James 1994), within the Deutoromycetes are *Beauveria bassiana* that affects *Tarsonemidae* (Peña et al. 1996) and *Hirsutella kirchneri* that affects *Abacarushystrix* mites, *Eutetranychus orientalis*, *Hemisarcoptes coccophagus*, *Panonychus citri*, *Phyllocoptruta oleivora* (Minter et al. 1983; Sztejnberg et al. 1997; Cabrera and Domínguez 1987), another fungus is *Paecilomyces eriophytis* affected by *Polyphagotarsonemus latus* (Peña et al. 1996), and another is *Rickia* sp. that affects *Lobogynium* sp. and *Hirstionyssus* sp. (Poinar Jr and Poinar 1998). *Hirsutella* is one of the most abundant and important fungi for pest insect control in the field. It includes approximately 90 species that are capable of infecting and parasitizing a wide variety of invertebrate tales such as mites, nematodes, and insects, many of which are considered major pests.

Fungi species belonging to the genus *Hirsutella* are capable of infecting and parasitizing a wide variety of pathogenic invertebrates. Its development on the host produces a grayish yellowish mycelium with low percentages in the formation of conidia. The morphogenetic characterization of *Hirsutella* has been carried out using conserved regions of rRNA called ITS, which has allowed to reveal homologies with important biocontroller genera such as *Beauveria* or *Cordyceps*. The biocontrolling properties of the fungus act on diverse species of mites and insects that cause diseases in crops of agronomic importance. In mites, the ability to colonize and control species such as *Aceria guerreronis* (damage to coconut fruits), *Acalitus vaccinii* (blueberry sprout mite), *Tetranychus urticae*, and *Calacarus heveae* (rubber tree pathogen) has been recorded. The fungus *Hirsutella* pathogenicity has been found to be due to complex metabolic toxins that develop during the vegetative phase, such as “hirsutellins” in *H. thompsonii*.

Among the mass production processes, *Hirsutella* shows high degrees of pathogenicity on mites such as *Aceria guerreronis*, *Tetranychus urticae*, and *Brevipalpus* sp., during the stages of conidiación. Rosas (2003) highlights the pathogenic potential of *H. thompsonii* on mites of the following families: Eriophyidae, Tetranychidae, Tenuipalpidae, Tarsonimidae, and Brevipalpidae, so with its three varieties, this fungus is considered the most important for the natural regulation of arthropods. A plague *Hirsutella* has been isolated from different host mites in tropical and temperate regions. *Hirsutella* is one of the most abundant and important entomogenous fungi, and play an important role in the biocontrol of pest insects. The fungus *Hirsutella* includes three important species: (1) *H. thompsonii,* (2) *H. gigantea*, and (3) *H. citriformis*. This genus has been one of the most difficult members for identification among all major genera of fungal entomopathogens largely because of the huge number of species and high variability among these species. *Hirsutella* spp. have been found efficient against nymph and adults of red spider mite, adults. the varroa mite (*Varroa destructor*) of honey bees, coconut eriophyid mite (*Aceria gueneronis*). The attack mode of fungi *Hirsutella* sp. penetrates into the mites mainly through the legs, which later on forms hyphal bodies in chains in the hemolymph.

The pathogenicity of the fungus *Hirsutella* strains against target or Diana pests is an important characteristic and its ability for mass production. One of the most important advantages is they have a restricted host range and are harmless to non-target microorganisms. Therefore, it might be used within integrated pest management programs, the disadvantages of biopesticides are that they have a relatively short shelf life that can be for a few weeks and are highly sensitive to the environmental conditions. New techniques need to be developed that will help manage the pests in better ways since the present pathogenesis mechanism of entomopathogens is slow and needs improvement.

On the other hand, in a study reported by Cabrera et al. (2005) six-monthly samples were taken from August 1997 to January 1998 to identify the fungus species associated with the tarsonemid mite *Steneotarsonemus spinki* (Smiley 1967), which infests rice, at five locations of Cuba. The species found included *Hirsutella nodulosa* Petch, 1926, *Sarocladium oryzae* Sawada, 1922, and three species belonging to the genera *Penicillium* Link, 1809, *Cladosporium* Link, 1816, and *Cephalosporium* Corda, 1839, as well as at least one unidentified fungus species. The fungus *H. nodulosa* parasitizing *Steneotarsonemus spinki* constitutes the first record of such association for Cuba, and probably for the entire world. *Sarocladium oryzae* was isolated from 70% *S. pinki* transferred to Saboureaud Glucose Agar medium, supporting the hypothesis that the mite is the main vector of this phytoparasitic fungus. The fungus *H. nodulosa* caused tarsonemid mortality near 71%. The *S. oryzae* fungus is strongly related to *S. spinki*, which is considered its main carrier.

In another study, the variability of *Hirsutella thompsonii* strains was reported, isolated from phytophagous mites from three terrestrial systems in the State of Colima, México. Between 1999 and 2004, ten strains of the fungus *Hirsutella thompsonii* Fisher were isolated from infected acari, in 9 localities of 3 of the 11 terrestrial systems found in the state of Colima, Mexico: Coastal Plain of Tecomán, Coastal Plain of Cuyutlán, and Valley of the Armería river of the 10 strains, 5 belong to var. thompsonii, whose growth is fluffed mycelial, slightly tall, gray to greenish-gray, and 5 to *var.* sinematosa, with a flat appearance, white to yellow. The hosts were two-spotted spider mite (*Tetranichus urticae*), Eriophidae (*Aceria guerreronis* and *Phyllocoptruta oleivora*) and Brevipalpidae (*Brevipalpus phoenicis*), *Citrus* spp. being the host of the acari in 8 of the 10 isolations. Of the 10 strains, only one HtM130 strain of *H. thompsonii* var. thompsonii displayed exudate formation in the sporulation phase, characteristic that gives it greater potential for implementation in management strategies for the control of phytophagous acari. Strain coloration and growth are not correlated with the terrestrial system where they were found, nor to prevailing climatic conditions in the system. This contribution demonstrates the importance of assessing the presence of native natural enemies, before introducing commercial microorganisms or isolations from elsewhere so as not to affect the natural regulatory systems (Rosas-Acevedo and Sampedro-Rosas 2006).

1.3 Nematophagous Fungi

Nematophagous fungi (NF) are one of the main natural antagonistic microorganisms of parasitic nematodes on plant and animal (Nordbring-Hertz et al. 1982, 2006) and they have been determined more than 700 species of NF (Zhang et al. 2011; Li et al. 2015). These cosmopolitan organisms in nature find themselves in a saprophytic way and pose the ability to convert into carnivores (de Freitas Soares et al. 2018). Many species of fungi (HN) have the ability to modify their nutritional strategy to cope with environmental variation, or more often, the presence or absence of a host, e.g., phytopathogenic fungi are a classic example of this versatility, as they switch from phytopathogenic to saprophytic modalities when their hosts die and are transformed into organic matter. This versatility enables them to survive until new living hosts with which to resume pathogenic activity become available (Souza and Rocha de Brito 2017).

The NF is divided into four groups according to the mechanism of action against nematodes (Yang & Zhang 2014). The first group conforms to the NF forming the tramp belonging to the phyla Zygomycota and Ascomycota as examples of these NF are found: *Cystopage* sp., *Arthrobotrys* spp. and *Dactyellina* spp., the structures for infecting nematodes that develop NF that fall within this group are mainly adhesive hyphae, adhesive networks, adhesive knobs and non-constricting rings (Moosavi and Zare 2012).

The second group is made up of the endoparasitic fungi that are grouped into the phyla Oomycota, Chytridiomycota, Blastoclamidiomycota, and Ascomycota, some examples of NF cases and their mechanism of action are: *Catenaria* (constricting rings), *Dreshmeria* spp. (Dijksterhuis et al. 1991), *Hirsutella* spp. (adhesive conidia), and *Haptoglossa* spp. “Gun cell” injection (Beakes and Glockling 1998). In the third group, they find themselves with the NF egg and female parasitic fungi that belong to the phyla Oomycota, Ascomycota, and examples of these NF and their mechanism of action: *Cordyceps* (Appressoria), *Nematophthora* (Zoospores), and *Pochonia* (Appressoria) (Ghahremani et al. 2019). *Pleurotus ostreatus* and *P. eryngii* showed the ability in reducing of population the nematode Haemonchus contortus (Comans-Pérez et al. 2021). In the fourth group are found the NF toxin-producing fungi that belong to the phyla Basidiomycota and examples of these NF and their activation mechanism are: *Pleurotus ostreatus* (Toxic droplets) (Arizmendi et al. 2014), *P. djamor* (Toxic droplets), *P. pulmonarius* (Toxic droplets), *P. eryngii* (Toxic droplets) (Pineda-Alegría et al. 2017; Cuevas-Padilla et al. 2018), and *Coprinus comatus* (spiny structures) (Luo et al. 2004), among others.

The isolation of the NF was based on soil, leaf litter, feces of ruminants: sheep, calves, and buffaloes, in respect of a study carried out by Ojeda-Robertos et al. (2019), loss NF from the feces of water buffalo (WB) and soil from southeastern Mexico were isolated, and there in vitro predatory activity against *Haemonchus contortus* infective larvae (L3) (HcL3) was assessed. Four NF isolates corresponding to *Arthrobotrys oligospora*, var. *microspora* (strains 4–276, 269 and 50–80), and one identified as *A. oligospora*, var. *oligospora* (isolates 48–80) were obtained from WB feces. From the soil, five isolates were isolated; three corresponded to *A. musiformis* (Bajío, Yumca, and Macuspana isolates), and two isolates were identified as *A. oligospora* (Comalcalco and Jalapa de Méndez isolates). The predatory activity of isolates from WB feces ranged between 85.9 and 100%. Meanwhile, the fungi from the soil ranged between 55.5 and 100% ($p \leq 0.05$). The NF obtained could have important implications in the control of parasites of importance in the livestock industry.

Another study reported by Castañeda-Ramírez et al. (2016) determined the morphological taxonomy of 18 nematophagous fungi (NF), as well as their in vitro predatory activity against *H. contortus* infective larvae (L3). Fungi were classified into six genera and 3 species, the most common of which were *Monacrosporium eudermatum* and *Arthrobotrys oligospora*. The sequenced five NF isolates used ITS4 and ITS5 primers. These sequences showed identity with sequences from the NCBI database (98–99%). In contrast, alignments among the same genera and species demonstrated 83–97% identity. Polymorphisms observed between *Arthrobotrys* and *Monacrosporium* appear to be associated with differences in biological function, nonspecific mutations, evolutionary processes, feeding behavior, predatory activity, and microecosystems.

On the other hand, a study reported by Rodríguez-Martínez et al. (2018) aimed to evaluate the in vitro lethal activity of the NF *Clonostachys rosea* against five nematodes species belonging to different taxa. Two groups of 35 Petri dishes (PD) each were divided into 5 series of 7 (PD). Group 1 (series 1, 2, 3, 4, and 5) contained only water agar; meanwhile, group 2 plates (series 6, 7, 8, 9, and 10) contained *C. rosea* cultures growth on agar. Every plate from the two groups was added with 500 nematodes corresponding to the following genera/specie: *H. contortus*, *Caenorhabditis elegans*, *Rhabditis* sp., *Panagrellus redivivus*, and *Butlerius* sp. After 5-day incubation at room temperature, free (nontrapped) larvae were recovered from plates using the Baermann funnel technique. Recovered nematodes were counted and compared with their proper controls. Results show a reduction percentage of the nematode population attributed to the fungal lethal activity as follows: *H. contortus* (L3) 87.7%; *C. elegans* 94.7%; *Rhabditis* sp. 71.9%; *P. redivivus* 92.7%; and *Butlerius* sp. 100% ($p \leq 0.05$). The activity showed by *C. rosea* against the *H. contortus* can be crucial for further studies focused on the biocontrol of sheep hemonchosis, although the environmental impact against beneficial nematodes should be evaluated.

The edible mushrooms, medicinal, and wild mushrooms are the three major components of the global mushroom industry. Combined, the mushroom industry was valued at $ 63 billion in 2013. Cultivated, edible mushrooms are the leading component (54%) accounting for $ 34 billion, while medicinal edible mushrooms make up 38% or $24 billion and wild mushroom account for $ 5 billion or 8% of the total (Royse et al. 2017).

The NF have great potential in biotechnological application against various pests. In an extensive review by Castañeda-Ramírez et al. (2020), an overview of the use as sustainable tools for the control of parasitic nematodes affecting agriculture and livestock industry is shown. Nematodes are organisms living in the soil and animals' guts where they may live as parasites severely affecting economically important crops and farm animals, thus causing economic losses to worldwide agriculture. Traditionally, parasitic nematodes have been controlled using commercial pesticides and anthelmintic (AH) drugs. Over the years, nematodes developed resistance to the AH drugs, reducing the usefulness of many commercial drugs. Also, the use of pesticides/anthelmintic drugs to control nematodes can have important negative impacts on the environment. Different NF have been not only used as food but also studied as alternative methods for controlling several diseases including parasitic nematodes. The nematocidal activity of NF assessed their potential use as sustainable tools for the control of nematodes affecting agriculture and livestock industry.

Among the forms of administration of the HN are: cereal grains, mineral blocks, energy blocks, alginate pellets, boluses of controlled release, and multi-nutritional pellets (CM). The application form of *Duddingtonia flagrans* for the control of livestock parasites is through oral administration (Aguilar-Marcelino et al. 2017). The NF *D. flagrans* has been studied and its antagonistic activity of animal parasitic nematodes has been demonstrated both in vitro and in vivo tests, and may be a biological tool in the control of livestock parasites (Mendoza de Gives et al. 2019).

Chlamydospores of *D. flagrans* have been incorporated into CM by adding molasses to make them tastier to sheep. In addition, an optimal amount of protein and energy has been added in the formulation for the elaboration of the CM. Once the MCs are ingested by the sheep, the chlamydospores pass through the animals' gastrointestinal tract and are eliminated along with the feces into the environment where they germinate and form their traps with which they capture and kill the larval stages of parasites to finally feed on their tissues. Under field conditions, *D. flagrans* has been proven to significantly reduce infection of grasslands with ruminant gastrointestinal nematodes (Mendoza de Gives et al. 2018).

Recently, the effect of supplementation of *D. flagrans* chlamydospores in Saint Croix sheep was evaluated on the *H. contortus* larvae population in feces and on weight gain. The results show that the use of *D. flagrans* using the CM as an administration vehicle can contribute significantly in providing a nutritional requirement similar to that of a commercial feed and also strengthen the immune system of sheep of the Santa breed. Cruz, in addition to reducing the *H. contortus* population in fecal cultures of sheep supplemented with MC in a range of 42.1–84.3%; it is important to note that the study was carried out in confined conditions, future studies should be carried out in field conditions and combine with other control methods (Aguilar-Marcelino et al. 2017).

Mycosynthesis of metallic nanoparticles or myconanotechnology (MNT) is the use of fungi in HN for the synthesis of NPs. The ability of filamentous fungi to grow on low-cost, low-nutrient substrates, as well as their ability to produce a wide variety of commercially useful secondary metabolites, have been of interest in the use of these microorganisms in NP production. Furthermore, for large-scale synthesis of nanoparticles in bioreactors, filamentous fungi are the best agents for biomass production compared to algae and bacteria, since HN mycelium can withstand flow pressure, agitation, and other conditions in this equipment. Recently, nanoparticles of NF *Duddingtonia flagrans* have been successfully produced (Silva et al. 2017).

1.4 Mycorrhiza

At present, 1.5 billion ha of the globe's land surface is used in crop production (arable land and land under permanent crops) (FAO 2003). Globally, agricultural production will need to expand by 60% by 2050 to increase world demand population (8.9 billion people) and productivity (Alexandratos and Bruinsma 2012) and be more resistant and resilient to extreme climate events. Lipper et al. (2014) stated that agriculture needs to get climate-smart. Sustainable agricultural systems have been defined as an alternative that could be used to solve food production in an ecological way through decrease fertilizer use, reducing input costs, and prevent environmental pollution (Siddiqui and Pitchel 2008; Harrier and Watson 2004). This type of agriculture develops new practices that are not harmful to the environment (Lichtfouse et al. 2009; Yadav et al. 2020b, c, d).

Microbial communities play an important role in sustainable agriculture and in the integrated soil fertility management. They can provide stability and productivity in agroecosystems reducing the input of chemical fertilizers and pesticides (Philippot et al. 2013; Bender et al. 2016). A key functional group of soil microorganisms is the arbuscular mycorrhizal fungi (AMF) that have shown to increase crop productivity and the ecosystem sustainability (Van der Heijden et al. 2008), enhancing plant performance and soil quality (Thirkell et al. 2017). The majority of agricultural crops, such as wheat, rice, corn, potato, tomato, onion, pulses, and soybean, have the potential to form AMF as root symbionts (Hijri 2016). In the symbiosis plant-AMF, the fungi facilitate uptake and translocate some mineral nutrients from the soil, alleviating nutrient deficiency, and increasing plant health and yield (Jansa et al. 2006; Hijri 2016).

AMF can be used such as *biofertilizers* improving plant nutrition, as *bioregulators* interfering in the phytohormone balance of host plants influencing plant development, and as *bioprotector* alleviating the effects of environmental stresses (Rouphael et al. 2015). AMF as a *biofertilizer* are important since these symbioses could decrease the use of agrochemicals (Chen et al. 2018). Mycorrhizal fungi play a vital role in nutrient cycling and productivity of crops (Smith and Read 1997) altering the quality and quantity of soil organic matter and the kinetic properties of the root were enhancing its nutrient uptake (Ryglewicz and Andersen 1994). They are also environmentally friendly fertilizers and do not cause the pollution (Sadhana 2014).

Several plant hormones, such as strigolactones, gibberellin, and auxin, have a role in the regulation of AM symbiosis influencing in plant development (AMF as *bioregulators*). Plant hormones are signaling-regulators in physiological processes between plants and microbes (Metting 1993; Ruyter-Spira et al. 2015; Weijers and Wagner 2016). Strigolactones are involved in the pre-symbiotic growth of the fungus, auxin is required for early steps of fungal growth and the differentiation of arbuscules and gibberellin modulates arbuscule formation (Van de Velde et al. 2017; Liao et al. 2018). Mycorrhizal plants may differ in their response to stress depending on the interaction of AMF-plants-environmental conditions. AMF symbiosis as *bioprotector* can protect host plants against stress conditions (Aggarwal et al. 2011). Abiotic stresses cause extensive losses to agricultural productivity. AMF symbioses are recognized to increase host tolerance to abiotic stresses such as mineral depletion, drought, salinity, heavy metals, and heat (Barea et al. 2011; Jeffries and Barea 2012). These fungi are an alternative for hostile environmental that limits crop productivity worldwide (Barea et al. 2011). Besides, AMF root colonization can provide protection against plant pathogens (parasitic fungi and nematodes) (Newsham et al. 1995; Akhtar and Siddiqui 2008; Saldajeno et al. 2008; Lax et al. 2011).

Many factors affect the mycorrhizal development, diversity, and AMF community's composition in horticulture and agriculture soils (Grant et al. 2005; Hartmann et al. 2015). For example, conventional agricultural practices may reduce AMF density while organic low-input system increased AMF activity contributing to the sustainable agriculture (Basu et al. 2018; Panwar et al. 2008). However the effects of agricultural practices on AMF composition and mycorrhizal functioning depend on the environmental context (soil type, fertility, pollution, climate, and microbial communities) (Jansa et al. 2006).

In order to develop production strategies in sustainable agriculture, it is necessary to produce high-quality inoculum for successful root colonization with AMF (Barea et al. 2016). For every crop, the best AM fungus should be selected in order to provide particular benefits such as crop nutrient capture to growth and stress resistance (Püschel et al. 2017). AM fungal inoculation is based on their roles to improve water uptake (Augé 2004), soil fertility, and soil aggregate stability (Rillig et al. 2015; Rillig 2004). The applications of commercial AMF inoculums to crops under agricultural conditions are summarized in Weber (2014). Based on it, some AM fungal inoculant products can improve plant uptake of nutrients thereby increasing the use of efficiency of applied artificial fertilizers. As Baum et al. (2015) stated, the future challenge in the production of vegetables will be optimizing combinations of crop plant-AMF inoculum, inoculation methods, and soil/substrate properties for mycorrhizal establishment. In sustainable agricultural systems it is important to consider the development and establishment of "soil-root-AMF interaction" as well as to determine the influence of soil physical and chemical factors and the symbiosis efficacy as a *biofertilizer*, *bioregulator,* or *bioprotector*.

1.5 Dark Septate Fungi

Currently, sustainable agriculture management has involved the use of many biological agents as a biofertilizer to support the productivity of agricultural plants based on its function as a plant growth promoter, soil organic compound decomposer, facilitator of the availability of nutrients, and controlling plant diseases, one of which is the use of dark septate endophytic fungi (DSE) (Jumpponen and Trappe 1998; Usuki and Narisawa 2007; Mahmoud and Narisawa 2013; Surono and Narisawa 2017, 2018). Although the use of DSE fungi is not as popular as mycorrhizal fungi, the exploration of DSE fungi is increasingly attracting many researchers to investigate DSE fungi in the biodiversity, the role of ecology, and their potential ability to support sustainable agricultural practices both in the subtropics to the tropics to minimize the use of agrochemicals and improve soil health and plants. Initially, most of the studies on DSE fungi were related to the results of isolation from various forestry plants, especially conifers in temperate regions, and investigated their ability to symbiosis with forestry plants such as *Pinus sylvestris*

(Grünig and Sieber 2005), *Picea rubens* (Wilcox and Wang 1987), *Picea abies* (Lukešová et al. 2015), *Chamaecyparis obtuse* (Surono and Narisawa 2017), and *Pinus tabulaeformis* (Chu *et al*., 2019). DSE fungi can be isolated from one specific target plant, but based on the results of tests both in vitro and in vivo many DSE fungi species are capable of symbiosis with various plants in a wide spectrum or DSE does not have a special host plant so DSE fungi can be inoculated for various plants (Khastini et al. 2012; Diene et al., 2014). DSE fungi can colonize non-mycorrhizal plants that are not symbiotic with arbuscular mycorrhizal fungi such as plant families from Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae, Polygonaceae, Juncaceae, Cruciferae, and Amaranthaceae (Usuki and Narisawa 2007; Liu et al. 2017). The potential of the DSE fungi is very beneficial to be used in enhancing the productivity of various kinds of agriculture plants because the host plants are not specific so that they can be efficiently implemented in the field. Also, DSE fungal inoculant production will be easier and more efficient than arbuscular mycorrhizal inoculant that require host plants in propagation because arbuscular mycorrhizal fungi cannot be propagated individually without a host plant (Mandyam and Jumpponen 2005; Malusá et al. 2012). The use of DSE fungi inoculants in plants is also quite efficient. It can be inoculated in the nursery phase so that when the plants are ready to be planted in the field (Dalimunte et al. 2019). DSE fungi remain in the plant tissues even though the plants are moved to the field.

However, DSE fungi are a group of endophytic fungi that are mostly Ascomycota that have dark septates, can colonize both intra- and intercellular root tissue without causing disease symptoms (Jumpponen and Trappe 1998; Rodriguez et al. 2009; Diene 2009; Diene et al. 2013). DSE fungi play a role in facilitating the absorption of nutrients in host plants and can increase the tolerance of host plants to adverse environmental conditions such as in high acid and saline conditions (Jumpponen 2001; Wilson et al. 2004; Silvani et al. 2008; Mahmoud and Narisawa 2013; Usuki and Narisawa 2007). DSE fungi were known to have a symbiosis with about 600 plant species from 320 genera in 114 families, including non-mycorrhizal plant species (Jumpponen and Trappe 1998; Sieber 2002; Addy et al. 2005; Grünig and Sieber 2005; Smith and Read 2008). Isolation and selection of DSE fungi from the natural environment is increasingly being done to obtain new species that have never been reported before (Rodriguez et al. 2009).

Various species of DSE fungi are reported to be able to associate with agricultural plants with the potential ability to increase growth and protect host plants from pathogens (Table 1.1). As the report varies, the potential for using DSE inoculants to support sustainable agriculture will be very promising. *Phialocephala fortinii*, which is the most well-known DSE fungus species, which is generally symbiotic with forestry plants, is also able to promote the growth of agricultural plants such as *Asparagus officinalis* and suppress the severity of Fusarium disease in organic growing media (Surono and Narisawa 2017, 2018). DSE species, *Veronaeopsis simplex*, is capable of symbiosis with *Brassica campestris* and controlling Fusarium wilt (Khastini et al. 2012) and has the potential to be used as a cesium bioremediation agent in soils contaminated with heavy metals by involving symbiotic activity with *Solanum lycopersicum* and *Brassica campestris* so that it can be used as an

Table 1.1 DSE fungi that have the potential as a promoter for the growth of agricultural plants

DSE fungal species	Agricultural plants	Reference
Phialocephala fortinii	Asparagus *(Asparagus officinalis)*	Surono and Narisawa (2017)
Ramichloridium cerophilum	Chinese cabbage (*Brassica campestris)*	Xie et al. (2016)
Pseudosigmodea ibarakiensis	Chinese cabbage (*Brassica campestris)*, cucumber (*Cucumis sativus*)	Diene et al. (2013)
Veronaeopsis simplex	Chinese cabbage (*Brassica campestris)*, tomato (*Solanum lycopersicum*)	Khastini et al. (2012); Diene et al. (2014)
Cadophora sp.	Melon (*Cucumis melo*)	Khastini et al. (2014)
Harpophora oryzae	Rice (*Oryza sativa*)	Su et al. (2013)
Helminthosporium velutinum	Sorgum (*Sorgum bicolor*)	Diene et al. (2010)
Cadophora sp., Coniothyrium sp., Corynespora cassicola, Pyronellaea sp., Phaeosphaeria sp., Boeremia sp.	Soybean (*Glycine max*)	Rothen et al. (2017)
Cladophialophora chaetospira	Strawberry (*Fragaria ananassa*)	Harsonowati et al. (2020)
Scolecobasidium humicola	Tomato (*Solanum lycopersicum*)	Mahmoud and Narisawa (2013)
Leptodontidium orchidicola	Tomato (*Solanum lycopersicum*)	Andrade-Linares et al. (2011); Surono (2014)
Periconia macrospinosa	Tomato (*Solanum lycopersicum*)	Yakti et al. (2018)
Nectria haematococca	Tomato (*Solanum lycopersicum*)	Valli and Muthukumar (2018)
Paraphoma sp., *Embellisia chlamydospora*, and *Cladosporium oxysporum*	Maize (*Zea mays*)	Li et al. (2019a, b)
Exophiala pisciphila	Maize (*Zea mays*)	Li et al. (2011)
Gaeumannomyces cylindrosporus	Maize (*Zea mays*)	Yihui et al. (2017)
Rhizopycnis vagum	Chili (*Capsicum annuum*)	Manalu et al. (2020)
Cladosporium sp.	Physic nut (*Jatropha curcas*), *Philippine tung* (*Reutealis trisperma*)	Ahadi et al. (2019)
Acrocalymma vagum and Scytalidium lignicola)	Alfalfa (*Medicago sativa*)	Hou et al. (2020)
Melanconiella elegans	Cowpea (*Vigna unguiculata*)	Farias et al. (2019)

effort to clean up heavy metals polluted land in an environmentally friendly manner (Diene et al. 2014). DSE fungus, *Pseudosigmoidea ibarakiensis*, is capable of symbiotic with *Brassica campestris* and stimulates its growth under ultra acidic conditions (Diene et al. 2013). DSE species, *Ramichloridium cerophilum*, are also symbiotic with *Brassica campestris* (Xie et al. 2016). Several studies report that

DSE also forms a symbiotic relationship with *Zea mays* in supporting growth such as DSE fungi *Paraphoma* sp., *Embellisia chlamydospora*, and *Cladosporium oxysporum* (Li et al. 2019a, b), *Exophiala pisciphila* (Li et al. 2011), and *Gaeumannomycesa cylindrosporus* (Yihui et al. 2017). The symbiotic relationship between DSE fungi and *Vaccinum corymbosum* (blueberry) (Sadowsky et al. 2012), *Solanum tuberosum* (Das and Kayang 2010), *Saccharum officinarum* (Claassens et al. 2018), and *Hevea brasiliensis* (Dalimunte et al. 2019) have also been reported, through colonization mechanism that causes the performance of these host plants that can grow well compared to control plants. *Oryza sativa* is also capable of symbiosis with several DSE species such as *Harpophora oryzae* (Su et al. 2013), *Cladosporium* sp. (Surono et al. 2018), and *Leptodontidium orchidicola* (Surono 2014). DSE fungi are also capable of symbiosis with fruit plants such as *Cucumis melo* (Khastini et al. 2014) and *Fragaria ananassa* (Narisawa et al. 2002; Harsonowati et al. 2020). In *Glycine max* growth, DSE fungi that form symbiosis to promote growth are *Cadophora* sp., *Coniothyrium* sp., *Corynespora cassicola, Pyronellaea* sp., *Phaeosphaeria* sp., and *Boeremia* sp. (Rothen et al. 2017). The DSE species, *Helmintosporium velunitum*, is capable of symbiosis with the *Sorghum bicolor* which is a plant that can be used as a biofuel (Diene et al. 2010). Like *Brassica campestris*, *Solanum lycopersicum* is also an agriculture plant easy to associate with DSE fungi such as *Leptodontidium orchicola* (Andrade-Linares et al. 2011; Surono 2014), *Scolecobasidium humicola* (Mahmoud and Narisawa 2013), and *Periconia macrospinosa* (Yakti et al. 2018). Thus, the range of host plants on agricultural plants associated with DSE fungi is increasingly being reported with potential as a growth promoter; in fact, some studies report that DSE fungi are effective in promoting the growth of agricultural plants under organic conditions and pathogenic challenges (Mahmoud and Narisawa 2013; Surono and Narisawa 2017, 2018). The ability of DSE fungi to degrade organic sources of N, P, and K in growth media and transfer nutrients, both macro- and micronutrients to host plants, has the potential to be utilized in sustainable agricultural production (Surono and Narisawa 2017; Vergara et al. 2018; Usuki and Narisawa 2007). Although DSE fungi were reported to be symbiotic with 600 plant species (Mandyam and Jumpponen 2005), efforts to explore DSE fungi in various plants need to be continued, such as DSE fungi exploration in the tropics. In tropical regions of Indonesia, DSE fungi have been reported to be capable of symbiosis with *Oryza sativa, Zea mays, Jatropha curcas, Capsicum annuum,* and *Solanum lycopersicum* (Surono et al. 2018, 2019; Zaffan et al. 2018; Dalimunte et al. 2019; Manalu et al. 2020).

Indeed, the role and function of the DSE fungi is almost like mycorrhizal fungi, even under certain extreme conditions the DSE fungi replace the role of the mycorrhizal fungi in its function to enhance the adaptation of host plant growth in extreme environments such as high salinity, low pH, and high heavy metal content (Ruotsalainen et al. 2004; Mandyam and Jumpponen 2005) although the pattern of colonization within the roots of the host plant shows a distinct difference, especially the form of microsclerotia as a characteristic of most DSE fungi (Jumpponen and Trappe 1998). Therefore, the symbiotic ability of DSE fungi can be used to support host plants that are intolerant of extreme environmental conditions into plants that can tolerate extreme conditions in the growing environment so that these plants can be planted in extreme environments where conditions without inoculation DSE fungi cannot grow normally. In general, microbes, especially DSE, are very effectively utilized as plant growth-promoting agents under abiotic stress conditions (Schulz and Boyle 2005). DSE fungi were distributed as root colonization fungi in almost all parts of the world from temperate to tropical regions and were often found in high abundance in environments where abiotic stress is very strong (Rodriguez et al. 2009; Knapp et al. 2015) although the information is still limited. Selecting DSE fungi that can increase plant tolerance to abiotic stress is very important because abiotic stress is a major factor that limits plant development and productivity (Santos et al. 2017).

In general, the association of DSE fungi through the colonization of host plant tissue and the formation of microsclerotia is important to enhance the host's ecological adaptability by increasing tolerance to biotic and abiotic stress (Jumpponen and Trappe 1998; Schulz and Boyle 2005; Sieber and Grünig 2013; Hou et al. 2020). Several studies related to DSE fungi have focused on the interaction effects of DSE fungi isolated from various plants, mainly as biotic and abiotic stress controllers so that host plants can grow well without any stress and disease symptoms (Narisawa et al. 2004; Su et al. 2013). Mandyam and Jumpponen (2005) stated that DSE fungi are more commonly found in depressed environments than arbuscular mycorrhizal fungi. DSE fungi are predominantly found and associated with plants that grow in stressful and nutrient-limited environments (Lugo et al. 2009; Newsham et al. 2009). Dark septate endophytes (DSE) are ascomycetous fungi that are rich in melanin and their presence and diversity is abundant in stressful environments (Berthelot et al. 2019). Melanin expression in DSE fungi can be an indicator for controlling abiotic and biotic stress conditions (Henson et al. 1999; Mugerwa et al. 2013). Melanin in DSE fungi protects damage to cell structures in stressful environments by functioning as an antioxidant agent and heavy metal ion binder (Ban et al. 2012; Santos et al. 2017).

Research on DSE fungi to promote plant growth in biotic and abiotic stress conditions is still limited (Arnold et al. 2003; Knapp et al. 2015; Ban et al. 2012). One abiotic stress that limits plant growth is soil acidity (Kochian et al. 2004). On the other hand, soil acidity plays an important role in the formation of the fungal community (Bonfim et al. 2016). To date, the effect of DSE fungi in promoting plant growth under environmental stresses such as low pH has only been reported by Diene et al. (2013), Surono (2014), and Surono (2017). Diene et al. (2013) stated that *Pseudosigmoides ibarakiensis* could stimulate the growth of *Brassica campestris* in ultra acidic conditions (pH 3) in the absence of damage or death of plants inoculated with that DSE fungus. Surono (2014) reported that the DSE fungus species, *Leptodontodium orchidicola*, was significantly able to promote the growth of *Solanum lycopersicum* and *Oryza sativa* under acidity conditions and high aluminum concentrations (Fig. 1.2). The DSE fungus, *Phialocephala fortinii*, is also able to increase the growth of *Asparagus officinalis* in various organic nutrients in soil acidity stress (Surono 2017). Surono et al. (2019) succeeded in isolating the DSE fungi from plants that grow in the acidic sulfate soil ecosystem of Kalimantan,

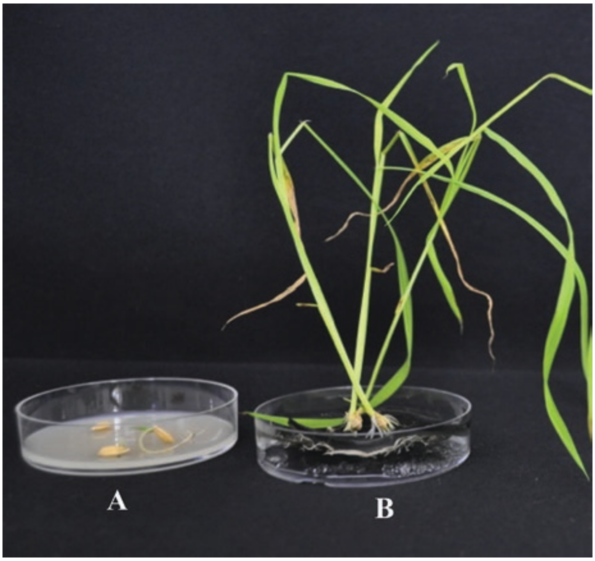

Fig. 1.2 Inoculated rice plant by dark septate endophytic fungus, *Leptodontidium orchidicola* (**b**), compared to control plant (un-inoculated plant) in acidic condition (pH 4) (Surono 2014)

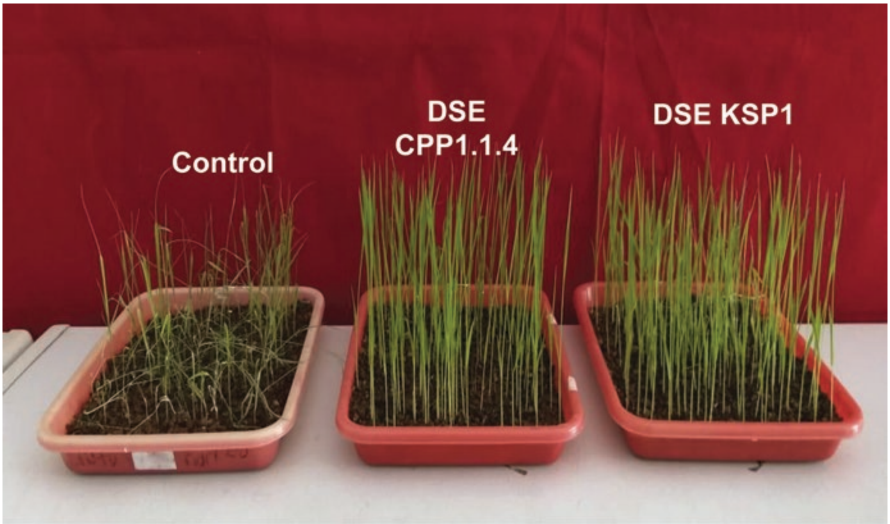

Fig. 1.3 Upland rice seedlings (var. Situ Patenggang) associated with DSE fungal isolates CPP1.1.4 and KSP1 grow better than controls in Ultisols soil media which have a pH of 4

Indonesia, which is in symbiosis with *Eleocharis dulcis* that grows predominantly in tropical swamp areas under pH 3. In another experiment (Surono et al. 2018), by inoculating DSE fungi in upland rice seeds and then planted in growth media using Ultisols soil with high acidity conditions (pH 4), upland rice seedlings could still grow normally and healthier than control plants (Fig. 1.3). Postma et al. (2007)

reported that the colonization of DSE fungi in plants was positively correlated with low soil pH compared to mycorrhizal fungi. They suggested that DSE fungi were able to replace the role of arbuscular mycorrhizal fungi at very low pH (Göransson et al. 2008; Alberton et al. 2010). Based on this limited information, further studies are needed regarding the role of the DSE fungi to encourage plant growth under acidic conditions. It is hoped that the selected DSE fungi that have a role as a plant growth promoter under low pH in vitro and in vivo can be used to overcome the problem of acid soils in the field in the future.

Climate change affects several factors related to the occurrence of drought that causes a very serious impact on agricultural productivity (Waqas et al. 2014; Kour et al. 2019a; Singh et al. 2020b). Therefore, technology to improve plant tolerance and adaptation to keep producing optimally in these conditions is needed, one of the potential technologies used is the symbiotic technology of DSE fungi. Under drought stress conditions, several species of DSE fungi can have a positive effect on the host plant by increasing biomass and active compound content so that host plants can adapt to the environment such as DSE fungus, *Acrocalymma vagum* (He et al. 2019). In conditions of water deficit, *Phialophora* sp. and *Leptosphaeria* sp. improved root biomass, nutrient concentration, and antioxidant enzyme activity in host plants (Li et al. 2019a, b). DSE fungus, *Nectria haematococca*, could induce *Solanum lycopersicum* tolerance to drought stress and increase the growth under water deficit stress which was indicated by increasing plant height, stem girth, and plant biomass (Valli and Muthukumar 2018). DSE fungi were also able to increase the tolerance of *Oryza sativa* to stress due to water deficiency (Santos et al. 2017). The interaction model of DSE fungi with host plants in drought conditions contributes to the understanding and utilization of DSE fungi ecological functions in drought stress or water deficit conditions (Li et al. 2019a, b). In the interaction of plants and DSE fungi related to the adaptation of host plants to drought stress and water deficit, DSE fungi stimulate increased antioxidant activity, catalase, or certain compounds in host plants that promote host defense and adaptation to drought stress conditions (Santos et al. 2017; Waqas et al. 2014). In addition to improving the defense system, DSE fungi melanin also affects the anticipation of these stresses (Postma et al. 2007). The content of melanin in the DSE fungal hyphae provides several selective advantages for being able to survive in a variety of extreme environmental stress conditions, such as in environments contaminated by heavy metals, acidic soil, saline land, and drought (Postma et al. 2007; Bonfim et al. 2016).

Salinity has now become a major threat to sustainable agricultural production due to rising global temperatures and the rate of evaporation from the land that often changes so that agricultural plants are faced with these conditions of stress salinity (Waqas et al. 2014; Yadav et al. 2020a). Endophytic fungi, including DSE fungi, have the potential to increase plant tolerance under conditions of salinity stress, although specifically for high salinity stress, the use of DSE with its symbiosis in host plants has not been widely reported. There are only limited publications regarding the potential use of DSE symbiotic to stimulate host plant growth under high salinity stresses such as the report of Farias et al. (2019) which stated that *Vigna unguiculata* inoculated with DSE fungus, *Melanconiella elegans* was able to increase nutrient uptake of N and P in plants and the rate of net photosynthesis despite the stress of high salt content in the growing environment. Mateu et al. (2020) succeeded in isolating DSE fungi from *Phragmites australis* that can improve the survival of *P. australis* seeds under high concentrated salt pressure both in the laboratory and in greenhouse testing. The challenge going forward is to isolate DSE fungi from plants that grow in high salinity ecosystems such as coastal areas, such as mangroves, which can grow and develop in high salinity conditions that can be symbiotic with agricultural plants that through their symbiotic can increase tolerance and production on high salinity land.

Another important function of the DSE fungi is its ability to remediate heavy metal and pesticide contamination (Diene et al. 2014; Spagnoletti and Chiocchio 2020; Manalu et al. 2020). The ability of DSE fungi as a remediator can be utilized to clean up land contaminated with heavy metals and pesticides in an environmentally friendly manner so that the land can be re-utilized for sustainable agricultural cultivation practices. Even DSE species such as *Pseudosigmoidea ibarakiensis* I.4–2-1, *Veronaeopsis simplex* Y34, and *Helminthosporium velutinum* 41–1 can remediate Cesium in polluted environments through its symbiotic mechanisms with agricultural plants such as *Brasssica campestris* and *Solanum lycopersicum* (Diene et al., 2014). The DSE fungi, *Acrocalymma vagum* and *Scytalidium lignicola*, which are inoculated on *Medicago sativa* can increase root growth and nutrient absorption, change soil Cd concentrations, and facilitate plant growth and survival under Cd stress conditions (Hou et al. 2020). Another agriculture plant that is often used as plant indicator for bioremediation, *Zea mays*, inoculated with DSE fungus, *Gaeumannomyces cylindrosporus*, was able to significantly increase the height, basal diameter, root length, and biomass under high Pb stress conditions (Yihui et al. 2017). Inoculation *of Zea mays* with DSE fungus, *Exophiala pisciphila*, resulted in a significant decrease in cadmium phytotoxicity and an increase in corn growth, one of which was due to the triggering mechanism of the antioxidant system (Wang et al. 2016). Ahadi et al. (2019) reported that *Cladosporium* sp. KSP.1 was able to increase the growth of *Jatropha curcas* and *Reutealis trisperma*, colonization, and Pb uptake in planting media using soil contaminated with Pb, even to anticipate the Pb stress conditions, the DSE formed colonization in the form of microsclerotia which dense in the roots of *Reutealis trisperma* (Fig. 1.4). DSE fungi isolated from pesticide-contaminated chili cultivation areas are also able to remediate pesticides with active ingredients of mancozeb (Manalu et al. 2020) so that the DSE fungi have the potential to clean agricultural land contaminated with mancozeb and support the growth of vegetables, especially *Capsicum annuum* in a sustainable manner. DSE fungus, *Cochliobolus* sp., is tolerant of pesticides with active ingredients glyphosate and carbendazim in agronomic doses, even at doses that are increased to 2 and 10 times the agronomic dose (Spagnoletti and Chiocchio 2020). Reports on the use of DSE fungi for remediation of pesticide contamination are still limited, but based on research by Manalu et al. (2020) and Spagnoletti and Chiocchio (2020), DSE fungi have the potential to remediate pesticide contamination and needs further investigation.

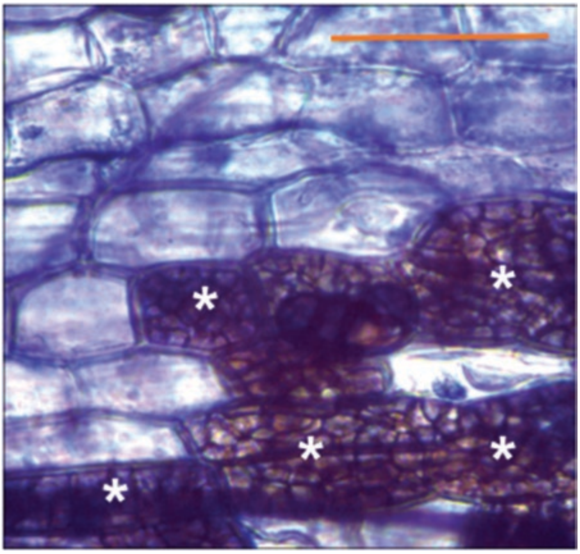

Fig. 1.4 Colonization of fungus DSE fungus, *Cladosporium* sp. KSP.1, at the roots of *Reutealis trisperm* in the treatment of 100% tailings contaminated with Pb. Asterisk: microsclerotia. Bars: 50µm

Interestingly, the ability of DSE fungi to suppress the growth and attack of agriculture plant pathogens has begun to be investigated to take advantage of its symbiotic role in protecting host plants from plant diseases (Narisawa et al. 2002; Khastini et al. 2012). Various DSE fungi species have been reported to have a role in protecting host plants from plant pathogen attacks and their potential is promising to be a biocontrol agent for sustainable agriculture (Khastini et al. 2014; Surono and Narisawa 2018; Dalimunte et al. 2019; Zaffan et al. 2018; Harsonowati et al. 2020). DSE fungi such as *Heteroconium chaetospira* and *Phialocephala fortinii* have been reported to be able to suppress *Verticillium* wilting in *Solanum mengolena* (Narisawa et al. 2002). Khastini et al. (2012) reported that *Veronaeopsis simplex* was effective in suppressing *Fusarium* disease in *Brassica campestris* even though the dual culture DSE testing of the species did not show an emphasis on the growth of *Fusarium oxysporum*. DSE fungi are also effective for controlling *Fusarium* disease in fruit plants such as *Cadophora* sp. which controls *Fusarium* wilt in *Cucumis melo* (Khastini et al. 2014) and *Cladophialophora chaetospira* which stimulate fruit growth and production on *Fragaria ananassa infected* with *Fusarium oxysporum* (Harsonowati et al. 2020). Surono and Narisawa (2018) also reported that *P. fortinii* was able to have a symbiosis with *Asparagus officinalis* in organic growth media while suppressing *Fusarium* asparagus disease. In forestry plants, *Phialocephala sphareoides* are used to suppress the growth of *Heterobasidion parviporum* on Norwegian spruce plants (Terhonen et al. 2016). DSE fungus *Harpophora oryzae*, which are associated with *Oryza sativa*, have the potential to inhibit blast disease caused by *Pyricularia oryzae* (Su et al. 2013). In tropical regions such as Indonesia, DSE fungi were reported to be able to control tropical plant diseases such as *Fusarium* wilt in *Solanum lycopersicum* (Zaffan et al. 2018), white root rot in *Hevea brasiliensis* (Dalimunte et al. 2019), and blast disease in *Oryza sativa* (Surono et al.

2018). DSE fungi, *Phialophora mustea, Leptodontidium* sp., and *Cadophora* sp. can suppress the growth of pathogenic fungi such as *Pythium intermedium, Phytophthora citrinola*, and *Heterobasidion annosum* (Berthelot et al. 2019). DSE fungi are also able to suppress the growth of *Ganoderma boninense* that causes basal stem rot disease in *Elaeis guineensis* (Fig. 1.5). Attention to the potential of DSE in controlling plant diseases, further exploration and assessment of appropriate inoculation techniques are needed so that DSE fungi can be applied effectively and efficiently to reduce the incidence and severity of plant diseases while increasing the growth of the host plants. The mechanism in inhibiting the growth of pathogens and protection of host plants from the pathogen attack of each DSE fungus varies by species, such as *Veronaepsosis simplex* in dual culture. The testing does not inhibit the growth of *Fusarium oxysporum* but effectively protects *Brassica campestris* from the severity of *Fusarium* disease (Khastini et al. 2012), whereas *Phialocephala fortinii* was able to suppress the growth of *Fusarium oxysporum* in dual culture testing and was also able to inhibit the severity of *Fusarium* disease in *Asparagus officinalis* (Surono and Narisawa 2018). Another mechanism is to produce secondary metabolites such as sclerin and sclerotinin by *Phiaocephala europaea* that can suppress the growth of pathogens such as *Phytophthora citrinola* (Tellenbach et al. 2013). DSE fungus *Cadophora* sp. is able to produce isochromanones, which have

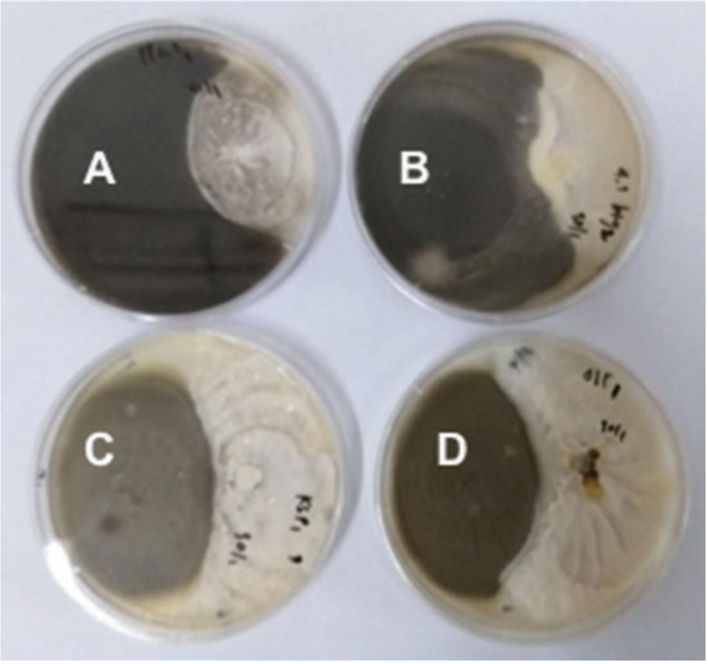

Fig. 1.5 DSE fungal species, *Curvularia* sp. PP 2.3 (**a**), *Curvularia* sp. 4.1 (**b**), *Cladosporium* KSP.1 (**c**), and *Cladosporium* sp. OLT (**d**) suppress the growth of *Ganoderma boninense* in dual culture test

antimicrobial activity (Rusman et al. 2018). In general, endophytic fungi can produce bioactive compounds to inhibit the growth of pathogens, in addition to their role in increasing tolerance and resistance of host plants to abiotic and biotic stresses (Yu et al. 2010).

Generally, the application of DSE fungal inoculants as biofertilizers to support sustainable agricultural productivity to succeed needs to pay attention on the several things such as the selection of appropriate DSE fungi as active ingredients of organic fertilizers with tested functional abilities and inoculation techniques on target plants. Selected DSE fungus as an active ingredient of biological fertilizers must have been selected in the laboratory, greenhouse, and fields with the ability to stimulate the growth of host plants with good quality control (Herrmann and Lesueur 2013), especially in environments affected by abiotic and biotic stresses. Also, inoculation testing of various target plants needs to be done, whether the selected DSE fungus has a specific host or has a broad spectrum of host plants including the various stressed environment conditions (Berruti et al. 2016). Of course, if the selected DSE fungus has a broad spectrum of host plants it will be useful and efficient in its production and application, compared to DSE which is only limited to its host plants. Appropriate inoculation techniques will also be a success factor in the use of DSE-based biological fertilizers because inoculation is the key to the symbiotic process between DSE and target plants from early plant growth to harvesting time. Some reports state that DSE fungi are very effective inoculated in the seed germination and nursery phases that make host plants healthy from the beginning of growth and until production (O'Callaghan 2016; Dalimunte et al. 2019). Another important factor is the quality of DSE fungal inoculants as controlled biofertilizers in both the production and use processes in the field so that no contaminants emerge in DSE biofertilizers (Herrmann and Lesueur 2013).

1.6 Endophytic Fungi

The use of endophytic fungi-based biological fertilizers is now increasingly promising to support sustainable agriculture because of its multifunctionality and positive impact on the agricultural environment (Zheng et al. 2016; Li et al. 2016). Environmental issues including climate change have suppressed the use of excessive agrochemicals to prevent environmental damage and enhance the role of microbial resources on degraded agricultural land (Santos et al. 2012; Latz et al. 2018). Endophytic fungi become an alternative to support sustainable agriculture because of its use as an active ingredient of environmentally friendly fertilizer, namely biological fertilizer (Mahanty et al. 2017; Rana et al. 2019b), although people tend to be more familiar with Rhizobium and mycorrhizal inoculants which are already popular. Based on various research results, endophytic fungi are effective for inoculation in agricultural host plants with functions as a supplier of nutrients, controlling biotic and abiotic stresses, and other functions when not symbiotic with their host plants in a life cycle, namely by switching roles as decomposers of organic materials that help decompose nutrients which changes from an unavailable form to a form available to host plants (Card et al. 2016). Therefore, endophytic fungi have the ability to symbiotic mutualism with their host plants, but in certain phases, they can change their function as saprophytes or decomposers in certain environmental conditions that also have benefits in maintaining natural balance (Jia et al. 2016; Xia et al. 2019).

The types of endophytic fungi vary in the natural environment, but to support sustainable agriculture endophytic fungi need to be selected that function as plant growth promoters, control plant diseases, and restore land contaminated with heavy metals and pesticides for inoculants that can be produced and applied in the field in a broad scale (Fernandes et al. 2015). Many signaling of fungi enhanced the growth and development of plants (Al-Ani et al. 2020b). An example of endophytic fungi that is widely used is *Piriformospora indica* which can encourage its host plants to grow under conditions of biotic and abiotic stresses by facilitating nutrient uptake and modulation of phytohormone so that plants can grow well under various stress conditions (Johnson et al. 2014). *Piriformospora indica* has the ability to symbiosis with a wide range of crops such as *Oryza sativa, Zea Mays, Triticum aestivum, Brassica campestris*, and *Saccharum officinarum* (Johnson et al. 2014) so they can be utilized on a broad scale to support agricultural sustainability. *Piriformospora indica* which is inoculated with *Glycine max* significantly increases dry weight, nutrient content, resistance to abiotic stress, rhizobial biomass and stimulates positive interactions with rhizobia (Bajaj et al. 2018). *Piriformospora indica* also symbiosis with vegetable plants such as *Solanum lycopersicum* with a positive effect on increasing shoot and root biomass, fruit biomass, and decreasing *Verticillium dahliae* attack in hydroponic culture (Fakhro et al. 2010).

Another endophytic fungus, *Pichia guillermondii*, is also able to increase plant growth, yields, and resistance to the biotic stress of its host plants such as tomato, citrus, and chili pepper (Basha and Ramanujam 2015; Sangwanich et al. 2013; Xia et al. 2019). Endophytic fungus, *P. guilliermondii*, has the potential to produce helvolic acid as an antimicrobial pathogen with strong inhibitory activity in the germination of *Magnaporthe oryzae* plant pathogenic spores with IC50 value of 7.20μg/mL (Zhao et al. 2010). Thus, endophytic fungus such as *Piriforspora indica* and *P. guilliermondii* tend to be explored and used as inoculants because of their wide and unlimited range of host plants in symbiosis with only one agricultural crop.

As one of the main food plants in the world, rice plants are associated with various kinds of endophytic fungi, one of which is *Phomopsis liquidambari* which has a function in promoting rice plants and reducing the use of nitrogen fertilizer (Yang et al. 2014). With the inoculation of *P. liquidambari* on rice plants, the activity of nitrate reductase and glutamine synthetase is increasing (Yang et al. 2014). In a symbiotic relationship with rice plants, *P. liquidambari* can increase the content of organic compounds in root exudates, ammonia oxidizers and N-fixers in the rhizosphere environment which has implications for increasing the level of nitrification and reducing the input of N fertilizer to increase rice productivity in a sustainable manner (Yang et al. 2015). *Phomopsis liquidambari* is also indicated to stimulate the mineralization of organic matter and NH_4^+ release (Chen et al. 2013). *Phomopsis* *liquidambari* symbiosis with rice has the potential to degrade phenanthrene absorbed by plants through changes in the phenanthrene-degrading gene that correlates with the activity of phenanthrene-degrading enzymes in the roots of rice plants (Fu et al. 2020). *Phomopsis liquidambari* in addition to symbiosis with rice plants is also associated with peanuts (*Arachis hypogaea*) with the ability to increase peanut yield, nodulation, and N_2 fixation of *Bradyrhizobium* strains by increasing the expression of phenolic and flavonoid synthesis-related genes (Xie et al. 2019).

Apart from being a supplier or provider of nutrients for host plants, endophytic fungi can be considered as potential biological competitors against plant pathogenic fungi because they can live in almost all plant tissues from roots to leaves (Silva et al. 2018; Rojas et al. 2020), with the ability to inhibit the attack of plant pathogens through mechanisms such as the production of antimicrobial compounds and induce host plant resistance (Latz et al. 2018; Rojas et al. 2020). Its function is not only as a promoter for the growth of its host plant, but endophytes usually also have other abilities to make plants fit and resistant to pests and plant diseases. Such symbiosis occurs in wheat plants with various endophytic fungi with *Sarocladium strictum, Anthracocystis floculossa, A. floculossa*, and *Penicillium olsonii* which have the potential to control Fusarium head blight caused by many *Fusarium* spp. especially *Fusarium graminearum* so that wheat plants can continue to produce even if they are attacked by these pathogenic fungi (Rojas et al. 2020). Endophytic fungi of wheat plants can also be used as bioremediators of heavy metal contamination such as endophytic fungi, *Penicillium ruqueforti* which are inoculated in wheat plants grown in soil contaminated by Ni, Cd, Cu, Zn, and Pb have a positive effect on plant growth, nutrient uptake, low heavy metal concentrations in plant biomass through the production of indole acetic acid (IAA) so that it has the potential as phytostabilization of heavy metals (Ikram et al. 2018). Endophytic fungus, *Serendipita indica,* can stimulate the growth of sweet basil (*Ocimum basilicum*) in lead- and copper-contaminated soils and even increase concentrations of linalool and eucalyptol essential oils in these plants (Sabra et al. 2018). Endophytic fungus, *Mucor* sp., which are inoculated in Brassicaceae such as *Arabidopsis arenosa* stimulate higher N uptake and show significant catalase activity under heavy metal stress conditions such as Zn, Cd, and Pb (Domka et al. 2019). It could enhance the crop production by microbial technologies (Aguilar-Marcelino et al. 2020a).

Endophytic fungus, *Epicoccum nigrum*, is a fungus that has a diverse host plant (Lugtenberg et al. 2016). Endophytic fungus, *E. nigrum*, can increase the growth of potato plants, tuber production, reduce the severity of blackleg disease through a mechanism of increasing phenolic content, superoxide dismutase, catalase, ascorbate peroxidase, and glutathione peroxidase (Bagy et al. 2019). The symbiosis between *Epicoccum nigrum* and sugar cane can increase the root system biomass and control the pathogen in the phylloplane environment (Fávaro et al. 2012). *Epicoccum nigrum* associated with *Zingiber officinale* produces antimicrobial compounds that suppress the attack of plant pathogenic fungi, *Ustilago maydis* by producing Pretrichodermamide A (Harwoko et al. 2019). *Epicoccum nigrum* also symbiosis with fruit plants such as grapevine (*Vitis vinifera*) with potential as a growth booster and controlling pathogens (Martini et al. 2009).

Another genus of fungi known as endophytic fungi is *Acremonium* such as *Acremonium zeae* which is symbiotic with corn plants that can function as biocontrol of *Aspergillus flavus* and *Fusarium verticillioides*. In addition to this, endophytic fungi can produce Pyrrocidine A that showed a high activity against *Candida albicans* (Wicklow et al. 2005). Endophytic fungi, *Acremonium ochraceum*, and *A. strictum* reduce *Fusarium* wilt incidence and severity in tomato plants by increasing resistance to the disease (Grunewaldt-Stöcker and von Alten 2003). *Acremonium* sp. is symbiotic with *Panax notoginseng* by colonizing the roots, increasing resistance to root rot, saponin, indole acetic acid, and jasmonic acid (Han et al. 2020). *Acremonium* spp. also symbiotic with date palm plants with their antagonistic ability against *Fusarium oxysporum* f.sp. *albedinis* by reducing attacks by 87% (El-Deeb and Arab 2013). Secondary compounds of endophytic fungi can reduce the growth and development of plant pathogens (Al-Ani 2019a, b). The discoveries of patents of endophytic fungi and *Trichoderma* were very interesting (Al-Ani 2019c, e).

Based on the description above, endophytic fungi have the potential to have a symbiotic relationship with various crops with a positive impact. Endophytic fungus originating from *Curcuma longa* namely *Trichoderma harzianum* can suppress rhizome rot and leaf blight diseases caused by *Pythium aphanidermatum* and *Rhizoctonia solani* (Vinayarani and Prakash 2018). *Trichoderma harzianum* can induce maize plants resistant to maize chlorotic mottle virus (SCMV) (Kiarie et al. 2020). *Penicillium simplicissimum* and *Leptosphaeria* sp. derived from cotton roots can suppress the incidence and index of *Verticillium* wilt disease that occurs in cotton plants both in greenhouse and field experiments and even increase cotton production so that endophytic fungi are potential as biocontrol agents (Yuan et al. 2017). *Trichoderma* and non-pathogenic *Fusarium* produced several volatile compounds affecting *Foc*TR4 (Al-Ani and Salleh 2010; Al-Ani et al. 2013a, b; Al-Ani and Albaayit 2018a, b). *Trichoderma* showed the ability in controlling plant diseases such as leaf diseases, root diseases, stem diseases, and fruit diseases (Al-Ani and Mohammed 2020; Sharma et al. 2019).

The multifunctional potential of endophytic fungi to support agricultural sustainability needs to be explored and formulated to produce inoculants that can be utilized to promote sustainable agriculture production because it will reduce the use of various agricultural agrochemicals such as inorganic fertilizers and pesticides (Santos et al. 2012; Mahanty et al. 2017; Rana et al. 2019c). Appropriate inoculation technology in stimulating symbiosis with target plants needs to be assessed so that their use is right on target, especially for field applications. The use of biological fertilizer based on endophytic fungi is very important to mitigate the negative impacts of environmental change of agrochemical application because its use is following environmentally friendly rules that have an impact on the restoration of environmental conditions, especially degraded land into an environment that can be utilized for sustainable agricultural cultivation (Singh et al. 2011). Besides, the use of biological fertilizer based on endophytic fungi is expected to increase the effectiveness and efficiency of the use of biological fertilizers because it is expected to be applied once at the beginning of plant growth and can still live in its host plant tissue even though it is moved to various places in the field (Rana et al. 2020b). Indeed, 13 fungi genera that classified into two big groups Ascomycota and Basidiomycota (the classification can advantage from Wijayawardene et al. (2020)) comprised *Trichoderma*, *Pestalotiopsis*, *Curvularia*, *Cladosporium*, *Phomopsis*, *Chaetomium*, *Penicillium*, *Phoma*, *Alternaria*, *Fusarium*, *Colletotrichum*, *Aspergillus,* and *Diaporthe* that more endophyte could modify in the chemistry of the medicinal plants (Al-Ani 2019f).

1.7 Edible and Inedible Mushroom

Wild mushrooms have been part of the human diet for several centuries due to their nutritional and organoleptic characteristics: taste, texture, and smell (Román et al. 2006). Likewise, the medicinal use of mushrooms has a long history in countries such as China and Japan, where for thousands of years there has been an inherited tradition that considers the consumption of mushrooms beneficial to health. In addition to being recognized as a nutritious food, some fungi are also an important source of bioactive compounds with medicinal potentials, such as phenolic compounds, sterols, and triterpenes (Wasser 2010).

Furthermore, the scientific understanding of edible and non-edible fungi present in our environment is increasing. This is not only from a nutritional and medicinal point of view, but also related to the role they can play when used as an alternative to chemical pesticides and other toxic substances. This role contributes to the development of sustainable agriculture, which helps to improve environmental quality, economic output, and human life.

In recent decades, there has been an enormous increase in the use of agrochemicals worldwide to maximize food production for a rapidly growing human population (Campos et al. 2019). Regarding the above, Geiger et al. (2010) stated that during the last 50 years, agricultural intensification led to the extinction of many species of wild plants and animals, which has profoundly changed the functioning of agroecosystems, since agricultural intensification involves the loss of elements of the landscape the enlargement of farms and excessive use of fertilizers and pesticides.

Unfortunately, the indiscriminate use of and excessive dependence on synthetic pesticides in agriculture has led to the accumulation of residues toxic to human health, the environment, and the development of strains resistant to pests and pathogens (Lengai et al. 2020). Furthermore, pesticides affect soil enzymes, which are essential catalysts that govern soil quality (Campos et al. 2019). Given these challenges, there is a greater interest in using alternative substances to synthetic agrochemicals that present a lower risk to the environment and human health, producing more food in a clean and sustainable way.

The use of chemical fertilizers can be reduced by applying mycopesticides in the field, where this type of control quickly destroys most insects and focuses on reducing disease and damage to crops (Manivel and Rajkumar 2018). In response to the growing market for these biopesticides, it must be ensured that they are available in sufficient quantities and that they maintain quality and effectiveness over time, which requires the development of economical and efficient production methods (Jaronski and Mascarin 2016). Most of the fungi produced commercially for biological control belong to the Order Hypocreales. These include *Beauveria bassiana*, *Metarhizium* spp., *Isaria fumosorosea*, and *Lecanicillium* spp., which are used for pest control in various crops (Lacey 2017). An advantage of micropesticides compared to many bacterial and all viral biopesticides is that they do not need to be ingested to exert their biocontrol action.

Another alternative is mycoremediation, a form of bioremediation in which fungi are used to break down toxic compounds, including chemical pesticides. A large number of fungal species have demonstrated incredible abilities to degrade a growing list of hazardous toxic industrial waste products and chemical pollutants to a less toxic or non-toxic form (Prakash 2017). Its application falls into two categories: in situ and ex situ, where in situ methods treat contaminated soil where it is located, while ex situ processes require excavation of the contaminated soil before they can be subjected to bioremediation. The most used fungi as microremediators are: *Pleurotus ostreatus*, *Rhizopus arrhizus*, *Phanerochaete chrysosporium*, *P. sordida*, *Trametes hirsuta*, *T. versicolor*, *Lentinus tigrinus*, and *L. edodes* (Purohit et al. 2018). In this sense, the edible species of the genus *Pleurotus* are considered the most popular and widely cultivated varieties around the world, since they not only stand out for their nutritional and therapeutic properties, but also for their high biosorption potential due to their extensive biomass, accumulating high levels of heavy metals (Kapahi and Sachdeva 2017).

The use of microremediation methods for the protection of plants reduces the need for the use of pesticides; consequently, research in decontamination processes is essential to find a solution to some of the environmental problems caused by toxic residues generated by human activity. In addition, it is clear that to avoid an even worse scenario, large-scale changes must occur in the way industries and the populations manage and carry out their activities (Pavlovskaia 2014). The degradation of many substrates, including those that are toxic, can be achieved through the metabolism of fungi, key organisms for sustainable agriculture in the future.

1.8 Mycoherbicidal

Weeds are unnecessary plant growth among important crops without human intervention. In nature, many fungi could affect on these weeds. Some fungal pathogens can utilize biocontrol weeds as an alternative of chemical herbicides (Al-Ani et al. 2020a). *Phytophthora palmivora* showed the ability to control the weed *Morrenia odorata* that grow in Florida (Charudattan 2005). Waipara et al. (2005) detected three plant pathogens *Colletotrichum gloeosporioides*, *Phomopsis* sp., and *Sclerotinia sclerotiorum* possibly used against weeds. *Alternaria conjuncta* and *Fusarium tricinctum* are plant pathogens for dodder (Hopen et al. 1997). *Fusarium tricinctum* is used to biocontrol against two weeds *Cytisus scoparius* (Broom) and *Ulex europaeus* (Gorse) (Morin et al. 2000). *Fusarium oxysporum* infected and controlled *Cannabis sativa* (Tiourebaev et al. 1999). *Colletotrichum dematium*, *Phomospsis amaranthicola*, and *Alternaria cassiae* showed high efficacy in controlling three weeds *Crotalaria spectablis* (Showy crotolaria), *Amaranth* spp. (Pigweed), and *Senna obtusifolia* (Sicklepod) (Chadramohan et al. 2002).

However, fungal metabolites are a good mechanism affecting the physiology of weeds and growth. Fungi can produce many types of mycotoxins that affected on organisms such as weeds. The plant pathogens for weeds can produce several phytotoxins affecting on plant metabolisms (Walton 1996). Some fungi produced phytotoxins affecting the processes of weed physiology (Cimmino et al. 2015a, b). *Fusarium tricinctum* produced tricothecenes (Morin et al. 2000). *Ascochyta caulina* could produce three herbicidal toxins included Ascaulitoxin, ascaulitoxin aglycone, and trans-aminoproline (Vurro et al. 2012). Two fungal pathogens including *Ascochyta caulina* and *Phoma chenopodiicola* can control weeds of *Chenopodium album* by producing phytotoxins (Cimmino et al. 2014). The metabolites of three fungi such as *Drechslera biseptata*, *D. australiensis*, and *D. holmii* affected on plant vigor of weed *Rumex dentatus* L. by reducing the shoot biomass, seed germination, and root biomass (Akbar and Javaid 2013). *Aspergillus* sp. produced many mycotoxins (Attitalla et al. 2010a, b). The culture filter of two *Aspergillus* species *A. flavus and A. parasiticus* showed herbicidal activity and affected the root and shoot of weed *Parthenium hysterophorus* (Javaid et al. 2014). Cochliotoxin (new phytotoxin) was produced by weed fungal pathogen *Cochliobolus australiensis* showing high activity toxicity against buffelgrass (Masi et al. 2017). Bioproducts of mushroom have more effect on plant pathogens (Castañeda-Ramírez et al. 2020).

1.9 Conclusion

Fungal showed capability importance for general health and our environment. The beneficial fungi possessed very interesting mechanisms that must be utilizeed as possible in agriculture sustainability. The useful fungi are determined according to some features such as producing secondary metabolites, and enzymes, as well as, other mechanisms. These mechanisms have very clear efficiency for controlling plant enemies in the fields. The prevalence of useful fungi is very wide in soil and rhizosphere that indicates the ability for providing nutrients important for plant growth and development. The capability of useful fungi can degrade the complex elements to be available and easy absorption by the plant roots. This case can prepare nutrients for the plant which is leading to useful interaction between plant and fungi by excretion nutrients for fungi. Some useful fungi increase the surface of the root that reflects an increase of absorption for nutrients.

Therefore, it must use molecular technologies such as PCR, DNA sequencing, DNA microarrays, RT-PCR, and cytogenetic methods to study the feature of the genome for useful fungi. Many analyses such as GC-MS/MS, HPLC-MS/MS, IM-MS, IT-MS, UPLC-MS/MS, NMR, Hs-SPME, FTIR, Proteomic, and LC-MS-MS can be utilized to determine the secondary compounds, phytohormones, and enzymes that are more useful to producing biofertilizers and biopesticides. The usage of two or three methods is necessary for detecting some important characteristics of some useful fungi which can select isolate containing more interesting properties to be a strain. In this case, we can utilize this strain in the manufacture of biopesticides, or biofertilizers, or both of them.

Indeed, the detection of new useful fungal strains showing high efficacy in controlling plant pathogens, pests, and weeds for exploitation is a requirement. We must work on developing the fungal metabolites and utilizing the mycotoxins target-specific toxicity as compounds to target weeds, pests, and nematodes by improving bioherbicidal, biopesticides, and nematicidial. Finally, the wide study on the benefit of fungi is more necessary to reach the levels possible for an alternative to the synthetic chemical used in the manufacture of pesticides. The interesting role of useful fungi is to save our environment from the residue of chemicals that potential can detect in food and feed, as well as, damages for the ecosystem generally. The influence of synthetic chemicals is increasing through appearing resistance into the plant pathogens, weeds, and pests against chemical pesticides. But we must don ot forget the efforts made in using the biological factors but did not reach the required level. The possible is using useful fungi as biological factors around 99% as an alternative to the chemical pesticides in the ecosystem, that will lead to empty environment of residue of synthetic chemical.

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Chapter 2
Arbuscular Mycorrhizal Fungi: Interactions with Plant and Their Role in Agricultural Sustainability

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Contents

2.1 Introduction

Arbuscular mycorrhizae (AM) are geographically ubiquitous soil-borne microorganisms that establish a mutualistic symbiosis with the vast majority of terrestrial plants. They develop symbiotic mutualisms with roots of about 70–90% of vascular plant species (Smith and Read 2010). AM fungi are the members of an ancient phylum, Glomeromycota. They are the most abundant type of fungi found in the soil contributing 5–36% of the total soil biomass and about 9–55% of the soil microbial biomass (Olsson et al. 1999). AM fungi are obligate biotrophs, which need a host plant to complete their life cycle (Bago and Bécard 2002). However, the real

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mechanisms of their occurrence, diversity and dispersal under natural conditions are still obscure. The significance of mycorrhiza in sustainable agriculture has been established already several years ago, but the importance of these in the level of application was recognized only recently. AM fungi play a pivotal role in enhancing plant growth and fostering plant diversity. They also improve soil structure by forming extensive networks of hyphae in the soil. Therefore, AM fungi are important in organic farming systems and other sustainable agriculture practises. The sustainability concept in agriculture focus on increasing the productive capacity of the soil, to efficiently recycle the nutrients and organic matter and to minimize the required energy and resources. Sustainability demands effective utilization of nutrients by plants. This process can be facilitated through mycorrhizal associations (Jeffries and Barea 2001).

In plant-AM fungi association, a bidirectional trade of nutrients takes place between plant and AM fungi through extensively branched haustoria, termed arbuscules. The plant supplies up to 20% of carbon to the AM fungi from its fixed photosynthates, while AM fungi support the plant for the uptake of water, phosphates and other mineral nutrients available in soil. The development of AM fungi is accompanied by novel class of plant hormones, known as strigolactones, which act as the signalling molecule between the symbionts. Strigolactones produced by the plant root stimulate metabolism and branching in AM fungi, which in turn initiates the symbiotic association. AM fungi also play a key role in increasing host plant's resistance to root pathogens and tolerance to abiotic stresses (Smith and Gianinazzi-Pearson 1988). Mycorrhizal symbiosis is a keystone to plant diversity and productivity as they influence nearly all metabolic processes of the plants (Bonfante and Genre 2015). Owing to their diverse functionality and host benefit interaction, utilizing mycorrhizal inoculants in sustainable agriculture and forestry has major potential for maintaining plant growth and development.

2.2 Characteristics of AM Fungal Symbiosis

In the rhizosphere, AM fungi live in symbiosis with plant roots, forming intraradical (hyphae, arbuscules, vesicles) and extra radical (hyphae, spores) structures. They are one group of the beneficial soil mycobionts associated with plants that colonize roots and form the mycelial network to facilitate nutrient uptake and plant growth. The benefits acquired through AM-plant symbiosis can be physiological, nutritional or ecological. AM fungi have been widely utilized in agriculture, vegetation restoration and horticulture for around two decades. Mycorrhizal network, also termed as common mycorrhizal network (CMN), is one of the primary element of terrestrial ecosystem, which has substantial effect on different plant communities, especially on invasive plant species such as *Lythrum salicaria* (Pringle et al. 2009). The host plants get rewards of symbiosis based on the identity of AM fungal species (Facelli et al. 2010; Hoeksema et al. 2010). In plant-mycorrhizal symbiosis, fungal hyphae in the rhizosphere reach out in the soil and transport phosphorous, nitrogen and other essential nutrients to the plants (Yadav et al. 2020c).

AM fungi improve plants tolerance to different stress environments which may be biotic or abiotic via beneficial changes in their morphological and physiological traits (Feddermann et al. 2010; Hashem et al. 2015; Plassard and Dell 2010). A major challenge faced by the mycorrhizologist is to understand the signalling mechanisms and the colonization process of the extremely symbiotic AM fungi towards its host plants. AM fungi cannot be cultured in the absence of a host plant and that is the reason why they are often known as obligate biotrophs, which always need symbiotic relation with its host plant to complete its life cycle. It is an accepted fact that during the long run of evolution, AM fungi lost some of its carbon-fixing capabilities and the genetic machinery that supports their symbiotic relationship with the plants and became completely dependent on the host plant for a fixed carbon supply. A pragmatic evidence for this hypothesis is still lacking, but several indirect approaches to the study of this relationship have been developed. AM fungi are natural biofertilizers in soil as they nurture the growth of many terrestrial plants. Furthermore, AM fungi-enriched soil is remarkably more fertile and forms constant masses with significantly higher extra-radical fungal mycelium. Glomalin-related soil protein (GRSP), which is found abundantly in hyphae and spores of AM fungi, that helps to sustain the water content in soil subjected to diverse stress conditions which in turn regulates water frequencies between plants and soil thus triggers the plant growth and development (Wu et al. 2014). Glomalin and its relative compounds protect the soil from dehydration by enhancing soil aggregation and thus augmenting the water holding capacity in soil (Sharma et al. 2017). Therefore, researchers encourage the use of AM fungi as biofertilizers in sustainable crop improvement (Barrow 2012). Upon AM fungi inoculation, plant growth related functions such as photosynthesis, uptake of water and CO_2 assimilation increases (Chandrasekaran et al. 2019).

An ecologically important association is detected in the rhizosphere between leguminous plants and rhizobia. This mutualistic relation is induced by a fungal factor called Myc, which is analogous to the rhizobial signalling molecules such as Nod factor. Myc factors are the AM fungal signals that stimulate and establish AM symbiosis in leguminous and other mycotrophic plant species. However, it is still unclear whether the Myc factors are induced by plant root generated strictolactones. Besides acting as stimulators of AM fungal symbiosis, Myc factors function as plant growth regulators (Maillet et al. 2011). Myc factor has been considered the ideal candidate for biofertilizers in green technology applications. For example, leguminous seeds treated with Myc factor and Nod factors increased yields of crops such as pea, alfalfa and soybean. Compared to Nod factors, Myc factors have much broad spectrum of activity that results in the form of improved mycorrhization in plant roots, which in turn facilitate a better uptake of water and nutrients and resistance to various stresses by the host plant. For large-scale application, an efficient synthesis and production of Myc factors by bacteria has already been developed (Maillet et al. 2011). In order to implement the Myc factors in agriculture, a detailed investigation is needed to understand the biological activity and specificity of Myc factors on the host plant, as well as the optimal conditions required for its application (López-Ráez and Pozo 2013).

2.3 Taxonomy/Phylogenetic Classification

Mostly, AM fungi form deep monophyletic branches within the fungi and are very diverse that they are ranked with a taxonomic phylum, Glomeromycota, which currently comprises of approximately 200 species distributed among 14 genera (Schüβler et al. 2001; Stürmer 2012). The phylum Glomeromycota contains all known AM fungi which are co-evolved with their host plants, which originated in the terrestrial habitat during the Ordovician period more than 430 million years ago. Molecular studies performed later in the 1990s validated the finding that the AM fungi originated at a time between the Ordovician and the Devonian period (Helgason et al. 2007; Simon et al. 1993). Excitingly, the mycorrhizal association existed before plants had evolved its true roots (Bonfante and Selosse 2010). According to the phylogenetic analyses of SSU ribosomal RNA gene sequences, AM fungi have been moved from the Zygomycota to a new phylum Glomeromycota (Schüßler and Christopher 2011). The conventional taxonomy of AM fungi works on the basis of its morphological features of the hyphae, spores and the layers of the cell wall (Morton and Msiska 2010). However, the evaluation of the actual distribution patterns of Glomeromycota assemblages in all ecosystems requires further scrutiny (Lee et al. 2013).

Recent revolutions in the molecular techniques have enabled re-evaluation of the taxonomy and systematics so that many robust classifications of AM fungi have been introduced. New classification systems are introduced based on morphological and ontogenic characters of AM fungal spores, as well as consensus nucleotide sequences (SSU, ITS, LSU, β-tubulin and nrDNA) (Błaszkowski et al. 2014; Oehl et al. 2011). Based on the latest classification, the phylum Glomeromycota comprise of four orders (Diversisporales, Archaeosporales, Paraglomerales and Glomerales), which consist of 11 families, 25 genera and approximately 250 species (Redecker et al. 2013). Goto et al. (2012) proposed a new classification based on combined molecular and morphological studies. Recent studies of root samples using next-generation sequencing indicate that the number of species may be several magnitudes higher than what is known to date (Chen et al. 2018).

2.4 Beneficial Aspects of AM Fungi

Various benefits acquired by plants establishing symbiotic association with AM fungi are mainly due to the well expanded extra radical mycelium produced by AM fungi, which take up the nutrients and other essential elements from the rhizosphere zone within the bulk soil and transfer it to the host plant root in exchange for carbon

(Rastegari et al. 2020a, b; Yadav et al. 2020c). Compared to plant root hairs, AM hyphae are longer and thinner, which help them to move greater distances from the root and to get into soil pores which are unreachable to plant root hairs. It has been estimated that for each centimetre of colonized root by AM fungi, there will be an increase in the volume of soil explored by 15 cm^3. This value can increase up to 200 cm^3 depending on the environmental factors involved (Sieverding et al. 1991). The ratio of the length of AM fungal hyphae to that of roots in soil is expected to be 100:1 or greater (George et al. 1995). With effective colonization of AM fungi in the plant roots together with the ability of extra radical mycelium to transport nutrients is one of the well-known advantage of mycorrhizal formation, especially those nutrients (e.g. phosphorus) that have mobility limitations in soil. In addition, the extra radical hyphae provide greater stability of soil structure by enmeshing soil particles and by producing substances that bond soil particles together. AM fungi contribute substantially to the formation and aggregation of smaller soil particles into larger macro-aggregates (Rillig and Mummey 2006).

AM fungi provide many other benefits to host plants other than nutrient acquisition. These include stress alleviation to abiotic and biotic factors, such as pathogenic tolerance, water stress, drought, tolerance to toxic heavy metals, pH, salinity and adverse temperature (Singh et al. 2020; Singh and Yadav 2020). AM fungi association increases the efficiency of N fixation by legumes and a better plant performance following transplantation shock (Campanelli et al. 2013; Chen et al. 2018; Meddad-Hamza et al. 2010). AM plants show physiological and morphological changes, especially when they are growing in stressful conditions. This results in the form of modifying some essential growth regulators, such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and jasmonic acid, that help the host plant to have a better adaptation and homeostasis with the changing environmental conditions (Cameron et al. 2013; Foo et al. 2013). Reports state that even with a weaker AM fungi colonization, gene expression in plants can be altered. For an example, with a very weak AM fungal colonization, the mechanism involved in the expression of inorganic P (Pi) transporters get changed (Poulsen et al. 2005). Studies suggested that there will be an increase in the average yield of the crop plants upon increased AM fungal colonization (McGonigle and Fitter 1988).

2.5 Commercial Application of AM Fungi

The beneficial attributes of AM fungi have raised the possibility of their commercial application. In the last few decades, AM fungi market has increased and diversified with more patented products becoming available (Devi et al. 2020). Globally, the leading producers are located in the United States, China and India. In the last decade, the Indian market has seen remarkable progress in biofertilizer production (Chen et al. 2018). The European market is the leading marketplace for mycorrhizal-based biostimulants. As per surveys, the companies involved in producing and marketing AM fungi products are growing every year. In 1990s, the number of companies selling AM fungi products was 10; it has reached to 75 firms in 2017. The main areas of AM fungi application include agriculture, landscaping, forestry, horticulture, restoration of degraded land, soil remediation and research. Sometimes mycorrhizal inoculants are available in the form of mixed inocula, which have different strains of AM fungi and rhizobacteria or PGPR (Kour et al. 2019). The cost of mycorrhizal inoculation for potato field was estimated to be $135 per hectare in the United States (Hijri 2016). AM fungi inocula are nowadays utilized as biofertilizers for sustainable agriculture applications, but a larger volume of inocula production is possible only through conventional pot culture methods. AM inocula are available as spores, root fragments of plants colonized by AM fungi, or the combination of the two or by the incorporation of mycelium. The cultured or isolated inocula are usually mixed with a carrier material in either solid or liquid form and applied directly to the soil or plants. Mostly used carrier materials include perlite, clay, sand, vermiculite, soilrite and glass pellets.

Other alternative methods for AM inocula production include soil-free aeroponics systems (Jarstfer and Sylvia 1995), nutrient film (Elmes and Mosse 1984) and root organ culture (Mugnier and Mosse 1987), though they are not cost-effective, and large-scale production with them is poorly developed. Though AM fungi have been reported as excellent biofertilizers, their large-scale production and inoculation are not practical and achievable for a large-scale agriculture application because they are strictly produced by conventional pot culture method. The application has been limited more on the production of high-value nursery stocks, gardening practices and research purposes. Another aspect is the need for diverse communities of AM fungi in the product, as different species perform differently in the soil upon inoculation. This again influenced by various environmental conditions. Furthermore, research evidence suggests that different species of AM fungi vary in their ability to increase the crop yield and nutrient transport to its host plant (Rai 2006).

2.6 Significance of AM Fungi in Natural Habitats

The degree to which a plant benefits from AM fungal symbiosis mainly depends on the environmental conditions. AM fungi have always been considered important plant symbionts in natural habitats with poor soil conditions. Plant root cells with arbuscules receive more nutrients due to considerably increased contact surface (Alexander et al. 1989). Another way to absorb nutrients is through AM fungal hyphae networks. The AM fungi-colonized plants seem to develop special pathways and mechanisms to improve their nutrient uptake. Therefore, it is likely that the plants with AM fungi symbiosis thrive better than the non-AM plants in habitats with low nutrient contents. Phosphorus is one of the main nutrients, that is made available for plants in the form of phosphate via AM fungi symbiosis (Karandashov and Bucher 2005; MacLean et al. 2017). Studies have shown that apart from phosphorus, other nutrients are being transferred to plants via AM fungal symbiosis. Although the mechanisms and pathways of phosphate transfer are well studied, more information is needed to understand how AM fungi help plants in acquiring the necessary nutrients such as potassium (Garcia and Zimmermann 2014), nitrogen (Correa et al. 2015), sulphur (Casieri et al. 2012), and some micronutrients like as zinc (Smith et al. 2010) and Fe (Ouledali et al. 2018). Therefore, AM-associated plants in natural habitats with low nutrient availability have the advantage of easier access to nutrients compared to non-AM associated plants (Yadav et al. 2020a, b). When it comes to competition over the resources, AM-associated plants might have a better survival rate.

Under natural conditions, plants are always subjected to various environmental stresses, which have negative impact on plant growth and development and it often leads to a threat on their survival (Ruiz-Lozano 2003). AM fungi have been found in various environments. It is believed that apart from the nutrient exchange, they could assist plants in surviving some environmental stress such as salinity or drought in arid and semiarid areas. In arid lands, when plants are under stress due to low water availability, they undergo anatomical, physiological and metabolic adaptations (Bray 2004; Rossi et al. 2013). Some plant species evolved in the land to avoid drought while others tolerate it through certain dodges. In this respect, AM fungi play a crucial role in plants to develop tolerance to drought via root symbiosis (Rapparini and Peñuelas 2014). In such symbiosis, host plants attain an integrative drought response by achieving either a tolerance or avoidance strategies, which help the host plant to well adapt with the situation (Ouledali et al. 2018; Rapparini and Peñuelas 2014; Ruiz-Sánchez et al. 2010). AM fungi can enhance drought resistance of their host plants through affecting the physiological nutrient uptake, hormone balance, osmotic adjustment and antioxidant systems (Wu and Zou 2017). P nutrition enhancement (Bethlenfalvay et al. 1988; Sweatt and Davies Jr 1984), increasing water uptake capacity by hyphae (Zou et al. 2015), and longer roots (Bryla and Duniway 1997) in AM-associated plants are additional AM benefits assisting them in overcoming drought stress. In general, plant growth strongly gets affected by drought, while AM fungi symbiosis significantly mitigates the negative effects of drought stress in plants. Recent studies show that AM fungi are more common in drier/non-irrigated soils compared to irrigated soils in certain plant species (Landolt et al. 2020). It is believed that in root, microbial symbiosis, such as AM, could be the most important factor in the resistance of some tree species to drought (Calvo-Polanco et al. 2016).

Several studies have investigated the role of AM fungi symbiosis in saline environments (Pan et al. 2020; Sonjak et al. 2009; Wang et al. 2004). Presence of excess salt in the soil affect the water and nutrient uptake efficiency of plants resulting in disrupting the distribution of ions channels at the cellular level. This will create an osmotic and ionic imbalance in the plant cells and thus negatively influence the plant growth mechanisms (Saxena et al. 2017). Adaptations to high levels of salinity in AM fungi-colonized plants include improvements in host photosynthetic potential, water use efficiency, nutrition and tolerance to ion toxicity, as well as several metabolic adaptations. The metabolic adaptations facilitated by AM fungi include higher K^+/Na^+ ratios in host tissues, improved maintenance of ion homeostasis and the accumulation of essential amino acids such as glycine, proline, betaine or soluble sugars that improve osmotic adjustment (Porcel et al. 2012). Thus, AM fungi found in natural saline environments could help with plant salt resistance in agricultural plants as well. Being the most severe abiotic stress, soil salinity affects the plant growth and production worldwide. Therefore, the application of such fungi could be of importance regarding crop production in saline environments. Similarly, AM fungi symbiosis offer temperature stress resistance in host plant via increasing its nutrient and water uptake efficiency, improving the photosynthetic capacity, increasing the osmolyte accumulation and reducing the oxidative damage by producing more secondary metabolites (Zhu et al. 2017). These changes could help the host plant to overcome stress caused by temperature extremes. AM fungi could also have alleviating effects on heavy metal stress in plants growing in the polluted habitats. This adverse effects caused by elevated levels of heavy metals can be mitigated by increased water and nutrients uptake and production of plant hormones, changes to root activities, including heavy metal uptake, or indirectly via interactions with the other soil microbes (Garg and Pandey 2015; Miransari 2010; Miransari 2017; Vangronsveld et al. 2005).

The success of host plant survival under harsh and stressed environment depends on the AM fungi habitat adaptation and co-evolution with its host plants (Meharg and Cairney 1999; Querejeta et al. 2006). Plants that do not have the adaptive mechanisms to survive the environmental stress are more likely to depend on AM fungi symbiosis for survival. For example, in a recent study conducted by Pan and co-authors (Pan et al. 2020) indicated that glycophyte plants are more dependent on AM fungi symbiosis than halophyte plants to tolerate the saline environments. Interspecific differences in the tolerance of AM fungi to environmental stress and their different reaction to the stress when forming symbiosis with different plant species highlight the importance of AM fungi studies in the natural environments. Indeed, AM fungal diversity and composition are significantly affected by the environmental variables such as plant community composition (Krüger et al. 2017) and functional groups (Gui et al. 2018), climatic changes (Xiang et al. 2016), properties of the soil (Abdedaiem et al. 2020; Carballar-Hernández et al. 2017; Gai et al. 2012; Yang et al. 2016), as well as practices used in management (Binet et al. 2013; Borriello et al. 2012; Higo et al. 2013; Lu et al. 2018; Uibopuu et al. 2009). These changes in the AM fungi community could lead to substantial changes in their effects on plant communities. Understanding the factors affecting the AM fungi communities in different habitats is essential to understand and predict their role in ecosystem services under future climate changes and the consequences.

2.7 The Significant Role of AM Fungi on Crop Health

The AM symbiosis offers several benefits to host plants and their surrounding habitats. They boost plant defence against soil pathogens, increase abiotic stress tolerance, heavy metal tolerance, and adaptation to climate changes (French 2017). During stress conditions, especially in drought, the stress tolerance in plants can be increased by AM fungi symbiosis. This leads to a higher amount of sugar substances such as trehalose and mycose in the host plant tissues. Such substance improves the plant tolerance against biotic and abiotic stress by producing secondary substances, which in turn improve cellular structures, cell wall and lipid bilayers (Lunn et al. 2014). Inoculation of vascular plants with ectomycorrhizal and AM fungi activates the production of trehalose in root cells of host plant (Müller et al. 1995), which improves the carbohydrate metabolism in plants via changing the amount of starch or sugar in the plant tissue (Wagner et al. 1986) and provides stress tolerance benefits to the host plants via mutual symbiosis. For example, under extreme drought, the cells of the AM-associated plants have a better chance to become intact and return back to normal under favourable environmental conditions (Wingler 2002).

Studies show that AM fungi induced defence against bacteria, other pathogenic fungi, nematodes, and insects (Jung et al. 2012). This defence mechanism mostly results from increased plant secondary metabolism followed by the AM fungi symbiosis. Such secondary metabolites are alkaloids and phenolic compounds, which can be found in the trichomes and vacuoles of the AM-associated plants that can improve the plant tolerance against pathogens and insects (Champagne and Boutry 2016). During a pathogenic attack, internal and external hyphae sense the pathogen metabolic compounds in the soil surrounding the roots. AM fungi then warns the host cell by producing short chitooligosaccharides (Cos) and lipo-chitooligosaccharides (LCOs) (Bonfante and Genre 2015; Zipfel and Oldroyd 2017); this message then transmits from cell to cell in the host plant via plasmodesmata.

Another significant feature of AM fungi is heavy metal tolerance in crops. AM fungi have chitin and melanin compounds attached to the cell wall, which could form a chemical chain with the unfavourable elements in the rhizosphere soil around the roots (Eisenman and Casadevall 2012). The melanin compounds in fungi protect them from harsh environmental conditions (Zhdanova et al. 2000). A clear mechanism behind the role of AM fungi in metal tolerance is unclear. It is believed that AM fungi use multiple mechanisms to immobilize metal ions. In the case of some ectomycorrhizae, these ions are stored in the cell wall, cytoplasm, and vacuole. It is suggested that AM fungi also alters the host metabolisms to respond to metal toxicity. For example, *Funneliformins mosseae* increased the metallothioneins in the *Festuca sp*. plants that have been growing in a soil with high nickel contamination by transcription of the related genes (Shabani and Sabzalian 2016).

AM fungi are cosmopolitan in distribution and their diversity has been detected in all major ecosystems across the globe (Davison et al. 2018; Öpik et al. 2013). Some AM fungi isolates are reported to have restricted distribution in natural communities (Rosendahl et al. 2009). The diversity of AM fungi is reported in arctic regions, deserts in the Arabic peninsula, tropical forest and even in the higher Himalayas (Al-Yahya'ei et al. 2011; Liu et al. 2011; Lovelock et al. 2003; Varga et al. 2015). The presence of cosmopolitan AM fungi species indicates that they are highly adaptable and have a great impact on the environment.

Another important service provided by AM fungi to both natural and agricultural systems may be the improvement of the soil structure. AMF hyphae which colonize in and around the plant root form a dense hyphal network, which highly interacts with the soil particles due to increased surface that is in contact with the soil. The glycoprotein referred to as glomalin improves the soil structure by affecting the soil aggregates (Singh et al. 2013). Glomalin improves the soil quality, when it is produced by spores and hyphae of AM fungi in the roots and their surrounding soil. They act as a very stable carbon sink and decrease the organic carbon degradation via improving the soil aggregation, thus functioning as carbon sequestration in soil (Rillig et al. 2001). AM fungi also benefit plant growth via higher water retention capacity by improving soil qualities, especially for plants growing in arid/semiarid areas or soils with low water availability (Chen et al. 2018). Nutrient leaching is another major problem faced in agriculture, which results in loss of soil fertility and groundwater pollution (Cavagnaro et al. 2015). Inoculation with AM fungi improves soil structure and facilitates storage of nutrients in the aggregates of mycorrhizal soil, thus benefiting plant nutrient and water availability (Querejeta 2017). AM fungi also alter the available nutrient in the soil by creating closed nutrient cycles, which provide long-term soil fertility (Cavagnaro et al. 2015). The beneficial effects and the role of AM fungi in plant growth and development are depicted in the Fig. 2.1.

2.8 Application of AM Fungi in Agricultural and Horticultural Crops

Majority of the agriculture crops are found to be potential hosts for AM fungi, and inoculation with AM fungi increases their productivity and fitness (Begum et al. 2019). AM fungi induce plant tolerance to environmental stress by interfering with

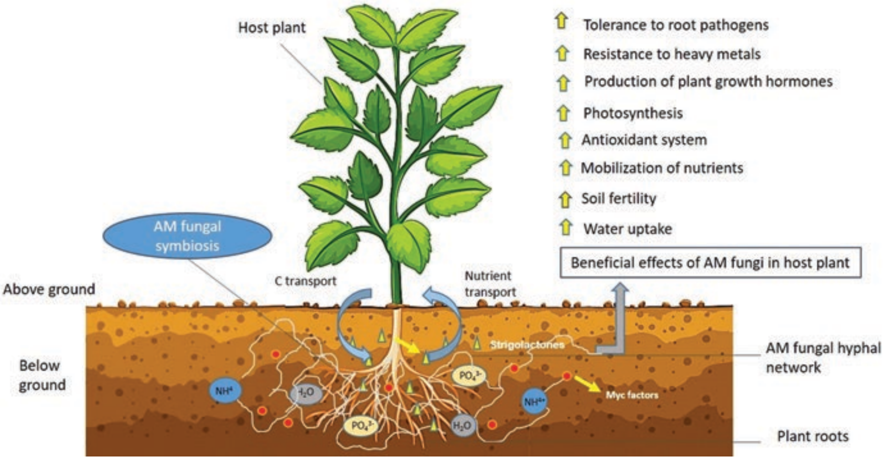

Fig. 2.1 The beneficial role of AM fungi in plant growth and development

phytohormone balance. AM fungi absorb and translocate minerals from the soil layers that are out of plant root zone and alter the secondary metabolisms leading to better metabolic trait. AM fungi also increase the root development and surface absorbing capability of host plants (Paszkowski and Gutjahr 2013). AM fungi have been widely used in agriculture and horticulture field applications. The success of AM fungi application always depends on external factors, that is management strategies such as weed control, pruning, ploughing and fertilizer usage (specially P), that interfere with AM fungi composition and colonization in the rhizosphere (Chen et al. 2018), as well as on the selection of an effective strain of AM fungi and the host plant (Njeru et al. 2015). In addition, selecting the ideal AM fungus is important for every crop (Njeru et al. 2015; Rouphael et al. 2015). The adaptation of plants produced by cuttings and micropropagation is a critical task in horticulture. Most horticulture practices involve sterile in vitro micropropagation production, but during the time of weaning, it can cause large losses. AM fungi inoculation is found to be an alternative solution to improve plant growth and nutrient uptake during the early stages, which results in larger products with higher commercial values (Schubert and Lubraco 2000). Many studies have reported the significance of AM fungi in the development of fruit seedlings in early stages. For example, in a study conducted by Schubert and Lubraco (2000), apple seedlings growth characteristics significantly improved by the AM fungi symbiosis.

Arbuscular mycorrhizal inoculation is profitable in agriculture. Large-scale production of AM fungi and coating seeds with them is the most suitable method of application. Many crop varieties have been significantly affected by inoculation with AM fungi (Ortaş et al. 2017). Researchers have suggested two main approaches of using AM fungi; inoculum production in the field and developing cultural practices that improve the native population of mycorrhizal fungi (Roy-Bolduc and Hijri 2011). A meta-analysis on potato carried out in 231 different crop fields of Europe and North America revealed a significant increase in tuber growth rate and size after inoculation with *R. irregularis* (DAOM 197198) (Hijri 2016). The average crop yield in the trials was 3.9 tons/ha, which constitute 9.5% of total crop yield. With an estimated profitability threshold of 0.67 tons/ha increased yield, nearly 80% of the trials were found to be more profitable. Though cultural methods often improve the effectiveness of native mycorrhiza, they do not create the best specific AM fungi-plant symbiosis for commercial production. This can be an important challenge when solving food security issues. Similarly, the selection of a suitable host plant is another concern (Ortas 2015; Ortas and Ustuner 2014). Targeted studies of mycorrhizal fungi could be a cost-effective option to solve these problems (Ortaş et al. 2017). In addition, profitability can be further increased by using AM fungi application to decrease fertilization without a decrease in yield (Chen et al. 2018).

2.9 Role of Subsoil AM Fungi in Sustainable Agriculture

AM fungal biomass abundance varies in the soil based on the soil depth and plant root length. AM fungi root colonization levels vary with soil depth. Almost half of AM fungi biomass is located below 30 cm (Higo et al. 2013). The AM fungi communities below 30 cm differ from the ones in topsoil both phylogenetically and morphologically (Säle et al. 2015). Growing evidence suggests that some AM fungal taxa are defined and limited by different soil layer characteristics (Sosa-Hernández et al. 2018b). According to a pot experiment with elevated CO_2 levels performed by Rillig and Field (2003), there was no change in the AM fungi in the topsoil (up to 15 cm), while there was remarkable increase in AM fungi in the subsoil (about 15–45 cm soil layer), indicating that top and subsoil communities have differential reactions to above ground environmental variables. Evidence of AM colonization in deeper soil layers (4–8 m) was reported in many tree species such as honey mesquite (Virginia et al. 1986) *Acacia*, and *Eucalyptus* (de Araujo Pereira et al. 2018). Altogether, AM fungal associations of deeper layers are often overlooked, which are probably highly valuable for management and production improvement (Kour et al. 2020).

AM fungi communities in the subsoil are abundant and unique and contribute to better plant production and ecosystem services (Higo et al. 2013; Sosa-Hernández et al. 2018a). Reduced pore size, higher soil compaction, and lower oxygen availability make the subsoil different from topsoil (Lynch and Wojciechowski 2015). Subsoil AM fungi are expected to follow a high-stress resistance life cycle. As such, deeper soil AM fungi produce more long-lived hypha, as well as optimized resource use efficiency, representing an advantageous carbon cost/benefit investment for the plants. Plants may receive more benefits in return for every unit of carbon they provide for the AM fungi in the subsoil compared to that of topsoil. Subsoil AM fungi have a considerable role in soil formation (Leake and Read 2017) and weathering via various indirect mechanisms (Taylor et al. 2009). Deeper soil layers have lower biological activity, higher clay content and usually contain higher amounts of primary minerals with great potential for mineral weathering and nutrient availability.

AM fungi symbiosis expands the soil space that is reachable by their host root, which is known as the mycorrhizosphere (Linderman 1991), and this likely results in higher microbial activity in the subsoil. This alliance between the plant roots, AM fungi, and the associated soil microbial community has the potential to improve the soil structure, especially in shallow soils where the parent material or the bedrock is close to the root system.

2.10 Role of AM Fungi in Reforestation and Landscaping

Forest disturbances are created by human activities and make a dramatic change in the habitat, vegetation and soil. The disturbed habitats are usually described by aboveground and belowground diversity (Helgason et al. 1998). Utilization of AM fungi in reforestation and landscaping is a promising approach as the degraded and eroding lands can regain functionality with AM fungi. In arid regions, juvenile trees are vulnerable to stress conditions such as heat, drought and nutrient deficiency. With mycorrhizal associations, this critical phase can be overcome. An example is the mycorrhizal inoculation mediated increase in fitness and survival of young argan trees, which are considered endangered species in their original habitats due to excessive harvest (El Abbassi et al. 2014). Another approach by Ouahmane et al. (2007) was the inoculation of young cypress trees with a mixture of indigenous AM fungi, which were isolated from a natural site of *C. atlantica*, which increased the chances to form a symbiosis between AM fungi that is adapted to drought environments and the host plant.

AM fungi inoculation was a successful approach as it increased the growth and survival of these trees in the arid environment. AM fungi inoculation is in fact a suitable, sustainable and cost-effective approach in reforestation. AM symbiosis is considered to be a critical asset in preventing soil erosion, especially in sandy soil ecosystems (Moradi et al. 2017). The rhizosphere of mangroves species belonged to nine genera in the west coast of Goa yielded a variety of AM fungi (Sridhar 2005). Appropriate vegetation builds the ecosystem in favour of existence and interactions of flora, fauna and microbes. The costal ecosystem is a habitat for many inhabitants and provides food, fodder and bioactive compounds (Sridhar and Bhagya 2007).

2.11 AM Fungi Promote Bioremediation of Contaminated Soils

AM fungi act as a sequester of toxic compounds from the environment as a form of bioremediation. They prevent heavy metals from travelling past the plant roots (Rajkumar et al. 2012). Though heavy metals play a significant role in some biological cycles occurring in plants, but excess amount of these heavy metals can have adverse effects in plants. AM fungi can store the heavy metals in their vacuoles. In some cases, AM fungi increase the heavy metal tolerance of plants instead of decreasing the uptake of heavy metals by plants (Ferrol et al. 2016). Thus, AM fungi play an essential role in modulation of plant heavy metal accretion in different ecosystems, and they are considered a key factor in phytoremediation and micronutrient uptake by crops growing in polluted soils.

Heavy metal toxicity in plants results from the excessive uptake of elements from the polluted soil. The effect of AM fungi on plant growth and tolerance to heavy metals in a polluted soil depends on the fungal species, plant species and heavy metals involved in the consortium. Many case studies reported that the toxic effect by heavy metals decreased in plants mainly due to the reduction of their concentrations in the soil, which in turn resulted in higher uptake of P and the enhanced growth of mycorrhizal plants (Chen et al. 2003). However, plant growth improvement induced by the AM fungi symbiosis is not always related to the metal concentrations in plant tissues or in the soil. For example, a study carried out by Lingua et al. (2008) reports that Cu and Zn mitigation was found in white poplar trees colonized by *R. irregularis or F. mosseae* even when the metal concentrations was higher in the host plants. AM fungi often increase heavy metal accumulation in roots, but their effects on the heavy metal concentration in aboveground organs of host plants are not remarkable.

AM fungi have been reported to decrease Zn uptake and allocation of heavy metals in above and below ground organs of red clover and tomato when in symbiosis with AM fungi on Zn-polluted soils (Li and Christie 2001; Watts-Williams et al. 2013). AM fungi spores and their abundance in roots are lower in heavy metal contaminated soils than unpolluted soils. Usually, the native AM fungi of polluted environments are more resistant and efficient at improving heavy metal tolerance of plants compared to native AM fungi that are found in non-polluted areas. For example, the *Rizhophagus irregularis* Br1 ecotype isolated by Hildebrandt et al. (1999) from the soil under *Viola calaminaria* plants shown to be more effective in inducing heavy metal tolerance on a variety of plants (tomato, maize and *M. truncatula*) than an ecotype of the same species isolated from a non-contaminated soil (Kaldorf et al. 1999).

2.12 AM Fungi and Abiotic Stress Tolerance

AM fungi could alleviate plant's response to different types of stresses or a combination of stresses that include salinity, drought, nutrients, heavy metals and temperature. Under stress conditions, reactive oxygen species (ROS) will be generated in host plants. Based on the severity, there will be an increase in ROS species, which in fact harmful to the metabolic activities of the plants (Bauddh and Singh 2012). The plants' cellular mechanisms fight against the reactive oxygen species (ROS) by the production of several enzymes, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) (Ahanger and Agarwal 2017). Upon inoculation with AM fungi, the plant adaptability to stress get increased by processes such as increase in mineral nutrient uptake, improved photosynthetic rate and accumulation of osmoprotectants, increased antioxidant enzyme activity and manipulation in the rhizosphere ecosystem (Yin et al. 2016). A study by Duc and co-authors revealed that inoculation with *Scolecobasidium constrictum* in tomato plants which were set for a combined treatment of salinity and drought showed improvement in water uptake, stomatal conductance and biomass production compared to non-inoculated plants (Duc et al. 2018).

The AM fungi are capable of significantly enhancing plants' tolerance to stress conditions and improve the plant growth and yield even under stress (Latef and Chaoxing 2014). Under stress conditions, AM fungi mediate alterations in the phytohormone level and up-regulate its antioxidant system. However, different mechanism of AM fungi action towards alleviating stress in plants depends on the stress type and the AM fungal species. For example, mechanisms such as production of phytochelatins, compartmentation and sequestration of toxic ions, and expression of stress proteins can be specific and show significant changes with AM species involved. The hydraulic conductivity changes occurring in the roots under salt stress can improve osmotic stress tolerance of the plant to a considerable level (Evelin et al. 2009). An investigation made by Zhang et al. (2018) shown that AM fungi made a remarkable influence in castor bean growing under saline condition by altering the levels of some essential plant metabolites and by altering the gas exchange traits. Thus, AM fungi offer a considerable importance in the production and management of different potential crops prone to stress conditions with high nutritional quality.

However, to achieve the benefits offered by AM fungi, an extensive study is necessary to unravel the role of AM fungi in neutralizing the effects of combined stresses.

2.13 Conclusion

AM fungi and their importance regarding plant growth, production and their effect on stress tolerance and nutrient uptake of their host have been studied during past decades. However, much remains to be investigated regarding their interaction with other root-colonizing microorganisms (e.g. endophytes) and the natural soil microbiome. Habitat adaptation and co-evolution with the host plant certainly need more attention from the scientists. Identifying the specific AM fungal species which have adapted to environmental stress in different habitats might be of value regarding agricultural production, especially under salt and drought stress, as well as poor soils. AM fungi contribute a major role in carbon sequestration through various mechanisms. But a thorough investigation is needed to study the mechanism of AM fungi-associated links between C fluxes in soil and the nutrient exchange to the host plants. AM fungi inoculation has shown to enhance crop productivity in many agriculture crop varieties. AM fungi inoculated alone or in combination with other microbial inoculants such as PGPR also help in alleviating plants against different stress conditions. In order to have better crop productivity, it is necessary to understand the AM fungi mediated cellular modulations in the tolerance mechanisms and the phenomenon by which the signals are transmitted to regulate plant performance. In order to promote sustainable agriculture, the use of synthetic fertilizers needs to be replaced with AM fungi inoculants which in fact recover the soil fertility and increase the crop productivity through its beneficial functions. Thus, the multiple benefits offered by AM fungi decipher its significant services in natural ecosystem as well as in sustainable agriculture.

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Chapter 3
Plant Growth-Promoting Endophytic Fungi from Different Habitats and Their Potential Applications in Agriculture

Neetu Singh, Archana Singh, and Praveen Dahiya

Contents

3.1 Introduction

Endophytic microorganisms as the name indicate generally colonize internal tissues of host plants without any harm or apparent loss to their host. Endophyte owes its origin to De Bary (1866) who first coined the term. It is comprised of two Greek words, endon means inside and phyte refers to plants (Suman et al. 2016). Most of the groups in the plant kingdom such as bacteria, actinomycetes, mycoplasma and fungi are reported as endophytic microbes (Arnold 2008). Endophytes are known for most intimate linkage with their host plants especially involved in beneficial or symbiotic associations. Plant and microorganism association may have evolved

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>400 years ago for which evidence of such association found in the fossils of stems and leaves (Kusari and Spiteller 2012; Verma et al. 2017). During the process of evolution, there were drastic changes in adaptation of plants from aquatic to terrestrial habitat under abiotic pressure in which, however, endophytes adapted their microenvironment accordingly or through genetic modification (Arora and Ramawat 2017). All types of microorganisms that belong to different categories of fungi or bacteria have been reported as endophytes, out of which most frequent are fungal endophytes (Strobel and Daisy 2003; Kour et al. 2019b; Yadav et al. 2020d).

The first endophyte of fungal category was recognized by Leveillé in 1846 in wheat leaves. The research for more than 100 years suggests that most of the plants are in symbiotic relationship either with mycorrhizal fungi and/or with other fungal endophytes (Petrini 1986; Noel and Nicholas 2004). Unlike mycorrhizal fungi that live symbiotically in cortical tissues of the roots and grow around the root zone, other fungal endophytes grow either intercellularly or intracellularly in the plant tissue and may inhabit stems, leaves, branches, twigs, bark, fruits and seeds (Torres et al. 2011); scales, resin canals and meristems (Arora and Ramawat 2017); petioles, flowers (Kumar et al. 2019a, b). However, some fungi are highly specific to certain genera/family of plants (Rana et al. 2019b).

The spectrum of fungal endophytes differs in space, time and function within a host plant also. For example, alfalfa plants are colonized by distinct fungi in leaves, stems and roots (Lugtenberg et al. 2016). Endophytic microbes can enter directly through the vertical seeding method and indirectly by horizontal transmission generally from the soil (Rana et al. 2019c). In terms of mutualistic benefits, they increase the availability of nutrients to the plant, improve the capability of tolerance in the plants for abiotic or environmental stress, act as protectant against biotic stress by pests and pathogens, help in plant growth and will also produce some allelochemicals helpful in reducing the growth of unwanted plants/weeds (Firakova 2007; Torres et al. 2011; Bamisile et al. 2018). Our knowledge about the potential of endophytic fungi, their use as biological agents in reducing abiotic and biotic stress, which in turn enhanced crop productivity, is highlighted in chapter. Furthermore, the need of fungal endophytes as microbial resources in future for maintaining sustainability in agriculture is also discussed.

3.2 Functional Grouping of Fungal Endophytes

Categorization of fungal endophytes is based on the differences in taxonomy, host range, tissue specificity, ecological functions, and transmission and colonization pattern into two major groups (Schaechte 2011). First group/class is the clavicipitaceous endophytes or C-endophytes known for their host specificity infecting some grasses mainly belong to grass family poaceae in tropical and temperate regions. They are often vertically transmitted through seeds (Saikkonen et al. 2002). C-endophytes include *Balansia* sp. and *Epichloë* sp., which represent limited number of clavicipitaceous species in grasses (Rodriguez et al. 2009; Khiralla et al. 2016).

These C-endophytes are highly beneficial in improving plant growth under abiotic stress including herbivory through producing some toxic chemicals harmful for grazing animals (Aremu et al. 2017; Stone et al. 2004). However, benefits of C-endophytes depend on the species of host, their genotype and environmental conditions (Saikkonen et al. 2002; Nair and Padmavathy 2014). The second group of non-clavicipitaceous endophytes or NC-endophytes are commonly found in vascular and non-vascular plant species (Rodriguez et al. 2009; Jain and Pundir 2017). Table 3.1 represents highly diverse and comprised of species belonging to three divisions: Pezizomycotina and Agaricomycotina of class Ascomycota and Pucciniomycotina division of class Basidiomycota (Faeth and Sullivan 2003). They have an ability to colonize host plants by both types of transmission, vertically and horizontally.

The endophytic colonization ranges from transmission via seeds, vegetative planting material, from the rhizosphere and phyllosphere, the surrounding environment of host plants. Endophytic fungi enter via seeds known as true endophytes. In horizontal transmission, entry of fungal spores of endophytes is possible through surrounding soil or air near the host plant (Zabalgogeazcoa 2008; Yadav 2019; Lata et al. 2018; Rana et al. 2020a). The already established endophytes may transmit from one generation to another generation via seeds (Lata et al. 2018). However, NC endophytes can be categorized into three classes 2, 3 and 4 on the basis of biodiversity, patterns of colonization, mode/mechanism of transmission in host and their role in environment (Lugtenberg et al. 2016), while the first group or C endophytes has just one class. NC-endophytes have been the most extensively researched and capable in enhancing growth of host plant in adverse environmental conditions or habitat-specific stresses such as pH, salinity, temperature and water. NC-endophytes, being rich in species and ecological diversity, can be recovered from tropical to tundra regions (Rodriguez et al. 2009). The fungal endophytes of class 2 belong to Ascomycota and Basidiomycota, and generally associate in aerial parts or above-ground tissues algae, bryophytes, pteridophytes, conifers, angiosperms; however, in biomes, they range from warmer zone to cooler zone communities (Khiralla et al. 2016).

Class 3 endophytes also present in rich diversity and form localized infections in the leaves of trees in tropical zones besides other lower plants (Lugtenberg et al. 2016). Fungal endophytes of class 4 termed as the 'dark-septate endophytes' (DSE)

Table 3.1 Classification of fungal endophytes after Rodriguez et al. (2009)

	Clavicipitaceous	Non-clavicipitaceous		
Criteria	Class1	Class 2	Class 3	Class 4
Host range	Narrow	Broad	Broad	Broad
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal
Colonized tissue(s)	Shoot and rhizome	Shoot, root and rhizome	Shoot	Root
Colonization	Extensive	Extensive	Limited	Extensive
Biodiversity	Low	Low	High	Unknown
Fitness benefits[a]	NHA	NHA & HA	NHA	NHA

[a] Among benefits, Non-habitatadapted (NHA) such as growth promotion and drought resistance/tolerance are common among all fungal endophytes; Habitat-adapted (HA) benefits result from habitat-specific selective pressures under high/low pH, salinity and temperature

having melanized dark septate hyphae are often members of class ascomycetes, colonize roots of host plants and referred as facultative biotropics. They are ubiquitous in a wide range of ecosystems, especially common in cooler zones and found in the roots of more than 600 plant species (Rodriguez et al. 2009; Newsham 2011; Lugtenberg et al. 2016). NC-endophytes have been recovered from almost all types of ecosystems (Wani et al. 2015). The diverse role of endophytes and their potential applications in agriculture (Arnold and Lutzoni 2007; Vasiliauskas et al. 2007) are gaining importance among ecologists, botanists, plant protectionists and scientists in field of biotechnology all over the world.

3.3 Biodiversity of Endophytic Fungi

The world's terrestrial biodiversity on earth are represented by tropical and temperate rainforests, which covers 60% of ecosystem. Most of the plant species are associated either to one or more microorganisms in their tissues (Anitha et al. 2013; Rana et al. 2019a; Yadav et al. 2020b, c). These fungal endophytes in terms of interaction with their hosts depend on abiotic and biotic stress under natural environmental conditions, besides experimental factors. Usually one or a few species dominate the community, while most of the species are rare and specific to their host (Torres et al. 2011). Fungal endophytes reported from higher latitudes are relatively characterized by few fungal species in comparison with tropical regions where species of fungal endophytes are more in number. These endophytes are predominantly members of ascomycetes that appear to be ubiquitous in nature, easily recovered from vegetation of warmer and cooler zones besides mangroves in coastal regions (Lugtenberg et al. 2016); extreme cold regions – arctic, alpine and xeric environments (Ali et al. 2018). *Xylariaceous* fungal endophytes of class Ascomycetes are less host specific (associated with one host or a group-related species) and commonly found in tropical and subtropical regions. However, endophytes in temperate regions are known for host specificity, while fungal endophytes in tropical zones are less specific in selection of host plants. It is evident from available literature that fungal endophytes worldwide comprise more than million species (Chhipa and Deshmukh 2019; Kumar et al. 2019a, b), which provides a significant bio/genetic resource for research in enhancing productivity and further application in agriculture fields.

3.4 Fungal Endophytes and Their Function in Agriculture

Fungal endophytes possess significant role in agriculture as it results in increase in the yield of crop, shoot and root biomass, and resistance to various biotic and abiotic (salinity, drought, metal, temperature) stresses. Endophytes can increase the growth and yield of plant by production of secondary active compounds that can help defend the plant against various pathogens. Endophytes play vital role in their colonization in the plant host by production of extracellular enzymes. They act as plant growth promoters by producing phytohormones and will help the plant to survive in contaminated soils (Eid et al. 2019; Rana et al. 2020b). Endophytes are also influencing the population dynamics, functioning of ecosystem and plant community diversity (Arora and Ramawat 2017). These possess significant function for human health due to the production of several bioactive compounds. For agriculture crops growing in natural or stress conditions, endophytic fungus may be used as future biological inoculants/biocontrol agents for sustainable agriculture and environments. Table 3.2 shows the role of various fungal endophytes reported in different field crops.

3.4.1 Fungal Endophytes Conferring Biotic Stress Resistance

Endophytic fungi can grow and survive in healthy crop plants and are able to protect the plant against various pathogens via production of phytoalexins and ecological occupation. Fungal endophytes lead to the upregulation of secondary metabolites such as antioxidant defence enzyme system, phytohormones and phenolic compounds that can protect the plants from various biotic stresses. Various fungal endophytes conferring biotic stress resistance includes *Piriformospora indica*, *Trichoderma* species, *Colletotrichum* species, *Epicoccum nigrum*, entomopathogenic fungi, etc., that are helping in the crop growth and yield. Some of these serving as major fungal endophytes with immense applications in sustainable agriculture are as discussed below.

3.4.1.1 *Piriformospora indica*

A root colonizing filamentous fungus *Piriformospora indica* was isolated from various xerophytes rhizosphere from Thar dessert, India, by Verma et al. (1998). *P. indica* belongs to the Sebacinales order in Basidiomycota, which can colonize both monocot and dicot plants including *Hordeum vulgare* (barley) and model plants such as *Nicotiana tabacum* (tobacco) and *Arabidopsis thaliana* (Johnson et al. 2014). The endophyte *P. indica* can form beneficial symbioses with plants and therefore possess potential function in agriculture, horticulture and floriculture (Oberwinkler et al. 2013; Johnson et al. 2014; Gill et al. 2016). This endophyte is famous for its broad host range and can provide several benefits to host such as improved plant growth under nutrient stress conditions and resistance to the various biotic and abiotic stress conditions. The endophytic fungus *P. indica* can interact with various plant species. It can colonize the root of plants, and the fungal hyphae will enter the roots by root hairs. It leads to the formation of pear-shaped chlamydospores in the root hairs and will further proceed in the rhizodermis cells. The fungus will grow in the root cortex tissue (Oelmüller et al. 2009). The fungus does not

Table 3.2 Role of some fungal endophytes reported in different field crops

Name of crop	Fungal endophytes	Activity in host plant	References
Cotton (*Gossypiumhirsutum*)	*Acremonium alternatum Cladosporium cladosporioides*, *Chaetomium globosum Paecilomyces* sp. *Alternaria tenuissima Epicoccum nigrum*, *Drechslerella dactyloides*, *Exserohilum rostratum*	Antagonists against plant pathogens	Ek-Ramos et al. (2013)
Cotton (*Gossypiumhirsutum*)	*Paecilomyces* spp. *Lecanicillium lecanii*, *Beauveria bassiana*	Entomopathogenic	Sword et al. (2012)
Soybean (*Glycine max*), Tobacco (*Nicotiana. tabacum*), Wheat (*Triticum aestivum*), Corn (*Zea mays*)	*Beauveria bassiana*	Entomopathogenic	Russo et al. (2015)
Rice (*Oryza sativa* L.)	*Fusarium oxysporum, Cladosporium cladosporioides Chaetomium globosum, Penicillium chrysogenum*	Mycoparasitic activities against rot pathogens	Naik et al. (2009)
Maize (*Zea mays* L.)	*Acremonium zeae*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Saccharomyces cerevisiae, Trichoderma koningii, Colletotrichum graminicola*, *Fusarium verticillioides*	Reported but not studied	Orole and Adejumo (2011)
Chili pepper (*Capsicum annuum* L.)	*Penicillium* (in seedling stage), *Fusarium* (in flowering stage) *Colletotrichum, Fusarium*, Alternaria, and *Xylaria* (in fruiting stage)	Antagonistic activity against fungal pathogens (*Phytophthora capsici*, *olletotrichum acutatum*, and *Fusarium oxysporum*) of chili pepper	Paul et al. (2012)
Black pepper (*Piper nigrum)*	*Ceriporia lacerata Annulohypoxylon nitens Daldinia eschscholzii*, *Diaporthe* spp., *Phomopsis* spp., *Fusarium* spp.	Mycoparasitic against rot fungus *Phytophthora capsici*	Sreeja et al. (2016)

(continued)

Table 3.2 (continued)

Name of crop	Fungal endophytes	Activity in host plant	References
Solanaceous vegetables	*Fusarium oxysporum*	Most potent anti-oomycete activity against late blight and several oomycete pathogens	Kim et al. (2007)
Chinese cabbage (*Brassica campestris*)	*Scolecobasidium humicola*	Plant growth promoter in nitrogen enriched soil	Mahmoud and Narisawa (2013)
Amaranth (*Amaranthus* sp.)	*Trichoderma harzianum*	Mycoparasitic activity against leaf blight pathogen *Rhizoctonia solani* Plant growth promotor	Uppala (2007)
Common bean (*Phaseolus vulgaris*)	*Aureobasidium pullulans*	Highest colonization in seedling stage	Parsa et al. (2016)
Sugarcane (*Saccharum officinarum* L.)	*Trichoderma virens*	Antagonistic against pineapple disease pathogen, *Ceratocystis paradoxa*, owing to the production of Endochitinases	Romao-Dumaresq et al. (2012) and Singh et al. (2008)
Sugarcane (*Saccharum officinarum* L.)	*Epicoccum nigrum*	Mycoparasitic against *Fusarium verticillioides*, *Colletotrichum falcatum*, *Ceratocystis paradoxa*,	Fávaro et al. (2012)
Sugarcane (*Saccharum officinarum* L.)	*Aspergillus niger*, *Trichoderma atroviride*	Antagonistic activities	Robl et al. (2013)

invade till the middle part of root beyond the endodermis and the structure of fungal were investigated using acid fuchsine-lactic acid red. Fungal endophytes conferring biotic and abiotic stress resistance is shown in Fig. 3.1.

The endophyte *P. indica* promotes plant growth and seed production, nutrient uptake, increases biomass production and confers resistance to pathogens, and thus has been considered a biocontrol fungus (Lugtenberg et al. 2016; Ali et al. 2018). Root colonizer *P. indica* can control different prevalent diseases in plants such as *Fusarium* and *Verticillium* wilt, black root rot, powdery mildew, yellow leaf mosaic, eyespot, cyst nematode, Rhizoctonia root rot and leaf blight in various crop plants (Arabidopsis, barley, maize, tomato and wheat). The important mechanisms involved in the protection of biological plant disease by *P. indica* include (a) competitiveness amongst each other in terms of habitat and nutrients, (b) antibiotics production and (c) induced resistance and mycoparasitism (Waller et al. 2005). The endophytic fungus *P. indica* confers disease tolerance in crop barley via a separate mechanism involved. The symbiotic crops can survive the necrotrophic root pathogens because of higher levels of antioxidation (glutathione-ascorbate) mechanism (Waller et al. 2005). In barley, *P. indica* can help tolerate the salt stress, increases the grain yield and show resistance against necrotrophic and biotrophic fungus such as *Fusarium culmorum* (root rot) and *Blumeria graminis*. Similarly, valuable effects

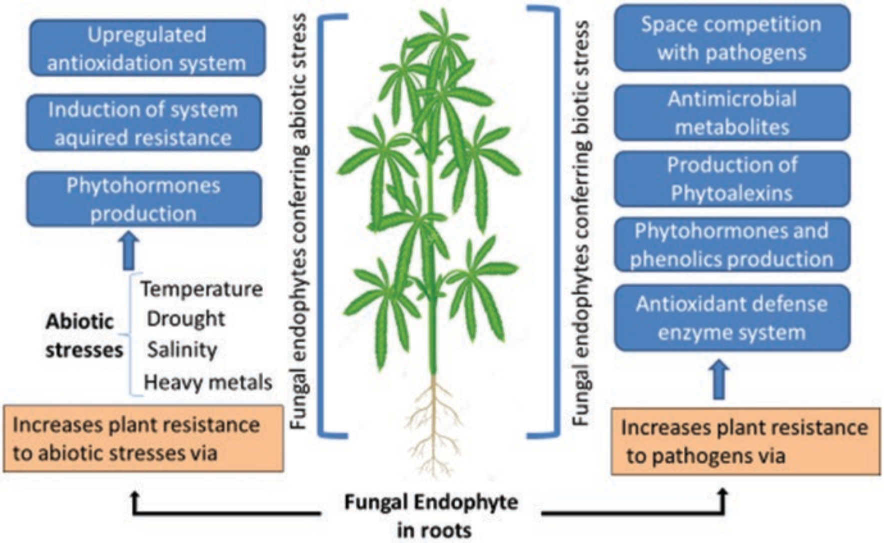

Fig. 3.1 Figure showing fungal endophytes conferring biotic and abiotic stress resistance

are observed in case for other plants such as rice, maize and wheat (Oelmüller et al. 2009). Qiang et al. (2012) demonstrated systemic resistance towards various foliar pathogenic organisms by interaction with endophyte *P. indica*. Tomato plant inoculated with *P. indica* will colonize the roots of plant and results in 20% increase in the leaf biomass and decreases the Verticillium wilt disease (caused by *Verticillium dahliae*) by more than 30% (Fakhro et al. 2010).

3.4.1.2 *Epicoccum nigrum*

Endophyte *Epicoccum nigrum* belongs to phylum Ascomycota and is known to have biocontrol function for various plant pathogenic organisms such as *Sclerotinia sclerotiorum* in sunflower crop (Pieckenstain et al. 2001), *Pythium* in cotton (Hashem and Ali 2004), phytoplasma in apple trees (Musetti et al. 2011) and *Monilinia* spp. in crops such as nectarines and peaches (De Cal et al. 2009; Larena and Melgarejo 2009; Larena et al. 2005; Mari et al. 2007). It also acts as an important sugarcane endophyte, which is known to provide resistance against phytopathogens by secondary metabolite production.

Fávaro et al. (2012) studied the antagonistic property in case of sugarcane endophyte *E. nigrum* strain P16 tested for various phytopathogens. Endophyte *E. nigrum* was found to colonize the sugarcane crop asymptomatically and resulted in higher root system biomass. Endophyte was also found to reduce the growth of plant pathogens (*Ceratocystis paradoxa, Colletotrichum falcatum, Fusarium verticillioides and Xanthomomas albilineans*). The bioactive compounds were found to be produced in the initial and advanced stage of growth of *E. nigrum.* Similarly, biocontrol potential of *E. nigrum* was reported against *Pseudomonas savastanoi pv. savastanoi* (Psv), leading to olive knot and reduced psv growth by Berardo et al. (2018). Chhipa and Deshmukh (2019) observed the biocontrol activity of sugarcane isolate *E. nigrum* against fungal pathogens *Pythium* and *Sclerotinia sclerotiorum* in cotton and sunflower crops. It possesses antibacterial potential against *Phytoplasma* and *Monilinia* species in apple, nectarines and peach. The sugarcane endophyte produces various anti-microbial compounds such as epicorazines A-B, epirodines A-B, flavipin, epicoccines A-D, epipiridones and epicocarines. Out of these, flavipin and epicorazines A-B compounds are mainly linked with biocontrol activity of *E. nigrum* (Brown et al. 1987; Madrigal et al. 1991; Madrigal and Melgarejo 1995).

3.4.1.3 *Trichoderma* sp.

Trichoderma species are well known as saprophytic fungi acting as biocontrol fungi since decades where few isolates are acting as endophytic plant symbionts. These can colonize the roots of plant, twigs and stem and thus induce plant resistance. Antibiosis, mycoparasitism, competition for nutrients and space were the primary biocontrol mechanisms, but recently induced systemic resistance (ISR) was found to be more significant mechanism involved (Singh et al. 2011; Talapatra et al. 2017; Sharma et al. 2019). Endophytic *Trichoderma* strains are not only serving as potential biocontrol agent but can also promote growth of plants and provide resistance towards various abiotic stresses.

Hosseyni-Moghaddam and Soltani (2013) reported the antifungal potential of fungus *Trichoderma* strain (*T. koningii* CSE32) against various pathogenic fungi such as *Spencermartin siaviticola, Diplodia seriata, Phaeobotryon cupressi* and *Pyricularia oryzae*. The results also highlight the antibacterial and antifungal properties, and cytotoxic potential possessed by endophytic *Trichoderma* isolate is also reported by several others (Sivasithamparam and Ghisalberti 1998; Harman et al. 2004). Park et al. (2018) reported the antifungal activity of *T. citrinoviride* which was isolated from mountain cultivated ginseng against pathogenic fungus including *Cylindrocarpon destructans, Botrytis cinerea*, *Pythium* spp., and *Rhizoctonia solani*.

Various secondary metabolites were produced by the endophyte *Trichoderma* sp., and these metabolites serve as the defence activator factor in plants such as tomato, canola and pea (Chhipa and Deshmukh 2019). *Trichoderma* is acting as a bio-controller and a biofertilizer agent, which can replace the harmful agrochemical use in agriculture. Plant growth-promoting activity of *Trichoderma* species tested on the seedlings of crop yerba mate has shown that various isolates of *Trichoderma* (*T. atroviride* LBM 112, *T. stilbohypoxyli* LBM 120 and *T. koningiopsis* LBM 219) increase the crop yerba mate dry weight. The native isolated strains showed improved yield of the crop and antagonistic activity against phytopathogens. Both the isolates *T. atroviride* strain LBM 112 and *T. stilbohypoxyli* strain LBM 120 possess biocontrol activity and PGP potential as they showed activities such as chitinase, endoglucanase, production of siderophores and phosphate solubilization (López et al. 2019).

3.4.2 *Fungal Endophytes Conferring Abiotic Stress Resistance*

At present, changing climate is the biggest problem faced by the entire world. Fungal endophytes support the crops in managing the biotic and abiotic stress factors, thus helps in reducing the effects of changing climate on the cultivation of agricultural crops (Rodriguez et al. 2008). Increasing soil salinity, drought, decrease in availability of water and increasing temperature are some of the growing challenges for growth of crops faced across the globe (Egamberdieva and Lugtenberg 2014; Kumar et al. 2019a, b; Yadav et al. 2015).

Abiotic stresses represent a significant threat to agricultural crops and their productivity. Various medicinal and crop plants are reported to nurture the fungal endophytes, which will provide protection from infectious agents and help the plants adapt under abiotic stress conditions. Fungal endophytes help the plants to adapt under diverse abiotic stress factors such as extreme temperatures, high salinity, heavy metal stress and drought by induced systemic resistance (ISR), bioremediation and biocontrol mechanisms (Yadav 2019; Rastegari et al. 2020a, b).

3.4.2.1 Salinity Stress Tolerance

Soil salinity is adversely affecting the plant metabolism, agriculture production and environmental health. Increase in the concentration of salt ion in soil reduces the uptake of water by roots, which lead to accumulation of toxic salts in the plant cells (Hussain et al. 2018; Yadav et al. 2020a). It is estimated that by the year 2050, 50% of arable land available will be facing the salinity issue (Parande et al. 2013). Fungal endophytes have significant function in managing salinity stress by increasing the antioxidant enzymes and photosynthesis, producing ACC deaminase enzyme and via phytohormone production mainly auxins.

Due to increase in the detoxifying enzyme potential and photosynthetic pigment concentration in barley and rice, endophyte *Piriformospora indica* can reduce the salinity stress (Waller et al. 2005; Jogawat et al. 2013). *Fusarium culmorum* strain FcRed1 isolated from dunegrass provides salt stress tolerance capacity to both monocot and eudicot plants such as rice and tomato, whereas strains isolated from plants in non-coastal areas are not showing any salinity resistance (Rodriguez et al. 2008). Hussain et al. (2018) reported significant improvement in salt stress and the yield in crops such as cucumber, clover, mungbean, maize and tomato when inoculated with AMF (arbuscular mycorrhizal fungi). This significant improvement is due to improved osmoregulation by proline accumulation, reduced NaCl level and phosphate acquisition. Fungal endophytes such as *Penicillium* sp. and *Phoma glomerate* reported to increase the plant biomass and accumulation of potassium, calcium and magnesium in cucumber plants and are also observed to decrease the toxicity due to sodium when the plant is facing salinity and drought stress (Lata et al. 2018). Endophyte *Aspergillus flavus* strain CHS1 showed salt stress tolerance in *Glycine max* via stimulation of antioxidative enzymes and endogenous hormone levels in the host (Lubna et al. 2018). Table 3.3 represents different fungal endophytes conferring abiotic stress tolerance in plants.

Table 3.3 Endophytic fungus conferring abiotic stress resistance in various agriculturally important host plants

Fungal endophyte (species/strain)	Host plant	Abiotic stress	References
Piriformosporaindica	*Hordeum vulgare*	Salinity stress	Baltruschat et al. (2008)
Trichoderma sp.	*Theobroma cacao, Hordeum vulgare*, *Brassica rapa* subsp. Pekinensis	Salinity and Drought stress	Chhipa and Deshmukh (2019)
Curvularia protuberate	*Lycopersicon esculentum*	Temperature stress	Rodriguez et al. (2008)
Paecilomyces formosus LWL1	*Oryza sativa* subsp. Japonica	Temperature stress	Waqas et al. (2015)
Chaetomium globosum and *Penicillium resedanum*	*Capsicum annum*	Drought stress	Khan et al. (2014)
Penicillium brevicompactum	*Hordeum vulgare*	Drought stress	Chhipa and Deshmukh (2019)
Piriformospora indica	*Brassica rapa* subsp. Pekinensis	Drought stress	Sun et al. (2010)
Penicillium roqueforti Thom	Triticum	Heavy metal stress	Ikram et al. (2018)
Exophialapisciphila	*Zea Mays*	Heavy metal stress	Wang et al. (2016)
Acrocalymma vagum	*Nicotiana tabacum*	Heavy metal stress	Jin et al. (2017)

3.4.2.2 Extreme Temperature Heat and Cold Tolerance

Extreme temperature stress leads to extensive denaturation due to damage to cellular proteins, which will ultimately result in cell death, whereas low temperature results in weakened metabolism due to changes in protein structure, inhibition of enzyme reactions and interactions among macromolecules (Andreas et al. 2012). Endophytes increases the adaptation of various crop plants under extreme temperature stress conditions by decreasing the cellular damage, increasing the rate of photosynthesis and accumulating different metabolites such as phenolic compounds, proline and starch. Fungal endophytes conferring biotic and abiotic stress resistance is shown in Fig. 3.1.

Fungal endophyte *Curvularia protuberate* isolated from *Dichanthelium lanuginosum*, a grass species grown in the geothermal soils of Lassen Volcanic and Yellowstone National Parks, can provide tolerance to high soil temperatures in the range of 38–65 °C (Ali et al. 2018). The survival of grass species in such high heat condition is due to its link with the fungal endophyte *C. protuberata* and its mycovirus *Curvularia* thermal tolerance virus (CThTV) as reported by Lata et al. (2018). The *Curvularia* sp. not only provide thermal tolerance to the grasses but also help tolerating high temperatures in case of many other crop plants such as tomato, watermelon and wheat (Chhipa and Deshmukh 2019). Ali et al. (2018) isolated a thermophilic endophytic fungus with high sequence homology (92%) with

Thermomyces species. Fungal endophytic strain CpE isolated from *Cullen plicata* (desert plant) roots provides resistance to heat stress in cucumber. This tolerance in cucumber is due to the build-up of saponins, flavonoids, total sugars, proteins and antioxidant enzymes.

3.4.2.3 Drought Stress Tolerance

Drought stress can suppress growth, development and productivity in plants and the plants need to adapt this stress in order to survive. Various biochemical and physiological responses are induced in plants due to drought stress, which includes closure of stomata, decrease in photosynthetic rate, reduced germination rate, suppression of growth and osmotic stress (Kour et al. 2019a). Endophytes supports plants during drought stress conditions by enhancing the plant metabolism to produce amino acids (polyols and prolines), soluble sugars and plant secondary metabolites such as alkaloids (Jain and Pundir 2017).

Endophytic fungus has been shown to confer drought tolerance and enhances biomass and growth in agricultural crops. Endophytes *Curvularia protuberata* strain Cp4666D and *Fusarium culmorum* strain FcRed1 showed drought tolerance in tomato plant. Both the fungal strains were isolated from geothermal soil and Costal Beach (Rodriguez et al. 2008). Rice, tomato and various grasses such as dune and panic associated with endophytic fungus utilize less quantity of water and showed increased biomass when compared to non-symbiotic counterparts (Lata et al. 2018). The fungal endophyte *P. indica* can decrease the drought-induced decrease in the photosynthetic capacity, chlorophyll and thylakoid protein denaturation (Sun et al. 2010).

3.4.2.4 Heavy Metal Stress Tolerance

Heavy metal toxicity leads to 25–80% of loss of cultivated crop as it is one of the most important abiotic stress factors. Heavy metals are highly toxic to roots of crop plants, and they result in defective root system development. In order to counteract the heavy metal toxicity, plants adopt to various molecular and biochemical mechanisms such as antioxidant enzyme activity, hormonal regulation, transporters and metal chelators. Moreover, plants possess antioxidant defence enzymes such as glutathione reductase, superoxide dismutase, guaiacol peroxidase and catalase, which reduces the heavy metal toxicity, and glutathione and ascorbate (non-enzymatic antioxidants) behave similarly.

Endophyte *Aureobasidium pullulans* strain BSS6 was found to be efficient in enhancing the cucumber plant tolerance to heavy metal stresses by increasing the antioxidant defence enzymes and regulating the soil enzymatic activities (Ali et al. 2019). Significant increase in the enzymes such as catalase, peroxidase and reduced glutathione, and inhibition of lipid peroxidation was observed under heavy metal stress conditions. The endophyte fungus was able to increase the chlorophyll pigment, carotenoid content and improved growth in cucumber plant (Ali et al. 2019). Fungal endophyte *Gaeumannomyces cylindrosporus* was isolated from mine tailings, and colonizing the roots of maize shows tolerance to heavy metal lead. The fungus significantly increases the height, length of roots, seedling biomass and photosynthetic rate under metal stress conditions (Yihui et al. 2017).

3.5 Conclusion and Future Prospects

The indiscriminate use of pesticides and fertilizers adversely affect the agro ecosystem and cause threats to consumers also; second, an increasing population and higher rate of environmental degradation are further points of concern for scientists in various fields. In order to improve soil crop and human health, the application of endophytic fungi as biological agents either alone or as component of IPM packages will be a better option and remedial alternative against chemical farming that has been gaining importance in recent years due to their potential properties in managing abiotic and biotic stresses imposed by surrounding environment and destructive insect pests and/or pathogens, respectively, on host plats by various mechanisms. In addition to that, endophytic fungi have shown great potential in promoting crop growth and productivity. Fungal endophytes have been proven to be a safe and cost-effective option for attaining sustainable farming owing to their ability to produce growth hormones and other essential compounds that help in promoting the growth of host plants. Such microorganisms are of immense importance and attempts would be taken towards their application in sustainable agriculture in the near future. They could, therefore, be biotechnologically manipulated to improve the productivity and sustainability of agricultural yields.

However, there are some challenges in the green revolution, which could be fulfilled by establishing consortia constituting potent strains of fungal endophytes from local regions and other different ecological habitat that could be a biological tool in disease management packages besides crop productivity. Fungal consortia of locally isolated fungal endophytes will be a source for our farming community of particular region, which should be explored and applied in fields in order to minimize the dependency of growers on pesticides under conventional farming leading towards improving soil, crop and human health. Furthermore, investigations are required for searching new endophytic fungi from nature, enhancing fertility of soil in an ecofriendly way. An advanced research is diverted towards detailed study of either community or function of fungal endophytes through using biotechnological tools (omics) is also recommendable. Molecular techniques are also recommended for studying the endomicrobiome. Further studies are required to establish the fungal consortia constituting potent strains of fungal endophytes from local origin that could be applicable in location specific disease management. Further investigations are required for discovery of novel endophytic fungi for enhancing fertility of soil in an ecofriendly way. Molecular techniques are recommended for studying the endomicrobiome. Consortia of fungal endophytes will be proven a source for our farming community, especially indigenous isolates of bioagents grown on agro-waste should be explored and applied, which would further minimize the dependency of growers on pesticides under conventional farming leading towards improving soil, crop and human health.

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Chapter 4
Fungal Diversity and Ecology: Major Challenges for Crop Production in Present Climate Scenario

Arindam Chakraborty, Jatinder Singh, and Hina Upadhyay

Contents

4.1 Introduction

Fungi are vital component of the modern-day ecosystem. They always have an essential position in the emergence and succession of different groups of land plants and animals (Loron et al. 2019). Fungi, one of the major groups of eukaryotes, have species richness between 1.5 and 7.1 million species (Dornburg et al. 2017). Since its recognition in the Linnaean taxonomy, the taxonomical ideas have undergone

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significant amendments, which were considered in 'Regnum Vegetabile' (Linnaeus 1767). Initially, based on morphological and reproductive traits, four major phyla were defined, namely, Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (Whittaker 1969).

Fungal taxonomy depends on the morphology and phenotypic characters and sometimes the characters are unstable so it provides partial systematic information. For this reason, other conserved characters are needed for more consistent fungal classification. Earlier the taxonomy of fungal communities were based on the morphological features only, but several fungi are unculturable so later on molecular techniques have turn out to be broadly used for its taxonomic detection (Tedersoo and Nilsson 2016; Tedersoo et al. 2018). In addition, these molecular techniques have transformed our perceptive about the phylogenetic relationships amongst several fungal species and have significantly changed the old classification system based on morphology (Tedersoo et al. 2018; Wijayawardene et al. 2018). Adhering to this precise identification of fungal species, it may be used for further ecological and functional traits of those taxa (Tedersoo and Smith 2017).

The kingdom fungi consist of nine major phyla that have been established by using molecular data and their mode of sexual spore production. They are Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Glomeromycota, Mucoromycota, Neocallimastigomycota, Opisthosporidia and Zoopagomycota. All these lineages together form a single clade of true fungi that share as a sister clade to the amoeboid protozoans (Tedersoo et al. 2018; Naranjo-Ortiz and Gabaldón 2019). During the last decade, 350 new families have been described within the fungal kingdom (www.stateoftheworldsfungi.org/2018/reports/SOTWFungi_2018_Full_Report.pdf).

4.2 History of Fossil Fungi

The most distinguished naturalist, Charles Darwin, noticed the mutualistic presence of fungi and some plant fossils (Smith 1884). Some years after in 1898, Meschinelli published a compendium on fossil fungi (Meschinelli 1898). Fungi/fungal spores in the sediments were a curiosity in the early days; however, several workers mentioned their full description. They also discussed how these fungi might have affected the performance of hosts and how it functions in the environment. During the end of the nineteenth century, a French palaeobotanist Bernard Renault published a series of papers (Renault and Bertrand 1885; Renault 1894, 1895a, b, 1896, 1903) from the Carboniferous cherts in France. These studies mentioned several microorganisms associated with the plants present in those rocks. These studies show the presence of fungus and its ecological significance. These type of studies was also done from the early Devonian (407 Million years) Rhynie chert (Kidston and Lang 1917, 1920a, b, 1921a, b). They also described various assemblages of fungi and fungus-like organisms. After a long gap, Pirozynski and Malloch (1975) mentioned that fungi played a major role in the evolution of terrestrial plants.

They initially hypothesised the new organism was produced from the mutualistic relationship among fungus and green alga. The studies on the microbial life in the Precambrian Period created an interest to know the microbial evidence in different ecosystems (Taylor 1993).

The history of fungi is still a mystery in the present world. More than 1 billion years ago, fungi diverged from the animal kingdom and made them more closely connected to us rather than plants. Still, there is a large gap in the fossil record that shows the connecting link. The earliest fungal fossil record is always debatable, which provides us with a clue of the life divergence. The oldest record is now traced to about 1010–890 million years. This fungus (*Ourasphaira giraldae*) is preserved in the shales of Arctic Canada (Loron et al. 2019). After this, there was another record published by Bonneville et al. (2020) from shales of Democratic Republic of Congo (715–810 million years ago). Since then in the recent times, it continued to survive with several new groups.

4.3 Fungal Classification Based on Habitat

4.3.1 Aquatic Fungi

The water bodies have several fungal forms. Most of the fungi have their life in water and some may have one stage of their life cycle in water and another dispersed in air. Several other groups have a transitory aquatic life, probably brought by wind or flood water from other areas. Rain plays a major role in the terrestrial fungal spores to be carried into the water. So it is tough to identify the real aquatic fungal spores. The useful characters for determining the real habitat of aquatic fungi, there are number of useful terms have been demarcated later on by various researchers. These fungi are available in different freshwater and marine water masses.

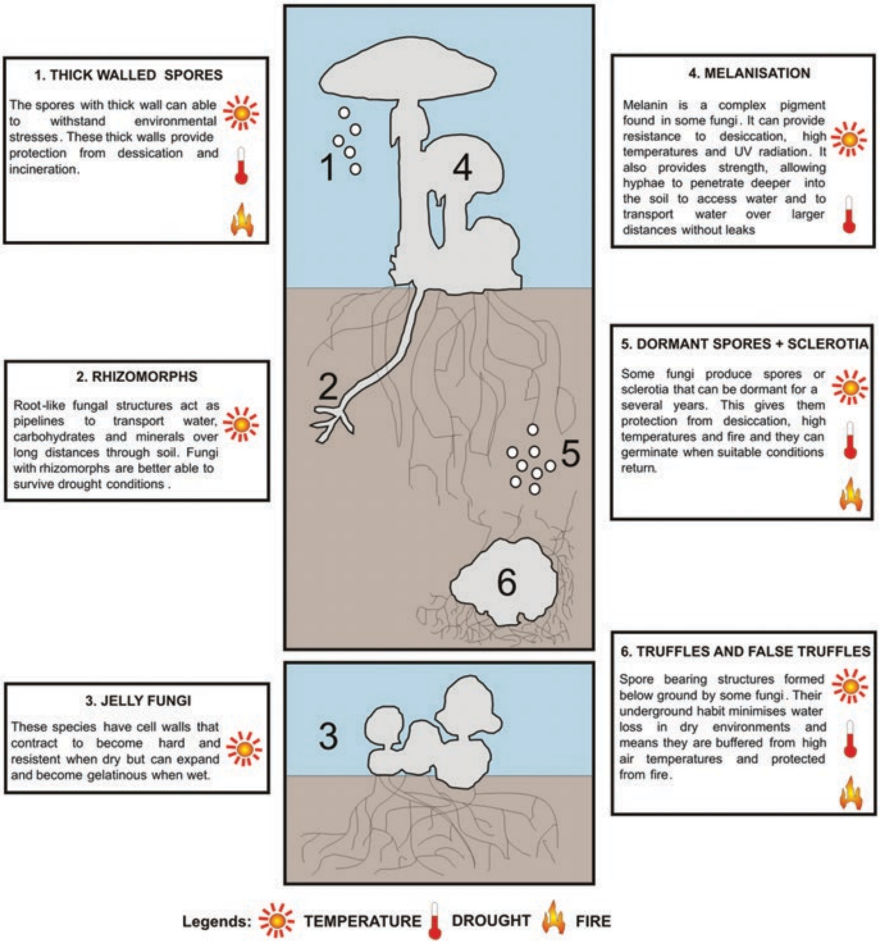
The diverse group of freshwater fungi has a vital role in the aquatic ecological food chain. These organisms break down the allochthonous organic material, namely, plant parts that provide 99% of the total energy back to the surface waters (Ittner et al. 2018 and references therein). So, the microorganisms and aquatic fungi together play an essential part in the freshwater food web. The freshwater fungi are classified into various forms (Goh and Hyde 1996; Shearer et al. 2007). Freshwater fungi mostly belong to Ascomycetes, Basidiomycetes, Chytridiomycetes and Glomeromycetes (Shearer et al. 2007). Later, a classification was proposed based on the functional traits rather than the phylogeny (Wurzbacher et al. 2014; Krauss et al. 2011). They introduced four groups based on the functional characteristics, namely, aquatic hyphomycetes, chytridiomycetes, glomeromycetes and yeasts. Most of these groups are monophyletic. The aquatic hyphomycetes and yeasts both belong to ascomycetes and basidiomycetes (Shearer et al. 2007), so they are differentiated by morphology. In addition to this, oomycetes form a separate group since they are non-fungal according to their morphology (Shearer et al. 2007). This classification is considered reasonable as all the groups are aquatic, and they have fungal-like ecological functions (Wong et al. 1998).

Marine fungi represent only a ~1% of the total known species (Jones 2011; Jones et al. 2015). Jones et al. (2015) mentioned that 1112 species are belonging to 472 genera marine fungi. Amongst these, most of them belong to Ascomycetes (Jones et al. 2015) with 805 species belonging to 352 genera. Most of these reports are from coastal habitats. Ascomycota, Basidiomycota, chytridiomycosis and basal fungal clades are found in the marine waters. They mainly help in the biogeochemical cycles (Jones 2000).

4.3.2 Terrestrial Fungi

Fungi are also present in the terrestrial realm. They are present mainly in the soil and on the land crops/plants. A vast majority of more than 80,000 fungal species that are known likely occur in the soil at some stage of their life cycle (Bridge and Spooner 2001). Fungi successfully live in the soil for its high plasticity and capability to cope up with diverse forms in response to unfavourable environment (Sun et al. 2005). They have several functions in soils such as degradation of dead plant material; propagules present as resting states for further reproduction in favourable condition. Present knowledge of fungal diversity present in the soil is mostly based on the observations of fruiting bodies or the laboratory cultures of fungi isolated from soils (Fig. 4.1). So, there are restrictions for detecting the true species diversity present in the environment. In terrestrial ecosystems, fungi play a significant role in nutrient and carbon cycling as pathogens, mutualists and saprotrophs (McLaughlin and Spatafora 2014). They also play an integral part in the process of nitrogen fixation, production of essential hormones, biological control against pathogens and protection against several natural extremities (Jayne and Quigley 2014; Baum et al. 2015; El-Komy et al. 2015). Several fungal species hold the ability to absorb the toxic metals such as cadmium, copper, mercury, lead and zinc through their fruiting bodies (Baldrian 2003; Kour et al. 2019b; Singh et al. 2020b).

Soil fungi are classified into three functional groups: (1) biological controllers, (2) ecosystem regulators and (3) decomposers (Gardi and Jeffery 2009). Ecosystem regulators help in soil formation and habitat alteration for other organisms by regulating the physiological processes in soil. The biological controllers control several pests, growth of other microorganisms and diseases (Treseder and Lennon 2015; Bagyaraj and Ashwin 2017). Fungi those live in land crops/plants can be advantageous, as well as pathogenic in nature (Devi et al. 2020). Beneficial fungi participate in different biological cycles such as decaying the dead plant materials by converting cells/tissues into nutrients that are later utilised by the plants growing in that area. Some also grow in a symbiotic relationship with the root of higher green plants known as mycorrhizal. Roots of most cultivated plants, e.g. corn, soybeans, cotton, peas, apples, citrus fruits and several others have mycorrhizal relationships with soil fungi. The mycorrhizae are highly beneficial and necessary for optimum growth of



1. THICK WALLED SPORES

The spores with thick wall can able to withstand environmental stresses. These thick walls provide protection from dessication and incineration. ☀ 🌡 🔥

2. RHIZOMORPHS

Root-like fungal structures act as pipelines to transport water, carbohydrates and minerals over long distances through soil. Fungi with rhizomorphs are better able to survive drought conditions . ☀

3. JELLY FUNGI

These species have cell walls that contract to become hard and resistent when dry but can expand and become gelatinous when wet. ☀

4. MELANISATION

Melanin is a complex pigment found in some fungi. It can provide resistance to desiccation, high temperatures and UV radiation. It also provides strength, allowing hyphae to penetrate deeper into the soil to access water and to transport water over larger distances without leaks ☀ 🌡

5. DORMANT SPORES + SCLEROTIA

Some fungi produce spores or sclerotia that can be dormant for a several years. This gives them protection from desiccation, high temperatures and fire and they can germinate when suitable conditions return. ☀ 🌡 🔥

6. TRUFFLES AND FALSE TRUFFLES

Spore bearing structures formed below ground by some fungi. Their underground habit minimises water loss in dry environments and means they are buffered from high air temperatures and protected from fire. ☀ 🌡 🔥

Fig. 4.1 Potential of different fungal structures and characteristics to mitigate some of the major climate change consequences

many plants. *Trichoderma* spp. are active biocontrol agents against the pathogenic fungi. *Arthrobotrys dactyloides* traps and parasitise the plant pathogenic nematodes. Some of the fungal species produce useful antibiotics and enzymes. *Penicillium* spp. produce the famous compound Penicillin-G that prevents from several bacterial infections by inhibiting the cell wall formation. The food processing industries such as bakery, brewery and milk products are based on fungi. In addition to this, some fungi (e.g. mushrooms) are important as daily food.

The pathogenic fungus creates a huge loss for human life and also to the environment. Wheat crops are commonly destroyed by the infectious fungal spores and causes wheat rust diseases (Yellow, leaf and stem rust, Table 4.1). The fungal-like organism *Phytophthora infestans* causes the potato blight disease. The epidemic of

Table 4.1 Effect of climate change in the incidence of fungal disease of common cereal crops

Name of disease	Causal organism	Favourable conditions	Symptoms
Stem rust (primarily affect wheat, but sometimes affect barley and rye)	*Puccinia graminis tritici*	Optimal temperature is above 20 °C, so it is important to associate acceptable air motions with high temperatures	Black elongated pustules containing teliospores formed on stems
Leaf rust (primarily affect wheat but, weakly pathogenic on barley, triticale and some species of goatgrass and wheatgrass)	*Puccinia triticina*	Mild winters and warm springs are likely to become more widespread, making common problem earlier in the season	Orange to brown pustules on the green leaf region are seen in the early-sown crops
Stripe rust (primarily affect wheat and barley)	*Puccinia striiformis* var. *tritici*	Temperature: less than 18 °C (optimum 6–12 °C) with minimum 3 hours of leaf wetness (e.g. dew) for new infections to occur. Once infection is established the fungus can survive short periods of temperatures more than 40 °C	The older leaves present in the lower part of plant have yellow stripes of pustules above the leaf surface
Blackspot (Peas)	*Mycosphaerella pinodes, Phoma medicaginis* var. *pinodella, Phoma koolunga*, *Ascochyta pisi*	During wet weather the disease may spread rapidly	Purplish-black discolouration and streaking of the lower stem Conspicuous spotting of the leaves and pods also occurs Leaf spots are small, irregular and dark-brown in colour Spots on the pods combine to form large, sunken, purplish-black areas Infected seeds may be discoloured and appear purplish-brown
Common Bunt/ Stinking smut (Wheat)	*Tilletia caries, Tilletia laevis*	Wind-blown spores, particularly from late harvested crops, can contaminate neighbouring fields that may present bare soil ready for next crop planting.	The flag leaves of infected plants have yellow streaks and plants with short, dark grey-green ears and slightly gaping glumes may be stunted. Grain discoloration and odour

(continued)

Table 4.1 (continued)

Name of disease	Causal organism	Favourable conditions	Symptoms
Covered smut (Barley)	*Ustilago hordei*	Infection is seed borne.	Kernels are replaced by dark-brown smut spores. Smutted heads are hard and compact.
Loose smut (Barley) (Wheat)	*Ustilago nuda* *Ustilago tritici*	Seed borne disease: needs to infect the seed in the previous growing season so that it be a pathogen of the plant in the current growing season. During flowering weather conditions influence the amount of time the florets stay open and hence the time the plant becomes vulnerable to infection. Wind and moderate rain, as well as cool temperatures (16–22 °C) are ideal for the dispersal of spores	Smutted grain heads that contain masses of black or brown spores
Powdery mildew (Wheat) (Soybeans) (Onions)	Different species of fungi in the order Erysiphales mainly *Podosphaera xanthii* *Blumeria graministritici* *Microsphaera diffusa* *Oidiopsis taurica*	High humidity, temperatures range from 5 °C to 30 °C while 15 °C is optimal with relative humidity above 95%. Sucking insects transmits the disease	White powdery spots on leaves and stems. The lower leaves are the most affected
Yellow rust/stripe rust (Wheat)	*Puccinia striiformis tritici*	Optimal temperatures: 10–15 °C and high relative humidity required for spore germination and its growth that are dispersed by air	Yellow-coloured stripes are produced parallel along the leaf venations

powdery mildew occurs in wheat, soybeans, onions etc. by different fungi of Erysiphales (Table 4.1). *Plasmopara viticola* affects grape vineyards and causes great loss. In addition to these, fungi also produce highly toxic and carcinogenic chemicals affect humans in the present and the past. For example, *Aspergillus* spp. can grow on the corns and toxins are filled in the seeds. When these corns are taken by the humans, it attacks the liver.

4.3.3 Human Pathogenic Fungi

Human health is impacted by several microorganisms, among which the pathogenic fungi are also one. The skin and nail infections are the most common fungal infections that are caused by dermatophytes (*Epidermophyton* sp., *Microsporum* sp. and *Trichophyton* sp.). These represent one of the most common forms of human disease that affects 20–25% of the entire world's population (Havlickova et al. 2008). Certain fungal species (*Aspergillus*, *Candida*, *Cryptococcus* and *Pneumocystis*) can also cause several invasive diseases in humans, which kills ~1.5 million people/year (Brown et al. 2012). These fungal infections can be acquired environmentally by *Aspergillus* sp. and *Cryptococcus* sp. or endogenously by *Candida* sp. *Aspergillus fumigatus* is a species complex composed of ten species found in the decaying vegetation when the temperature becomes 50–55 °C. These species are thermophilic, so they are adapted to these high temperatures. In human beings, it affects the lungs when the spores are inhaled. This disease may be very contagious.

In contrast, *Candida albicans* is a dimorphic fungus that is present in the mouth, digestive tract and vagina of perfectly healthy humans. These species cause severe infections on skin, nails, mouth, bronchial tubes and lungs. Chromoblastomycosis is mostly tropical to subtropical diseases that are caused by several fungal species mainly inhabiting the soil or on rotting vegetation. They enter the human body through the foot by wounds from bare-foot walking. *Coccidioides immitis* which causes Coccidioidomycosis (Valley Fever) is endemic to the southwest region of the United States, northern Mexico, and some areas of central and South America. Infections occur following travel to one of these areas. The disease occurs by the inhalation of spores, that causes respiratory disease in animals and people. Later on, this may spread from lungs to other parts of the body by bloodstream and create pathologic changes, e.g. skin lesions. Sometimes this disease may proceed to a complicated stage and remains inactive for several years that may reappear later.

4.4 Major Challenges in Crop Production in Present Climate Scenario

Agriculture is strongly influenced by weather and climate. The farmers often deal with the weather and its year-to-year variability. They usually adapt to the local environment in the form of established infrastructure, local farming practice and individual experiences. So, climate change may, therefore, impact on crop production and the organised aspects of farming systems (Fig. 4.2).

In the tropical region, the most challenging part is to adapt the modes of production to the climatic variability with trying to ease the risks and the vulnerability of production systems. It is essential to reduce the negative impacts of agriculture on the environment, or else we would continue to increase the likelihood of adverse

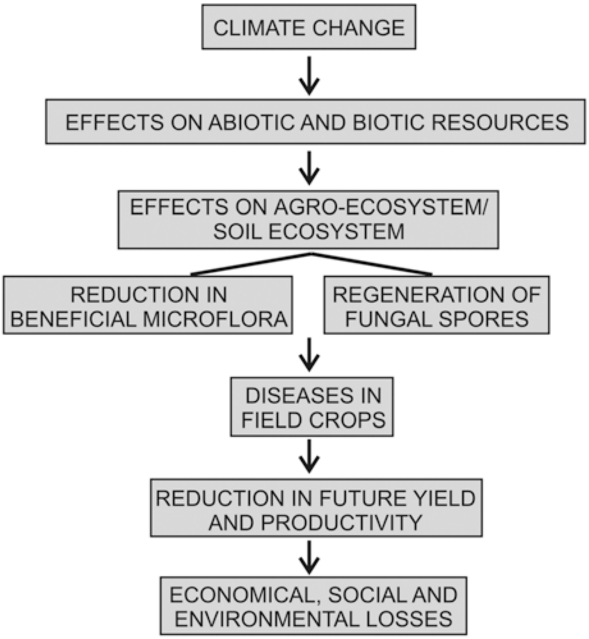


Fig. 4.2 Effect of climate change on agro-ecosystem and economic loss of future yield

events (cyclone/storms, droughts, heavy rainfall, etc.). For promoting efficient and sustainable agricultural production, the national policies must be revised accordingly. It is also essential to identify the risk factors and develop suitable indicators to assess the sustainability of farming systems. This development of risk indicators will allow understanding the impact of threats, but a well-organised communication system must also accompany this with significant scope. This process can minimise the loss of producers from adverse events. For this reason, it is vital to strengthen the agricultural service institutions that will implement new conservation techniques, diversification of production activities and technological adoption.

One of the major limitations for agricultural practices in the tropical region is the fluctuations of watering for plant production and the limitations of soil quality suitable for plant types. Majority of the tropical soils, especially in humid areas, are suffering from acid soil infertility and low organic matter content. The ability to capture and exchange water for lands is mostly dependent on organic matter. This soil organic matter content can not only boost productivity and crop quality; however, it can also counteract the growth of atmospheric greenhouse gases. This tropical environment is suitable for several pests, weeds and diseases. Global warming may increase the insect populations that decrease the yields of staple crops such as wheat, corn and soybeans (Fig. 4.2). The warmer temperature causes faster growth rates for plants and increases the metabolic rate and number of breeding cycles for the insects. This can be checked by several measures with prior detection of the exact cause. The pesticides and synthetic inputs are majorly used in agricultural practices. These uses must be done in a systematic control where the farmer must have prior knowledge because if they are not used correctly, there may be resistance to the active ingredients or chemical groups that are in use.

These climatic changes could affect agriculture in several ways:

1. The productivity of crops is affected in terms of quantity and also its quality.
2. The agricultural practices are changed by changes in water use in the irrigation and the use of herbicides, insecticides and fertilisers.
3. The environmental effects cause more intensity of soil drainage (leading to nitrogen leaching) and soil erosion that indirectly reduces the crop diversity.
4. The rural space is decreasing with rapid urbanisation, by which the cultivated lands are decreasing.
5. Adaptation by organisms can become more or less competitive where humans have developed more competitive organisms such as flood-resistant and salt-resistant rice varieties.

Only technological research alone is not sufficient for addressing future issues related to climate change. Strong policies are necessary for carrying out sustainable agriculture. Some of the major causes of low productivity are as follows:

(i) The overpopulation caused by climate change may also dwindle natural resource. This may cause poor and precisionless agricultural practices.
(ii) During the worse hit climatic times, the inconsistent and poor implementation of pro-climate policies creates a problem for different decisions.
(iii) Widespread corruption in the system and poor leadership of the society.
(iv) Reduced funding for research and technology development, as well as poor commercialisation of the important products to the local community.

4.5 Climate Change Impacts and Its Effect on Plant Diseases

Environmental conditions play a significant effect on disease incidence on field crops and stored grains that range from sporulation in pathogens and its growth with virulence gene expression in stressed situations. There are numerous direct and indirect effects on the health of the crops after interactions with global change drivers. Under this climate change, there must be more diversity of management strategies by participatory approaches to interdisciplinary sciences (Pautasso et al. 2012). They also reported that further research is required on climate change effect on the crop production in different parts of tropical and subtropical regions. The research must also hover on multiple factors of climate change and its close relationship with endophytes, viruses and mycorrhiza using the long-term and large-scale data sets. Similarly, the negative impact of climate change is expected while the vulnerability of biotic agents increases, that is seen between plants and animals (Lonsdale and

Gibbs 2002). So, there is little doubt that in future, the disturbance patterns may increase and more frequent problems may occur (Marçais and Wargo 2000).

The production and dispersal of microbial inoculums in crop fields are well known to be critical for disease epidemics. Amongst them, fungi are also prone to several diseases. Fungi spread through the air by its spores and fasten on the leaves of the plant body. Some of them even live in the soil that may move into the plants through roots. Some fungal species are most damaging plant pathogens including *Phytophthora* sp. that are the causative agents of potato late blight disease (Fig. 4.3).

Fig. 4.3 Potential effects of global climate change on fungal diseases and insect-pests in field crops and crop production

4.5.1 Changes in Mean Climate

Locally, the agricultural and farming practices are influenced by long-term mean climate change. The local farming communities have appropriate experience to produce a particular group of crops that are productive under that local environment. If the mean climate changes from the current scenario, then adjustments to the current practices are required to maintain productivity. The higher seasonal temperatures considerably impact the productivity, incomes from it and food security (Battisti and Naylor 2009). Different groups of crops show different sensitivities to warming. If mean seasonal temperature increases, then the harvest time of many crops lessens that further reduces the final crop yield. In the tropical and arid regions, where the atmospheric temperatures are close to the physiological maxima for crops, higher temperatures are more immediately damaging by increasing heat stress and water loss by evaporation. The temperature of 28 °C in the mid-latitudes increases wheat production by nearly 10%; however, at low latitudes the similar amount of warming may decrease the yields by the same amount (Gornall et al. 2010). These warming are more in the high latitudes; however, little increase in temperature in the lower latitudes may have a more considerable impact (Gornall et al. 2010), since agriculture in higher latitudes is less. The changes in precipitation patterns have considerable impact on agriculture, as water plays an important need. Around 80% of total agriculture is rain-fed, thus the changes in rainfall pattern will influence the crop production (Olesen and Bindi 2002; Tubiello et al. 2002; Reilly et al. 2003).

4.5.2 Climate Variability and Extreme Weather Events

4.5.2.1 Temperature

Increases in temperature associated with climate change across the globe have created a problem for crop production by having diseases (Beal Cohen et al. 2020). The increment of climate variability is also affecting their total yields. The extremely high temperatures in the summer with the prolonged heatwave in the tropical Indian subcontinent and even in the higher latitudes have contributed widespread trouble in world cereal markets (Battisti and Naylor 2009). The short-term temperature extremes may also cause a problem if they coincide with the key stages of crop development (Fig. 4.3). If the temperature rises more than 32 °C for a few days at the flowering stage of the crops, then it drastically reduces the yield (Wheeler et al. 2000).

4.5.2.2 Drought

Demand for water to society has always been an important need. Rainfall is the primary source for agricultural practices. Most of the tropical countries such as India and other parts of Southeast Asia's economy depend on the pattern of monsoonal rainfall. If there is no normal rainfall in the country, there is a drought condition which affects the growth of crops (Kour et al. 2019a). Since the soil water level also goes gradually downwards, providing water to the croplands is hard for the farmers. In this case, there is a huge loss for the farmers. Sometimes there is a deficiency in the soil moisture that increases water stress condition in plants.

Charcoal rot disease, also known as dry weather wilt, is caused by fungus *Macrophomina phaseolina* and affects corn, sorghum, soybean, sunflowers and dry beans. This fungal species has a widespread distribution and broad host range. Hardened fungal survival bodies known as Microsclerotia are formed inside the infected crop tissues. The microsclerotium survives for years in dry soil but only a few weeks in the wet soils.

This disease can be managed by reducing the plant density that reduces the stress, use of resistant varieties and hybrids, and crop rotation to non-host plants (e.g. wheat).

Aspergillus ear rot is another plant disease in the drought-prone and non-irrigated fields. This disease affects mainly the cornfields. *Aspergillus flavus* and *A. parasiticus* cause this disease, and it produces a chemical compound 'aflatoxin' that can be toxic for humans and animals that consume the contaminated grains. The pathogen *Fusarium* exists in soil and crop residues for a longer time. Plant stress due to drought is one of the foremost factors that increase the severity of diseases by this microbe. They are associated with several important diseases of corn, wheat and soybean that cause significant loss. Under drought conditions, Fusarium stalk rot, Fusarium ear rot and kernel rot diseases of corn are produced by *Fusarium verticillioides*, whereas the wheat root diseases caused by *Bipolaris sorokiniana* and Fusarium crown rot disease caused by *Fusarium* spp. are more severe under high atmospheric temperature. The root infections by these fungal species can cause severe reduction in crop yield in the dry soils. In the soybean plants, drought reduces the sudden death syndrome (SDS), but on the other side, it favours the Fusarium infections such as Fusarium wilt. These *Fusarium* diseases can be reduced by minimising stress and injury through herbicides, foliar diseases, damages caused by drought or hails, optimising the fertility level of the soils, minimising soil compaction, using fungicide-treated seed while sowing, crop rotation.

The root and crown rot diseases in wheat caused by several different fungi are generally overlooked due to invisible symptoms on the outer surface of the crop. The fungus *Bipolaris sorokiniana* causes the common root rot disease that is characterised by dark brown to black necrotic lesions on the roots, internodes and stem. In dry areas, dryland foot rot disease develops by a dark brown lesion on the entire stem.

These diseases of wheat can be managed by following ways:

1. The fields must be ploughed regularly to reduce stress due to inadequate moisture.
2. Excessive nitrogen fertilisation must be avoided in dry conditions as it promotes vegetative growth, which depletes the available water present in the soil. This water stress for the plants causes several diseases.
3. Rotation of crops such as planting non-cereal crop may reduce fungal diseases.
4. Pathogen-free and fungicide-treated seeds must be sown.
5. Drought-tolerant varieties of crops may also reduce these diseases.

4.5.2.3 Heavy Rainfall/Flooding

Crop production is also impacted by too much water in the soil. Heavy rainfall events that lead to flooding wipes out the cultivable field and the excess water affects water logging in agricultural fields, anaerobic condition and reduced plant growth (Singh et al. 2020a). The humidity also increases during the rainy season, which provides a suitable condition for microbial activities (Fig. 4.3). Moisture conditions increase the spread of weeds and weed biomass, which is a menace for crop production. The soil-borne microbial diseases and pests such as certain mycotoxins, e.g. aflatoxin, patulin, and ochratoxin increase with the increase in moisture content. The temperature fluctuation and changing climate are directly influenced by the worsening of crop and its annual produce. When the water content in the atmosphere increases, it helps the fungal species to regenerate from the spores present in the agricultural soils, which further affects the crops growing in it. While there is excessive rainfall, the atmospheric moisture increases, that led to foliar fungal diseases in wheat.

For managing these types of condition, the agricultural field must be designed in such a way that water drainage is well planned. The water present in the field must be equally distributed and excess water drains off to the unused land or nearby canals/rivers.

4.5.2.4 Tropical Storms

These storms are often termed as a cyclone in the tropical region. It is a low-pressure system over the tropical and sub-tropical waters with controlled convection and specific surface wind cyclonic circulation (Holland 1993). The societal and economic implications of these tropical storms can be high in the countries that have high population density in tropical and subtropical regions. In India, we are more prone to this type that resulted in increased farming in the coastal areas, which is at risk from flooding. The seawater inundation in the coastal region is another problem in coastal irrigation. This may be managed by producing salt-resistant plants such as salt-resistant paddy varieties. These high-speed winds can spread the fungal spores over thousands of kilometres for diseases in wheat such as the stem rust disease caused by pathogen *Puccinia graminis* (Velásquez et al. 2018).

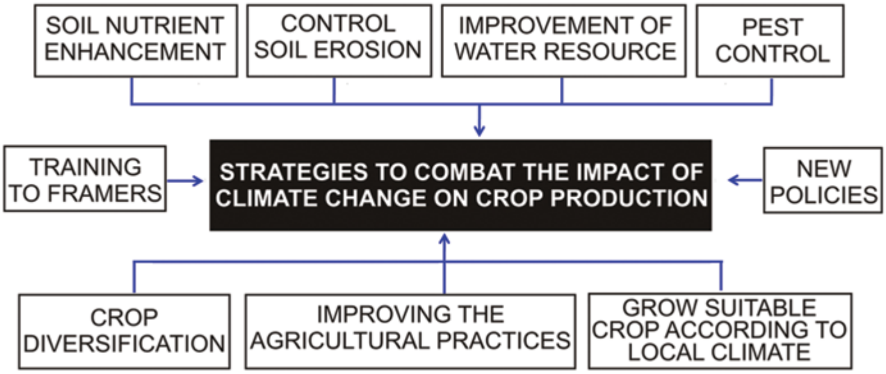


Fig. 4.4 Strategies for sustainable disease management in the agricultural field

We know that many studies are focussed on the negative impacts of tropical cyclones in agriculture. However, sometimes it can also bring benefits by bringing a large portion of the annual rain in some arid regions in the tropics (Fig. 4.4).

4.6 How to Control the Common Plant Fungal Diseases

The common plant fungal diseases can be controlled by the following ways:

1. The plant varieties must be chosen for production with their known disease resistance and also grow the plants in suitable areas where it will get its requirements from the environment. If the matches are poor in this case, the plants come to stress and several diseases occur, which dispose of the plant.
2. Irrigation must be done wisely. Overhead watering to the plants may disturb the powdery mildew spores; however, it encourages several waters spread pathogens. Water must be given to the plants close to the ground so that leaves are not wet, and it must be carried out early in the morning so that the excess moisture may dry by evening.
3. The air circulation and light penetration in and around plants must be increased through trimming and proper spacing of the plants. Thinning the plants or rearranging the surroundings may also help in this case.
4. Prune the infected parts of the plant and dispose of the debris. Always the healthy tissues must be cut back so that no disease is there.
5. The instruments by which these trimming are done must be sterilised by wiping with common household disinfectant as these instruments may spread diseases from one plant to the other.

4.7 Future Prospects of Global Agriculture and Its Solution

In this regard, we have to manage the whole agro-ecosystem, with the ability to map the vulnerable areas. By addressing these sensitive areas, we have to choose fields where high-quality yield may be achieved. This can be achieved by using sensors and applying precision that will make this workable and economical in wide areas. Sensors may provide the data of soil pH, soil electrical conductivity (EC), organic matter and others. Using drones in this situation can only locate inputs where they are required. Due to global warming, frequent occurrence of the drought has become more intense and is supposed to wreak havoc in Africa, southern Europe, Middle East, most of America, Australia and Southeast Asia. Their impacts are felt more likely due to the increase in water demand, population growth, urban expansion and environmental protection in many cases. Moreover, drought results in crop loss and also loss of pasture.

Reviews done in the past reveal that climate change is indeed a challenge, and it needs immediate attention as it is backed up by several problems such as agriculture, forestry, landscape management and nature conservation. For these reasons, it is essential to understand the interconnections among climate change and other devices of global change that is affecting the plant health. Efficient land planning, control of the irrigation, integrated nutrient management, weed management and pest management play an important role when a farmer is well trained to adapt due to climatic conditions and can make an alternate effort to manage the agro-ecosystem sustainably. Pest is a persistent problem for farmers and its suppression is a valuable service to the environment. Future annual crop production and food security for the whole world will be achieved effectively by maintaining a healthy, perennial plant cover and mulching, increasing soil health through organic cultivation.

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Chapter 5
Phosphate-Solubilizing Fungi: Current Perspective and Future Need for Agricultural Sustainability

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5.1 Introduction

It is anticipated that world population will reach 9.7 billion by 2050, wherein Southeast Asia is the main contributor (Forbes 2017). The current dynamics of demographics suggest that the population in India will even surpass China by 2022. With our limited and shrinking agricultural land resources, the impetus is largely on the development of innovative and sustainable ways of transforming agriculture to feed the ever-increasing population. Currently, around 58% of the rural households depend upon agriculture as their principal source of livelihood agriculture is one of the largest contributors to GDP (gross domestic product) (IBEF 2017). According to Central Statistics Organization (CSO), the share of agriculture and allied sectors is expected to be 17% of the gross value added (GVA) in 2016–17 at 2011/12 prices. Green revolution ushered the successful implementation of Industrial Agriculture fueled by large-scale use of synthetic agro-chemicals and chemical fertilizers. The unavailability arising from modern agriculture is due to increase in cost of cultivation and rising food prices, both of which have to be extensively compensated by government which makes the economics of its perusal extremely costly and inefficient.

Phosphorus (P) is an essential macro-nutrient for plant growth and development. In spite of having an ample presence in the soil, its bioavailability is very low (Kour et al. 2020b). Mostly it is present in the form of the insoluble complexes and only 0.1% of the total P is reported to be present in the soluble form (Farhat et al. 2009; Tomer et al. 2016). Unfortunately, it is among the least mobile and most unavailable soil nutrient for the plants. Its solubility is reported to depend on several factors, namely, organic matter, pH, active sesquioxides, lime and nature and content of clay (Kour et al. 2019a). Soil pH is the important determinative factor and pH 6.7 is considered ideal for the same (Mehrvarz et al. 2008; Selvi et al. 2011). In tropics, it is observed to present as the inorganic compounds, i.e., iron–aluminum compounds (under acidic condition) and calcium compounds (under neutral to alkaline conditions) (Mehrvarz et al. 2008; Selvi et al. 2011). During summer and rainy seasons in the tropical countries including India, pH was found to go up to 10.5 units, salt level up to 2% temperature between 35 and 45 °C, which largely affects the mobility of the nutrients in the soil (Nautiyal 2000). Further, the major portion of the chemical fertilizers (75–90%) when applied to the agricultural fields get transformed into an insoluble oxide/silicate forms by reacting with Al^{3+}, Ca^{++}, Zn^{++}, Fe^{3+}, Co^{++}, etc.

(Selvi et al. 2011). This conversion decreases the efficiency of the fertilizers and ultimately increases the input cost for the agriculture. In this scenario, PSM provides a sustainable alternative to supplement the P to the crops. Application of PSM has shown up to 40% reduction in the need of chemical fertilizers when applied alone (Tomer et al. 2017) (Rajwar et al. 2018). This ability of the microorganisms has opened the new doors toward the exploration of microbial technologies in the agricultural sector.

India possesses a remarkable potential for the development of organic farming practices due to its agro-climatic conditions (Charyulu and Biswas 2010; Giri et al. 2015). Although India is one of the largest producers for agricultural commodities of the world, the productivity index in comparison to world benchmarks is extremely low. Shrinking agricultural land sizes are one of its major causes as the average plot size in India has fallen from 2.7 hectares in 1970 to under 1.2 hectares today (Economist 2015). Also, due to lack of proper education and awareness, there have been indiscriminate practices of chemical fertilizers and pesticides across the Indian Subcontinent thereby creating huge loss of natural soil productivity. It has been reported that excessive application of agro-chemicals leads to loss of soil fertility due to increase of salt content and thereby impacting on consumer's health (Swapna 2013). Based on numerous studies conducted, it is imperative that a transformation of large-scale conventional agriculture is required which in turn will need modification of biotic and abiotic factors in order to fulfill the agricultural demand of the future.

Replacement of chemical phosphatic fertilizers with PSM is the need of the hour to propagate organic input-based agriculture for improvement of overall human and environmental health. PSMs are the microbial inoculants or biological active products with formulations containing one or more beneficial strains of fungi or bacteria in an easy to apply and efficient carrier material which either add, conserve, or mobilize phosphate in soil (Mazid and Khan 2015; Dash et al. 2019). PSM-based biofertilizers are easy to use, non-toxic, and cost-effective (Kour et al. 2020c). They either manufacture the nutrients required by crops from soil or atmosphere or mobilize the nutrients pre-existing in soil media in forms most absorbable by crops. They have also been reported to act as biocontrol agents by conducting antagonistic activities against phytopathogenic bacteria. An example of one such activity is interference in the bacterial quorum sensing system. However, the primary function of PSM is reportedly for plant growth enhancement from which it excises more than one mechanism (Fig. 5.1) (Rani et al. 2013; Suyal et al. 2014a).

PSMs can solubilize the insoluble P complex into the bioavailable form through chelation, ion-exchange reactions, and acidification (Fig. 5.2). Several microbial groups including bacteria (*Pseudomonas, Thiobacillus, Azotobacter, Erwinia, Serratia, Agrobacterium, Arthrobacter, Bacillus, Enterobacter, Flavobacterium, Bradyrhizobium, Salmonella, Micrococcus, Alcaligens, Streptomyces, Chromobacterium,* etc.), cynabacteria (*Calothrix braunii, Westiellopsis prolific, Anabaena variabilis,* etc.), and fungi (*Aspergillus, Penicillium, Arthrobotrys, Trichoderma,* etc.) are known to solubilize the rock phosphates.

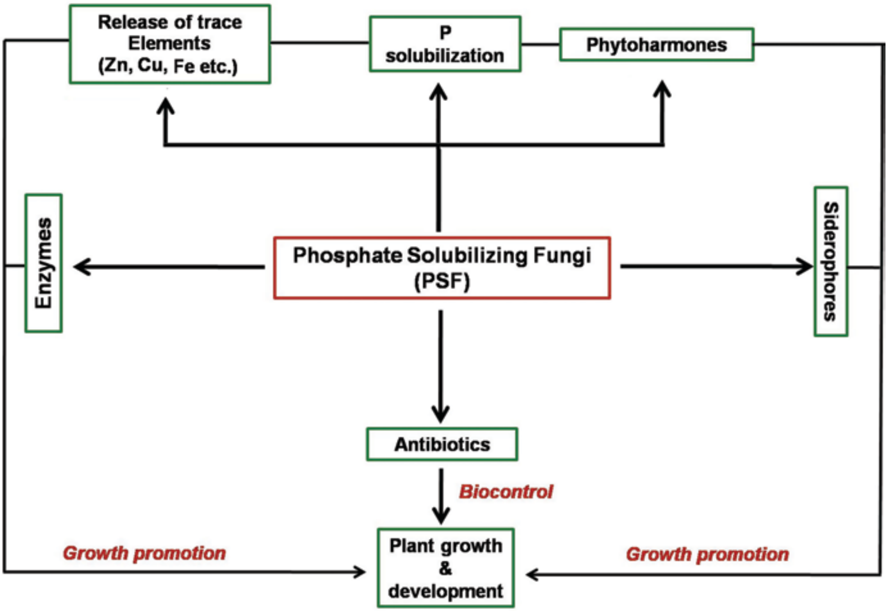

Fig. 5.1 Role of phosphate-solubilizing fungi in plant growth development

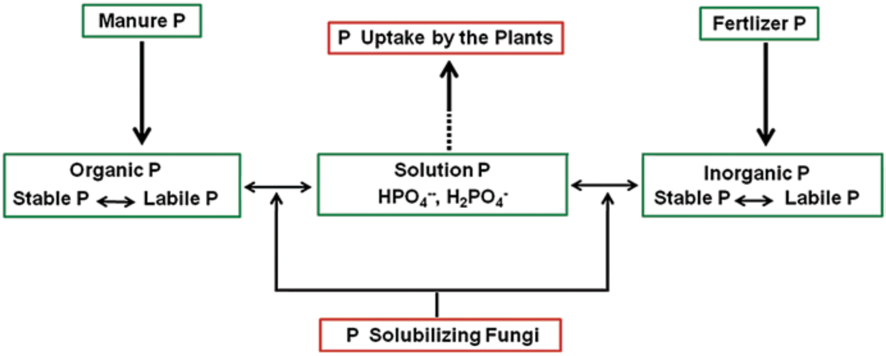

Fig. 5.2 Depiction of P-solubilization mechanism of the fungi

Among the fungi, *Aspergillus* and *Penicillium* are the most predominant genera which have shown their potential for P solubilization. Yu et al. (2005) have reported the P solubilization by *Penicillium oxalicum* and *Aspergillus niger* in liquid culture. Recently, Kalayu (2019) has reviewed several PSF, namely, *Aspergillus tubingensis, A. sydawi; A. ochraceus; A. versicolor, Penicillium bilaii, P. citrinum, P. digitatum; P. lilacinium; P. balaji; P. funicolosum, P. oxalicum, P. simplicissimum; P. rubrum, Arthrobotrys oligospora, Trichoderma viride*, *Rhizopus, Fusarium*, and *Sclerotium*.

5.2 Rhizospheric P-Solubilizing Fungi

Soil is the natural media that support vegetation by providing nutrients and other essential elements for growth. The thin area of soil surrounding the roots of the plants is known as rhizosphere. It is directly affected by root exudates and hence is rich in soil-related microorganisms. Root exudates are the compounds secreted by roots in its immediate proximity. The nature of microbial community in the rhizosphere is directly affected by the constituents of these root exudates. Exudates are majorly constituted by ions, sugars, aromatic and aliphatic acids, volatile aromatic compounds, vitamins, peptides, proteins, enzymes, plant hormones, alcohols, ketones, olefins, and urea. Root exudates contribute to 40% of the net fixed carbon by plant photosynthesis containing almost 200 different types of compounds. Root exudates perform ecological interactions with the soil microbial community by releasing signaling molecules, attractants, and stimulants. Moreover, they can be used by plants in their defense against various pathogens (Baetz and Martinoia 2014; Kobae 2019; Kour et al. 2019b).

The nature of the root exudates varies from one place to another because of the impact of various biotic and abiotic factors. The change in the nature of these exudates also changes the microflora of rhizosphere. Besides it, they also help the plant to compete with surrounding plants and promote plant–microbe symbiotic interactions (Yadav et al. 2017; Rajwar et al. 2018; Yadav et al. 2019; Rai et al. 2020). The microbes use these root exudates as a substrate and also contribute some of the metabolites that are absorbed by the plant to fulfill its nutritional requirements. Among the rhizospheric PSF, mycorrhizae are the most important groups of the microorganisms (Remy et al. 1994; Ezawa and Saito 2018). They are also known as fungal roots. Mycorrhizae are well efficient in the nutrient absorption from the soil, especially P (Harrison and van Buuren 1995; Harrison et al. 2002; Fonseca and Berbara 2008; Hart et al. 2017). They may be ectomycorrhiza (*Leccinum, Hebeloma, Lactarius, Suillus*, etc.) or endomycorrhizae (*Rhizophagus irregularis, Acaulospora, Gigaspora, Glomus, Entrophospora,* etc.) (Jansa et al. 2008; Kikuchi et al. 2016; Kobae 2019).

5.3 Mechanism of P-Solubilization

PSM employs the following three mechanisms (McGill and Cole 1981) to solubilize P: (a) by releasing compounds such as hydroxyl ions, protons, siderophores, organic acids, and CO_2 that assist the breakdown and solubilization of complex molecules; (b) biochemical mineralization by the discharge of extracellular enzymes; and (c) by releasing phosphorous during substrate degradation.

Further, on the basis of the nature of the substrate, the P-solubilization mechanisms can be explained as follows:

5.3.1 Organic

Three groups of enzymes are involved in the release of organic phosphorous from soil (Kaur et al. 2017).

5.3.1.1 Nonspecific Acid Phosphatases (NSAPs)

These are dephosphorylated phosphoester or phosphoanhydride compounds of organic matter. Phosphomonoesterases (phosphatases) are the most common among them. Acid phosphatases have been found to be present in several fungi, such as *Aspergillus, Penicillium, Fusarium,* and *Neurospora* (Shahab et al. 2009). These phosphatases were produced in media containing an inorganic nitrogen source [$NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3] and a very low concentration of inorganic phosphate (Pi). The fungal strain *Humicola lutea* 120-5 utilizes the phosphoprotein casein through biosynthesis of extracellular enzymes: acid proteinases (Aleksieva and Mutafov 1997; Aleksieva and Peeva 2000) and acid phosphatases (Micheva-Viteva et al. 2000). In some cases, mineralization of natural phosphorus and phosphate solubilization can exist together. Inoculation either only with phosphate solubilizer or with other potential rhizospheric organisms has been very much achieved (Ahemed and Kibret 2014).

5.3.1.2 Phytases

These are the enzymes having an ability to release at least one phosphate group from the phytic acid, a fixed organic form of P (Suyal and Tewari 2013a, 2013b; Kour et al. 2020a). The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC–IUB) distinguish two classes of phytate degrading enzymes, 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.28), initiating the dephosphorylation at the 3 and 6 positions of phytate, respectively (Guilan et al. 2009); completely hydrolyzing to inositol and inositol monophosphate.

Microbial phytase activity was most frequently detected in fungi. Mostly phytase producers are filamentous fungi, especially from the genus *Aspergillus*, *Penicillium,* and *Mucor*. Phytase from *A. niger* group is considered most active. Over 200 fungal isolates belonging to the genus *Aspergillus*, *Mucor, Penicillium*, and *Rhizopus, Trichoderma* have been tested for phytase production (Soni et al. 2010; Rawat et al. 2009). The phytase and its applications have recently been well reviewed by Sharma et al. (2020).

Several strains of yeasts, the eukaryotic fungi, contain biologically valuable proteins (40–60%), vitamin B-complex, important trace minerals, and several unique "plus" factors, such as ability to enhance P bio-availability (Sharma et al. 2020). It is reported that among yeasts, extracellular phytases are produced by *Schwanniomyces castellii* (Segueilha et al. 1992), *Arxula adeninivorans* (Sano et al. 1999), and

S. cerevisiae (Veide and Andlid 2006). Intracellular phytase occurs in several yeasts such as *Saccharomyces cereviseae* (Man-Jin et al. 2008; Iefuji et al. 2009) and *Cryptococcus laurentii* (Pavlova et al. 2008). Baker's yeast *S. cerevisiae* is generally recognized as safe (GRAS, defined by U.S. Food and Drug Administration) for food production, and has been widely used for the production of food-grade phytase (Veide and Andlid 2006; Yasoda et al. 2007).

5.3.2 Inorganic

The following are two main theories in this aspect:

(i) Acid production theory
(ii) Proton and enzyme theory

As per acid production theory, PSMs produce organic acids such as oxalic, fumaric, glyoxalic, malic, citric, gluconic, succinic, alpha-ketobutyric, 2-ketogluconic, and tartaric acid which lower the pH (Puente et al. 2004; Rodrigues et al. 2004). Its amount and type vary from fungus to fungus. Lowering of pH of the filtrates of PSMs is because of these organic acids (Rani et al. 2013). Fasim et al. (2002) observed the role of microbes in the solubilization of zinc oxide and phosphate through gluconic acid and 2-ketogluconic acid production (Table 5.1).

Proton and enzyme theory states that a group of enzymes such as esterase are responsible for the phosphorous solubilization from compounds containing organic phosphate. According to this theory, phosphorous solubilization, besides the

Table 5.1 Organic acids produced by P-solubilizing fungi

Fungi	Acids
Aspergillus candidus, A. flavus, A. niger, A. terreus, A. wentii, Fusarium oxysporum, Penicillium sp., *Trichoderma isridae, Ttrichoderma sp.*	Lactic, maleic, malic, acetic, tartaric, citric, fumaric, gluconic
A. flavus, A. candidus, A. fumigatus	Glutaric, oxalic, tartaric
Penicillium oxalicum	Malic, gluconic, oxalic
Aspergillus flavus, P. canescens	Oxalic, citric, gluconic, succinic
Penicillium rugulosum	Citric, gluconic
A. niger	Succinic, citric, oxalic, gluconic
Penicicllium variabile, Penicillium rugulosum, Penicillium radicum	Gluconic
A. awamori, A. foetidus, A. terricola, A. amstelodemi, A. Tamari	Oxalic, citric
A. japonicus, A. foetidus	Oxalic, citric, gluconic, succinic, tartaric
P. simplicissimum, P. bilaji	Citric, oxalic
A. awamori, P. digitatum	Succinic, citric, tartaric
Chaetomium nigricolor	2-Ketogluconic

generation of acid, involves release of protons in association with ammonium assimilation (Shahab et al. 2009). Other than these two systems, phytohormones such as indole acetic acid, cytokinin, and gibberellin also aid phosphate solubilization. Formation of chelating agents such as H_2S, CO_2, mineral acids, and siderophores also has indirect effect on phosphate solubilization (Shahab et al. 2009).

5.4 Genetics of P-Solubilizing Microbes

Generation of organic acids is likely to be involved in mineral phosphate solubilization in all the PSM including fungi. The genes mandatory for these acid productions were anticipated to affect this feature. A few genes involved in acid phosphatase have been represented (Rossolini et al. 1998). For example, the *acp*A gene communicates an acid phosphatase showing ideal activity at pH 6, with extensive substrate specificity (Reilly et al. 2006). Furthermore, broad-spectrum acid phosphatases containing class A gene *pho*C and class B gene *nap*A, separated from *Morganella morganii*, are extremely encouraging. Besides this, a little is known about the mechanism involved in the biochemical systems required for the union of the GDH-PQQ halo enzyme and the region for the variation in some microorganisms among constitutive and inducible phenotypes.

The conceivable inducers that show promising halo enzyme activity are glucose, gluconate, mannitol, and glycerol. Gluconic acid is synthesized by a mechanism involving direct oxidation of glucose through two key proteins, namely, membrane-bound quinoprotein and glucose dehydrogenase (GDH) (Kim et al. 1997; Patel et al. 2008). GDH requires pyrroloquinoline quinone (PQQ) as a cofactor, which is the product of a *pqq* operon comprised of six genes (*pqq*A, B, C, D, E, and F) in *Klebsiella pneumonia, Enterobacter intermedium* 60-2G, and *Rahnella aquatilis* (Kim et al. 1998, 2003). PQQ is essential for the formation of holoenzyme which leads to the production of gluconic acid from glucose. Han et al. (2008) have shown that the absence of 2-ketogluconic acid, due to inactivation of *pqq* genes in *Enterobacter intermedium* 60-2G, leads to insolubility of hydroxyl-apatite. PCR studies were conducted in *S. marcescens* CTM 50650 strain (Farhat et al. 2009) to check the presence of genes involved in the expression of MPS via activation of the direct oxidation pathway of glucose (GDH encode by *gdh* and *pqq* genes involved in the biosynthesis of the required PQQ cofactor). Rodrıguez et al. (2000), Rajwar et al. (2018), and Joshi et al. (2019) have reported *pqq* genes in the diazotrophs. A gene of phosphatase enzyme in *Burkholderia cepacia* is known to encode an outer membrane protein which increases the P transport within a cell (Rodrıguez et al. 2000). Two nonspecific periplasmic acid phosphatase genes (*nap*D and *nap*E) from *Sinorhizobium meliloti* were also cloned (Deng et al. 2001).

A MPS gene (*gab*Y) was isolated from *Pseudomonas cepacia* and its expression was studied in *E. coli* HB 101. Babu-Khan et al. (1995) have identified 396 *gab*Y ORFs of *P. cepacia* and evaluated their expression in *E. coli* K-12. They have found that this strain had synthesized apo-GDH but PQQ. Furthermore, JM109 (pSLY4) and JM109 (pGAB1) were found to synthesize 10-fold more gluconic acid in the presence of 1 mM PQQ. In another study, a genetic construct using pKT230 and pMCG898 was prepared by Rodriguez et al. (2000), encoding PQQ synthase gene (responsible for MPS) from *Erwinia herbicola* and was transferred to *Pseudomonas* sp. and *Burkholderia cepacia* IS-16. The positive recombinant clones were able to produce higher insoluble phosphate in comparison to their respective wild type strains. A 7 kb fragment from *Rhanella aquatilis* was cloned by Kim et al. (1997) and transferred to *E. coli* strains so that hydroxyapatite-solubilization ability can be conferred and hence induce the production of gluconic acid. Presence of two open frames ORF1 and ORF2 and a partial ORF were revealed in nucleotide analysis. Among them, ORF2 encodes a protein of 44 kDa which has remarkable sequence resemblance to *pqq*E of *Klebsiella pneumonia, E. herbicola* and *Acinetobacter calcoaceticus* and were revealed in nucleotide analysis. Further, a 10 kDa protein was found to encode by ORF1 which has shown a strong sequence resemblance to the *pqq*D of *A. calcoaceticus* and *K. pneumoniae. E. coli* can produce GDH, without PQQ, and thus, does not produce GA.

The cloned 1.8 kb locus encodes a protein that shows striking resemblance to the gene III product of a *pqq* synthesis gene complex from *Acinetobacter calcoaceticus,* and to *pqq*E of *K. pneumoniae* (Liu et al. 1992). It has been observed that DNA fragment from *E. herbicola* worked as PQQ synthase gene. Further, few *E. coli* strains may possess cryptic PQQ which were supposed to complement by this ORF. These observations have revealed that although acid production is an essential way of P solubilization, it cannot be considered the only way to perform that. Numerous genes are reported which are responsible for solubilization of the insoluble phosphate. A *pcc* (phosphoenolpyruvate carboxylase) gene from *Synechococcus* was found to involve in P solubilization. To release Pi from the organic complexes, microorganisms have developed a specific system which possesses the alkaline and acid phosphatases. The genetic regulation of these enzymes has been studied. Under P limiting conditions, several genes are observed to induced and initiate the *pho* regulation, namely, *pho*A (for alkaline phosphatase), *pho*B (a positive regulator or an activator), *pho*T, *pst*S, and *pst*B, etc. They all constitute *pho* box (Torriani and Ludtke 1985; Makino et al. 1989; Ezawa and Saito 2018). PhoR protein regulates the Pho regulation both negatively and positively with excess and limited phosphate, respectively. Pho M is another protein showing inhibitory effect on the product of PhoR, into an inactive form, PhoM. In presence of Pi, PhoU exhibits a negative control.

The Pst-Pho U region constitutes an operon with a transcription attenuator between Pho S and Pho T (Wanner 1987). As organic acid production is among the key mechanisms of P solubilization, it is assumed that any change in structure/function of the respective genes will affect this property. In this scenario, genes of P uptake have been studied thoroughly in several PSM. It has been observed that *Sinorhizobium meliloti* possess at least two P transport systems – high- and low-affinity transport systems. The high-affinity system is observed to encode by the *pho* CDET operon, whereas low-affinity system is known to encode by orfA-pit operon. These genes are regulated by PhoB activator. In case of P-sufficient conditions, *Pho*B becomes inactive and thus *pho*CDET genes are not expressed. Under P deficiency, *Pho*B becomes activated and thus *pit* permease system (low-affinity system) is suppressed while *pho* CDET system gets activated and predominantly acts as P transport (Bianco and Defez 2010). *pst*SCAB homologs have been found in some microorganisms that are known to serve as high-affinity P transporters (Behera et al. 2014). Recently, Ezawa and Saito (2018) have reviewed the genetics of P solubilization by arbuscular mycorrhizal fungi. The group has reported an SPX domain in the proteins which are involved in Pi homeostasis in eukaryotes.

5.5 Applications of Genetic Engineering for Potential Bioinoculants Development

PSF performance mainly depends on its potential to colonize under a certain habitat. Plate counting and most-probable-number techniques have been used for the study of fungal communities in the rhizosphere. It is considered that less than 1% of the microorganisms in the environment can generally be cultured by standard culture techniques. Spatial heterogeneity and culturing inability are the major limitations for identification of the fungus (Kirk et al. 2004, Mummey et al. 2006). Spatial heterogeneity occurs due to the temporal and spatial variability during the sampling. Moreover, improper sampling and handling may also affect the results. On the other side, culturing inability arises due to the lack of the suitable growth media. Therefore, microbial habitats, their interactions, and growth requirements need to be studied properly to overcome this problem.

Molecular biology techniques are extensively used for characterizing microbial community structures in different environments. Cloning and sequencing techniques are commonly used techniques to determine microbial community structure. Besides them, hybridization and probing techniques can also determine the same with the advantage that they are less time-consuming, however require a sufficient knowledge of the community to select the appropriate target sequence. Some other techniques such as ribosomal intergenic spacer analysis (RISA) and amplified ribosomal DNA restriction analysis (ARDRA) can be used to study PGF colonization or community structure. ARDRA and RISA have been used in the analysis of mixed bacterial populations from different environments. ARDRA can be used for taking an overview of genotypic changes occurred in the community over time. However, RISA provides a method of microbial community analysis for comparing differing environments or treatment effects without any kind of biasness imposed by culture-dependent approaches. In brief, RISA involves PCR amplification of an intergenic spacer region (ISR). These molecular techniques have greater quantitative efficiency and can be further extended to characterize PGF under in situ conditions.

Knowledge of the fungal genes governing the production of organic acids would make it possible to transfer the phosphate-solubilizing ability to various other microorganisms that are competent of colonizing a particular rhizosphere. As clear from the earlier discussions, rhizosphere competence is a most important factor that determines the fate of success or failure of microbial inoculant. The rhizosphere has various amounts of carbon sources that can be utilized by the heterogeneous microbial communities in soil to produce various types of organic acids. Oxidative metabolism of glucose by glucose dehydrogenase (GDH) produces gluconic acid; glucose dehydrogenase (GDH) requires pyrroloquinoline quinine (PQQ) cofactor. Therefore, genes involved in the transport/biosynthesis of PQQ can be cloned from various microbes and transferred to the other (Bruto et al. 2014). If the genes involved in PQQ biosynthesis are transferred to *Trichoderma* sp. that possess apo-GDH and that is rhizosphere competent too, the resulting *Trichoderma* strains will show both phosphate-solubilizing activity as well as biocontrol activity. Similarly, Ambrose et al. (2015) have successfully characterized salicylate hydroxylase gene from the fungal endophyte *Epichloë festucae*.

5.6 Available Approaches and Methodologies to Study P-Solubilizing Microbes

For identification and characterization of the rhizospheric fungi, two different approaches can be explored, namely, culture-dependent and -independent (Soni et al. 2016; Suyal et al. 2019a, 2019b). Culture-dependent approaches involve the culturing of the fungi in the lab followed by their morphological characterization, carbon source utilization pattern, plasmid fingerprinting, FAME (fatty acid methyl esters) analysis, PLFA (phospholipid fatty acid analysis), DNA microarray, MLST (Multilocus sequence typing), mass spectrometry, etc. Unfortunately, all the fungi are not culturable and therefore, need another approach known as culture-independent approach. It involves metagenomics and other genetic fingerprinting techniques which provide a profile of the whole community and do not rely on the culturing of the fungi. These methods are rapid, accurate, and easy to perform. Furthermore, these methods involve the isolation of DNA directly from the soil samples followed by its restriction digestion, cloning, and metagenomic library construction (Goel et al. 2017). These shotgun clones can further be subjected to activity screening. The culture-independent approach also involves the in situ identification of microorganisms by FISH (fluorescent in situ hybridization) and PCR based identification by using different phylogenetic markers.

5.6.1 Morphological Characterization

Several morphological characteristics are useful in the fungal identification and characterization, namely, hyphal structure, mycelial growth, pigmentation, spores, etc. Morphological characterization is really rapid, easy, and does not need any sophisticated instrument. But it has several drawbacks too because the morphological expressions are dependent upon environmental factors (Li et al. 2009).

5.6.2 Biochemical Characterization

5.6.2.1 Carbon Source Utilization Patterns

The evaluation of carbon source utilization efficiency of the microorganisms is one of the oldest methods used for their identification and characterization. This technique is considered fast, reproducible, and cost-effective. The Biolog identification system is a commonly used microbial identification method based on their ability to oxidize a panel of 95 different carbon sources (Morgan et al. 2009). Thus, metabolic profile of the microorganisms is prepared and compared. The major disadvantage of this method is its biasness for cultivable microbial communities. Nevertheless, results may also vary according to the growth conditions of the microorganisms and inoculum density. Frąc et al. (2016) have developed a fast, accurate, and effective Microplate Method (Biolog MT2) for the detection of *Fusarium*.

5.6.2.2 FAME and PLFA Analysis

For many years, microbial lipids have been routinely used for their own identification. The two most common methods which are being used for this purpose are FAME and PLFA. FAME is rapid but indiscriminate while, PLFA is precise but time-consuming. These methods involve the analysis of the microbial fatty acids and identify them on the basis of signature molecules. Signature fatty acids are known to make a relatively constant proportion within a cell and can be differentiated among major taxonomic groups of the microorganisms (Frostegard and Baath 1996; Siles et al. 2018). Therefore, any variation in the fatty acid profile of the microorganisms represents the change in the microbial community structure. This technique is precise, high-throughput, and cost-effective with higher resolution capacity (Nelsona et al. 2010). The limitation of this technique is that cellular fatty acid composition depends on growth conditions, media, and temperature used to grow the organism, thus, may lead to misinterpretation.

5.6.3 Molecular Characterization

5.6.3.1 PCR-Based Methods

Random Amplification of Polymorphic DNA (RAPD)

It is a PCR-based fingerprinting technique. RAPD markers are the DNA fragments produced from the random amplification of the genomic DNA using single primer of arbitrary nucleotide sequence. After purifying the genomic DNA, PCR amplification can be done by using randomly designed primers (Clerc et al. 1998). By selecting the primers and amplification conditions judiciously, all such pairs of sequences represented in the genome result in a set of fragments that is characteristic of the species or strain from which the DNA was prepared. These fragments are resolved by gel electrophoresis. The band pattern generated in the analysis represents genome characterization of a respective microbial strain. Recently, Hassan et al. (2019) have used this technique for rapid identification of the *Trichoderma* sp.

Amplified Fragment Length Polymorphism (AFLP)

It is a variation of RAPD technique, and able to detect polymorphic restriction sites without prior sequence knowledge using PCR amplification. Restriction enzyme (RE)-digested genomic DNA can be used as a template for PCR amplification. The primers contain the recognition sites of the RE as well as additional "arbitrary" nucleotides that extend beyond the restriction sites (Blears et al. 1998; Bertani et al. 2019). The fixed portion gives the primer stability and the random portion allows it to detect many loci. The amplified fragments are separated and visualized on denaturing polyacrylamide gels. This multiple-locus fingerprinting technique is highly sensitive and robust and has been evaluated for genotypic characterization of the fungi (Kathuria et al. 2015). Furthermore, it has higher reproducibility, resolution, and sensitivity compared to other techniques.

Repetitive Sequence-Based PCR (Rep-PCR)

Microbial genomes possess several low-copy-number repeated sequences, namely, rRNA operons, tRNA genes, insertion elements, etc. These sequences contribute to the evolution of the genome and function through DNA rearrangements. It also helps in creating the genetic fingerprints. Therefore, Rep-PCR fingerprinting is considered a well-established technique for microbial diversity analysis and identification (Shin et al. 2012; Masanto et al. 2019). This method is based on PCR-mediated amplification of DNA fragments located between specific interspersed repeated sequences in microbial genomes. It has high resolution, but results may vary due to the PCR biasness.

Multiple Locus Variable Number Tandem Repeat Analysis (MLVA)

It is a molecular technique which explores the natural variation in the number of tandem repeats found in the multiple loci of the microorganism. This method is extensively used for molecular typing of the microorganisms (Johansson et al. 2006). In this technique, variable number tandem repeats (VNTR) loci are subjected to PCR amplification followed by amplicon sequencing. The amplicon size is used to assess the number of repeated units in each locus (Singh et al. 2019). Thus, total numbers of repeats of the VNTR loci are combined and used to prepare the MLVA profile, which can be compared for the fungal identification. This technique has high resolution and accuracy but often imperfect repeats containing mutations are encountered which affect the reproducibility of the results.

Multilocus Sequence Typing (MLST)

This technique identifies the microorganisms by analyzing the internal fragments of house-keeping genes present in multiple loci (Maiden et al. 1998; Gaiarsa et al. 2019). These fragments are then sequenced and compared. Fragments that differ are designated as separate alleles and thus the relatedness of the microorganisms is displayed in terms of their phylogenetic relationships.

Single-Strand Conformation Polymorphism (SSCP)

It is a conformational difference in single-stranded nucleotide sequences of identical length (Schwieger and Tebbe 1998). These nucleotide sequences with different confirmation can be separated by the gel electrophoresis technique. Moreover, the gel patterns thus observed can be used for fungal identification and characterization. The change in single nucleotide in the amplified region is sufficient to produce the distinct PCR-SSCP patterns. This technique is rapid and convenient for mutational analysis and allelic variance (Martynov et al. 2019). Problem in reproducibility is the major limitation for this SSCP technique.

Denaturing Gradient Gel Electrophoresis (DGGE) and Temporal Gradient Gel Electrophoresis (TGGE)

These two techniques are the well-known techniques for the microbial ecology analysis and involve both PCR as well as polyacrylamide gel electrophoresis (Rajwar et al. 2018; Rawat et al. 2019). The metagenomic DNA is amplified using GC clamp containing primers and allowed to separate on a polyacrylamide gel. The denaturation of the amplicons is achieved by urea and formamide in DGGE, while temperature in case of TGGE. The amplicons get denatured and separated on the basis of their nucleotide sequences. Thus, a profile can be generated which can be compared further for assessing the microbial diversity within a respective sample (Kumar et al. 2014). These techniques are fast and labor-intensive. However, primer selection, electrophoresis conditions, and PCR reactions require optimization to achieve reproducibility.

5.6.3.2 Restriction Enzyme-Based Methods

Pulsed-Field Gel Electrophoresis (PFGE)

This technique can be used for the separation of DNA fragments under the influence of an electric field by changing their directions periodically on the gel matrix. It is a powerful genetic fingerprinting technique to construct a genome amp of the microorganisms (Basim and Basim 2001; Kwon et al. 2019). Microbial DNA can be restricted digested using RE and allowed to separate through gel electrophoresis. However, the direction of the electric field is changed continuously to get a discrete band pattern. These patterns are then compared and matched with the available databases for the identification of the microorganisms.

Restriction Fragment Length Polymorphism (RFLP) Analysis

RFLP is a genetic fingerprinting technique that explores variations in homologous DNA molecules. It involves the restriction digestion of DNA followed by gel electrophoresis (Osborn et al. 2000; Florek et al. 2019). Digested fragments are then transferred from the gel matrix to the nitrocellulose membrane. The predesigned probes are then subjected to the hybridization with the membrane-bound DNA fragments. RFLP is considered very sensitive for microbial identification. However, incomplete restriction digestion of the DNA molecules may change the results.

Ribotyping

It involves the identification of microorganisms based on the restriction digestion of rRNA coding genes. In case of bacteria 16S rRNA genes are used for this purpose (Suyal et al. 2015a, b, 2019b), while intergenic transcribed spacer (ITS) regions are frequently used for ribotyping of the fungi (Suyal et al. 2013a). Furthermore, 18S rRNA genes can also be used for fungal identification (Goes et al. 2012). This technique is among the most powerful genetic fingerprinting techniques and is being used extensively worldwide. It is highly accurate and reproducible along with a high level of resolution.

Plasmid Fingerprinting with Restriction Enzymes

Plasmid is an extrachromosomal, covalently closed, double-stranded circular DNA molecule. Besides the bacteria, these are well known in the members of several fungal genera, namely, *Absidia, Agaricus, Alternaria, Claviceps, Epichloe, Erisyphe, Fusarium, Saccharomyces*, etc. Plasmids can be isolated, restricted digested, and allowed to separate on gel electrophoresis to get a unique pattern (Owen 1989; Qin et al. 2019). These patterns are then compared for interpreting the fungal relatedness. It is a rapid, popular, easy, and cost-effective technique. However, plasmid instability is a major drawback for this genetic fingerprinting technique.

5.6.3.3 Hybridization-Based Methods

Fluorescent In Situ Hybridization (FISH)

This technique is extensively used for microbial identification under culture-dependent as well culture-independent approaches. It can detect the complementary DNA sequence within a chromosome with the help of a fluorescence tag (Amann et al. 2001; Witchley et al. 2019). For this purpose, fluorescent probes are designed which are actually the complementary sequence of the desired DNA fragment. The probe: DNA binding is detected by the fluorescent microscopy technique. This technique is highly reproducible with good resolution ability. Cellular permeability, sensitivity, target site specificity, and accessibility are the major concerns with this technique.

DNA Microarray

A DNA microarray can be defined as the collection of small DNA spots on a solid surface. It can be identified as a DNA chip, biochip, or gene chip (Wang et al. 2002). Initially, this technique was used for expressional analysis of the genes; however, nowadays, it is frequently used for microbial identification and characterization. In microarray, randomly fragmented microbial genomes are allowed to hybridize to with the microbial genome spotted on a solid surface (DNA chip) (Ye et al. 2001; Nilsson et al. 2019). Resulting hybridization profiles are then analyzed and compared. This genetic fingerprinting has shown high reproducibility, accuracy, and resolution. However, it is considered laborious to perform.

5.6.3.4 Protein-Based Characterization

Serotyping

Serotypes are the microbial strains with distinct immune cells and antigenicity. Thus, the identification and characterization of the microorganisms based on their serotypes is known as serotyping. Cell surface antigens are the major determinative factor for the serotyping. This approach involves western blotting, immunoprecipitation, ELISA, and other immunological techniques to generate the serotyping profiles which are then compared to get an idea about the genetic relatedness among the microorganisms (Li et al. 2006; Akins and Jian 2019).

Mass Spectrometry (MS)

It offers high-throughput, robust, and sensitive way of microbial identification and characterization. Fungal proteins can be extracted, purified, and allowed to mass spectrometric analysis for their detailed characterization (Demirev and Fenselau 2008; Welker 2011). In the field of microbial proteomics, MS can be used for both gel-based as well as gel-less approaches. In recent years, two-dimensional-gel electrophoresis (2D-GE) coupled with MS having ionization with matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) have shown its potential under gel-based proteomic approach (Soni et al. 2015; Suyal et al. 2014b, 2017). However, under gel-less approach, liquid chromatography analysis coupled with MS (LC-MS) is in great demand (Suyal et al. 2018, 2020). Both the approaches produce a profile of the microbial proteins which is then used to compare and characterize the respective strains.

5.6.3.5 Enrichments Methods

Bromodeoxyuridine (BrdU) Method

This method can be used to identify a metabolically active population within a niche (Sebastián and Gasol 2019). In this technique, BrdU (a labeled nucleotide) is added to the system and microbes are allowed to grow (Yin et al. 2000). Metabolically active individuals will incorporate BrdU into their nucleic acid and thus identified by using a label. This strategy is widely used in the bioremediation, especially for the isolation of the fungi which can use xenobiotics, heavy metals, and other compounds.

Stable Isotope Probing (SIP)

It is also an enrichment method in which ^{13}C-labeled substrate is provided to the microorganisms. Metabolically active microorganisms incorporate ^{13}C in their DNA and thus, make it denser than normal DNA. Density gradient centrifugation can be used to separate both the DNA which can be analyzed further with the help of the specific primers (Achouak and Haichar 2019). Therefore, SIP offers broad opportunity to study microbial communities and can be expanded further to stable isotopes of nitrogen and/or phosphorus (Buckley et al. 2007).

5.7 Conclusion and Future Prospects

To meet the ever-increasing demand of food due to population pressure, green revolution came into existence. It, however, brought remarkable gain in food production but with unnoticed concerns for sustainability due to disproportionate use of chemical fertilizers. Moreover, future reliability on chemical fertilizers will persist to cause loss in soil fertility, pollution, and a lot of saddle on the fiscal system. Therefore, biofertilizers are being promoted alone or with combination with fertilizers. This integrated approach is vital to improve crop productivity and to maintain soil fertility.

PGFs not only exhibit plant growth promotion but they are also effective in bioremediation by detoxifying detrimental pollutants such as pesticides and heavy metal pollutants. Nevertheless, they are potential biopesticides, as they can control a wide variety of phytopathogens. In the case of controlled soil conditions, remarkable enhancement in yields of different crop plants has been reported through PGF applications. But soil is an unpredictable natural ecosystem. Efficacy of PGF in crop yield may vary under laboratory, greenhouse, and field trials, and therefore, the desired results are sometimes not achieved. Besides it, climatic variations influence the effectiveness of PGF. However, their performance can be optimized through acclimatization according to the prevailing natural soil environment. In the current scenario, where there is global reluctance toward genetically modified food crops, PGF-based farming practices might be an excellent alternative. This is a technology which is easy to access even to the farmers of developing nations including India. Thus, this trend of least possible input of chemicals in sustainable agricultural systems may help to achieve the food reliance for an ever-growing population. Further research in this perspective will widen the horizon of our knowledge and enable us to understand microbial responses to the diverse environments.

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Chapter 6
Fe-Chelating Compounds Producing Fungal Communities and Their Applications

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Contents

6.1 Introduction

Fungi can produce many compounds and among the compounds are siderophores (chelating of Fe), the Greek "siderophores," which means "iron carriers or carriers," the semolecules are natural products that currently have a broad biotechnology (Renshaw et al. 2002), these compounds possess the ability to bind metal ions with a high affinity, additionally a complex formation of these natural chelators with mostions and metalloids, thus linking a potential development of biotechnological products forth area of ecology and agriculture (Kramer et al. 2020; Winkelman 2007). Siderophores are low molecular weight molecules that are in a range of 500

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to 1500 Da, and are synthesized by different microorganisms such as bacteria (Kour et al. 2020; Santoyo et al. 2009), particularly Gram-negative and fungi, for example, *Verticillium dahlia* (Barash et al. 1988), *Candida* sp., *Aspergillus fumigatus* (Hass 2014), *Mucor* sp., *Fusarium roseum*, *Ustilago sphaerogena* (1998a, 1998b; An et al. 1997; Ardon et al. 1997), *F. oxysporum*, *F. oxysporum* f. sp. *cubense* (FocTR4), and *F. fujikuroi* (Al-Ani 2017b), *Trichoderma* sp. (Anke et al. 1992; Al-Ani 2017b), edible mushroom such as Pleurotus (Castañeda-Ramírez et al. 2020; 38. Comans-Pérez et al. 2021), and monocotyledonous and grass-type plants (phytosiderophore), presenting functional groups that coordinate Fe ions with high affinity and specificity.

The main structural characteristics that determine the selectivity of a siderophore by the ferric ion are the type, number (Denticity), and spatial distribution of the metal-binding groups, approximately a number of 500 siderophores have been identified, the diversity of the group functionalities that coordinate the ferric ion is limited due to the need for donor groups, for example, oxoanions, to coordinate the most oxidized form of iron. In this way, most siderophores can be grouped as follows: hydroxamates, catecholates, α-hydroxycarboxylic acids, and mixed siderophores.The semolecules are generated in response to the low of availability of iron in the environment, and its relationship for this elemental low sits uptake from compounds that are present in the environment and from protein from host organisms such as transferrin or ferritin. On the other hand, the synthesis of the semolecules increases when the microorganisms are in conditions of iron shortage, the high affinity of the semolecules for iron facilitates the uptake of this metal from compounds such as ferric hydroxide, and proteins from the host organism such as transferrin or ferritin (Crowley et al. 1987, 1991).

The complexes that siderophores form with iron in the soil are efficiently assimilated both by the microorganism that produces them and by other microorganisms that inhabit them. Various studies have shown that the concentration of this complex is high to promote plant nutrition. Likewise, radical exudates, particularly phenolic compounds, have an important effect on the proliferation of siderophores-producing microorganisms in the rhizosphere of plants, especially in conditions of low iron availability.

The types of transport of siderophores in microorganisms such as bacteria and fungi have the ability to use exogenous or heterologous ferri-siderophores (Hider and Kong 2010). Regarding iron deficiency worldwide, it represents a serious problem, where in places with a high concentration of salts it limits the availability of this important element. On the other hand, due to their capacities to sequester iron, microbial siderophores and phytosiderophores (plants) constitute a fundamental study area of the nutrition of plants that develop in soils with low iron availability. The development of plants with a high capacity to produce siderophores should be implemented in plant improvement programs.

One of the alternatives would be isolation and fungi with the ability to produce siderophores that have the capacity to sequester or capture this element to contribute to the nutrition of various crops, particularly those that are lacking this element (Yehuda et al. 1996). Currently, the applications of protein engineering to the biosynthesis of siderophores open the possibility of the natural diversity of the semolecules, through the directed design of new assembly lines.

The strategies are based on the addition, deletion, reorganization of domains and modules, alterations in the adenylation domains that affect the specificity of monomer selection, and combination of compatible subunits of different assembly lines. Currently, siderophores of a microbial nature have been characterized and are also used in various areas such as human medicine, industry, and environmental studies. However, the characterized siderophores represent only a small fraction of the total, with the field of siderophores largely unexplored. For this reason, the objective of this chapter is to study the chelating compounds of Fe that produce fungal communities and their applications.

6.2 Fungal and Production of Chelating Compounds

Industrial contamination and the indiscriminate use of chemical fertilizers in the soil with heavy metals (HM), which are incorporated into rivers, plants, animals, and foods, produce an alteration of the trophic chain producing potential risks to nature and society (Waisberg et al. 2013). The chelating mechanisms of plants are different from those of fungi and bacteria. A wide variety of fungi and bacteria produce organic acids as natural chelating agents of HM, contributing to mitigate the toxicity of these metals in living organisms (Seneviratne et al. 2017). Iron is an essential transition metal for living beings that intervenes in vital biological processes, including oxygen transport, electron transference, enzymatic reactions, aerobic metabolism, photosynthesis, and nitrogen fixation. The availability of this element influences the success or failure of pathogenic and symbiotic microorganisms in invading an organism or colonizing a given environment (Litchman 2010; Singh et al. 2020a).

Iron is almost insoluble at neutral or alkaline pH, due to which most fungi and microorganisms have developed a highly specific and efficient system to acquire iron (Kornitzer 2009; Zeng et al. 2018). This consists of synthesizing siderophores, which are chelating compounds with high affinity for ferric iron (Fe^{3+}) that act specifically as chelating agents that sequester iron in the presence of other metals and reduce it to (Fe^{2+}), which is much more soluble and useful in nutrition, forming complexes that may be taken into the cell by active transport (Kraemer 2004). Most of the siderophores found in fungi are hydroxamates classified into four structural families—rhodoturulic acid, the fusarinins, the coprogens, and the ferrichromes (Al-Fakih 2014; Garnerin et al. 2017; Winkelmann 2002).

Gluconic, oxalic, acetic, and malic acid have been reported to solubilize heavy metals by soil microbes (Gube 2016). Fomina et al. (2005) showed that over secretion of oxalic and citric acid with strong metal chelating properties provided high tolerance to toxic metals and soluble minerals of Cd, Cu, Pb, and Zn in *Beauveria caledonica*; the data suggested that oxalic acid was the main agent of mineral transformation. It is also known that the oxalate crystals produced by the ectomycorrhizal (ECM) fungi *Hebeloma velutipes*, *Piloderma byssinum*, *Paxillus involutus*, *Rhizopogon roseolus*, *Suillus bovinus*, and *S. variegatus* immobilize and detoxify heavy metals (Gadd et al. 2014).

A study by Kaewdoung et al. (2016) showed that the oxalate crystals produced by two wood-rotting fungi, *Fomitopsis* cf. *meliae* and *Ganoderma* aff. *Steyaertanum*, favored the tolerance and transformation of heavy metals into less toxic forms, for example, zinc sulfate ($ZnSO_4 \cdot 7H_2O$) into zinc oxalate dihydrate ($C_2O_2Zn \cdot 2H_2O$); copper sulfate ($CuSO_4 \cdot 5H_2O$) into copper oxalate ($C_2CuO_4 \cdot xH_2O$); cadmium sulfate ($3CdSO_4 \cdot 8H_2$) into cadmium oxalate trihydrate ($C_2CdO_4 \cdot 3H_2O$); and lead nitrate ($Pb(NO_3)_2$) into lead oxalate (PbC_2O_4). ECM fungi accumulate soil heavy metals adequately (Gadd et al. 2012), promoting the dynamism of plants in environments with metallic alterations (Fig. 6.1), because the metal-tolerant ectomycorrhiza function as an impediment to the passage of metals into plant tissues and also simulate the response of plants to abiotic stress (Colpaert et al. 2011; Khullar and Reddy 2018).

An evaluation of the in vitro production of chelating compounds of heavy metals using ECM fungi collected in pine plantations in southern Chile found that isolates of *Scleroderma verrucosum*, *Suillus luteus* and two isolates of *Rhizopogon luteolus* produced a number of metal-chelating compounds (oxalic, citric, succinic, and malonic acids) when cultivated in Melin-Norkrans-modified (MMN) liquid medium (Machuca et al. 2007). A recent study showed that ectomycorrhiza may be used in phytoextraction and phytostabilization of sites affected by heavy metals and reduce the detrimental consequences of these metals in host plants, decreasing their concentrations and improving the nutritional state of plants (Tang et al. 2019). This is

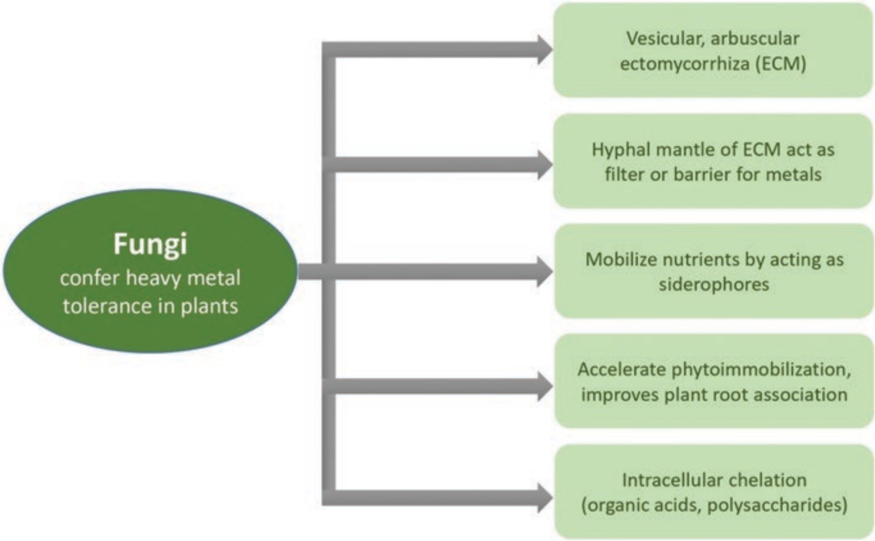

Fig. 6.1 Fungi and metal tolerance in plants

important for the production and characterization of chelating agents of heavy metals with biotechnological potential.

6.3 Type of Siderophores Produced by Fungi

Siderophores (Greek: "iron carriers") are organic compounds defined as "ferric-ion specific chelating agents" with low molecular weight synthesized by fungi under low iron stress (Matzanke 2011; Neilands 1995) helping to maintain iron homeostasis (Das et al. 2007), chelating ferric ions in the surrounding solution (Watkinson 2016).

Siderophores can be classified into three groups depending on the chemical nature of the oxygen ligands for Fe^{3+}: (a) catecholates and phenolates, (b) carboxylates, and (c) hydroxamates (Haas et al. 2008). The fungal siderophores names are based on their iron charged forms. The principal groups are the hydroxamate and the catecholate-type (Jalal et al. 1984). The basic structural unit of the hydroxamate siderophores is N^{δ}-acyl-N^{δ}-hydroxy-L-ornithine and there are four hydroxamates families classified as: rhodotorulic acid, fusarinines, coprogenes, and ferrichromes (Renshaw et al. 2002). The simplest hydroxamate, rhodotorulic acid, consists of two N^{δ}-acyl-N^{δ}-hydroxy-L-ornithine units (Winkelmann 1992). The ferrichromes (ferricrocin), one of the large families of hydroxamate siderophores, were isolated from low-iron cultures of many fungi (Emery and Neilands 1961). They are cyclic hexapeptides consisting of three N^{δ}-acyl-N^{δ}-hydroxy-L-ornithine and three amino acids (glycine, serine, or alanine). Coprogen is a linear tri-hydroxamate composed of three units of N^{δ}-acyl-N^{δ}-hydroxy-L-ornithine. The fusarinines (fusigens), from *Aspergillus, Penicillium*, and *Fusarium* species, can be monomers, linear dimers or trimers, or cyclic trimers. The acyl group is an anhydromevalonic acid (5-hydroxy-3-methyl-pent-2-enoic acid) residue (Winkelmann 1992).

The major groups of soil fungi and types of siderophores are shown in Table 6.1. Members of Mucoromycota (ex-Zygomycetes) do not produce hydroxamate-type siderophores (Comensoli et al. 2007; Winkelmann 1992). They store iron in their cells by using the iron-binding protein called rhizoferrin. This molecule is a polycarboxylate siderophore originally isolated from the fungus *Rhizopus microsporus* var. *rhizopodiformis* (Drechsel et al. 1991). For Ascomycota and Basidiomycota, the siderophores produced are hydroxamates (Drechsel et al. 1992; Leong 1986) being classified into four structural families: fusarinines, coprogens, ferrichromes, and rhodotorulic acid (Table 6.1). There are exceptions like the brown-rot fungus *Wolfiporia cocos*, a basidiomycota member used in Chinese medicine, has also been reported to have secretion of catecholates and hydroxamates chelators (Arantes and Milagres 2008).

In mycorrhizal fungi, few siderophores have been described due to the difficulties of cultivation, under iron limitation, in pure culture. Ericoid, ectomycorrhizal, and the ectendomycorrhizal fungi produce hydroxamate (ferrichromes) siderophores (Table 6.1) (Haselwandter 1995; Haselwandter et al. 1992; Haselwandter

Table 6.1 Some examples of the major groups of soil fungi and the types of siderophores produced

Group of fungi	Type of siderophores	Examples of fungi	References
Mucoromycota	Rhizoferrin	*Rhizopus microspores* var. *rhizopodiformis* *Mucor mucedo* *Phycomyces nitens* *Chaetostylum fresenii* *Cokeromyces recurvatus* *Cunninghamella elegans* *Mycotypha africana* *Mortierella vinacea* *Basidiobolus microsporus*	Van der Helm and Winkelmann (1994), Drechsel et al. (1991, 1992, 1995) and Thieken and Winkelmann (1992)
Ascomycota	Rhodotorulic acid	*Epicoccum purpurescens* *Histoplasma capsulatuma* *Stemphilium botryosum*	Frederick et al. (1981), Burt (1982) and Manulis et al. (1987)
	Coprogens	*Curvularia lunata* *Epicoccum purpurescens* *Fusarium dimerum* *Histoplasma capsulatum* *Neurospora crassa* *Stemphilium botryosum* *Penicillum chrysogenum* *Trichoderma* spp.	Van der Helm and Winkelmann (1994), Frederick et al. (1981), Van der Helm and Winkelmann (1994), Burt (1982), Van der Helm and Winkelmann (1994), Manulis et al. (1987), Charlang et al. (1981) and Baakza et al. (2004)
	Ferrichromes	*Aspergillus* spp. (*A. nidulans*) *Epicoccum purpurescens* *Microsporum* spp. *Trichophyton* spp. *Neurospora crassa* *Trichoderma* spp.	Charlang et al. (1981), Frederick et al. (1981), Bentley et al. (1986), Mor et al. (1992), Van der Helm and Winkelmann (1994) and Baakza et al. (2004)

(continued)

Table 6.1 (continued)

Group of fungi	Type of siderophores	Examples of fungi	References
	Fusarinines	*Aspergillus* spp. *(A. fumigatus)* *Epicoccum purpurescens* *Fusarium* spp. *Histoplasma capsulatum* *Paecilomyces* spp.	Winkelmann and Huschka (1987), Frederick et al. (1981), Diekmann and Zähner (1967), Burt (1982) and Van der Helm and Winkelmann (1994)
Basidiomycota	Rhodotorulic acid	*Rhodotorula mucilaginosa* (yeast)	Andersen et al. (2003)
	Ferrichromes	*Ustilago maydis* *Rhodotorula minuta* (yeast) *Ustilago sphaerogena* (yeast)	Ardon et al. (1997, 1998a, 1998b), Matzanke (1990) and Emery (1966)
	Catecholate and hydroxamate	*Wolfiporia cocos*	Arantes and Milagres (2008)
Ericoid mycorrhizal fungi	Ferrichromes (ferricrocin)	*Hymenoscyphus ericae* *Oidiodendron griseum*	Haselwandter et al. (1992) and Haselwandter et al. (1992)
	Fusarinines	*Rhodothamnus chamaecistus*	Haselwandter et al. (1992)
Ectendomycorrhizal Fungus	Ferrichromes	*Wilcoxina mikolae* *Wilcoxina rehmii*	Prabhu et al. (1996)
Ectomycorrhizal fungi	Ferrichromes	*Cenococcum geophilum* *Hebeloma crustuliniforme* *Suillus granulatus* *Suillus luteus*	Haselwandter and Winkelmann (2002), van Hees et al. (2006) and Haselwandter et al. (2011)
	Fusarinines	*Laccaria laccata* *Laccaria bicolor* *Suillus granulatus* *Suillus luteus*	Haselwandter et al. (2013) and Haselwandter et al. (2011)
	Coprogen	*Suillus granulatus* *Suillus luteus*	Haselwandter et al. (2011)
Arbuscular mycorrhizal fungi	Rhizoferrin (Glomuloferrin)	*G. etunicatum* *G. mossae* Unidentified *Glomus* sp.	Winkelmann (2017)
Dark septate fungi	Ferrichromes (ferricrocin)	*Phialocephala fortinii*	Bartholdy et al. (2001)
Orchidaceous mycorrhizal	Ferrichromes (basidiochrome) Ferrichromes	*Ceratobasidium* and *Rhizoctonia* *Nigritella nigra*	Haselwandter et al. (2006)

and Winkelmann 2002). Recently, in the ectomycorrhizal fungi, *Cenoccum geophillum* (Ascomycota; Haselwandter and Winkelmann 2002) and *Hebeloma crustuliniforme* (Basidiomycota; van Hees et al. 2006) were isolated ferricrocin. In the basidiomycota *Laccaria laccata* and *L. bicolor* the principal siderophores reported are fusigen, coprogen, ferricrocin, and small quantities for tri-acetyl fusarinine (Haselwandter et al. 2013). Until 2008, it has not been established if arbuscular mycorrhizal fungi produced siderophores since they cannot be grown independently of plant tissue (Haselwandter 2008). In a recent work, Winkelmann (2017) showed the release a rhizoferrin-derived siderophores (glomuferrin) from arbuscular-mycorrhizal fungi using *Tagetes patula nana* plants inoculated with *Glomus* spores (*G. etunicatum*, *G. mossae*, and unidentified *Glomus* sp.). Arbuscular mycorrhizal fungi seem to respond to low-iron stress by an increased excretion of glomuferrin, a carboxylate type siderophores. In dark septate root endophytes of *Phialocephala fortinii* type, the ferricrocin is released as the main siderophore (Bartholdy et al. 2001). The orchidaceous mycorrhizal fungi released ferrichrome as principal siderophore, although a novel structure was described as a basidiochrome, a linear tris-hydroxamate siderophore (Haselwandter et al. 2006).

Although not all fungi produced siderophores, they can transport iron into the cell utilizing a ferric reductase or can release large amounts of the relatively weak chelating agent citric acid (Watkinson 2016). Most fungi help to cover a wide range of natural environments to overcome adverse local conditions of iron solubility (Winkelmann 2007) produce a variety of siderophores.

6.4 Application of Siderophores Compounds in Agriculture

The mention to utilize microbial strains and any compounds in agriculture is returned to relate it with many organisms. We must use several methods to control plant pathogens, pests, and weeds which are possible in getting high yields (Thakur et al. 2020). The methods are including chemical, physical, and biological control agents. The chemical method is best from other methods in controlling the plant enemies (plant pathogens, pests, and weeds) speedily. Definitely, the synthetic chemicals used in the manufacture of pesticides have side effects for organisms and the environment. In addition, the continuous sprays the chemical pesticides in the fields leading to appear resistance against their pesticides and the high cost of its manufacture. Therefore, they are looking for alternative methods of high efficacy in the fields and near to the effect of the chemical method such as biological control agents (Aguilar-Marcelino et al. 2020b) included several virus (Sharma et al. 2020), bacteria (Al-Ani 2017a; Rai et al. 2020), fungi such as *Trichoderma* (Al-Ani 2018b, 2019c; Al-Ani and Mohammed 2020; Sharma et al. 2019), Entomopathogenic fungi (Al-Ani 2019d; Al-Ani et al. 2018), endophytic fungi (Al-Ani 2019e, 2019f; Al-Ani and Furtado 2020; Rana et al. 2019a), and non-pathogenic *Fusarium* (Al-Ani 2019a; Al-Ani and Salleh 2010), plant (Al-Ani et al. 2020a), and nematode (Al-Ani et al.

2020a), as well as, utilize the natural product (Adetunji et al. 2020; Al-Ani et al. 2012; Jatoi et al. 2020; Mohammed et al. 2012).

Interestingly, biological control agents in controlling plant enemies are by using different mechanisms comprising, (1) induce resistance (Al-Ani 2006; Al-Ani and Al-Ani 2011) and defense (Al-Ani 2018a) in plant, (2) competition (Al-Ani 2018a), (3) mycoparasitism (Al-Ani 2018a), (4) antibiotic (Al-Ani 2019b), (5) production of volatile and nonvolatile compounds (Al-Ani 2019a; Al-Ani and Albaayit 2018a, 2018b), and (6) siderophores (Al-Ani 2017b). PGPR, non-pathogenic *Fusarium*, and *Trichoderma* showed high efficacy in control of *Fusarium oxysporum* f.sp. *cubense* tropical race 4 by producing volatile compounds and siderophores (*Foc*TR4) useful for Agricultural sustainability (Al-Ani et al. 2013a, 2013b; Al-Ani and Albaayit 2018a, 2018b; Mohammed et al. 2011, 2013, 2014; Singh et al. 2021). *Trichoderma* is potential controlling the plant bacteria pathogen, and plant parasite nematodes (Al-Ani et al. 2020b; Sarker et al. 2020). All these mechanisms such as producing the siderophores are so interesting, which have attracted the curiosity of the researchers by using the best methods to reduce injury as much as possible and as an alternative to chemical pesticides.

On the other hand, different species of fungi (Wijayawardene et al. 2020) are producing siderophores and these fungi included harmful fungi and beneficial fungi. Harmful fungi are producing the problematic compounds such as mycotoxins that affect the living cells and also iron-chelating (Attitalla et al. 2010a, 2010b; López-Díaz et al. 2018). These fungi are producing phytotoxins such as fusaric acid (López-Díaz et al. 2018), and causing a disease for plant as plant pathogen. Some fungi as non-pathogen for plants are producing phytotoxins but these fungi can affect on the harmful plants as weeds (Amalfitano et al. 2002). During spraying of phytotoxins, extraction of *Colletotrichum gloeosporioides* on seven different weeds showed several effects such as stunted, severely burned, and severely damaged without killed (Ohra et al. 1995). Phytotoxins can be a type of siderophores to have an activity for iron chelation (Ohra et al. 1995). Production of siderophore by plant pathogens is necessary for pathogenicity. Inability of plant fungal pathogen to get iron is leading to reduce or lose the pathogenicity (Renshaw et al. 2002). The ability to produce many useful compounds such as siderophores is mentioned previously. The siderophores produced by fungi showed the influence on plant such as development and growth, as well as, plant protection (Al-Ani et al. 2020b; Aguilar-Marcelino et al. 2020a). Indeed, the importance of Fe-chelating compounds for agriculture can be determined in two very interesting points, as follows:

6.4.1 Biocontrol of Plant Pathogens

Siderophores have wide applications in environmental sciences and medicine. With respect to agriculture, they are used to improve soil fertility and biocontrol (Vellasamy et al. 2015). There is substantial evidence to believe that siderophores can control phytopathogenic microorganisms inhibiting pathogen growth or metabolic activity (Riquelme 1996). In the biological control mechanism, the significant role of siderophores to reduce the Fe availability has also been demonstrated for rhizobacteria (Beneduzi et al. 2012; Kloepper et al. 1980). There are studies regarding the siderophores produced by pseudomonads in the biological control of plant pathogen such as *Fusarium oxysporum* (Schippers et al. 1987) and *Gaeumannomyces graminis* (Voisard et al. 1989). Other bacterial species besides pseudomonads can be used as biocontrol agents such as *Bacillus subtilis* in the biocontrol of *F. oxysporum* (Yu et al. 2011).

Fungi can produce different types of siderophores that have been suggested to be an environmentally friendly alternative to hazardous pesticides (Devi et al. 2020; Schenk et al. 2012). The rhodotorulic acid produced by the yeast *Rhodotorula glutinis* improved the biological control of blue rot caused by *Penicillium expansum* in harvested apples (Calvente et al. 1999; Chand-Goyal and Spotts 1996). The role of siderophores in biocontrol is still unresolved. For example, the antagonistic of *Trichoderma* strains are not correlated to a type or amount of siderophores. *Trichoderma* strains have been shown to produce coprogen and ferricrocin siderophores (Anke et al. 1991). *Trichoderma asperellum* produced siderophores that controls *Fusarium* wilt (Segarra et al. 2010). Besides, siderophores produced by *Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma harzianum* were found to increase the shoot and root lengths of chickpeas (*Cicer arietinum*) (Yadav et al. 2011).

With respect to mycorrhizal fungi, arbuscular mycorrhizal sorghum plants have been observed with a higher concentration of Fe compared to non-mycorrhizal plants (Caris et al. 1998). The nutrition of ectomycorrhizal plants is known to depend on fungal siderophores (Van Schöll et al. 2008). The most important factor in biocontrol by siderophores is the availability of iron in the medium, since this regulates the siderophore production (Buyer and Sikora 1990). More investigation on the use of siderophores produced by fungi in the biocontrol of plant pathogens is needed.

6.4.2 Enhancing the Plant Growth

Iron deficiency is a limiting factor for plant growth; it affects crop yield negatively (Kobayashi and Nishizawa 2012). Lack of Fe also makes young leaves acquire photosynthetic activity that produces biomass reduction (Briat et al. 2007). Application of Fe as ferrous sulfates or chelates significantly increases the growth and yield of peas (Thapu et al. 2003), chickpeas (Kumar et al. 2009), and other crops. These applications proved Fe plays an important role in promoting plant growth.

The chelating capacity of siderophore metals has been studied in many biotechnological areas, including agriculture (Renshaw et al. 2002). The association that is established between a fungus and its host enhances various characteristics of plants, promoting growth and biomass production due to its influence on photosynthesis and improvement of their health status (Baynes et al. 2012; Mei and Flinn 2010).

Siderophores may act as biocontrol agents against damage caused by phytopathogens and substitute dangerous pesticides, which explains their role in bioremediation (Saha et al. 2016).

Endophytes, also called endosymbionts, are a group of microorganisms that colonize plant tissues. A large number of fungal and bacterial genera have able to colonize the intracellular and/or intracellular areas of plants (Singh and Dubey 2015; Rana et al. 2019b; Yadav et al. 2020c). Endophytic fungi may be highly resistant to the presence of heavy metals, even with chelating activity, which may allow remediation of contaminated soils and regulation of the toxicity that these produce in plants (Khan and Lee 2013; Yadav et al. 2020a, 2020b).

The endophytic fungi of arbuscular mycorrhiza (AM) (Table 6.2) (Fig. 6.2) are a heterogeneous group of the Glomeromycota division that form a symbiotic relation with 90% of superior plants (Bonfante and Genre 2010). These fungi produce a number of bioactive compounds that include phenolic acids, alkaloids, terpenoids, tannins, saponins, steroids, and quinones (Gouda et al. 2016); they also allow the development of biopesticides, promoting good growth of plants and resistance to environmental stressors, including Fe (Feng et al. 2017; Jalgaonwala et al. 2011; Philippot et al. 2013).

Table 6.2 Effective strains of fungi that form associations and promote plant growth

Species	Crop	References
Glomus versiforme	Chickpea (*Cicer arietinum*)	Alloush et al. (2000)
Glomus intraracides	Pepper (*Capsicum annuum*)	Martin and Stutz (2004) and Beltrano et al. (2013)
Dive versiformes	White clover (*Trifolium repens*)	Lu and Wu (2017)
Glomus intraradices	Rangpur lime (*Citrus limonia*)	Nogueira and Cordoso (2006)
Glomus caledonium *Glomus mosseae, Glomus intraradices* or *Glomus versiformes*	Cucumber (*Cucumis sativus*)	Ortas (2010) and Wang et al. (2008)
Rhizophagus irregularis	Wheat (*Triticum aestivum*)	Perez-de-Luque et al. (2017)
Glomus mosseae	Garlic (*Allium sativum*)	Sari et al. (2002)
Glomus intraradices and *Glomus mosseae*	Maize (*Zea mays*)	Lone et al. (2015)
Glomus intraradices and *Glomus mosseae*	Potato (*Solanum tuberosum*)	Lone et al. (2015)
Glomus sp.	Onion (*Allium cepa*)	Shuab et al. (2014)
Glomus mosseae, Glomus versiforme and *Paraglomus occultum*	Peach (*Prunus persica*)	Wu et al. (2011)
Rhizophagus irregularis	Tomato (*Solanum Lycopersicum*)	Khalloufi et al. (2017)

Source: Ahmad et al. (2019)

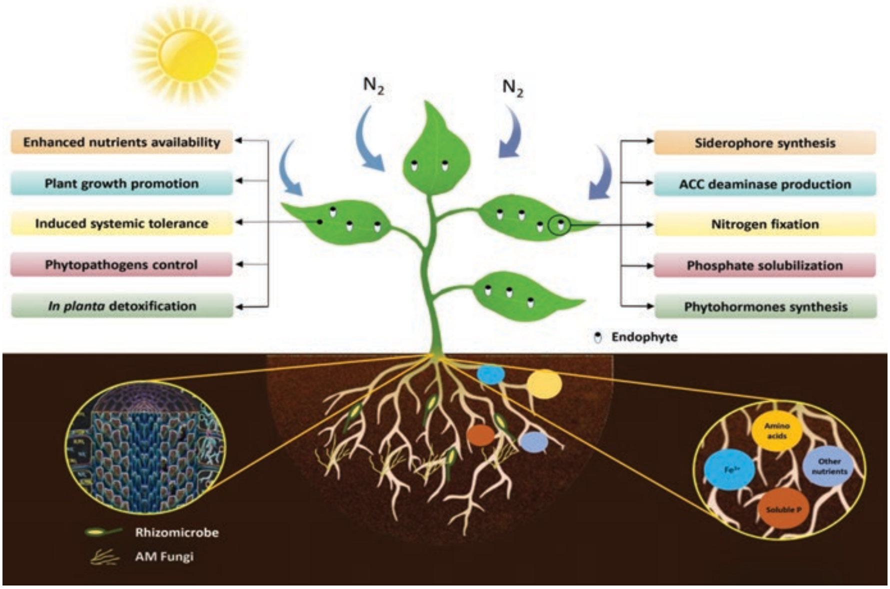

Fig. 6.2 Mechanisms of plant-growth-promoting endophytes (Adapted from: Feng et al. 2017)

The AM play an important role in promoting the absorption of Fe by the host plant. Lehmann and Rillig (2015) reported that inoculation with AM fungi (AMF) has a significant positive impact in the Fe nutrition of crops. However, greater mobilization of Fe in the rhizosphere of mycorrhizal plants may not represent a greater capacity of root absorption (Nogueira et al. 2007). This suggests that mycorrhizal colonization protects the host plant against excessive Fe toxicity, and consequently the AMF has a dual function in Fe absorption, which depends on the nutritional status of this metal in the plants.

The decrease in Fe stress in soils with mycorrhizae may be due to the mobilization of Fe in the rhizosphere and direction absorption of Fe by the extra-radicle hyphae of the AM, resulting in better Fe absorption. It is known that the AM may improve the capacity of the plant to absorb nutrients and minerals from the soil, thus plants inoculated with AM assimilate nutrients by an alternative way such as the intra-and extra-radicle hyphae (Smith and Read 2008).

In response to an environment with low Fe availability, AMF can excrete specific chelates that mobilize Fe nutrients. Glomaline is a glycoprotein that absorbs metals produced by AM hyphae (Rillig 2004); it has been demonstrated to be closely related to soil Fe, but the effect of glomaline on the availability and transferability of this metal is little known (Yongming et al. 2019). It is known that as well as being a component of the hyphal wall, it also contributes organic material to the soil, improves the stability of the aggregates present in soil, sequesters metallic cations of Pb, Cd, Cu, and Fe, and decreases their toxic effects, both for the mycorrhiza and the plants (Driver et al. 2005; Göhre and Paszkowski 2006).

The structural community of microbes and their diversity in the rhizosphere region are essential for the development, growth, and health of plants; the microbial community associated with a plant has been called its second genome, which is much larger than the genome of the plant (Berendsen et al. 2012). Microbes vary in number and diversity; they include dozens of thousands of species in fertile agricultural soils. Soil microbial communities usually include fungi, bacteria, algae, protozoa, nematodes, and microarthopods, each of which fulfills important roles such as the transformation of organic material and fixation of nutrients in the soil (Paul 2016; Raaijmakers 2001).

Beneficial soil–plant–microbe interactions are important and imply dynamic changes in the genome of the interacting partners by establishing a metabolic and signaling network. Two symbiotic plant–microbe associations have been widely studied in the last two decades—root nodule symbiosis and the association of AM (Kawaguchi and Minamisawa 2010). The beneficial interactions of plant and microbe may contribute to better health, growth, and productivity of the plants (Rastegari et al. 2020a, 2020b; Velmourougane et al. 2017; Verma et al. 2017). The stress tolerance induced in plant crops improves with beneficial microbe populations by the engineering of the rhizosphere or the use of microbial inoculants and/or their metabolites that can modify the soil microbiome (Velmourougane et al. 2017), which leads to increase of the productivity of the harvest and sustainability of the agro-ecosystem (Subrahmanyam et al. 2020).

The improvement of crops by inoculation with beneficial soil microbes in normal and stressful environments has been widely studied (Ahmad et al. 2019; Kour et al. 2019; Singh 2015; Singh et al. 2020b). However, the application of modern techniques to improve the yield of the soil microbes maybe key for the sustainability of agriculture by improving crop productivity, equilibrated nutrition, soil fertility, and stress tolerance in plants (Gouda et al. 2018).

6.5 Conclusion

Fungi are very important organisms living in different environmental conditions and interacting with many organisms. Therefore, fungi are producing many different types of secondary compounds necessary for living and competing with other microorganisms. Fungi can produce many important secondary compounds such as siderophores between problematic compounds (as phytotoxins) to beneficial compounds. Fungi showed the potential for producing several types of siderophore such as coprogens, fusarinines, rhodotorulic acid, and ferrichrome. The ability of fungi to produce siderophores is an activity in the uptake of iron essentially. This type of secondary compound produced by some biocontrol agents exhibits a significant role in the control of some plant pathogens especially soil-borne plant pathogens. The enhancement in the production of siderophores is necessary for use in the industry, especially in the domain of agriculture.

However, it is potential utilization of siderophores in agriculture instead of many synthetic chemicals such as pesticides and fertilizer. The determination of the ability of fungal strain (biocontrol agent) to produce siderophore is an important trait and can utilize this strain in the manufacturing of biopesticides and biofertilizers. The possible enhances the characterization of some strains of fungal biocontrol agents for producing more siderophores and tolerance for the unsuitable environment. The biotechnology method such as a transgenic method for fungal strain(s) producing siderophore can get strain(s) more tolerant for changes in environmental conditions, and confront different plant pathogens, as well as, the fungal strain(s) can promote plant growth by providing soluble ferrous available to the plant for uptake iron. In addition, the phytotoxins can be utilized as a bioherbicidal instead of chemical herbicides. Finally, the mechanism of producing siderophores by biocontrol agents is a more interesting tool that the potential to be instead of synthetic chemicals pesticides, and synthetic chemicals fertilizer. Therefore, it is enhancing the traits in producing siderophore under the different environments that increase in efficiency into using in agriculture.

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Chapter 7
Fungal Secondary Metabolites: Biological Activity and Potential Applications

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7.1 Introduction

Fungi can be found in almost any type of habitat. They compete with other creatures in order to survive. Some of the fungi are detritivores, while others form interkingdom communities to obtain food and development (Willis et al. 2019). Fungi are remarkable organisms, and in order to survive (Devi et al. 2020b), they have developed many protective strategies as well as interactions, one of which is the production of different types of compounds known as secondary metabolites (Devi et al. 2020a). These compounds protect them against various invasive predators, parasites, and diseases (Calvo et al. 2002). Traditionally, fungi are considered to be as contaminants which infect the human surroundings and cause various diseases. Fungi are known for causing serious illnesses such as late blight of potato, caused by *Phytophthora* sp. essentially becoming responsible for the potato famine in Ireland, and rice blast leading to the famous Bengal famine of 1943 (Ul Haq et al. 2020). The damage was not limited to plants only, cattle poisoning with ergot (mold) body formed by the mycelium of *Claviceps* species, and mycotoxins are also caused due to fungi (Riet-Correa et al. 2013). Despite of having some negative impacts of members of fungal kingdom, it is a known that they carry a vast range of metabolites that can be helpful and could have medical, industrial, environmental, and agricultural applications (Goyal and Ramawat 2017; Kour et al. 2019b). The idea of harvesting secondary metabolites fascinates many biotechnologist and emerging companies. This idea can be dedicated to the discovery of penicillin by Alexander Fleming. Penicillin is a metabolite produced by *Penicillin chrysogenum*. This particular discovery has paved the path for consideration of fungi as useful organisms.

By using the same method, other antibiotics such as chloramphenicol and streptomycin were later isolated from various fungal species. In general, today *Saccharomyces cerevisiae* or commonly known as Baker's yeast is essentially used for food production, and the same fungi often act as genetic model for lab testing, and in that effect, mycological studies are in their own niche growing ever since. In addition, *Pichia pastoris* and *S. cerevisiae* are utilized for the biopharmaceuticals production (Berlec and Strukelj 2013). Today, we have established that fungi contain many beneficial primary and secondary metabolites ranging from alcohol, organic acids, antibiotics, vitamins, pigments, immunosuppressant and immunomodulatory agents, and economically important proteins and enzymes (Sanchez and Demain 2017). Some of the examples are antibiotic such as penicillin and immunosuppressant such as cyclosporine derived from *Penicillium fellutanum*, *Tolypocladium inflatum*, and many more (Anjum et al. 2012).

Nevertheless, benefits of fungi are not just limited to pharmaceutical industry, and fungi are being actively utilized as biofertilizer, as feedstocks for biofuel production, and also as human food for consumption (Azizan et al. 2016; Rana et al. 2019a; Rastegari et al. 2020a, b; Yadav et al. 2020a, b). Search for the novel compounds is of high demand in the pharmaceutical industry, and this has proven to be endless as nature holds an incomprehensible data bank of compounds. The requirement of novel compounds can be regarded to the fact that microbes infecting humans are ever evolving, which eventually makes the existing solutions ineffective. Exploring the compounds for medicine purposes has been a challenge ever since the beginning. But one thing that is essential to understand is that fungal kingdom might hold the answers to the problems, and a lot of studies are proving that fungal metabolites are pharmacologically important and can act as a potential solution in different domains such as anticancer, immunosuppressant, antidiabetic, immunoregulator, antimicrobial, and antifungal. Therefore, in this chapter, various fungal secondary metabolites and their applications have been discussed.

7.2 Fungal Classification

In 1969, Robert Whittaker published the five-kingdom classification which separated fungi from the plant kingdom and gave a separate position to fungi in the kingdom classification. Prior to that, a two-kingdom classification was proposed by Linnaeus, based on whether an organism has the ability to move or not was only classified into two groups of animals and plants. The five-kingdom classifications were more widely accepted and is still the way of classifying all the organisms. A considerable amount of traits were recognized that were neither of animal kind nor of plant type, and this prompted R.H. Whittaker to propose a separate kingdom for fungi. Traits such as chitin cell wall and absorptive mode of nutrition were different as compared to plants which do photosynthesis hence are autotrophs and have cellulose cell wall. On the basis of different phylogenetic evidences, fungal kingdom was further subdivided into six broad classifications (Fig. 7.1).

7.2.1 Basidiomycota

Basidiomycota is a monophyletic group having more than 31,000 species, and around one third of fungi belong to this phylum, such as mushrooms, toadstools, puffballs, jelly fungi, bracket fungi, rusts, and smuts (Taylor et al. 2014). The phylum gets its name from the club-shaped sexually produced spore known as basidiospore or basidium (Rivera-Mariani and Bolaños-Rosero 2012). Genetic and molecular studies show that there is large diversity present within this group that yet to be discovered. They play vital role in functioning of ecosystem at different levels and able to degrade different components in wood. Basidiomycota is further

Fig. 7.1 Classification of fungal kingdom

subclassified into three subphyla: Pucciniomycotina, Ustilaginomycotina, and Agaricomycotina. Pucciniomycotina appear as the most basal subphylum with highest support, with *Mixiaosmundae* branching at the base of subphylum Ustilaginomycotina and Agaricomycotina, both of them branch as sister subphyla and are monophyletic (McCarthy and Fitzpatrick 2017).

7.2.2 Ascomycota

The phylum computes of the largest phyla of fungal kingdom along with Basidiomycota. Its sexually produced spores are housed in a sac-like structure called as ascus. For the same reason, the group is often attributed as sac fungi. Presence of ascus/sac is the defining feature of Ascomycota. The phylum consists of cup fungi, morels, bakers, and brewer’s yeast and truffles (Lutzoni et al. 2004; James et al. 2006). Further, it has major three subphyla: Taphrinomycotina, Saccharomycotina, and Pezizomycotina. Many ascomycete yeasts, that is, Saccharomycotina, are connected with insects for dispersal, and the fungi provide enzymes, vitamins, and other resources to host insects however; the fungi do not harm the host insects (Vega et al. 2009). Taphrinomycotina are parasitic or saprobic on vertebrates and plants (Taylor et al. 2014). Pezizomycotina are the most ecologically and morphologically complex subphyla of ascomycota.

7.2.3 Zygomycota

The fungi belonging to this category have coenocytic hyphae. Mostly they grow on terrestrial terrains. Zygosporangia is the characteristic structure in which spores formed by the fusion of two hyphae are stored. They are an ecologically heterogeneous, paraphyletic, or polyphyletic assemblage of predominantly terrestrial organisms, which are generally placed near the base of fungal tree of life. They reproduce asexually via nonmotile endospores formed in sporangiola, sporangia, or merosporangia or by the formation of arthrospores, chlamydospores, and yeast cells, and they reproduce sexually by the formation of zygospores in zygosporangia. Species such as *Mucor* and *Rhizopus* are the major known examples of this class (Moore et al. 2011).

7.2.4 Oomycota

This class of fungal kingdom consists of water molds. The name is derived from sexually produced oospore, which is a result of contact between male antheridia and the female oogonia. Examples include late potato blight and sudden oak death. Due to few major similarities, the class is now classified along with brown algae (Goyal and Ramawat 2017).

7.2.5 Deuteromycota

This class is inclusive of fungi which are known only to have asexual form of reproduction or we are yet to discover their sexual reproduction. These are also known as imperfect fungi. Most of the members are analogous to either Basidiomycota or Ascomycota class. *Alternaria, Colletotrichum*, and *Trichoderma* are the ones which are included in this taxon (Goyal and Ramawat 2017).

7.2.6 *Microsporidiomycota*

Microsporidiomycota are obligate intracellular parasite belonging to fungal kingdom. These are basically unicellular spore-producing fungi (Goyal and Ramawat 2017) and parasites of animals and protists. They are highly reduced in form and develop intimate association with certain parts of cells or organelles. *Encephalitozoon* are the human pathogens and infect kidneys, muscles, eyes, and sinuses. Not all of them are detrimental in nature; some of them also possess promising role in pest control without the use of pesticides.

7.3 Fungal Secondary Metabolites

The metabolites which are essential for growth and development are regarded as primary metabolites, while secondary metabolites are defined as those compounds which are not generally essential for the primary growth and development of an organism. Their absence often does not result in any observable phenotypic changes in the producing organism when grown in laboratory conditions. These small molecules possess important role in fungi such as defense and survival in external environment. These secondary metabolites are source of novel drug formulation. Studies prove that secondary metabolites act as antibiotics, antioxidants, antitumor, and antidiabetic agents. Bioassays of some of the compounds have also revealed their insecticidal properties. A large number of compounds have been isolated from fungi (Fig. 7.2).

7.3.1 *Polyketides*

Polyketides are the largest class among the fungal secondary metabolites. These compounds can be crystallized or can undergo reduction reaction, and they even undergo various step reactions and all this enables them to be a diverse group. The formation is by polyketide pathway in which condensation of acetyl-coenzyme A (CoA) and malonyl-CoA is catalyzed by type I polyketide synthases (Daley et al. 2017).

7.3.1.1 Aflatoxins

Aflatoxins are mycotoxin that are produced majorly by *Aspergillus* sp., and these species are able to produce an aflatoxin precursor sterigmatocystin, which is a carcinogenic compound. Aflatoxins are generally found in different agricultural commodities and are strongly regulated with different threshold limits depending on the

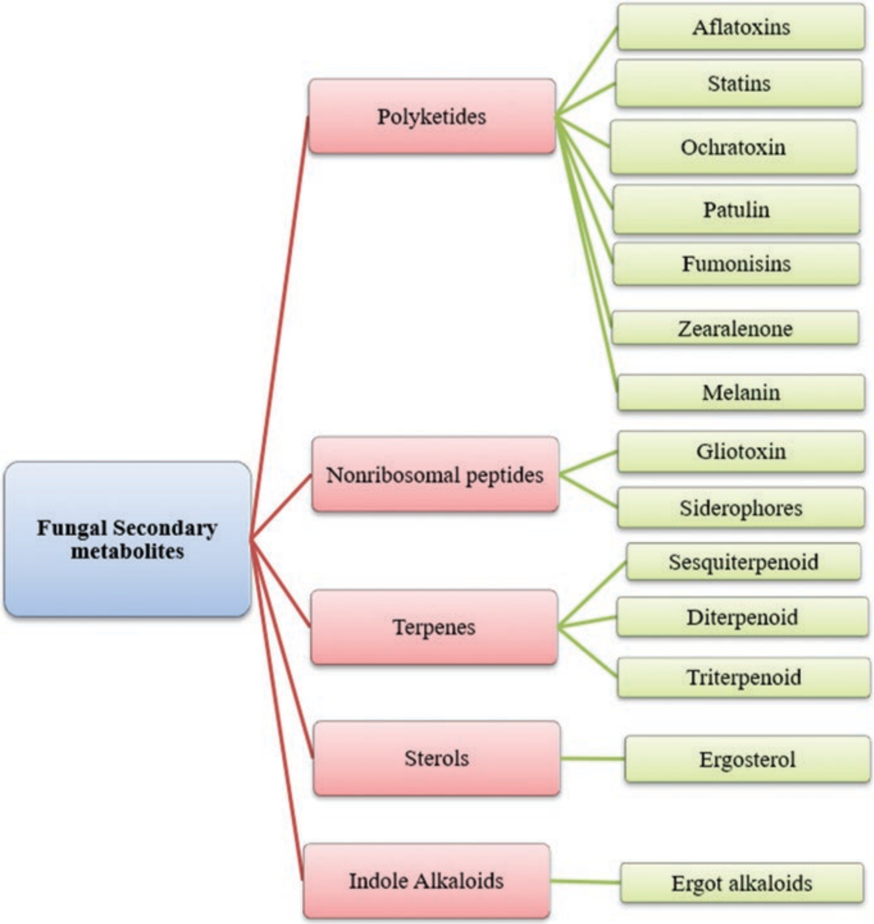

Fig. 7.2 Secondary metabolites of fungi

matrix (Pfliegler et al. 2020). *Aspergillus* sp. are particularly attracted to nuts and oils seeds, such as peanuts, walnut, corn, maize, and cotton seeds (Kumar et al. 2017). Aflatoxins are categorized as carcinogenic compounds as they include some of the most carcinogenic compounds ever isolated, such as B_1, B_2, G_1, and G_2. Among all, B_1 is the most toxic and carcinogenic obtained from *Aspergillus terreus*. Hepatitis B in conjugation with aflatoxin increases the probability of hepatocarcinogencity. They cause serious problems in human and animal, such as hepatotoxicity, teratogenicity, and immunotoxicity (Kumar et al. 2017). The toxic nature of these compounds links them to a number of diseases in both plants and animals. Illudin S isolated from *Omphalotus illudens* has the ability to glycolate the DNA, which makes it extremely toxic. Various aflatoxins producing species include *A.*

aflatoxiformans, *A. arachidicola*, *A. austwickii*, *A. cerealis*, *A. minisclerotigenes*, *A. mottae*, *A. pipericola*, and *A. texensis* (Pfliegler et al. 2020).

7.3.1.2 Statins

Statins are cholesterol-controlling compounds. One of the best studied compounds is lovastatin produced commercially using *A. terreus*. Statins usually show biological activity by influencing on 3-hydroxy-3-methylglutaryl-CoA reductase enzyme, which is essentially responsible for cholesterol formation. These are also referred as hypocholesterolemic agents. The inactivity of enzyme by statin lowers or nullifies the blood low-density lipoprotein cholesterol level (Adrio and Demain 2003).

7.3.1.3 Ochratoxin

Ochratoxin A is a mycotoxin produced by numerous fungal species such as *Aspergillus carbonarius, Aspergillus niger, Aspergillus ochraceus,* and *Penicillium verrucosum* (Bui-Klimke and Wu 2015). It was one of the first toxin that was discovered due to its capacity to infect human cells. It was isolated from *Aspergillus ochraceus*, but toxin is also found in other species such as *Aspergillus carbonarius* and *Penicillin verrucosum*. Toxin can also be found in grains such as corn, barley, oat, and wheat (Kolakowski et al. 2016). It is lethal to dogs, mice, pigs, and trout. It is an acute nephrotoxin, which causes necrosis in renal tubules and periportal liver cells. Other harmful effects include immunosuppression, embryo damage, and cancer induction (Bhalla 2019). Structurally, it is a pentaketide and derived from dihydrocourmarins which are similar to β-phenylalanine. The fungi species mainly invade plant products such as coffee beans, olives, grapes, nuts, and wines. Although ochratoxin A is a fat-soluble toxin and is not excreted, it can be transferred from animal sources too. If the fodder of animal is mold contaminated, it can build up in issues and circulatory system of animals.

7.3.1.4 Fumonisins

Both *Fusarium verticillioides* and *Fusarium proliferatum* are the major producers of Fumonisins. It is a mycotoxin which predominantly infects maize, corn, and sorghum (Smith 2018). It is a cause of porcine pulmonary edema and equine leukoencephalomalacia. They are structurally similar to sphingosine, essential phospholipid in cell membrane. Fumonisins are 20-corbon aliphatic chain with two ester-linked hydrophilic side chains. Coincidently, the toxicity of the discovered fumonisins B1 and B2 is also a result of competition in phospholipid metabolism. The main concern in pharmacology is their carcinogenic, genotoxic, and teratogenic effects rather than acute effects (Smith 2018).

7.3.1.5 Zearalenone

The toxin is produced by various species of *Fusarium* sp., including *F. cerealis*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, and *F. semitectum* (Zhang et al. 2018). Zearalenone is a heat-stable compound, and hence, it is found in open-field growing crops majorly cereals. The production of toxin is usually pre-harvesting, but vague handling of crop can lead to postproduction of the toxin too. Unlike Ochratoxin, Zearalenone is rapidly metabolized in the animal body, and hence, it is less likely to be transferred from animals to humans (Binder et al. 2017). It causes reproduction and fertility disorders in mammals. The concentration in some cases plays an important role. In cow's milk, zearalenone can be found if the mold concentration on the fodder is extensive. The toxin F-2 was isolated, and structurally, it is a resorcylic acid lactone. Prior to this discovery, *Fusarium* sp. has been a major cause for many mycotoxic outbreaks. Zearalenone is also referred as mycoestrogen for its estrogen-like activity. It has been reported to stimulate the growth of breast cancer cells, though seldom it is administered in cases of hypoestrogenic syndromes.

7.3.1.6 Patulin

Patulin is a toxin produced by *Penicillium*, *Aspergillus*, and *Byssochlamys*. This toxin is most common in apple-made products such as juices, compotes, cider, and baby foods (Zhong et al. 2018). Grapes, oranges, pears, and peaches are also contaminated with patulin. Patulin has various biological activities in both animals and humans. It is neurotoxic, genotoxic, and immunotoxic to rodents while teratogenic to chickens. Patulin causes distortion of DNA and induces mutations in it. On cellular level, many abnormal pathways can be observed, such as production of reactive oxygen species, cell cycle arrest, caspase-3 activation, PARP cleavage, and subsequent apoptosis (Kwon et al. 2012). In human intestine and kidney, production of reactive oxygen species induces mitochondrial apoptosis and causes endoplasmic reticulum stress (Kwon et al. 2012).

7.3.1.7 Melanin

Melanin acts in a protective nature for the fungi. It is produced using two pathways: one is polyketide pathway with malonyl-CoA as the precursor and other uses diphenolic compounds such as 3,4-dihydroxyphenylalanine as precursor (Belozerskaya et al. 2015). Melanin protects the possessing species of the fungal kingdom from the UV radiations and essentially helps in proliferation and growth of the fungi. The open-field stress is reduced by the melanin by photoprotection approach (Cordero and Casadevall 2017). Albino mutants (i.e., lacking melanin) of the same species of fungi did not grow in the open fields. Resting spores of species such as *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Wangiella dermatidis* consist of melanin, which helps them to remain dormant and act as perennation agents. Melanin also partially acts as a defense against host immune system. Melanin absorbs ROS secreted by macrophages and neutrophils. The pigment is considered to be critical for pathogenicity of many species of fungal kingdom (Garvey and Keller 2010).

7.3.2 Nonribosomal Peptides

Nonribosomal peptides are secondary metabolites which are synthesized by multidomain enzymes called nonribosomal peptide synthetases without the requirement of cell ribosomal machinery. They are naturally synthesized by fungi and produced via mRNA-independent process. They consist of both proteinogenic and nonproteinogenic amino acids. *Tolypocladium niveum* produced an immunosuppressant cyclosporine which is administered to patients undergoing organ transplant. Nonribosomal peptides possess wide range of bioactivities and pharmacological properties.

7.3.2.1 Gliotoxin

Gliotoxin was found as a contaminant in the process of fumagacin production when it was extracted from *A. fumigatus*. Structurally, gliotoxin constitutes disulfide bridge across the piperazine ring and is characterized as dipeptide. This structural integrity of the toxin allows it to interact with other proteins via disulfide bridge linking to cysteine residues of the protein. This interaction leads to the formation of reactive oxygen species (ROS). The formation of ROS is mainly responsible for its toxicity. ROS generation releases cytochrome c and promotes mitochondrial apoptosis, caspase production leading to cell death. It is known for its immunosuppression activity. Immune response suppression can be seen in many fronts such as inhibiting NF-κB factor, thereby suppressing inflammatory and cytokine responses, inhibiting phagocytosis, and blocking mast cell degranulation. All these events suggest that gliotoxin possesses protective mechanism in *A. fumigatus* from host environment (Kwon-Chung and Sugui 2009).

7.3.2.2 Siderophores

Siderophores are mycotoxin with low molecular weight and considered to be highly coordinated with iron. There are three structural families of siderophores: fusarinines, coprogens, and ferrichromes (Yadav et al. 2020c). On basic level, almost all fungal siderophores are hydroxamate types with few exceptions to this generalization. The basic unit is N^{δ}-acyl-N^{δ}-hydroxyornithine, whose precursor is L-ornithine (Renshaw et al. 2002). Siderophores formation occurs through nonribosomal peptide pathways. Role of siderophores is speculated in virulence of *A. fumigatus* as iron plays an important role in host–pathogenic interactions, and siderophores promote growth of hyphae in even iron-limiting environment (Garvey and Keller 2010). On the other hand, medically siderophores are being used to treat iron overload and aluminum overload conditions, although side effects come into play with the administration of these mycotoxins (Page 2019). They are also studied for anticancer properties in mice models and found to inhibit tumors in them. Actinides are elements which are mostly radioactive and carcinogenic, while siderophores enhances the excretion of actinides from body.

7.3.3 Terpenes

Terpenes are important bioactive metabolites produced by many fungi species. Structurally, terpenes are repetitive units of isoprene unit, both in linear and cyclic fashion. They are even categorized on the basis of number of isoprene units (C_5) present into diterpenes (C_{20}), hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), sesterterpenes (C_{25}), triterpenes (C_{30}), and tetraterpenes (C_{40}). Diterpenoid, triterpenoid, and sesquiterpenoid are the terpenes which possess various biological activities. This group of compounds is structurally well diverse, and this fact can be pointed to the ability of the compounds to undergo various catalytic modifications such as glycosylation, cyclization, redox reaction, and alkylation. The production of terpenes is observed by mevalonic acid pathway. Gibberellins, carotenoids, indolediterpenes, trichothecenes, and aristolochenes all are the examples of terpene class metabolites (Daley et al. 2017).

7.3.4 Sterols

Sterols are isoprenoid-derived molecules and major constituent of eukaryotic cell membranes. It is necessary for permeability, fluidity, and protein function. Therefore, they are required for growth of fungi. Ergosterol was discovered over 100 years ago in *Claviceps purpurea*. It has been considered as fungal sterol, and multiple pathways are involved in the formation of ergosterol. In some taxa, pathways are incomplete and, in some cases, result in the formation of other end-products. Ergosterol synthesis starts with acetyl-CoA and comprises of 20 steps (Alcazar-Fuoli et al. 2008). In a study two aromatic steroids were isolated from *D. concentrica*, that is, 19-norergosta-1,3,5,7,9,14,22-heptaene and 1-methyl-19-norergosta-1,3,5,7,9,14,22-heptaene (Qin and Liu 2004). Another study reported the presence of two steryl esters with a polyhydroxylated ergostane-type nucleus in *Tricholomopsis rutilans* (Wang and Liu 2005).

7.3.5 Indole Alkaloids

Alkaloids are the largest group which is of pharmacological importance. For indole alkaloids, tryptophan and dimethylallyl phosphate are the precursors, but sometimes, other amino acids are also used. The most widely studied group of indole alkaloids is ergot alkaloids. Initial findings suggested ergot alkaloids as toxins, but later other uses were also found. They are produced by *Claviceps purpurea* and its related species. Ergot alkaloids have the ability to induce abortion and promote uterine contractions (Schiff 2006). They also act as vasodilators which can reduce blood pressure by dilating the blood vessels. Ergots also inhibit noradrenaline and sclerotin. Fumigaclavines and fumitremorgens are also tryptophan-derived alkaloids synthesized by *Aspergillus fumigatus* (Goetz et al. 2011).

7.4 Function of Secondary Metabolites

The release of secondary metabolites aligns with either the fungal development or to counter any stress conditions including both biotic and abiotic stresses. These metabolites can change the course of development, survival, and interaction with other species both interkingdom and intrakingdom.

7.4.1 Protects from UV Damage

This functionality is usually devoted to the presence of a polyketide compound know as melanin. Melanin is a constituent part of spores and hyphae of the fungi. It is usually produced by either polyketide or l-3,4-dihydroxyphenylalanine pathway. Protection being the ecological role of melanin was seen in an albino mutant (i.e., lacking melanin) of *Cochliobolus heterostrophus*, which was unable to stand the sunlight and thus was not able to survive in open ground. However, other species which was having melanin in their spores was able to survive in the open ground. There are other metabolites which are also having the function of photoprotection. These compounds also protect the pathogenic fungi from the host immunity.

Melanin is a well-studied and widely used secondary metabolite in cosmetics, food coloring, human skin care products, and bioelectronics (Blachowicz et al. 2020). It is a brown-colored natural pigment localized in the cell wall of spores and hyphae of many fungi (Toledo et al. 2017). Melanin biosynthesis in fungus occurs through enzymatic or spontaneous polymerization of polyphenols, such as catechol, 1, 8-dihydroxy naphthalene (DHN), or dihydroxyphenylalanine (L-DOPA). The primary role of fungal melanization is microbial pathogenesis and protection against extreme environments, such as Arctic and Antarctic poles, high-temperature fluctuations in deserts, acidic pH, exposure to radiations, extraterrestrial conditions, metal-polluted areas, oxidative stress, low moisture, nutrient availability, hypersaline waters, elevated osmotic pressure, and so on. The pigmented fungi exhibit resistance to radiotoxicity compared to their albino counterparts. Melanin is defense armor for fungus to tolerate and resist toxic conditions (Gómez and Nosanchuk 2003). A notable property of melanin is to interact with electromagnetic rays, acting as a photoprotective and energy harvesting agent. Electromagnetic radiations are potentially hazardous when exposed to the human skin depending on the duration of exposure. Ionizing radiations or electromagnetic radiations, including gamma rays, X-rays, and ultraviolet rays, cause skin damages such as erythema, tanning, skin aging, degeneration of skin cells, fibrous tissue, and blood vessels. Prolonged exposure of UV rays is responsible for most nonmelanoma and melanoma skin cancer. The UV-A rays (320–400 nm) permeate the dermis causing mutations in the DNA, premature aging of the skin (wrinkles, fine lines, freckles, and coarse skin), and suppresses immunity. The UV-B rays (280–320 nm) penetrate the epidermis, causing erythema and sunburn – a vital factor responsible for melanoma skin cancer. The high-frequency electromagnetic radiations generate intermediate free radicals from biomolecules such as DNA and proteins (Ikehata and Ono 2011). The free radicals or reactive oxygen species (superoxide anions, peroxides, hydrogen peroxide, and hydroxyl radical) have unpaired electrons making them highly unstable. The ROS damages the cellular structure and function in the epidermal and dermal regions of the skin by oxidizing the proteins, carbohydrates, and lipids. Melanin absorbs and dissipates photons from ionizing radiations and thus acts as a photo protectant or a natural sunscreen for human skin. The complex, unorganized structure of melanized fungi absorbs the entire UV region (UV-A, UV-B, UV-C) of the electromagnetic spectrum. The same properties of melanin that prevent oxidative damages in the human skin protect pathogenic fungi against the host defense mechanism.

7.4.2 Defensive Role

In the open fields, competition for food and survival is not only interspecies but also interkingdoms. Fungi have developed mechanisms, either direct or indirect to survive in the environment. The first ever direct functionality of the mycotoxin was illustrated by Alexander Fleming. Penicillin, an antibacterial, was produced by *Penicillin notatum* to kill the surrounding bacteria such as *Staphylococci* present in the culture. Later, penicillin was regarded as the wonder drug. *Beauveria bassiana*, another fungus, contains a toxic metabolite arsenal to kill insects. The species also fills the insect with antibacterial so curb down the microbial competition. *Aspergillus flavus* produces aflatoxin, which is considered to be a group of carcinogenic compounds which increases the ability of a fungi to fight and survive with insect proximity by 26 times. Bacterial–fungal interaction is also beneficial for fungi in a number of cases. Some examples include that the endosymbiont relation of *Burkholderia rhizoxinica* and *Rhizopus microspores* is responsible for rice seedling blight, which becomes worse once the microbial toxin rhizoxin enters into the plant cells. Some other symbiont species also enhance the rhizoxin to increase its phytotoxicity to mutually benefit both organisms from the host. A complex gradient-depended effect is also seen with *Aspergillus*, where phenazine produced by *Pseudomonas aeruginosa* acts as an antifungal agent if present in high concentration, but within a certain concentration, phenazine is helpful to the fungi for sporulation (Macheleidt et al. 2016; Raffa and Keller 2019).

7.4.3 Protects from Toxic Natural Products

Fungi also need certain mechanisms to protect itself from toxins that are being produced by itself. That is where different self-protection strategies come in. The efflux pumps in GliA are important and provide protection from trichothecenes, cellular biosynthetic gene cluster intermediate transporters, detoxifying enzymes, and duplicate copies of target protein. Among all, duplicate copies are of high importance for human kind in drug discovery as the presence of duplicate function directly points toward a eukaryotic target. This is the main source of identification of many antifungal agents which are modified and used as an effective treatment for human invasive pathogens and pathogenic biofilms (Keller 2019).

7.4.4 Growth and Development

Although major development is coordinated by primary metabolites, some protective function is still depending on secondary metabolites which promote the development and reproduction of fungi. Fungi produce toxins such as fusarubins and furocoumarin which protect the sexual structures from predators and fungivores. Photoprotection of spores by melanin improves the chances of spore germination. Production of certain secondary metabolite inhibits other spore germination in the surrounding. This external behavior can be seen in *Penicillium* sp. (Calvo and Cary 2015; Keller 2019).

7.5 Biological Application of Fungal Secondary Metabolites

For the past few decades, scientists have tested secondary metabolites from over 10,000 fungal species for biological activity. There is an extensive history of using fungi folk medicine in Asian countries to promote health and longevity. Bioactive compounds from fungi offer vast and unexplored chemical compounds. The ability of fungi to acclimate to all niches of earth offers an understanding that the bioactive compound aids the survival of fungi. Decoding the biosynthetic gene clusters of fungi can help in finding unexplored bioactive compounds with beneficial

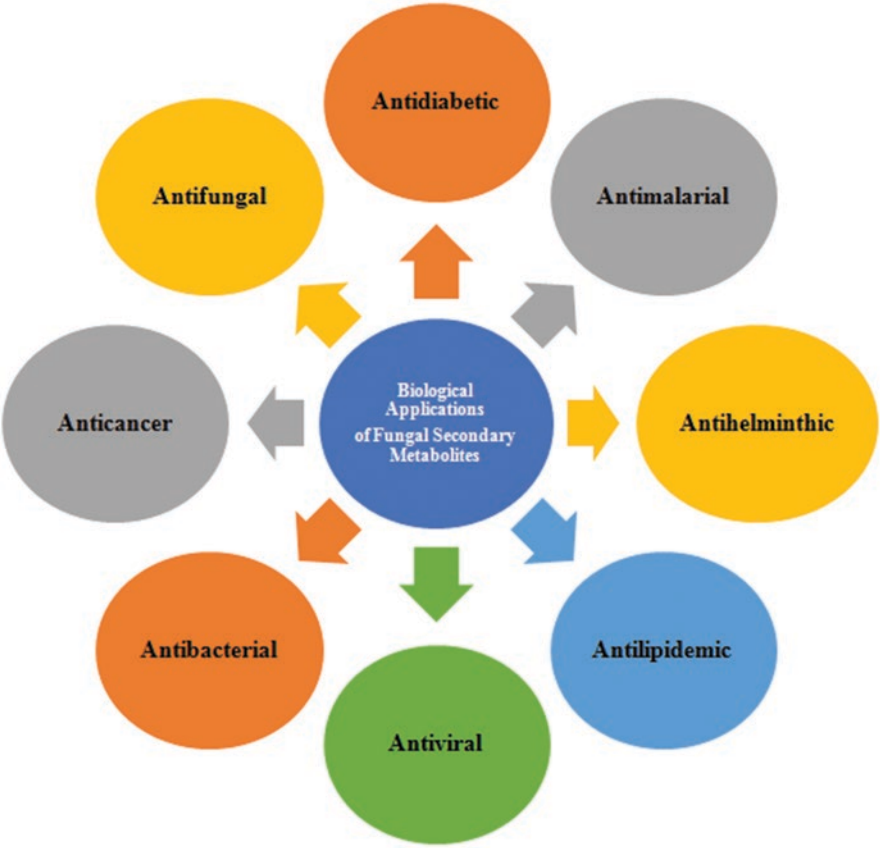

Fig. 7.3 Biological applications of fungal secondary metabolites

properties. Further investigation of bioactivity of secondary metabolites opened the gates of new era of antimicrobials from higher fungi. Pharmacological research of these bioactive compounds reveals that fungi possess various biological applications such as antibacterial, antiviral, antiprotozoal, antihelminthic, and antifungal activities. In recent decades, fungal secondary metabolites are being researched as potential leads in drug development, cosmetics, and crop protection in agriculture (Fig. 7.3).

7.5.1 Antibacterial Agents

The development of penicillin paved the way for development of antibacterial fungal metabolites. Penicillin is a bactericidal antibiotic that kills bacteria by inhibiting the action of enzyme transpeptidase, required for crosslinking of peptidoglycans in the bacterial cell wall synthesis. In the late 1940s, a team of Oxford scientists under Howard Florey developed a way to mass-produce penicillin during the outbreak of the Second World War, but the drug yields were very low (Florey 1944). To overcome this challenge, researchers implemented a deep-tank fermentation method, which resulted in the production of over 600 billion units of pharmaceutical-grade penicillin per annum in 1945 during the Second World War. Ernst Chain, a German origin British biochemist along with Florey, discovered the chemical composition and therapeutic role of penicillin. In 1945, Howard Florey, Ernst Chain, and Alexander Fleming shared a Nobel Prize in Medicine for their work with penicillin. There are five types of naturally biosynthesized penicillin viz., penicillin G, penicillin K, penicillin N, penicillin O, and penicillin V. The discovery of β-lactam antibiotics such as cephalosporin, monobactam, and carbapenem followed the success of penicillin (Ingolia and Queener 1989). Cephalosporin isolated from the aerobic mold, *Cephalosporium acremonium*, forms a significant class of β-lactam antibiotics. There are four generations of cephalosporins which are grouped based on their antimicrobial activity (Mehta and Sharma 2016). The previous β-lactam drugs were short-range antibiotics effective against few gram-positive bacteria; however, the new generation of β-lactam antibiotics exhibits broad range of action and is effective against a wide range of pathogenic gram-negative bacteria. There are other classes of antibiotics derived from fungi that show antibacterial activity by following either of the mechanisms, that is, inhibiting protein synthesis; inhibiting nucleic acid and folic acid synthesis pathways; permeabilizing cytoplasmic membrane; and interfering with cellular processes (Silver 2011).

Fusidic acid isolated from *Fusidium coccineum* has antibacterial activity against *Mycobacterium tuberculosis*, *Neisseria* sp., *Nocardia* sp., *Staphylococcus aureus*, and penicillin-resistant and methicillin-resistant *Staphylococcus aureus* (Dobie and Gray 2004). Retapamulin, extracted from an edible mushroom *Pleurotus mutilins*, was the first pleuromutilin topical antibiotic developed by GlaxoSmithKline and sold under the trademark names Altabax and Altargo. Retapamulin is useful in the treatment of impetigo. Alamethicin, an ion channel-forming peptaibol antibiotic, was isolated from *Trichoderma viride* (Dotson et al. 2018). Ongoing studies have reported that several polyketides, peptides, and sterols extracted from fungi possess antibacterial properties, although their mechanism of action is yet to be deciphered.

7.5.2 Antifungal Agents

Fungal infections can be mild skin infections (mycosis), or they may have life-threatening implications, as seen in the cases of aspergillosis and candidiasis. The most common form of fungal infections includes skin and nail infections. About 40 types of fungi (typically *Trichophyton*, *Microsporum*, or *Epidermophyton*) can cause fungal skin infections (White et al. 2014). They lead to dry red itchy patches on the skin. Nail fungal infections, technically called onychomycosis, lead to thick, discolored, cracked nails. The cause of these infections is soil yeasts such as

Candida parapsilosis, *Candida guilliermondii*, and *Candida albicans*. In recent years, nondermatophyte molds, for instance, *Fusarium* spp., and *Onychocola canadensis* are being increasingly detected as the causative agent for fungal nail infections. It is difficult to find a cure for fungal infections, and at present, only a handful of antifungal agents are available. Fungi synthesize some antifungals as secondary metabolites for their existence. Griseofulvin is one of the first naturally synthesized antifungals from *Penicillium griseofulvum*. It is a fungistatic drug used in the treatment of hair, skin, and nail fungal infections. Griseofulvin inhibits mitosis of fungal cells by binding with tubulin and hinders microtubule function (Gupta et al. 2004). In the case of nail and hair infections, griseofulvin binds with keratin and makes it resistant to fungal invasions. Cerulenin, an antifungal antibiotic, derived from *Cephalosporium caerulens*, inhibits fatty acid biosynthesis in yeasts.

Invasive fungal infections are life-threatening in immune-compromised patients. Healthy individuals can breathe in air with *Aspergillus* spores and remain unaffected. In case of immune-compromised individuals, spores of *Aspergillus* can cause aspergillosis. *Aspergillus* infections result in allergies and infections in lung and other organ. Similarly, *Candida* is present on the skin, mouth, throat, gut, and vagina without affecting a healthy individual. *Candida* can be precarious when it grows out of control or enters the bloodstream or internal organs. Fungal pathogens such as *Candida albicans* and *Aspergillus* can also cause hospital-acquired infections such as bloodstream infections, ventilator-associated pneumonia, urinary tract infections, and surgical site infections (Khan et al. 2017).

Echinocandins are the precursor leads of semisynthetic antifungal drugs against systemic infections such as aspergillosis and candidiasis. These drugs act by noncompetitively inhibiting the enzyme 1,3-β-D-glucan synthase required for the synthesis of fungal cell wall component β-glucan polymers. Echinocandin B isolated from *Aspergillus nidulans* was the first drug lead of class echinocandin (Denning 2002). Echinocandin B is the precursor of semisynthetic antifungals caspofungin (cancidas), anidulafungin (Eraxis), and micafungin (Mycamine). These three agents are approved by the FDA to treat candidemia, invasive candidiasis, and esophageal candidiasis. Echinocandins are administered with other groups of antifungals in combination therapy to cure aspergillosis. For instance, anidulafungin is used in combination with voriconazole to treat *Aspergillus* infections (Jeans et al. 2012).

Caspofungin is an intravenous drug used in empirical therapy of fungal infections in febrile patients with neutropenia and therapy of aspergillosis where patients are intolerant of conventional antifungal drugs (Shalhoub et al. 2014). Micafungin is used in the treatment of candidemia, candida peritonitis, and esophageal candidiasis. Micafungin is the only FDA-approved echinocandin drug for the prophylaxis of *Candida* infections in hematopoietic stem cell transplantation patients (Shalhoub et al. 2014). Pneumocandins are a closely related group of echinocandins isolated from *Zalerion arbicola* which are effective antifungals against *Candida* sp. and *Pneumocystis carinii* (Patil and Majumdar 2017).

7.5.3 Anticancer Agents

Scientists have examined the cytotoxic activity of fungal metabolites in the past few decades. Although these secondary metabolites were used since ages, their antitumor activity has been decoded in the past few years. For instance, in ancient China, Reishi, a medicinal fungus, served as a folk medicine to promote health and longevity, but in recent years, researchers have pointed out its antitumor mechanisms as well. The polysaccharide peptide (GI-PP) from Reishi is a potent angiogenetic and induces cell apoptosis by reducing the expression of Bcl-2 (antiapoptotic protein) and elevating Bax (proapoptotic protein) (Wachtel-Galor et al. 2011). A high dose of GI-PP alleviates the expression of vascular endothelial growth factor, and this explains the mechanism of antitumor activity of GI-PP. *Ganoderma lucidum*, a medicinal mushroom, has been demonstrated with apoptotic, antiproliferative properties. *G. lucidum* suppresses the migration of PC-3 cells, a highly invasive prostate cancer cell (Sohretoglu and Huang 2018). The water extract of *G. lucidum* was studied extensively compared to its alcohol extract. *G. lucidum* promoted the synthesis of $CD5^+$, $CD4^+$, and $CD8^+$ T lymphocytes (Lin 2005). In horses, *G. lucidum* was observed to elevate the production of specific antibodies. The alcohol extract of *G. lucidum* induced apoptosis in MCF-7 breast cancer cells in a dose- and time-dependent manner (Wu et al. 2012). The underlying mechanism is still not known, but the hypothesis suggests that it may be due to an increased expression of pro-apoptotic Bax protein. Another antineoplastic metabolite isolated from *G. lucidum* is a triterpenoid, lucidenic acid N. It is a potent cytotoxic metabolite against Hep G2 cells, with an IC50 value of 2.06×10^{-4} μM (Wu et al. 2001).

Maitake glycan, isolated from *G. frondosa*, is an SFDA-approved drug, clinically used to treat cancer, polycystic ovary syndrome, and impaired glucose tolerance (Rossi et al. 2018). The bioactive component of maitake glycan is β-glucan or the d-fraction composed of β-(1 → 6)-glucan main chain with β-1,3 branches as opposed to β-(1 → 3)-glucan as main chain with β-1,6 branches in the β-glucan of other fungi with antitumor properties (Rossi et al. 2018). Maitake glycan inhibits tumor growth by cell apoptosis via notch1/NF-B/p65-mediated caspase pathway, activating immune cell and regulating the production of cytokines. Paclitaxel is a potent drug for chemotherapy medication to treat pancreatic cancer, ovarian cancer, breast cancer, cervical cancer, lung cancer, and Kaposi's sarcoma (Singla et al. 2002). The WHO has included it in the World Health Organization List of Essential medicines. Initially, paclitaxel was derived from *Taxus brevifolia*; in 1993, researchers discovered paclitaxel in an endophytic fungus that inhabited in the Pacific yew, and, since then, it has been found in several endophytic fungi. In 2001, the National Institute for Health and Care Excellence approved the use of paclitaxel in the treatment of non–small-cell lung cancer, first-line and second-line treatment of ovarian cancer, and treatment of advanced breast cancer where anthracyclic chemotherapy fails. Paclitaxel is a cytoskeletal drug that targets tubulin. It stabilizes the microtubule polymer and prevents the spindle configuration of chromosomes in the metaphase, thus blocking the progression of mitosis. This triggers cell apoptosis at the mitotic checkpoint. Poricoic acid G, isolated from *Poria cocos*, showed cytotoxic properties against leukemia HL-60 cells (Ukiya et al. 2002). Experimental cancer drugs that target the enzyme farnesyl transferase to inhibit the activity of Ras protein in cancer cells such as 11,11′-dideoxyverticillin A, andrastin A, barceloneic acid A, and barceloneic acid B were isolated from marine *Penicillium* sp (Jin-Ming 2006). Other antitumor cytoskeletal drugs targeting tubulin include vinblastine and vincristine. Both these drugs are included in the World Health Organization List of Essential Medicines. Vinblastine was isolated from the endophytic fungi *Curvularia verruculosa* from the leaves of *Catharanthus roseus*. Fungal vincristine from *Eutypella* sp.-CrP14 was isolated from *Catharanthus roseus* (Kumar et al. 2013). These drugs are used in chemotherapy medication to treat small-cell lung cancer, acute lymphocytic leukemia, acute myeloid leukemia, Hodgkin's lymphoma, and neuroblastoma.

7.5.4 Antiviral Agents

Fungal secondary metabolites which possess antiviral properties are grouped in two categories based on their mechanism of action, that is, biological response modifiers (BRMs) and viral inhibitors. BRMs are compounds that modulate the host defense system by acting on a therapeutic target in a pathogenic disease. Most of the antiviral metabolites from fungus are BRM. For instance, styrylpyrone compounds, hispidin and hispolon, isolated from *Inonotus hispidus* were observed to show antiviral activity against influenza virus type A (H1N1 and H3N2) and B (Awadh Ali et al. 2003). A retrovirus requires three enzymes for replication in a host, namely reverse transcriptase, integrase, and protease. Antivirals target these three enzymes to inhibit viral activity by blocking virus replication. Hispidin caps its phenolic group with methyl ether and inhibits the activity of enzyme integrase required for viral replication (Roy 2017). *Ganoderma pfeifferi*, a medicinal mushroom, is a source of many antiviral metabolites. Triterpenes, ganodermadiol, lucidadiol, applanoxidic acid G, ganoderone C, lucialdehyde B, and ergosta-7, 22-dien-3α-isolated *Ganoderma pfeifferi* have shown antiviral properties against influenza virus type A (Mothana et al. 2003). Another group of triterpene metabolites lanosta-7, 9(11), 24-trien-3-one, 15; 26-dihydroxy, and ganoderic acid Y isolated from *G. lucidum* inhibits the growth of enterovirus 71. The fruiting bodies from *G. lucidum* such as ganoderiol F and ganodermanontriol inhibit the action of HIV protease enzyme. The chloroform extract of fruiting bodies *G. colossum* also inhibits the activity of HIV protease enzyme and ceases viral replication (El Dine et al. 2008). Krestin is a protein-bound polysaccharide extracted from Basidiomycetes with anti-HIV properties. It was reported to inhibit the activity of reverse transcriptase enzyme of avian myeloblastosis virus (Maehara et al. 2012).

7.5.5 Antilipidemic Agents

Antilipidemic agents are cholesterol-lowering drugs that inhibit the synthesis of cholesterol or low-density lipoproteins (LDL), decreasing fat accumulation in plasma and preventing atherosclerosis and thrombosis. Statins are a class of drugs primarily used to inhibit the biosynthesis of cholesterol (Endo 2010). The first statin drugs, mevastatin isolated from *Penicillium citrinum* and lovastatin isolated from *Monascus ruber* or *Aspergillus terreus*, were fermentation products (Subhan et al. 2016). Static acid is structurally analogous to enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, required in the biosynthesis of cholesterol by mevalonate pathway. Thus, statins act as competitive inhibitors of HMG-CoA and inhibit the synthesis of cholesterol, reducing the total and low-density lipoprotein cholesterol levels, and prevent the risk of coronary heart diseases. In a study, effect of rosuvastatin was evaluated on Coronary Atheroma Burden, patients with preexisting heart disease, where 40 mg/day of rosuvastatin was administered. It was observed that the drug reduced the levels of LDL cholesterol and elevated the levels of HDL cholesterol, and this led to a regression in the atheroma burden (Aly et al. 2011). Statins help in decreasing the incidences of strokes by ceasing atherosclerosis progression, stabilizing plaque, and improving endothelial functions (Pinal-Fernandez et al. 2018).

7.5.6 Antihelminthic Activity

Parasitic nematode infections in humans, plants, and animals are a significant cause of concern. They result in grave economic losses of crops and livestock. *Ascaris lumbricoides* and the hookworms *Ancylostoma duodenale* are common nematodes that infect humans. In the late nineteenth century, an antihelminthic agent, PF1022A, was isolated from *Mycelia sterilia*. PF1022A is a cyclic octadepsipeptide composed of alternating L-D-L configuration of 4N-methyl-L-leucines, 2 D-lactic acids, and 2 D-phenyllactic acids. PF1022A was initially tested against the roundworm *Ascaridia galli* in chickens. An oral administration of 2 mg/kg of PF1022A in chickens proved to inhibit the growth of *A. galli* (Von Samson-Himmelstjerna et al. 2000). PF1022A is not cytotoxic and does not affect the growth of gram-positive or gram-negative bacteria or fungi. The antihelminthic activities of PF1022A have been reported against *Haemonchus contortus*, *Ostertagia ostertagi*, *Toxocara canis*, and *Trichostrongylus colubriformis* and the intestinal nematode *Angiostrongylus cantonensis.* Fujisawa Pharmaceutical Co. Ltd. (Japan) has synthesized a semisynthetic drug derived from PF1022A known as emodepside, which is used in combination with praziquantel and sold under the tradename Profender® to treat nematode infections (Krücken et al. 2012).

7.5.7 Antimalarial Agents

Malaria caused by a female *Anopheles* mosquito is a life-threatening disease prevalent in Asian and African regions. As per the World Malaria Report, there were 228 million cases in 2018. Codinaeopsin, a fungal secondary metabolite, has been tested for its antimalarial properties against *Plasmodium falciparum* (Kontnik and Clardy 2008). 7-hydroxy-3,4,5-trimethyl-6-on-2,3,4,6-tetrahydroisoquinoline-8-carboxylic acid and 2,5-dihydroxy-1-(hydroxymethyl) pyridin-4, two alkaloid extracts isolated from endophytic fungi, have been reported to possess antimalarial activities (Elfita et al. 2011). Other antimalarial metabolites from fungi such as efrapeptins, zervamicins, and antiamoebin are under research.

7.5.8 Antidiabetic Activity

Many fungal metabolites are tested for its antidiabetic activity. A medicinal fungus, *Poria cocos* or *Wolfiporia extensa* Ginns, traditionally known as china root, is used as a folk medicine in China to treat diabetes. The compounds dehydrotumulosic acid, dehydrotrametenolic acid, and pachymic acid were isolated from the chloroform extracts of *P. cocos* (Kim et al. 2019). These three extracts showed different levels of insulin sensitizer activity. Dehydrotumulosic acid exhibits hypoglycemic properties. An alpha-glucose inhibitor, Aspergillusol A, isolated from a marine *Aspergillus*, has alpha-glucosidase inhibition property (Ingavat et al. 2009). Other compounds such as ternatin isolated from mushroom suppress hyperglycemia (Kobayashi et al. 2012). Few fungal isolates were studied, which act as DPP-4, alpha-glucosidase, and alpha-amylase inhibitors.

7.5.9 Fungicides and Insecticides

Fungal pathogens are responsible for some of the most devastating crop infections. They destroyed about a third of food crops annually, causing grave loss of economy. As per the Food and Organization of the United Nations, in 2009–2010, fungi induced losses in five staple crops, namely rice, wheat, maize, potato, and soybean. Similarly, insects destroy farm produce and can lead to famine. Fungal bioactive metabolites can be used as fungicides and insecticides to mitigate the losses (Rana et al. 2019b; Singh et al. 2020; Yadav et al. 2020c).

Strobilurins are a class of fungicides derived from β-methoxyacrylic acid. These are isolated from the Basidiomycetes genera that include *Crepidotus*, *Cyphellopsis*, *Filoboletus*, *Hydropus*, *Mycena*, *Oudemansiella*, *Strobilurus*, and *Xerula* and an ascomycete *Bolinea lutea* (Cooper et al. 2020). Strobilurins are effective against phytopathogenic fungi at concentrations as low as 10^{-8} to 10^{-7} M and exhibit minimal toxicity to mammalian cells (Cooper et al. 2020). Strobilurins prevent mycelial growth and spore germination by blocking the electron transport chain and suppressing fungal cell respiration. Strobilurins are single-target compounds, and thus plant pathogenic fungi have quickly developed resistance to them within 2 years of introducing them in the market. Nodulisporic A isolated from *Nodulisporium* sp. is an effective insecticide against fleas, where it blocks the glutamate-gated ion channel in invertebrates (Smith et al. 2000). Its analog N-tert-butyl nodulisporamide is administered orally to dogs and cats to control fleas and ticks. Kresoxim-methyl from BASF is another popularly used strobilurin fungicide available as mixture with other fungicides. For instance, to tackle fungal infection on cereals, kresoxim-methyl and fenpropimorph or epoxiconazole are used in combination, which are sold as Brio® and Allergo®, respectively (Aly et al. 2011). Azoxystrobin is a strobilurin fungicide from Zeneca registered for use on 55 crops in about 49 countries and marketed under the brand names Amistar® for cereals, Quadris® for grapevines, and Heritage® for turf (Camargos et al. 2016).

7.5.10 Immunosuppressants

Immunosuppressants are drugs that suppress the activity of the immune system. Immunosuppressive drugs are used to prevent rejection during organ transplantation and suppress allergic reactions, autoimmune disorders, and uncontrolled inflammation that might damage tissues and organs. Mizoribine (MZB), an imidazole nucleoside known by the generic name Bredinin, is an immunosuppressive drug isolated from fungus *Penicillium brefeldianum*. It is used in the treatment of lupus, IgA neuropathy, rheumatoid arthritis, and other rheumatic diseases. It is also used during renal transplantation, since compared to other immunosuppressive drugs, MZB is less toxic and has no drawbacks. MZB inhibits the synthesis of guanine nucleotide by inhibiting the activity of inosine monophosphate synthetase and guanosine monophosphate synthetase. It suspends DNA synthesis in S-phase of the cell cycle (Yokota 2002). Cyclosporine is a widely used class of immunosuppressants isolated from *Tolypocladium inflatum* (Yang et al. 2018). Using cyclosporine in organ transplantation surgery to prevent the rejection of bone marrow and in heart, kidney, and liver transplantation has been a revolutionary success, increasing the survival rates in transplant patients (Chinen and Buckley 2010).

Cyclosporines prevent cell apoptosis by binding with cyclophilin D, an integral part of mitochondrial permeability transition pore that regulates cell necrosis. This property of cyclosporines makes them the drug of choice in the treatment of neurodegenerative disease, cardiac hypertrophy, trauma, and ischemia–reperfusion injury (Yang et al. 2018). The binding of cyclosporine with cyclophilin D inhibits the activity of calcineurin, a protein phosphatase that activates T cells. Cyclosporines are the first class of nontoxic immunosuppressants that can selectively immunoregulate T cells (Yang et al. 2018). Gliotoxin, an anti-inflammatory drug, was isolated from fungal species such as *Aspergillus* sp. and *Gliocladium fimbriatum*. It is an immunosuppressive drug that prevents apoptosis in neutrophils, eosinophils, granulocytes, macrophages, and thymocytes. Gliotoxin prevents an inflammatory response and release cytokines by inhibiting the activity of nuclear factor-κB (NF-κB) (Fraga-Silva et al. 2019).

7.5.11 Miscellaneous Activity

In traditional medicines, fungi have been used to cure several health ailments. *Cordyceps sinensis* was discovered 2000 years ago in China in the Qing dynasty. Ethnomedical reports suggest that *C. sinensis*, an aphrodisiac, has antioxidant properties and down regulates apoptotic genes (Shashidhar et al. 2013). *G. lucidum* has also been used as a folk medicine in China, Japan, and South Korea. Extracts from *G. lucidum* have shown immunomodulatory effects that impact cancer cells. Apart from this, *G. lucidum* extracts have antiviral, antibacterial, and antitumor agents. Endophytic fungi have long been used for sustainable agriculture by developing a symbiotic plant–microbe interaction (Singh and Yadav 2020; Verma et al. 2017). Endophytic fungi can produce bioactive secondary metabolites such as iron chelators, flavonoids, phenolic acids, steroids, alkaloids, coumarin, quinones, lignans, peptides, terpenoids derivatives, phosphate solubilizers, insecticides, and nitrogen fixation compounds, which benefit the plant host (Rao et al. 2020; Kour et al. 2019a; Rana et al. 2019c, 2020). For instance, endophytic fungi *Gliocladium catenulatum*, inhabiting in Cacao seedlings, releases bioactive metabolites that act as insecticides. Endophytic fungi *Fusarium solani* inhabiting *Rheum palmatum* synthesize rhein, a potent antimicrobial compound. Fungi are also used for biotransformation of steroids commercially as they are comparatively feasible than chemical methods. Fungal biotransformation of steroids includes reactions such as hydroxylation, dehydrogenation, and sterol side-chain cleavage. *Aspergillus* species are used in the preparation of rostanes by degrading the C-17 saturated side chain of the sterol. Fungi such as *yeasts, Candida,* and *Rhodotorula* are used in the production of industrially important products such as amino acids, ethanol, enzymes, recombinant protein insulin, and vitamins. Further source and application of fungal secondary metabolites have been mentioned in Table 7.1.

7.6 Conclusion and Future Prospects

Fungi are the microorganisms that can inhabit extreme environmental conditions and are a reservoir of bioactive secondary metabolites. Their commercial production is still at a slow pace, and alternative methods for upscaling are the need of hour. Fungal fermentation has a great scope for producing therapeutic proteins, enzymes, and recombinant proteins. The fermentation process needs to be optimized to increase the production yield and find an economical method to purify the

Table 7.1 Source and application of fungal secondary metabolites

Drug/drug lead	Source	Uses	Reference
Lucidenic acid N	*Ganoderma lucidum*	Anticancer drug	Wu et al. (2001)
Poricoic acid G	*Poria cocos*	Anticancer drug	Ukiya et al. (2002)
Psilocybin	*Psilocybe mexicana*	Psychedelic drug	Passie et al. (2002)
Mizoribine	*Fusarium subglutinans*	Immunosuppressant	Yokota (2002)
Ganodermadiol, lucidadiol, applanoxidic acid G	*Ganoderma pfeifferi*	Antiviral	Mothana et al. (2003)
Cephalosporin	*Cephalosporium acremonium*	Antibiotic	Schmitt et al. (2004)
Ganoderic acid β	*Ganoderma lucidum*	Antiviral	Li and Wang (2006)
Pravachol	*Penicillium compactum*	Antilipidemic	Endo (2010)
Fingolimod	*Isaria sinclairii*	Multiple sclerosis	Chun and Brinkmann (2011)
Endocrocin	*Aspergillus*	Immunosuppressant	Lim et al. (2012)
Asparaginase	*Penicillium digitatum*	Anticancer drug	Shrivastava et al. (2012)
Zhankuic acid A	*Antrodia camphorata*	Anticancer drug	Lee et al. (2012)
Lentinan	*Lentinula edodes*	Anticancer drug Antilipidemic	Ina et al. (2013)
Nigrosporin B	*Nigrospora*	Antibiotic	Wang et al. (2013)
Caspofungin	*Glarea lozoyensis*	Antifungal	Maiolo et al. (2014)
Anidulafungin	*Aspergillus*	Antifungal	Maiolo et al. (2014)
Mycophenolic acid	*Penicillium stoloniferum*	Immunosuppressant	Patel et al. (2016)
Lysergic acid	*Claviceps purpurea*	Psychedelic drug	Das et al. (2016)
Proliferin	*Aspergillus proliferans*	Antibiotic	Woappi et al. (2016)
PGG glucan	*Saccharomyces cerevisiae*	Antitumor	Bashir and Choi (2017)
SSG glucan	*Sclerotinia sclerotiorum*	Antitumor	Bashir and Choi (2017)
Polysaccharides	*Ganoderma lucidum*	Antitumor	Li et al. (2018)

end product. The biosynthetic gene clusters (BGCs) in fungi are untapped. Researchers must explore bioinformatics tools and techniques to study the fungal secondary metabolomes to capture, characterize, and synthesize bioactive metabolites. Current challenges in fungal secondary metabolite research include replicating the existing BGC cluster, expressing proteins from cryptic BGC, and identifying BGC from unknown genes. Investigations on genomic, proteomic, and metabolomic can help gather the knowledge necessary for the future development of fungal secondary metabolites on the industrial level.

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Chapter 8
Fungal Communities as Biological Control Agents for Different Phytopathogenic Organisms

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Contents

8.1 Introduction

Biocontrol research is aimed at providing solutions for disease management in the agriculture sector. Certain organisms have been extensively studied as potential biocontrol agents, one of the most well studied of these is the fungal genus *Trichoderma*. *Trichoderma* spp. have great potential as biological control agents against a wide range of soil-borne plant pathogenic fungi (Sharma et al. 2019). Members of this genus produce a number of fungal cell-wall degrading enzymes including chitinases, 1,3-glucanases, proteases, mannanases, etc. *Trichoderma* spp. have been used increasingly in agriculture, especially in greenhouses where a significant proportion of soil-borne disease control products are *Trichoderma harzianum*-based. Not only

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is the genus known for its agricultural applications, it also has potential for soil bioremediation activity such as hydrocarbon pollutant removal (Knudsen and Dandurand 2014).

Biocontrol agents are involved in a variety of trophic and non-trophic interactive mechanisms including production of antifungal compounds, hyper-parasitism of pathogens and stimulation of host plant defences or competitive colonization of spermosphere and rhizosphere substrates. The first fungus to be registered with the EPA for control of a plant disease was *T. harzianum* ATCC 20476. A total of 12 fungi have been registered with EPA for control of plant diseases (Fravel 2005).

Many promising biological antagonists, mainly from the genus *Trichoderma, Aspergillus, Penicillium, Pseudomonas* and *Bacillus*, have been reported as effective antagonists against plant pathogens. The potential of *Trichoderma* spp. to parasitize, suppress as well as kill other plant pathogenic fungi has been recognized as an important mechanism for its success as a biological control. Some species of *Trichoderma* such as *T. asperellum, T. atroviride, T. virens* and *T. harzianum* are widely used as biological control agents of plant pathogens. Three important *Trichoderma* species, i.e., *T. virens, T. atroviride* and *T. harzianum*, are recognized for their ability to produce higher amounts of chitinolytic enzymes as compared to other fungi known to have similar biological control abilities.

8.2 Current Scenario

Plant-fungal diseases are one of the most important issues in agriculture and hindrance in food production in the world. Especially, *Penicillium* species such as *P. digitatum* and *P. italicum* are involved in post-harvest losses in citrus (Barkai-Golan 2001; Eckert and Eaks 1989). Other citrus pathogenic fungi include *Aspergillus* and *Alternaria*. *Aspergillus* sp. is the most common environmental fungi and can be isolated from citrus fruits, vegetables, tomatoes, corn, pistachios, etc. In citrus, *Aspergillus niger* produces brown rot and *Aspergillus flavus* creates albinism or virescence (Aboutorabi 2018).

Currently, more than two billion tons of pesticides are used in agriculture, every year, all over the world. These pesticides include fungicides, bactericides, herbicides, insecticides and others that are used to eliminate undesirable agents, mainly fungi, weeds and insects, which are considered crop pests, with the aim to guarantee a high yield. The cost of this practice is not only financial improvement, but also environmental improvement, because the excessive use of pesticides leads to water bodies, groundwater and soil contamination in addition to affecting human and animals' health due the toxicity, recalcitrance and the carcinogenic potential of many of these compounds. The biological control of pests has been recognized as an alternative to the use of pesticides (Thakur et al. 2020). Even though this is a considerably cheaper technology and less harmful to the environment, biological control practices are currently very scarcely used in relation to chemical pesticides (Rana et al. 2019a; Rana et al. 2019b). This fact mainly occurs because biological management is more specific and takes a longer time to achieve the desired results (Baron et al. 2019).

Biological control of plant pathogens is the key practice in the sustainable agriculture which strives to minimize the use of synthetic fungicides and to use as alternative management strategy to control soil-borne pathogens (Barea et al. 1993). Biocontrol agents can be defined as directed, accurate management of the common components of the agriculture ecosystem against pathogens (Azcon-Aguilar and Barea 1997). The number of biocontrol agents is commercially employed these days such as *Pseudomonas fluorescens, Bacillus subtilis, Trichoderma harzianum, T. viridae* and *Mycorrhizal fungi* (*Glomus* spp.), *Agrobacterium radiobacter* strain 84 and K1026, etc., for soil-borne pathogens. Among them, use of mycorrhizal fungi as biocontrol agent gained importance in integrated disease management programs (Rana et al. 2020; Rana et al. 2019c). Mycorrhizal fungi are a major natural occurring component of soil ecosystem and found associated with roots system of more than 80% of all terrestrial plant species. Mycorrhizal fungi are known to protect the plants against several soil-borne pathogens like *Fusarium*, *Pythium*, *Verticillium*, *Ralstonia*, *Macrophomina,* etc. (Singh et al. 2019).

8.3 Biological Control for Sustainable Agriculture

Biological control is defined as the introduction of an exogenous biological agent into the environment with an aim towards its permanent establishment to control the pests present therein over the long term (Kenis et al. 2017). The biological agent applied can be a parasitotic, a pathogen, or a predator of the organism that is causing economic loss (Hajek and Delalibera 2010). However, the growing problem related to the indiscriminate use of agricultural chemical inputs has been changing, which has led several organizations to begin debates and efforts to improve awareness regarding the need to reduce pesticide and fertilizer consumption and prohibit the use of products that have been shown to threaten food and occupational safety (Carneiro et al. 2015; Yadav et al. 2020a, b, c). This has resulted in greater visibility of biological control, which has led to the application of all the accumulated knowledge on this subject in the field.

Fungal diseases of insects have been known since 1834 when the Italian, Agostino Bassi, elucidated the fungi to be the cause of the white muscardine disease of silkworms. By using fungi as biological control agents is by no means a novel idea and was suggested by Pasteur and the American, Le Conte in 1874. This was followed by mass production of a fungus, *Metarhizium anisopliae* for the first time by Metschnikoff (1879) and Krassilstchik (1888). Fungi are unique among all the groups of insect pathogens, in that they can penetrate directly the cuticular barrier and do not have to be ingested. Given favourable environmental conditions, fungi will exert spectacular natural control of insect populations but usually epizootics occur only when pest populations are very high. Using fungi can prevent a build-up of pests to damaging levels, by artificial application of fungal propagules (Hall and Papierok 1982).

Biocontrol fungi (BCF) are beneficial organisms that reduce the negative effects of plant pathogens and promote positive responses in the plant (Devi et al. 2020). They do control diseases and in addition have other benefits, including amelioration of intrinsic physiological stresses in seeds and alleviation of abiotic stresses. They also improve photosynthetic efficiency, especially in plants subjected to various stresses. Finally, several fungi also increase nitrogen uptake efficiency in plants. As a consequence, plants treated with beneficial fungi may be healthier and have greater yields than control. Most of the early work on biocontrol of plant diseases by *Trichoderma* spp. revolves around the direct ability of these fungi to interact with soil pathogens. The specific mechanisms described are mycoparasitism, production of antibiotics and competition for nutrients in the rhizosphere. During the process of mycoparasitism, the fungi first locate target hyphae by probing with constitutively produced cell wall degrading enzymes (CWDEs) coupled with very sensitive detection of cell wall fragments released from target fungi. After the fungi come into contact, *Trichoderma* spp. attach and coil around and form appressoria on the surface of the host. Enzymes and antibiotic substances are produced that degrade the target hyphae and permit penetration of the *Trichoderma* strains. Both the enzymes and the antibiotics are strongly antifungal and are synergistic in their action (Shoresh et al. 2010).

8.4 Sources of Myco-Biocontrol Agents

Bioactive fungi have a wide host range, although there is considerable genetic diversity within species and some strains show a high degree of specificity. For example, *Metarhizium anisopliae* var. *acridum* is only effective against insects such as grasshoppers and locusts (Driver et al. 2000). Primary requirement for the use of an entomogenous fungus such as a myco-biocontrol agent is susceptibility to the insect on one hand and virulence of the fungus on the other hand. Deuteromycetes fungi have a broad host range, and in particular, *Metarhizium* and *Beauveria* are promising as myco-biocontrol agents with application as myco-insecticide. *Beauveria bassiana* and *Metarhizium anisopliae* are among the first entomopathogenic fungi being successfully used for myco-biocontrol of insect pest. The development of molecular biology techniques for entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae*, coupled with cloning of putative pathogenesis determinant gene, will create more potential candidates for managing the notorious insect pest population (Rana et al. 2008; Thakur et al. 2011).

8.5 Applications of Fungi in Biological Control

Among the wide variety of microorganisms that have reported to be potential and are being used in biocontrol practices, fungi are the most studied and applied (Schrank and Vainstein 2010). According to Thomas and Read (2007), the main reasons for their broad use are their efficiency in eliminating their hosts, their wide metabolic diversity that increases the chances of finding appropriate isolate for biocontrol, and their relative environmental safety, as they are primarily decomposers. Fungal species that are used in biological control include both basal and higher fungi. The basal fungi are the representatives of phylum *Blastocladiomycota*, such as *Coelomomyces,* and of the subphylum *Entomophthoromycotina*, such as species of the genera *Conidiobolus*, *Entomophthora*, *Erynia* and *Entomophaga*. Among the higher fungi, several species of the phylum *Ascomycota* (Subkingdom Dikarya) have been reported potential and are commercialized and applied in the field such as *Purpureocillium, Metarhizium, Beauveria, Cordyceps, Fusarium, Trichoderma*, etc. Before defining the use of a fungus as a commercial product, many characteristics must be observed, including its virulence, capacity to resist environmental stressors (ultraviolet resistance, temperature tolerance), mass production potential, sporulation capacity on low-cost alternative substrate, ability to cause infection under low humidity condition and specificity to the parasite of interest (Pourseyed et al. 2010).

Results of ecological studies on epizootic or enzootic infections by fungi, laboratory studies and field application studies indicate that fungi can be manipulated in two possible ways to control pests (Rastegari et al. 2020a, b). Firstly, they may be applied to achieve a ‘knock-down’ effect, either rapidly in the case of fungi producing toxins or more slowly, where a comparatively long ‘lag-phase’ between applications and death can be tolerated and where the target insect has a long life cycle relative to the infection cycle. Secondly, where an insect pest species has a rapid reproductive rate, as in the case of aphids or mites, the fungus must be able to spread, following application, more rapidly than the pest is able to reproduce (Latge and Perry 1980; Hall 1981). Ideally, it is desirable that a pathogen is able to recycle in the environment to control insect below economically or medically important thresholds on a long-term basis. The muscardine fungi, *M. anisopliae* and *Beauveria* spp., are the most intensively studied entomopathogenic fungi. *M. anisopliae* has been used to control several pests such as rhinoceros beetle, *Oryctes rhinoceros* (Latch and Falloon 1976; Anonymous 1978). The use of this fungus as an adjunct with a *Baculovirus* has virtually eliminated this major pest of palms in Polynesia (Bedford 1981).

8.5.1 Biological Control of Arthropods by Fungi

Insects are the main class of the *Arthropoda* phylum and are among the most diverse living being on the planet. Even though a small portion of this group contains species known as agricultural pest, they are responsible for causing considerable damage to crop, devastating approximately 20% of the global annual production (Schrank and Vainstein 2010). Most entomopathogenic fungi belong to the order *Hypocreales* (phylum *Ascomycota*) and *Entomophthorales* (subphylum *Entomophthoromycotina*) (Hibbett et al. 2007). Generally, entomophthoralean fungi are specific to their host and therefore present a relatively low risk of infecting beneficial insect, such as pollinators. While fungi of the *Hypocreales* order are less selective and infect a wider range of host (Roy and Pell 2000). Entomopathogenic fungi are useful for biological control of mosquitoes. *Beauveria bassiana* is an opportunistic pathogen, and its use against many arthropods is widespread.

The fungal strains have restricted host range, for example, *Aschersonia aleyrodes* infects only scaly insects and whiteflies, with individual isolates being more specific to target pests. Some species are facultative generalist pathogens, such as *Aspergillus* and *Fusarium*. Most species are obligate pathogens, often quite specific and rarely found, for example, many species of *Cordyceps*. Entomopathogens such as *M. anisopliae* and *B. bassiana* are well characterized with respect to pathogenicity towards several insects and have been used as myco-biocontrol biological control agents for agriculture pests worldwide (Singh et al. 2019).

8.5.2 Entomopathogenic Fungi

Entomopathogenic fungi are the first organism to be used for the biological control of pests. More than 700 species of these fungi from around 90 genera are pathogenic to insect. Most of them belong to genera *Deuteromycetes* and *Entomophthorales.* Some entomopathogenic fungi have restricted host ranges, e.g., *Aschersonia aleyrodes* infect insects with scales and whiteflies, while other fungal species have a wide host range. Entomopathogens such as *M. anisopliae* and *B. bassiana* are well characterized with respect to pathogenicity towards several insects, and they have been used as biological control agents for agriculture pests worldwide (Sandhu et al. 2012).

Entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* have been widely used against insects and hence these are being exploited as fungal biopesticide on a large scale. Both fungi are proved pesticides against many crop pests and farmers are well acquainted with their use on the field. Using chemical or synthetic pesticides as a single tactic in controlling an important and significant livestock pests has been proved as dangerous as their indiscriminate use have often resulted in problems such as pesticide resistance, pest resurgence, residual toxicity and imbalance in ecological equilibrium (Narladkar et al. 2015).

8.5.2.1 Mode of Action of Entomopathogenic Fungi

In many cases, the control of pathogen can involve direct interaction between fungi and plants. The fungi are able to act as plant pathogen antagonists, i.e., they can use several different mechanisms, such as the production of metabolites (antibiotics, volatile compounds – ammonia, cyanide, alcohols, esters, ketones, etc., or enzymes), competition (for space, C, N, or mineral source), parasitism, or the induction of systemic resistance in the plant or an increase in its growth response, resulting in a reduction in the pathogen's activities (Vega et al. 2009). The genus *Trichoderma*

(Hypocreales) is one of the best known because of its activities against plant pathogens. It includes cosmopolitan species commonly found in the soil. Member of this genus present rapid growth and their major role in nature is as primary decomposer. In addition, *Trichoderma* spp. have been targets of studies and are used for commercial exploitation due to their ability to produce antibiotics and several enzymes of interest and their potential as biocontrol agents (Anees et al. 2010). These fungi are able to inhibit the growth of phytopathogenic fungi by inducing plant resistance or by acting directly against the pathogen as an antagonist, mycoparasite/competitor (Howell 2003; Verma et al. 2007). Knowing how the fungus behaves in the presence of other plant-parasitic fungi is essential to improve the application of this microorganism. One of the main uses of *Trichoderma* spp. is in the fight against the basidiomycete *Rhizoctonia solani*, a known phytopathogen that causes disease in the roots of several plants. Currently, new studies are being developed to explore strain that have already been used extensively in biocontrol strategy. For example, studies involving *Metarhizium* and *Beauveria* species generally focus on their application in the control of insect and other arthropods.

Entomopathogenic fungi constitute the largest single group of insect pathogen among microorganisms. Such insect-killing fungi are very first microorganisms to be recognized as disease-causing agent in insect. Entomogenous fungi are effective myco-biocontrol agents for a number of crop pests. Different species belonging to order Lepidoptera, Coleoptera, Homoptera, Hymenoptera and Diptera are susceptible to various fungal infections.

The Infection Process

Fungi have a unique mode of infection; they reach the haemocoel through the cuticle or possibly through the mouth part. Ingested fungal spore do not germinate in the gut and are voided in the faeces. The death of the insect result from a combination of factor: mechanical damage resulting from tissue invasions, depletion of nutrient resource and toxicosis, and production of toxin in the body of insect.

Conidial Attachment with the Cuticle

The entomopathogenic fungi host location is a random event and attachment being a passive process that takes place with the aid of wind or water. Attachment of a fungal spore to the cuticle surface of a susceptible host represents the initial event in the establishment of mycosis. It was observed that dry spore of *B. bassiana* possesses an outer layer composed of interwoven fascicles of hydrophobic rodlet. This rodlet layer appears to be special to the conidial stage and has not been reported on the vegetative cell. The adhesion of dry spore to the cuticle was suggested to be due to nonspecific hydrophobic force imposed by the rodlet. Moieties like lectins have also been found on the conidial surface of *B. bassiana*. It was also observed that lectin could be involved in binding between conidia and the insect cuticle. When the pathogen reaches and adheres to the host surface, it proceeds with rapid germination and growth which are profoundly influenced by the availability of water, nutrients, oxygen as well as pH, and temperature, and by the effect of toxic host-surface compound. Fungi with a broad host range germinate in culture in response to a wide range of nonspecific carbon and nitrogen source. Entomopathogenic fungi with limited host range seem to have more specific requirements for germination.

Formation of an Infection Structure

Entomopathogenic fungi invade their host by infection process, penetration of the host cuticle or put pressure on cuticle by making appressorium and then penetrate by penetration peg. The cuticles have two layers: the outer epicuticle and the procuticle. The epicuticle is a very complex thin structure which is deficient in chitin but contains phenol-stabilized protein and has a covering of waxy layer containing fatty acids, lipids and sterol. The procuticle forms the majority of the cuticle and contains chitin fibril embedded into a protein matrix together with lipids and quinones. Protein may account for up to 70% of the cuticle. In many areas of the cuticle, the chitin is organized helically giving rise to a laminate structure. Entomopathogenic fungi, *B. bassiana* conidia, germinate on the host surface and differentiate into structure termed appressorium (Sandhu 1995).

Penetration of the Cuticle

Entomopathogenic fungi need to penetrate through the cuticle into the insect body to obtain nutrient for their growth and reproduction. Entry into the host involves both enzymatic degradation and mechanical pressure as evidenced by the physical separation of lamellae by penetrated hyphae. A range of extracellular enzyme that can degrade the major component of insect cuticle, including chitinases, lipases, esterases and at least four different classes of proteases, have been suggested to functions during the fungal pathogenesis. These fungi begin their infective process when spores are retained on the integument surface, followed by the formation of the germinative tube, and then the fungi start excreting enzymes such as proteases, chitinase, quitobiases and lipases.

8.5.3 Nematophagous Fungi and the Biological Control of Nematodes

The lack of specificity of the symptoms caused by nematode parasitism is a great challenge in diagnosing their presence in crops. A wide range of infestations are observed including wilting, discoloration, reduced vigour, nutrient deficiency, root lesions, reduced flowering, fruit loss, low productivity and even death. Necrosis may be observed in the aerial parts that are fast spreading while in the roots, galls, cysts and agglomerates of worms may occur (Degenkolb and Vilcinskas 2016). For the control of these worms, nematophagous fungi have been experimented with.

Nematophagous fungi are microorganisms that can change from saprophytic to carnivorous behaviour. This happens under unfavourable conditions of growth and the fungi are able to feed on nematodes. The infection strategies are highly developed. The nematophagous fungal conidia germinate on the body of their host, and the hyphae penetrate the nematode and infect it (Sexton and Howlett 2006). There are more than 700 nematophagous fungi belonging to phyla Ascomycota, Basidiomycota, Zygomycota, etc. (Li et al. 2015). In these, some are toxin-producing and some have attack devices or structures (Lui et al. 2009). An example of nematophagous fungi is *Purpureocillium lilacinum* which is one of the most studied and tested fungi in the biological control of nematodes. It is popular among researchers for its ability to parasitize even nematode eggs (Atkins et al. 2005). Another positive aspect related to *Purpureocillium* species is the production of secondary metabolites that are able to promote plant growth, such as phytohormones (gibberellins and auxins), and substances that facilitate their defence against the harmful effects of biotic and abiotic stresses (pH and salinity, for example) (Khan et al. 2012). Another example of the nematophagous fungi is *Pleurotus* sp. which are edible mushrooms cultivated commercially. *Pleurotus ostreatus* produces toxins that immobilize the nematodes before infecting those (Satou et al. 2008).

8.6 Fungal Compounds Involved in Induction of Plant Responses

Fungal proteins such as xylanase, cellulase and swollen in are secreted by *Trichoderma* species but seem to induce only localized plant reactions and necrosis. *Trichoderma* endochitinase can also enhance defence, probably through induction of plant defence-related protein. Other proteins and peptides that are active in inducing terpenoid phytoalexin biosynthesis and peroxidase activity in cotton, e.g., the small protein, SM1, which has hydrophobin-like properties, were found to be produced by strains of *T. virens*. Another class of elicitors of plant defence includes oligosaccharides and low-molecular-weight compounds. These are released from fungal or plant cell walls by the activity of *Trichoderma* enzymes.

8.7 Genetic Engineering Studies of Fungi

A more widespread use of fungi for myco-biocontrol will depend on the improvement of wild-type strains by combining characteristic of different strains and mutants. Two types of improvement could be considered: (i) improving the efficacy of the insecticide, by reducing the dose necessary to kill the insect, by reducing the time to kill the pest or decreasing crop damage caused by the pest by reducing the feeding time; and (ii) expanding the host range.

8.8 Conclusion

By using chemical or synthetic pesticides as a single tactic in controlling an important and significant livestock, pests have been proved as dangerous as their indiscriminate use have often resulted in problems such as pesticide resistance, pest resurgence, residual toxicity and imbalance in ecological equilibrium. The fungus *M. anisopliae* (ICIPE-30) and *B. bassiana* (IMI-391510) spores prove as efficacious in infecting and killing larvae of *Anopheles stephensi* and *Anopheles gambiae* under laboratory conditions (Bukhari et al. 2010). It has been demonstrated that infection of adult mosquitoes *Culex quinquefasciatus* with *B. bassiana* causes a significant reduction in their survival and disease transmission under field condition (Howard et al. 2010; Narladkar et al. 2015).

The advantage of using fungi as myco-biocontrol agent is as follows: (1) Their high degree of specificity for pest control. Fungi can be applied to control harmful insect pests without affecting beneficial insects and non-harmful parasites. (2) The absence of effects on mammal and thus the reduction of the hazard normally encountered with insecticide application, such as pollution of the environment. (3) The lack of problem caused to insect resistance and prolonged pest control. (4) A high potential for further development by biotechnological research. (5) High persistence in the environment provides long-term effect of entomopathogenic fungi on pest suppression (Sandhu et al. 2012).

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Chapter 9
Halophilic Fungal Communities: Current Research and Future Challenges

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Contents

9.1 Introduction

Salinity has been observed to be an important parameter for the growth of microorganisms. However, many microorganisms have been adapted to survive under high salt concentrations; these microorganisms are termed as halophiles. These halophiles can further divided into slight, moderate and extreme halophiles, depending on the salt concentrations they generally thrive in (Yadav et al. 2020). Literature studies have highlighted the presence of many diverse microorganisms even under such conditions. Previous reports suggested the presence of mainly the prokaryotes under hypersaline conditions, until Gunde-Cimerman et al. (2000) have detected the

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presence of eukaryotic organisms such as fungi in hypersaline waters of salterns. After that, various fungal species have been identified in different hypersaline areas (Butinar et al. 2005; Cantrell and Baez-Félix 2010). These fungi are called halophilic fungi and are supposed to be present in great diversity in these habitats. Further, very few studies have utilized the genetic and proteomic approach to identify these fungal species and their abundance in saline environments. PCR-based techniques (involving 16sRNA) have been used in few studies for the identification purposes (Baati et al. 2010). Few recent explorations on using genomic approach have proved the presence of high acidic amino acid residues in proteins as an adaptation to saline environment, which is also the feature of other halophilic prokaryotes (Fukuchi et al. 2003; Kis-Papo et al. 2014). Further, genomic/proteomic analysis revealed the presence of novel mechanisms in halophilic fungi to combat high salt concentrations (Ali et al. 2019).

Studies have also provided the evidence of biotechnological advantages of these halophilic species, and it has been reported that the hypersaline species produces variety of bioactive compounds of industrial importance (beta carotene and ectoine) (Lentzen and Schwarz 2006; Oren 2010). These species are capable of producing specific compounds in different conditions, that is, hemolysis, antibacterials and acetylcholinesterase inhibition (Sepcic et al. 2011). In a study, Xiao et al. (2013) have identified various cytotoxic compounds from halophilic fungi of *Aspergillus* sp. isolated from solar salterns of Shandong, China; the compounds such as ergosterol, rosellichalasin and cytochalasin E have been identified; and these compounds show effective anticancerous activity. Moreover, halophilic fungi have also been identified for the production of enzymes such as protease (Annapurna et al. 2012), amylase (Ali et al. 2014) and cellulose (Gunny et al. 2014). Further, many important information and biotechnological applications of halophilic fungi have been discussed and summarized in this chapter.

9.2 Application of Halophilic Fungi

9.2.1 Industrially Important Compounds

Extreme environments were once considered to have no life. However, research revealed that significant microbial diversity exists in these extreme conditions. Halophilic 'salt-loving' microorganisms generally show low nutritional requirements and are also resistant to high salt concentration along with the ability of maintaining a balance in osmotic pressure (DasSarma and Arora 2002; Roberts 2005; Yadav et al. 2019). This adaptation is generally based on their cellular feature of storing KCl (salt in strategy) or the accumulation of compatible solute (salt out strategy) (Corral et al. 2020). Advancement of culture techniques, molecular techniques and chemotaxonomic studies have profoundly helped us in understanding the diversity of halophilic microorganisms. These microorganisms also show diverse physiology, such as anaerobic, heterotrophic, phototrophic, and chemotrophic and chemoautotrophic (Oren 2008; Mesbah and Wiegel 2012). Adverse environment leads to the production of unique biomolecules with complex cellular biochemistry (Ibrar et al. 2020).

With the advance of biotechnological methods and culture of these halophilic microorganisms pave the way to get novel drug molecules for therapeutic purposes. A number of such medicinally and industrially important biomolecules were extracted from the halophilic microorganisms (Walter et al. 2011). These microorganisms are known to produce various pigments, proteins, enzymes, polymers and biofertilizers. Besides these applications, such microorganisms are also being used in the industries for various purposes, namely, fermented food products preservatives, fibre, plastics and biosensors (Dassarma et al. 2010).

Almost all living organisms including prokaryotes and eukaryotes produce some type of bioactive compound. These compounds possess different activities, namely, antimicrobial, anti-inflammatory, anti-allergic and anticancerous. Therefore, these compounds have become potential drug molecules. From the last four decades, the number of such bioactive compounds has been isolated from many species of bacteria and fungi acclimatized in extreme environments (Giddings and Newman 2015). The search of such novel microorganisms is of great importance. A number of studies were conducted in India and abroad to search these microorganisms inhabiting extreme environments. Novel features of these microorganisms make them a choice for the study of the bioactive compounds in them. Recent advancements in cutting edge biotechnology are helping in understanding the molecular mechanism by which these extremophiles produce new bioactive compounds.

Fungi are believed to be the second most diverse organisms on this planet with more than 3–5 million species (Blackwell 2011). So far, approximately 0.1 million species have been described, and out of this, only a minor proportion has been investigated for their pharmacological properties. *Aspergillus*, *Penicillium* and *Fusarium* are some of the regular producers of bioactive compounds. The process by which microorganisms synthesize these secondary metabolites is also being researched. Microorganisms show an interaction with their environment and also with the life form in the surrounding. Sometimes, it is a mutualistic interaction with some hosts. Fungal-derived secondary metabolites show diversity in their structure and biological activity (Devi et al. 2020). The era of fungal-derived medicine was started with the discovery of penicillin by Alexander Fleming in 1929. Later, the search for the fungal-derived antibiotics geared up, and the fungi are now considered as the ‘goldmine’. Griseofulvin produced by *Penicillium griseofulvum* and fusidic acid produced by fungi imperfecti are the examples of two such antibiotics.

In the domain Archaea, Bacteria and Eukarya, the halophiles are found with great diversity. The similar diversity is also present in the metabolic profile of these microorganisms. In present discussion, emphasis is given to the halophilic fungi. Fungi are also ubiquitous in nature and found in all known environments of the earth including the extreme environments. Report suggest that the fungi exists in the hypersaline environment (Gostinčar et al. 2011). Halophilic fungi are the best studied fungal extremophile found in salt lakes and salterns around the world (Buchalo et al. 1998; Casamayor et al. 2002). The classification of these extremophiles is based on different environmental variables such as pH, temperature, pressure and salinity (Gunde-Cimerman et al. 2004; Satyanarayana et al. 2005; de Oliveira et al. 2015). In the last decade, much emphasis is given to the extremophilic fungi due to the therapeutic applications of the bioactive molecules they produce. In the last decade, the fungi were discovered to be present in the hypersaline environment (Gunde-Cimerman et al. 2000). However, the role of fungi in such an environment is still being investigated (Gunde-Cimerman et al. 2004). The study conducted on the halophilic strains of bacteria in salt lake of Jaipur, India, also led to the discovery of novel bacterial products with medicinal properties (Jose and Jebakumar 2013). Similar studies were conducted in the Saline Saharan Soil and found the halophilic *Actinomycete* strain AH97 with broad-spectrum antibacterial and antifungal properties (Boudjelal et al. 2011). A number of rare *Actinomycetes* were isolated from different environments in search of novel bioactive compounds (Cai et al. 2009; Cavaletti and Marinelli 2017). Compared to halophilic bacteria, very few reports are available on the application of halophilic fungi in the production of bioactive molecules.

According to a study, the *Aspergillums* sp. is found to produce compounds with anticancer property. High salt concentration was further found to increase the production of these compounds (cytochalasin E, ergosterol and rosellichalasin) (Xiao et al. 2013). All of these compounds were researched for their efficacy against various cancerous cell lines. *Aspergillus* and *Penicillium* are the two main genera that dominate the hypersaline environment. Several stains of these species have been isolated from the saline environment. One of the strains of *Aspergillus* named 8Na is able to grow in a wide range of pH, temperature and salinity. These particular strains had antimicrobial properties against human pathogens.

In one of the studies conducted in Weihai, China, the ethyl acetate extract of halotolerant *Aspergillus* sp. from solar saltern found to produce three compounds (ergosterol, rosellichalasin and cytochalasin E(60-62)) with significant cytotoxic activity against human cell lines (RKO, A-549 and BEL-7402) (Xiao et al. 2013). The novel ceramide compound named N-acetyl-3, 5, 11, 18-tetrahydroxyoctadecyl-2-amine was isolated from another halotolerant fungus *Myrothecium* sp. GS-17 from saline soil in Gansu, China. Investigation showed that this compound has cytotoxicity against human cell lines (PC-3, HL-60 and MCF-7) (Liu et al. 2015). Anticancer potential of four *Aspergillus* strains from Sonoran Desert was investigated. The two new metabolites terrequinone A and terrefuranone along with four known compounds were extracted from these fungal strains (He et al. 2004). Studies also suggest that these metabolites have no direct role in the adaptation of fungi in the extreme environment. However, antimicrobial properties of these compounds indirectly give them an edge over other microorganisms to thrive in a limited nutrient environment. Much is still to be investigated regarding the growth and culture of these extremophiles in vitro. The research in the field of metagenomics of halophilic fungi and the new screening techniques will open up new commercial and industrial avenues. Finally, the adaptation of these extremophilic fungi to harsh environments needs to be explored.

9.2.2 Remediation of Pollutants

Increasing industrialization and world population cause negative impact on the environment (Dey et al. 2016; Gola et al. 2016b, 2020a). Multiple toxic contaminants such as dye, heavy metal, hydrocarbons, pesticides and antibiotics and their occurrence in above the threshold limits in soil and water have been reported by the multiple authors (Duncan et al. 2018; Addo-Bediako et al. 2018; Adeshina et al. 2019). The main cause of their occurrence in land and water is due to the discharge of partially or untreated wastewater directly into the nearby water bodies (river, lake, canal, etc.) or land. In addition to this, irrigation practice with contaminated wastewater due to the unavailability of fresh water cause production of contaminated vegetables and crops (Gola et al. 2016a). Consumption of such agricultural products possesses great health risk to the human health (Roba et al. 2016; Gola et al. 2017). To remediate the toxic contaminants, multiple physical, chemical and biological techniques have been developed with time (Lafi and Al-Qodah 2006; Gola et al. 2016a, 2019; Mathur et al. 2018; Heidari et al. 2019; Bahrami et al. 2019; Jain et al. 2020). However, biological methods showed upper hand in decontamination of toxic pollutants due to its economic value and production of less toxic by-product produced during the treatment process. Microbes play important role during biological wastewater treatment (Chawla et al. 2020; Gola et al. 2020b), and using halophilic fungi may provide an additional advantage. Most of the wastewater showed high salinity content, and halophilic fungi can easily survive this high salt condition. Hence, the present section discusses about the usage of halophilic fungi in the treatment of wastewater.

Jiang et al. (2016) investigated the phenol degradation capacity of halophilic fungi isolate (*Debaryomyces* sp.). However, phenol is a common pollutant in the effluent discharged by the multiple industries (chemical, paint, plastic and pharma). The fungal isolate was able to degrade phenol even at high pH (10) and salinity (15%), mimicking the actual wastewater conditions. It was observed that under optimized conditions, the fungal strain can degrade up to 100% phenol (initial concentration 500 mg/L and at pH 6) within 32 h. In addition to this, phenol degradation efficacy of the fungal strain does not change in the presence of toxic heavy metal ions (Zn and Mn) in the synthetic wastewater. Lu et al. (2017) examined the azo dye (Congo red) absorption capacity of marine isolated halophilic fungal strain (*Aspergillus niger* ZJUBE-1). The fugal pellets were used for the experiments, and up to 98% dye removal was observed from the synthetic wastewater with an absorption capacity of 263.2 mg/g. Further, no significant change was observed in the dye removal efficacy of the fungal pellets with the change in pH. Up to 88.66% and 98.7% dye removal was observed at pH 10 and 2, respectively. Moreover, UV-Vis spectrum of treated wastewater shows new peak that might be due to the degradation of Congo red during the absorption process. The appearance of new peak indicates towards the dye degradation ability of the *Aspergillus niger* ZJUBE-1 during the removal process. The presence of heavy metal ions in industrial effluent discharges has been reported worldwide (Bhattacharya et al. 2015; Gola et al. 2020a).

Lotlikar et al. (2018) examined the Cr removal ability of marine drive fungal strain (*Aspergillus sydowii*). Up to 26% removal was observed for the Cr at an initial concentration of 300 mg/L in the liquid broth. Further, analytical techniques such as scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS) indicate the morphological changes in mycelia along with the deposition of Cr inside the biomass of *Aspergillus sydowii*, indicating the active uptake of Cr ion from the liquid broth. Aydogan and Arslan (2015) investigated the dye removal potential of halotolerant fungal strain (*Aspergillus flavipes* MA25) via biosorption mechanism. The fungal strain was able to remove up to 100% dye from the unsterilized synthetic wastewater at an initial dye concentration of 0.2 g/L. Further, it was observed that the strain was able to remove the toxic dye at high salt concentration (15 g/L). In addition to this, the growth of the fungal strain was not affected by the increasing concentration of the dye (up to 04.4 g/L). *Aspergillus flavipes* showed great potential in treating the wastewater contaminated with the toxic dye.

Nazareth et al. (2012) isolated multiples fungal strains from the mangroves and salters region of Goa (India) on the basis of morphological appearance (colony appearance and spore colour). All the fungal species belong to genus *Aspergillus* (16 strains), *Penicillium* (9 strains), *Paecilomyces* (4 strains), *Fusarium* (2 strains), *Alternaria* (3 strains) and *Cladosporium* (1 strains). Out of all the above stains, species belonging to *Penicillium* genus showed maximum tolerance to salt concentration (17.5%). Further, maximum tolerance to lead (Pb: 10.0 mM) was observed with species belonging to genus *Aspergillus* followed by genus *Penicillium* that can tolerate Pb concentration up to 7.5 mM. While for copper, maximum tolerance was observed for species belonging to *Penicillium* genus followed by *Aspergillus* species. For cadmium metal, maximum resistance was showed by *Penicillium* species followed by species belonging to *Paecilomyces* species. It was concluded that tolerance against heavy metal was dependent on the metal ion present as well as the fungal species taken for the experiment. The above fungal stains showed great potential in remediating heavy metals (Pb, Cd and Cu) and can be optimized further to increase their heavy metal removal efficiency. Ferreira-Guedes et al. (2012) studied the pesticide (2,4-dichlorophenoxyacetic acid) degradation ability of fungal strain (*Penicillium chrysogenum*) isolated from the salt mine present in Algarve (Portugal). The degradation ability of fungal strain was tested with 2,4-dichlorophenoxyacetic acid (100 mg/L) as sole carbon source and at different salt concentration (0–5.9%). Up to 2% degradation was observed with 2% NaCl; however, degradation ability of the fungal strain increases with the decrease in initial concentration of 2,4-dichlorophenoxyacetic acid. Up to 14% degradation of 2,4-dichlorophenoxyacetic acid was observed at initial concentration of 40 mg/L, indicating the toxicity of the 2,4-dichlorophenoxyacetic acid at higher concentration. Further, it was observed that addition of different carbon source such as glucose, lactose and sucrose in the media increases the pesticide degradation ability of *Penicillium chrysogenum*.

The presence of additional carbon source increases 2,4-dichlorophenoxyacetic acid degradation up to 7%, 3% and 18% with glucose, lactose and sucrose, respectively. González-Abradelo et al. (2019) studied the biodegradation capability of two halotolerant fungi (*Aspergillus sydowii* and *Aspergillus destruens*) against xenobiotic compounds [PAHs (polycyclic aromatic hydrocarbons)]. Both these xenobiotic compounds have multiple negative affect on environment and have been reported to cause multiple health issues. Different concentration (60–240 ppm) of benzo-α-pyrene and phenanthrene (belonging to PAH) were taken for the degradation studies, and removal rate was observed at different time interval. It was observed that *Aspergillus sydowii* was able to degrade up to 99% and 97% of benzo-α-pyrene and phenanthrene, respectively, within the 12 days in synthetic wastewater.

On the other hand, degradation ability of *Aspergillus destruens* was on lower side as compared to *Aspergillus sydowii. Aspergillus sydowii* showed up to 97% and 55% degradation for phenanthrene and benzo-α-pyrene, respectively, during the 12-day experiment. After obtaining significant degradation of PAH in synthetic wastewater, experiments were performed on actual wastewater (collected from wood-processing biorefinery) contaminated with different PAHs (naphthalene 11.8 mg/L; acenaphthene 11.9 mg/L; fluorine 18.4 mg/L; anthracene 13.7 mg/L; phenanthrene 212.0 mg/L; fluoranthene 121.4 mg/L; benzo-α-anthracene 18.8 mg/L; chrysene 9.4 mg/L; pyrene86.8 mg/L; benzo-α-fluoranthene 8.4 mg/L; benzo[k]fluoranthene 2.9 mg/L; benzo(j) fluoranthene 3.2 mg/L; benzo-α-pyrene 3.3 mg/L). Up to 100% removal for all the PAHs was observed by both the *Aspergillus* strain, indicating the potential of halo-tolerate in remediating the xenobiotic compounds such as PAHs. From the above studies, it can be concluded that halophilic fungi can act as a good candidate in removal of toxic pollutant from the wastewater. However, all these studies are performed under optimized conditions and required validation at large scale, and hence, more studies are need in this regard. The next section discusses about the production of different enzymes using halophilic fungi.

9.2.3 Enzyme Production

Enzymes play an important role in healthcare, food, agriculture, textile, paper and pulp industries (Godfrey and Reichelt 1984; Madhu and Chakraborty 2017; Piotrowska-Długosz 2019; Yushkova et al. 2019; Abdelrazek et al. 2019; (Kour et al. 2019). Microbes can act as a good source of enzyme, and halophilic fungi enzymes can provide additional advantage as they can tolerate extreme conditions (Table 9.1). This section discusses about the various enzymes produced by the halophilic fungi under optimized condition.

Ali et al. (2014) reported α-amylase from *Aspergillus flavus* isolated from man-made saltern 35 KDa and specific activity was 131.02 U/mg. The enzyme kinetics showed *V*max and *Km* values of 8.36 U/mg and 6.33 mg/mL, respectively. The enzyme characterization studies showed the highest residual activity at pH 5 and temperature 60 °C showing the thermophilic nature of the enzyme. The residual activity increases from 30 to 60 °C, and after that, there was a sharp decline in the activity. Amylase showed extremophilic property as its residual activity increases from 5% to 25% of salt concentration and showed highest residual activity at 30%

Table 9.1 Enzyme produced by halophilic fungi

Enzyme	Species	References
Amylase	*Aspergillus gracilis*	Ali et al. (2014)
Amylase	*Aspergillus penicillioides*	Ali et al. (2015)
Cellulase	*Stachybotrys microspora*	Ben Hmad et al. (2017)
Cellulase	*Aspergillus flavus*	Bano et al. (2019)
Lipase	*Fusarium solani*	Geoffry and Achur (2018)
Protease	*Aspergillus flavus*	Annapurna et al. (2012)
Xylanase	*Aureobasidium pullulans*	Yegin (2017)

NaCl concentration. Ali et al. (2015) reported α-amylase from *Aspergillus penicillioides* and they were purified using column chromatography. The molecular weight of amylase was estimated to be 42 kDa and specific activity was 118.42 U/mg. The K_m and V_{max} values of enzyme kinetics are 1.05 μmol/min/mg and 5.41 mg/mL, respectively. The characteristic properties of enzyme showed a gradual increase in residual activity from pH 5 to 8 with the highest activity at pH 9 showing the alkalophilic nature of enzyme. α-amylase showed highest residual activity at temperature 80 °C, and after that, decrease in activity was found. In a similar study, Ali et al. (2014) extracted α-amylase from *Aspergillus gracilis* and showed best activity at pH-5 with 30% NaCl concentration at 60 °C. Further amylase activity was enhanced by the addition of $CaCl_2$, while $ZnCl_2$, $FeCl_2$ and EDTA showed the inhibitory effect. While enzyme incubated in the presence of detergent, 80% of activity was retained.

Ben Hmad et al. (2017) reported the novel cellulase (endoglucanase) from *Stachybotrys microspora* that showed specific activity 128.6 U/mg and molecular weight 55 kDa. The enzyme exhibited its optimum activity at pH 7 and temperature 50 °C. It showed thermostable property as it retained 100% activity from 30 to 50 °C and alkaline nature indicating stability from pH 5 to 9. The enzyme activity increases with increasing slat concentration and showed maximum activity at 30% salt concentration. In the presence of NaCl, the optimum activity of enzyme was observed at pH 8 and 70 °C. Enzyme was highly stable in the presence of 10% SDS. Bano et al. (2019) reported cellulase production from *A. flavus* and were purified using gel filtration column chromatography. The molecular weight of enzyme was 55 kDa and specific activity was 62.9 U/mg. The enzyme kinetics showed the K_m and V_{max} value 3.02 mg/mL and 37.87 mol/min/mg. The enzyme characterization showed the highest activity at pH 10 showing the alkaline nature of amylase, and after that, a sharp decline in relative activity was observed. With increasing temperature from 30 to 60 °C, the relative activity of enzyme increases and further decreases with increasing temperature. α-amylase showed the extremophilic nature as the relative activity increases with increasing salt concentration from 5% to 25%. It retained 100% relative activity at 20% NaCl concentration. Amylase showed >90% relative activity with addition of 2 mM of divalent cation, but with EDTA, relative activity was inhibited.

Geoffry and Achur (2018) reported lipase production from *Fusarium solani* using palm oil mill effluent. Using one factor at a time (OFAT), Plackett–Burman design and Face-centred central composite design (FCCD), medium components such as K_2HPO_4, $NaNO_3$ and Tween 80 were optimized. The activity of enzyme found using optimized medium components was 7.8 U/mL. The highest activity of lipase was observed at pH 8 and 9 showing the alkaline nature of enzyme. Annapurna et al. (2012) reported protease from *Aspergillus flavus* isolated from soil samples. Enzyme characterization revealed the optimum pH 6 from higher activity showing acidic nature and optimum temperature 57 °C showing thermophilic property of enzyme. Protease was activated in the presence of divalent cation $CaCl_2$ and inhibited by $HgCl_2$. Yegin (2017) reported extracellular xylanase *Aureobasidium pullulans* produced on wheat bran. The molecular weight of purified xylanase was 21.6 kDa. According to enzyme characterization, optimum pH of enzyme was 4 and retained 90–95% activity between pH 3 and 8. The optimum temperature was 50 °C, and a further increase in temperature above 50 °C led to decreases in enzyme activity. The half-life of the enzyme was estimated to be 47.15 min at 60 °C, and inactivation energy was 218 kJ/mol. The K_m and V_{max} values of enzyme kinetics were 19.43 mg/mL and 848.4 U/mL, respectively. Xylanase showed tolerance towards 10% ethanol and showed 100% relative activity with 0–20% NaCl concentration. It showed resistance towards metal ions and reagents such as Mg^{2+}, Zn^{2+}, Cu^{2+}, K^+, EDTA and β-mercaptoethanol.

9.2.4 Biofuel Production

Fungi are termed as oleaginous microbes as more than 20% of their biomass is composed of lipid (Wynn and Ratledge 2005). This lipid is mostly in the form of triglycerides (TAGs) and stored in intracellular compartments as a reserve supply of carbon and energy. TAG accumulation has been found to take place during late logarithmic phase with maximum production in stationary phase under limited nitrogen and excess carbon conditions (Ratledge and Wynn 2002). After biomass harvesting and lipid extraction, the derived lipid is converted into biodiesel using transesterification reaction.

Halophytic fungi are promising microbes for biofuel production because of the following advantages. (1) They have higher oil productivity than plants and vegetable oils and can be grown all year round. (2) They can be grown on inexpensive feedstocks such as agricultural waste, sugarcane distillery wastewater, sewage sludge, waste molasses and monosodium glutamate wastewater. (3) Fungi can be cultivated under controlled environmental conditions without any effect from weather fluctuations. (4) They have unique fatty acid profiles with the presence of certain groups such as γ-linolenic acid (GLA) that are absent or present in very low amount in other oleaginous microbes. (5) Fungi can be genetically modified to enhance lipid synthesis with certain genes expression (Dong et al. 2016; Patel et al.

Table 9.2 Various fungi with their lipid content

Fungal species	Lipid content (% w/w)	References
Mortierella isabellina	50.5	Harde et al. (2016)
Mortierella vinacea	66	Subramaniam et al. (2010)
Mucor circinelloides	19.6	Vicente et al. (2009)
Cunninghamella echinulata	46.6	Gema et al. (2002)
Mortierella alpina	31.1	Eroshin et al. (2000)
Mortierella ramanniana	42	Papanikolaou et al. (2017)

2020). Table 9.2 lists various fungi with their lipid content that demonstrated the potential for biofuel production (Table 9.2).

Bioethanol production using lignocellulosic biomass is a sustainable process with integration of waste utilization and biofuel production. Agricultural and forest residues, woody biomass and agricultural by-products are one of the most abundant lignocellulosic bio-resources present on earth. Lignocellulose consists of cellulose, hemicellulose linked together by β-1,4 sugar polymer, lignin, a small amount of pectin and nitrogenous compounds (Chen and Chen 2014). The degradation of this lignocellulosic biomass is one of the most important challenges to produce bioethanol from waste. Biofuel production from lignocellulosic biomass is generally a three-step process: pretreatment to remove lignin, using cellulases for hydrolysis of exposed cellulose to simple sugars and finally the fermentation of sugars to bioethanol or biobutanol. The lignin pretreatment often involves various physical and chemical processes which suffer from high-energy demand, usage of toxic chemicals, corrosion effects and contaminant discharge in the environment. Fungal treatment for lignin degradation and cellulase production is a promising green technology that has the potential to substitute toxic and energy intensive physical and chemical treatments.

Three groups of fungi have been known for lignin degradation: white-rot, brown-rot and soft-rot fungi. Out of these, white rot is effective in degrading both lignin and cellulose and thus helps in bioethanol production. Lignin breakdown by brown and soft rot has been found to be slow and incomplete. Table 9.3 shows the lignin removal efficiency of some white-rot fungi (Sánchez 2009).

9.3 Conclusion

With isolation of various halophilic fungi in diverse saline environments, the study of understanding the mechanisms of adaptations, identification, abundance and applications has already began. Researchers have used various proteomic and genomic tools to study the insight mechanisms used by halophilic fungi. Studies have highlighted many novel steps and modifications adopted by these fungal strains to survive and flourish under saline conditions. Further, research has also proved the tremendous potential residing in these fungal strains, various active metabolites and

Table 9.3 Lignin removal efficiency of some white-rot fungi

Fungal strain	Type of biomass	Culture technique used	Lignin removal (%)	Source
Trametes hirsuta	Corn stover	Solid state	71.99	Sun et al. (2011)
Pleurotus ostreatus	Rice straw	Submerged	41	Taniguchi et al. (2005)
Trametes versicolor	Rubber wood	Solid state	34.4	Nazarpour et al. (2013)
Cyathus pallidus	Corn stover	Submerged	38.1	Saha et al. (2016)

industrially important enzymes, and also the roles of isolated fungal strains in different bioremediation processes have also been recognized and established using advance scientific tools. The identified metabolites from halophilic fungi were found to be efficient antimicrobial, ***anticancerous and hemolytic agents. Further, in remediation processes (phenol degradation, heavy metal removal and improvement of saline soil) and other environmental issues, halophilic fungi have shown effectiveness. Moreover, with enormous potential highlighted by halophilic fungi, it is important to isolate and study more halophilic fungi for its wide-scale application in different sectors.

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Chapter 10
Extremophilic Fungi and Their Role in Control of Pathogenic Microbes

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10.1 Introduction

Biological ecosystems possess significant variation in environmental niches with different abiotic conditions. These abiotic conditions and factors differ within the ecosystem, resulting in the formation of specific zones termed “extreme habitats” (Grum-Grzhimaylo et al. 2016). Organisms which possess ability to survive in these extreme habitats are considered extremophiles. These habitats have extreme conditions of temperature, pressure, pH, salinity, nutrient concentrations, radiation, harmful heavy metals, and toxic compounds (Canganella and Wiegel 2011; Goswami and Das 2016; Moayad et al. 2017). Some of these territories had earlier been argued not to support microbial life; notable among them include hot springs, hydrothermal and geothermal vents, the cold Arctic, acidic conditions, saturated salt brines, and pressurized abyssal waters (Bendia et al. 2018; Gostinčar et al. 2010; Stetter 1999; Zhang 2016). Due to adaptation of these species to varying habitats, they could be classified as thermophiles (high temperature), psychrophiles (low temperature), acidophiles (low pH), alkaliphiles (high pH), piezophiles (high pressure), halophiles (high salt concentration), osmophiles (high concentration of organic solutes), oligotrophs (low concentration of solutes and/or nutrients), and xerophiles (very dry environment) (Seckbach and Rampelotto 2015; Gupta et al. 2014; Kour et al. 2019; Yadav et al. 2015c, 2020a).

Some of the conditions that trigger instrisic mechanisms in fungi are linked to a number of biochemical, structural, and spore-forming processes, thus enabling their survival in harsh environments. Also, besides being heterotrophic eukaryotic organisms and reproducing both sexually and asexually, they inherently possess diverse biotechnological, agricultural, and medical uses (Odoh et al. 2017b). For example, fungi from extreme environments are a rich source for novel natural products for pharmaceutical industries (Manimegalai et al. 2013; Zhang et al. 2018), biofuel synthesis (Javaid et al. 2019), industrial biotechnological applications (Cavicchioli et al. 2002; Hassan et al. 2019), and bioremediation of contaminated soil and sediments (Selbmann 2019). Over the years, extremophilic fungi such as *Trichoderma* sp. have been documented as efficient biocontrol agents against plant pathogens (Carreras-Villasenor et al. 2019; Nieto-jacobo et al. 2017; Kim et al. 2014; Sharma et al. 2019). Although extremophilic organisms have been shown to be present in all three domains of life (archaea, bacteria, and eukaryotes), the concept of extremophiles may be relative, as conditions that are “extreme” to one organism may be essential for the survival of another (Irwin and Baird 2004). Organisms that survive and thrive under conditions that are detrimental to the majority of other species have become a focus of increasing scientific attention over the last few years, with some astonishing discoveries of stress-tolerating mechanisms.

In recent times, some studies have directed their focus toward the exploration of extremophilic species (bacteria and fungi) while harnessing their role in biotechnological applications (Rastegari et al. 2020a, b; Yadav et al. 2020b, c). In medicine, fungal agents thriving under extreme conditions have been gaining prominence (Bari Kishor and Padalia 2015). Even though a number of these organisms are considered neutrophils (growing optimally in moderate conditions, e.g., 25–30 °C, pH 5–7), some of them have acquired intrinsic properties, enabling their growth in harsh environments (Grum-Grzhimaylo et al. 2013). Since the mid-1990s, discoveries of fungi growing in diverse extreme environments have broadened the study of extremophilic microbiology, which previously focused on the study of prokaryotic microorganisms. However, more insights about some of the eukaryotic adaptations have been given (Kogej et al. 2006). In this chapter, we tried to unravel extremophilic fungi and the mechanism of their adaptation in unfavorable conditions while also highlighting their application in the control of pathogenic microbes.

10.2 Extremophilic Fungi

Microorganisms living within extreme environments were previously restricted to prokaryotes (Gunde-Cimerman and Zalar 2014). The term “extremophile” was first used by MacElroy in 1974 to describe a broad group of organisms which lived optimally under extreme conditions, and the taxonomic range of these organisms has expanded from prokaryotes to all three domains—Eucarya, Bacteria, and Archaea (Zhang et al. 2018). The term “extremophile” according to Seckbach and Rampelotto (2015) also includes microorganisms growing in the presence of high metal concentrations or high doses of radiation. They may be found thriving from the frigid environments of the Antarctic to the superheated waters of the hydrothermal vents, from the bottom of 11-km deep ocean trenches to the high altitudes of the atmosphere, from acidic to alkaline (Stan-Lotter and Fendrihan 2012); some may grow in toxic waste, organic solvents, heavy metals, or in several other environments that are considered harsh and difficult to survive (Seckbach and Rampelotto 2015). For every extreme environmental condition investigated, extremophiles have shown that they not only can tolerate these conditions, but often require those conditions for survival (Rampelotto 2010). Fungi living in uneven environments have adapted to a number of factors such as pressure and temperature (Pettit 2011), or the alkaline pH and high salinity of soda lakes (Charlesworth and Burns 2016). In these environments, microbial inhabitants (fungi) become highly specialized with specific protein such as enzymes acting against adverse environmental denaturation.

Furthermore, a number of proteins sourced from extremophilic fungi have already been utilized in the industry for purposes as diverse as molecular biology reagents (Terpe 2013) or as common place as laundry detergents (Charlesworth and Burns 2016). Extremophilic fungi also present some biocontrol or bioremediation ability (Zhuang et al. 2010; Akpi et al. 2017b) as a result of their metabolic activities and tolerance to certain conditions (Akpi et al. 2017a). There is also a growing interest in sourcing these extremophilic fungi for natural products particularly those displaying antimicrobial activities (Pettit 2011; Charlesworth and Burns 2016). In understanding this section, we focused and laid much emphasis on classifications of extremophilic fungi.

10.3 Classification of Extremophilic Fungi

10.3.1 Acidophilic or Alkaliphilic Fungi

Acidophilic or alkaliphilic fungi are groups of organisms that thrive at the extremes of pH. They exhibit optimal growth below pH 3 and/or pH greater than 9 (Tiquia-Arashiro and Rodrigues 2016; Wiegel 2011). Found in various habitats across the globe, acidophiles flourish in sites of acid mine drainage, solfataric fields, acidothermal hot springs and fumaroles, coal spoils, and bioreactors (Hallberg et al. 2010; Reeb and Bhattacharya 2010), while alkaliphiles are isolated from normal environments such as garden soil. They proliferate in alkali thermal hot springs, shallow hydrothermal systems, sewage, and hypersaline soda lakes (Tiquia-Arashiro and Rodrigues 2016; Kanekar et al. 2012).

Acidophiles use a variety of pH homeostatic mechanisms that involve restricting proton entry by the cytoplasmic membrane and purging of protons and its effects by the cytoplasm. They have a highly impermeable cell membrane restricting proton influx into the cytoplasm to help maintain ΔpH. Because the membrane proton permeability determines the rate at which protons leak inward, the balance between proton permeability, proton influx through energetic and transport systems, and the rate of outward proton pumping determines cell-appropriate proton motive force (PMF). The second strategy involves the reduction of H^+ influx through transmembrane channels. In addition, generation of a Donnan potential through the accumulation of monovalent cations in the cytoplasm could also be used as an adaptive mechanism. The high intracellular cation concentration generates a positive charge gradient $\Delta\psi$, which inhibits$^+$ influx despite the favorable concentration gradient. K^+/H^+ antiporters with stoichiometries of >1:1 are employed to promote the formation of this Donnan potential. These antiporters and ATP-dependent H^+ pumps promote efflux of H+ and resist cytoplasmic acidification (Tiquia-Arashiro and Rodrigues 2016; Das et al. 2009; Enami et al. 2010). Alkaliphiles on the other hand live in an environment characterized with low H^+. Here, the organisms continuously neutralize the cytoplasm, enabling H^+ influx to drive ATP synthesis. One of the most prominent adaptations is the use of Na^+/H^+ and K^+/H^+ antiporters to move H^+ into and monovalent cations out of the cell (Krulwich et al. 2009; Mesbah et al. 2009; Mesbah and Wiegel 2011; Tiquia-Arashiro and Rodrigues 2016).

Furthermore, acidophiles have good applications in the metal industry for metal extraction from ores and as a source of gene products, for example, acid-stable enzymes with applications as lubricants and catalysts. They are also exploited for the synthesis of nanoparticles (Tiquia -Arashiro and Rodrigues 2016).

10.3.2 Xerophilic Fungi

Xerophilic fungi are yeast and molds that are capable of growing at low water activity or low equilibrium relative humidity. They are an important part of the indoor fungal community which has *Aspergillus* as one of the dominant genera (Visagie et al. 2017). These organisms have developed physiological aptitude that sustains the functioning of their biological pathways in environments with little or no water availability. External conditions of low water activity are sensed by membrane osmosensors, thus triggering xerophiles to accrue glycerol as a compatible solute to balance both internal and external osmotic pressure. This biological condition modifies the cell membranes to retain glycerol within the cell (Petterson and Leong 2011).

In nature, organisms such as *Aspergillus penicillioides* and *Aspergillus restrictus* are broadly distributed and are composed of trait which makes them significant for the built environment as well as the food industry. *Aspergillus* species are among the primary colonizers of building materials (Flannigan and Miller 2011) and are found in museums or libraries and on historic artifacts such as books, paintings, leather, softwood, and a variety of textiles and dried specimens (Cavka et al. 2010; Micheluz et al. 2015; Pinar et al. 2013; Pinar et al. 2015; Samson et al. 2010). The *Aspergillus* species has economic impact on the food industry owing to its ability to grow on stored grain, cereals, or preserved foods with high sugar (i.e., jams, maple syrup) or salt content (i.e., biltong, dried fish) (Samson et al. 2010; Odoh et al. 2017a).

Furthermore, xerophilic fungi produce many extrolites, exhibiting a wide range of biological activities (Gomes et al. 2012; Kanokmedhakul et al. 2011). For instance, compounds from *Aspergillus chevalieri* have shown to be active against the malaria parasite (*Plasmodium falciparum*), *Mycobacterium tuberculosis* and cancer cells (Kanokmedhakul et al. 2011). An antitumor compound has recently been reported from *Aspergillus cristatus*, while many compounds are known to be antioxidants (Visagie et al. 2017). Some xerophilic fungi produce mycotoxins, such as echinulin, flavoglaucin, and physcion, which are toxic to animals (Visagie et al. 2017; Greco et al. 2015). Xerophile microbes possess multifarious plant growth-promoting attributes and are highly effective as biofertilizers for soil health and sustainable agriculture (Yadav 2017).

10.3.3 Halophilic Fungi

Halophilic fungi are defined as those fungi which grow favorably in salt concentration or hyper saline environments. This is characterized by saturated salt concentrations (NaCl), with all inhabited species being tolerant to salt conditions (halophilic) (Gunde-Cimerman et al. 2018; Yadav et al. 2020a). Across the globe, these organisms have been isolated from environments with the salinity level above 1.7 M (Ali et al. 2014). In the past, fungi were not considered as part of hypersaline environments until they were first reported as an active inhabitant of solar salterns decades ago (Ali et al. 2014). Many species including *Aspergillus, Cladosporium, Pencillium*, and some yeast including those which were previously reported only to be of food contaminants have been isolated from hypersaline habitats (Cantrell and Baez-Félix 2010).

The mechanisms used by these salt-tolerant microorganisms to withstand the high salt concentrations and to adapt to changes in the salt levels are diverse. To maintain a high osmotic pressure inside the cells, processes such as "salt in" strategy (where osmotic balance is achieved by accumulating high concentrations of inorganic salts in the medium) and "compatible solute" strategy (which allows an enzyme to function effectively at a high concentration) are applied (Gunde-Cimerman et al. 2018; Casanueva et al. 2010; Klähn and Hagemann 2011; Oren 2011).

Even though the study of fungi in hypersaline environments is still gaining prominence, their application in biotechnology has advanced (Ali et al. 2014; Chamekh et al. 2019). In industries, halophiles (hypersaline surviving organisms) are primary sources of industrially important enzymes (Carex 2011) and used extensively in several fermentation processes (Gostinčar et al. 2011). Production of some bioactive compounds such as beta carotene and ectoine has been associated with some halophilic fungi (Lentzen and Schwarz 2006; Oren 2010). Findings have further revealed their role as biosurfactants, which enhances biodegradation, production of biorhodopsin for optical computing, and exopolysaccharides for efficient oil recovery and food additives (Ali et al. 2014; Akpi and Odoh 2017).

10.3.4 Thermophilic Fungi

Fungi that strive at elevated temperatures are called thermophilic organisms. They are also referred to as thermotolerant microbes. Typically, these fungi grow at a maximum temperature near 50 °C and a minimum below 20 °C (thermotolerant), while those that survive at 50 °C or above but do not grow at 20 °C or above are regarded as thermophilic (Thanh et al. 2019). Reports have shown that thermophilic fungi are a regular microbial component of self-heating decomposing hay. Thermophilic fungi are most commonly found in rapidly decomposing plant residues, in natural environments, where heat is generated through exothermic microbial activity (Thanh et al. 2019). Furthermore, most thermophilic fungi grow well at moderate temperatures and can be found in various substrates, including soils, composts, piles of hay, stored grains, wood chip piles, nesting materials of birds and animals, or in municipal refuse (Singh and Satyanarayana 2009).

Additionally, these fungi produce thermostable extracellular enzymes with useful applications. It is known that thermophilic activities are generally associated with protein thermostability. Therefore, proteins produced by thermophiles tend to be more thermostable than their mesophilic counterparts. The mechanisms of intrinsic thermostability of the protein that result in the molecular rigidity are related to a number of hydrogen and disulfide bonds, salt bridges, presence of hydrophobic amino acids, and the packing of external residues (Gomez et al. 2016; Mehta et al. 2016). Reports have shown that thermophilic fungi are harnessed for the treatment of cancer (Liao et al. 2012; Mehta et al. 2013). In an industrial scale, remediation of textile dyes, bioconversion of xylose to ethanol, degradation of crude oil, recovery of heavy metal, degradation of keratin, saccharification of agricultural residues, and diary product processing have all been done using thermophiles (Verma and Shirkot 2014; Sahni and Gupta 2014; Bajaj et al. 2014; Dimarogona et al. 2012).

10.3.5 Psychrophilic Fungi

Psychrophiles are microorganisms that colonized all permanent cold environments. They have been grouped into obligate psychrophiles (grow optimally at less than or at 20 °C) and facultative psychrophiles (optimal growth temperature of >20 °C) (Hamid et al. 2014). Psychrophilic fungi grow optimally at 15 °C or lower, while psychrotrophic fungi thrive well at temperatures above 20 °C (Hassan et al. 2016; Yadav et al. 2015a, b, 2016). This group of fungi has been found in cold habitats, such as Antarctica, Arctic regions, and cold deep sea environments (Hassan et al. 2016; Blanchette et al. 2010). There genera and species, e.g., *Thelebolus microspores, Lemonniera,* and *Tetracladium*, have been isolated from different regions of the Himalayas and India (Sati et al. 2014; Anupama et al. 2011). For instance, *Penicillium* species has been identified from soils, lakes, and historic woodlands and *Macroalgal thalli* in Antarctic regions (Loque et al. 2010). Also, some genera (*Leotiomycetes, Cladosporium, Trichoderma, Periconia*) are reported to be isolated from various polar and nonpolar cold habitats (Laura et al. 2013; Kostadinova et al. 2009).

In this cold environment, these species undergo many extreme limiting factors, including frequent freeze-thaw cycles, high salt concentration, low moisture content, extreme UV radiation, and low nutrient availability (Hassan et al. 2016). These are achieved via various physiological and ecological adaptation mechanisms (Anupama et al. 2011). Ruisi et al. (2007) reported production of antifreeze proteins, compatible solutes, trehalose, and other freeze tolerance mechanisms as some of the key adaptive mechanisms of psychrophilic fungi. Psychrophilic fungi produce cold-active enzymes, which remain active at low temperatures, and have great potential for industrial biocatalysis in terms of energy savings by lowering the required temperature of a reaction without sacrificing enzyme activity (Cavicchioli et al. 2011).

In the pharmaceutical, medical, and industries, enzymes (e.g., chitinase, endochitinase, lipase, laccase, ferulic esterase, beta-lactamase, esterase, peroxidase, and imidase) are used for hydrolysis of chitin to chitosan, chitooligosaccharides, and glucosamine. Besides being harnessed for biocontrol of mosquito larvae, they are also essential for the synthesis of antibacterial agents, antimicrobials, antioxidants, and as photoprotectants (Akpi and Odoh 2017; Cavicchioli et al. 2011; Jeon et al. 2009).

10.3.6 *Piezophilic Fungi*

These are fungi found predominantly in extreme barometric pressure. Piezophiles which are sometimes referred to as barophiles are high hydrostatic pressure-dependent. Fungi classified in this group have been identified from the deep-sea sediments (>3000 m depth) and the guts of bottom-dwelling animals (Zhang et al. 2018). Even though first evidence of piezophilic growth in mixed microbial cultures and recovering from the deep sea has been demonstrated, these molecular signs and traits were affiliated with the phylum Ascomycota, and a few belonged to the phyla Basidiomycota and Chytridiomycota (Nagano and Nagahama 2012).

However, many conditions such as low temperature, elevated hydrostatic pressure, and low nutrient availability are combined to make the deep sea an extreme environment (Jebbar et al. 2015), illustrated in their high-pressure, deep-sea hydrothermal vents study. In this habitat, geothermal activity occurs within the oceanic floor, resulting in widely varied pH, temperature, and varying nutrient availability, which in turn support a diverse and unique environmental niche (Charlesworth and Burns 2016). Reed et al. (2013), in their work, opined that piezophiles adapt to the high-pressure conditions, through their dense hydrophobic cores on proteins and via the formation of multimeric proteins. Finally, piezophillic enzymes play a key role in high-pressure sterilization of foods (Zhang et al. 2015), and their highly specialized cell membranes enable there adaptation through the incorporation of polyunsaturated fatty acids (Usui et al. 2012).

10.4 Mechanisms, Operation, and Dynamics of Extremophiles

Microorganisms (e.g., fungi and bacteria) are ubiquitous in nature, some of which possess unique qualities, genes, and properties enabling their survival. These organismal adaptations and mechanisms constitute a state by which they are able to biochemically and metabolically operate (Merino et al. 2019). For instance, some Fungi conidia or spores induced structures which support their growth in environments not conducive for typical fungal coenocytic cells (Blachowicz et al. 2019; Hussain et al. 2016). Although the presence of extremophiles and their physiological properties have long been studied, the whole genome sequence tool has enhanced the study of their underlying principles that enabled evolutionary and life existence, environmental pressures, and understanding of planetary bodies (Bertrand et al. 2015; Schulze-Makuch 2013; Kumar et al. 2018). Comparative genomic inquiry on extremophiles has shown sets of genes and proteins that empower necessary machinery in species; most importantly, organisms lacking these proteins cannot survive the same exposure. According to Kumar et al. (2018), these proteins safeguard the cell against a wide range of extreme exposures such as temperature, pressure, radiations, chemicals, drugs, etc. Also, besides the increasing understanding of extremophiles, the processes of their metabolic functions under extreme physical stress and how they evolve remain elusive (Lindgren et al. 2016).

10.4.1 High-Temperature Extreme

Thermophilic fungi are an assemblage of eukaryota that have developed strategies of growing at elevated temperatures (Czikkely and Bálint 2016; Salar and Aneja 2007). They are commonly found in soils and habitats with organic matter (Salar and Aneja 2006). It is thought that thermophilic fungi have been able to evolve over time, developing thermal resistance proteins, and genes, which confer in them the ability to thrive beyond mesophilic fungi temperature range. Thermo-tolerance species (*A. fumigatus*) expressed resistance genes. The *HIM-SKN7 gene*, which binds to the domain of heat shock transcription factors, helps in regulating various cellular functions (Hussain et al. 2016). Decreased genome size and shorter introns are linked to increased thermos tolerance as seen in thermophilic fungi. Van Noort et al. (2013) observed that the genomes of three thermophilic fungi; *C. thermophilum* (Cth), *T. terrestris* (Tte), and *T. heterothallica* (Tht) are significantly smaller than their close mesophilic relatives such as *Chaetomium globosum* (Cgl) and *Neurospora crassa* (Ncr); this possibly enhanced there stability in extreme temperature. Elsewhere, melanin contents are reported to associate with extremophilic fungi, thus serving as a protective shell against adverse conditions such as elevated temperature, heavy metals, UV radiation, and concentrations of salts, dryness, and desiccation (Pulschen et al. 2018; Nonzom and Sumbali 2015; Verma et al. 2017).

10.4.2 Low-Temperature Extreme

Fungi that flourish in cold and/or freezing temperatures (−20 and 10 °C) are known as psychrophilic or cryophilic fungi (Durán et al. 2019; Santiago et al. 2016; Wang et al. 2017). Predominantly, they are found in temperate regions such as polar ice, cold ocean water, and alpine snowpack (Goswami and Das 2016). At these zones, psychrophiles remain frozen most of the year. Wang et al. (2017), in their study, observed that thick pigmentation in the cell walls of psychrophilic fungi help them to withstand the harsh environmental conditions (cold) in Antarctic rock. Cryophilic fungi such as the Basidiomycetes inhabit these regions (Arctic and Antarctic) by producing antifreezing proteins (Tkachenko 2017). These proteins (AFPs) bind to ice crystals through a large complementary surface, creating thermal hysteresis while lowering the temperature for the organismal growth (Jia and Davies 2002). Gupta et al. (2014), in their work, opined that psychrophilic/cryophilic fungi enzymes have flexible structures of cold-active enzymes. Elsewhere, mechanisms of fungi survival in cold conditions have been suggested to include combination of strategies such as (a) increase of membrane fluidity at low temperatures (changing the composition of fatty acids), (b) increase in the intracellular trehalose and polyol concentrations and unsaturated membrane lipids, (c) compatible solutes (glycerol), (d) secretion of antifreeze proteins and enzymes active at low temperatures, (e) reduction of growth rates, (f) subcellular, molecular, and metabolic changes, and (g) formation of exopolysaccharides (Salvino et al. 2006; Wang et al. 2017; Merino et al. 2019). They are also protected against environmental stresses when found in exopolymeric substance (EPS).

10.4.3 Fungi Survival in Acidic or Alkaline Environments

Acidic habitats (pH < 3) represent some of the most extreme environments for microbial growth (Hujslová et al. 2014; Vylkova 2017). Organisms which are dominant in this habitat actively modulate the pH of their environment through the secretion of organic acid such as butyrate, oxalate, malate, citrate, gluconate, and succinate (Vylkova 2017). They also regulate the acidity level in their internal environment against its external concentration by actively pumping out the hydrogen ions fast enough to prevent damage of the DNA (Goswami and Das 2016; Hassan et al. 2019). Furthermore, Bi et al. (2016) reported that the extent of fungi adaptation in a pH-dependent environment depends on nutrient availability, organic acid synthesis, and the removal of ammonium ions from ammonium sulfate salt by the fungi.

In saline environment such as salterns, soda lakes, or alkaline soils, the surviving fungi are termed alkaliphiles or halophiles. They are extremophilic microorganisms growing optimally at high salt concentrations (Siglioccolo et al. 2011). Halophiles uses two key strategies to maintain proper osmotic pressure in their cytoplasm: these include (a) "salt-in strategy", which involves the accumulation of potassium and chloride with extensive adaptation of the intracellular macromolecules and (b) "osmolyte strategy" for the biosynthesis of organic osmotic solutes (Siglioccolo et al. 2011).Compatible solutes are low-molecular-mass organic compounds, which do not have net charge and can be accumulated in high (molar) amounts without negatively interfering with cellular metabolism (Silke et al. 2013). According to Silke et al. (2013), microorganisms grow and survive in saline habitats by bypassing high salt concentrations within the cytoplasm. They could also adjust by preventing water loss and plasmolysis through a specific cell wall construction and composition to pump out ions by "salting out" processes. Besides modification of the intracellular environment through the accumulation of non-toxic organic osmolytes, these organisms also involve proton electrochemical gradients, which serve as the driving force for the extrusion of Na^+.

10.4.4 Fungi Survival in an Environment of Extreme Pressure

The deep biosphere is often characterized with multiple extreme physicochemical conditions. Microbes which strive in pressure extreme conditions are known as barophiles or piezophiles (Dutta et al. 2019; Goswami and Das 2016; Gupta et al. 2014). Under this condition, densification of spatial organization of lipids occur, leading to cell damage (Jha 2014). Notably, barophilic groups have developed defenses to enable them flourish in extremes of pressure (Zhang et al. 2018). These mechanisms range from formation of dense hydrophobic cores on proteins and formation of multimeric proteins, which give tolerance to piezophilic species in high-pressure conditions (Charlesworth and Burns 2016). In addition, some fungi agents cope with hydrostatic pressure and temperature by reducing fluidity of their cell components. This is done through the increase of the packed fatty acyl chains that trigger the rise of the unsaturated fatty acids in their lipids (Phil 2017; Raghukumar et al. 2010; Oger and Jebbar 2010). Also, it is thought that pressure adaptation may be linked to change in cell physiology, which involves genes in osmoregulation (*omp*A), heat shock responses (*dna*K, *lon, gro*EL, *clp*PX, and others), periplasmic stress response (*omp*H), and cold shock responses (*csp*)(Dutta et al. 2019).

10.4.5 Fungi Survival in Extremely Dry Conditions

Microorganisms that grow in dry or low-water environments are called xerophiles (Gupta et al. 2014). Xerophiles grow at low water activity or low relative humidity. Some of the important fungal genera include *Aspergillus* (Visagie et al. 2017). Xerophilic fungi are predominantly found in desert environments. Typical examples are the black microcolonial fungi (MCF) and black yeasts, which are classified among the most stress-resistant eukaryotic organisms (Zakharova et al. 2013). Scientific inquiry into their strategies of survival under desiccating conditions has shown that xerophiles such as MCF jump desiccation by entering into dormancy—a restive state characterized by shutdown of metabolic activity. At this state, the organisms are still viable and capable of growth when normalcy returns (Huang et al. 2010; Nanguy et al. 2010).The mechanism of survival in *Xeromyces bisporus*, a xereophilic fungus, has been traced to the upregulation of glycerol synthesis genes and increased membrane fatty acid owing to decrease in water activity and modifications to phospholipids, sterols, and cell walls (Leong et al. 2015).

10.5 Role of Extremophilic Fungi in the Control of Pathogenic Microbes

10.5.1 Extremophilic Fungi as Bioagents

Extremophilic fungi can act as biological control for the suppression of damaging activities of some microorganisms often referred to as natural enemies (Jyoti and Singh 2016). One major area where the critical role of fungi as bioagents has been felt is in the control of plant pathogens/diseases. Here, some species (*Trichoderma*) which strive in extreme abiotic conditions possess capacity to confer disease resistance and stress tolerance to their hosts (Carreras-Villasenor et al. 2019; Nieto-jacobo et al. 2017; Singh et al. 2014). Interestingly, these organisms do this by acting as antagonists to various plant pathogens, thus preventing the severity and spread of the disease condition in different plants. The role of fungi as bioagents in agriculture has been gaining research attention owing to their application in sustainable agricultural production (Annapurna et al. 2018; Arya et al. 2017; Deshmukh et al. 2016).

10.5.2 Extremophilic Fungi as Biocontrol

The adaptation of fungi to extreme environments has allowed for the biosynthesis of novel natural products (Onofri et al. 2011). They colonize and develop distinctive defenses to survive and reproduce in their new environments. These extremophiles may be found in zones ranging from geothermal and humid soils in volcanic areas (Appoloni et al. 2008) to deep permafrost soils (Gilichinsky et al. 2007) and soils with low water activity and high salt concentration. Biological control of soil-borne plant pathogens by extremophiles is a growing area of plant pathological research (Bhattacharjee and Dey 2014). It involves the use of effective natural enemies of pests or plant pathogens to eliminate and/or control their population. Extremophilic fungi have been identified as biocontrol because of their ability to suppress phytopathogens and large number of foliar and soil-borne diseases (Odoh et al. 2020a). This disease control ability of extremophiles is possible via mechanisms such as pathogen antagonism, induced systematic resistance, mycoparasitism, signal interference, and competition for nutrients (Odoh 2017).

Predation and parasitism is also a major biocontrol mechanism used by *Trichoderma* species, an extremophilic fungus that has shown tolerance to unfavorable environmental conditions such as low nitrogen and water through enzymatic destruction of the fungal cell wall (Harman et al. 2004). The induction of plant resistance using nonpathogenic, pathogenic, or incompatible microorganisms is also a form of biocontrol mechanism that can be harnessed to improve plant/crop yield. Most times, multiple and diverse mechanisms are sorted and combined by the same fungi to suppress the proliferation of different phytopathogens including the production of volatiles, which have an important impact on soil microbiology.

Mycoparasitism, which is the direct attack of one fungus on another, can be divided into fungi with biotrophic or necrotrophic form of parasitism. This mechanism of biocontrol is usually a complex one involving series of events such as recognition, attack, penetration, and killing of the host depending on the form of parasitism involved. Biotrophic association in this case obtains nutrients from living cells while necrotrophics kill the host cells in advance and exploit their nutrients. In a study conducted by Purić et al. (2018), filamentous fungi isolated from soil and marine sediments in the Antarctic, an extreme environment, were assessed for their potential activity on phytopathogens (*Xanthomonas euvesicatoria* and *Xanthomonas axonopodis* pv. *Passiflorae*) that responsible for diseases in pepper, tomato, and passion fruit. The results of this study show that the bioactive compounds extracted from the fungi have the capacity to inhibit phytopathogens.

10.5.3 Biocontrol Mechanisms of Extremophilic Fungi

The use of fungi as biocontrol and alternative form of pest control on plants has long been documented (Fig. 10.1). They assist to maintain the quality of agricultural crops and minimize the undesirable and hazardous use of chemical pesticides. Plant diseases are the most important cause of economic loss to agricultural produce (Williams et al. 2017). However, these disease conditions or causing agents can be effectively managed and controlled through proper application and utilization of fungal base biocontrol. Most isolated species of extremophiles from rhizosphere are potential sources of antagonism against many soil-borne fungi such as *Fusarium oxysporum, Sclerotium rolfsii*, and *Rizoctonia solani*. They also produce antibiotics and antifungal toxic metabolites, i.e., trichodermin and viridian, inducing resistance activity against pathogens through enzymes such as glucanase, cellulose, chitinase, and protease, thus disintegrating the cell wall of the pathogen.

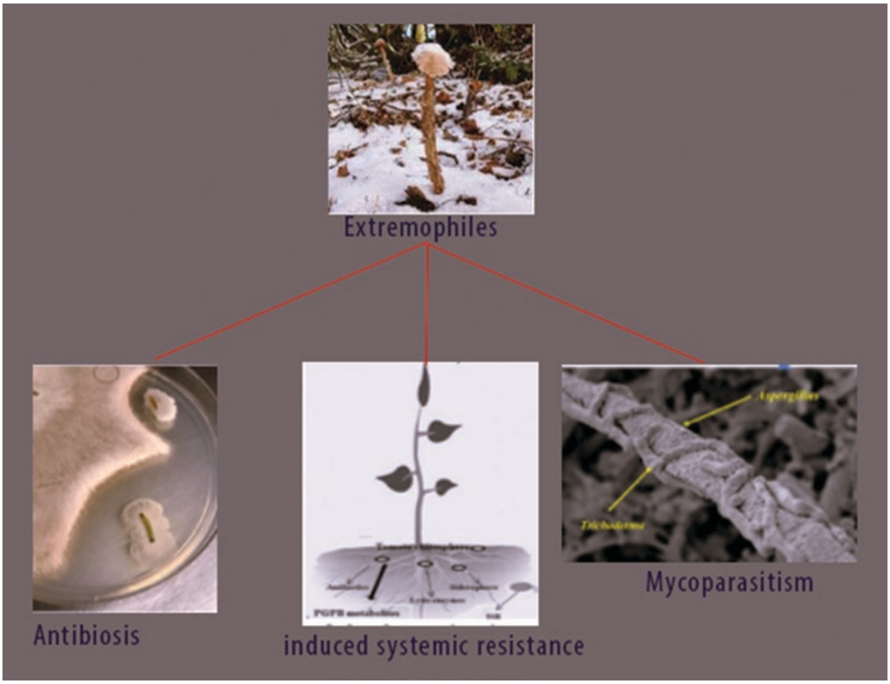

Fig. 10.1 Bio-mechanisms of action of extremophilic fungi on pathogenic microbes

10.5.3.1 Antibiosis

Antibiosis is a widely recognized and effective biocontrol approach employed by both plant growth-promoting bacteria and fungi in controlling soil-borne infections in crops. The word "antibiosis" describes the antagonistic effects between microorganisms. It is a biocontrol mechanism in which the antagonist produces substances (antibiotic, lytic enzyme, volatile substance, or toxin) that target and destroy the phytopathogen (Mousa and Raizada 2016). These antibiotics, which also function as microbial toxins, inhibit the growth of other microbes (Fig. 10.1). Antibiotics produced by microbes during abiotic/biotic stress are particularly effective against plant pathogens and the diseases caused by them (Thomashow et al. 2002; Odoh et al. 2019). Antibiotic production by fungi exhibiting biocontrol activity has regularly been isolated from *Gliocladium* and *Trichoderma* in extreme environments. They are also shown to exhibit significant in vitro activity against mycotoxin-producing *Aspergillus flavus* and *Fusarium verticillioides* (Wicklow and Poling 2009). Volatile compounds secreted by most fungi target a range of pathogens including the smut fungus *Ustilago hordei, F. oxysporum, Rhizoctonia solani* and *Phythium ultimum* while *Trichoderma harzianum* is shown to produce harzianopyridone as a mode of action during antibiosis (Mousa and Raizada 2016),

Table 10.1 Bioactive compounds isolated from extremophilic fungi

Type of compound	Biological activity	References
Alkaloids, peptides, and amides	Antiviral or antimicrobial Cytotoxic	Niu et al. (2017) Dalsgaard et al. (2005)
Quinones and phenols	Antimicrobial and cytotoxic Cytotoxic	Yao et al. (2014) Gao et al. (2016)
Xanthones	Antimicrobial	Fredimoses et al. (2014)
Polyketides	Antimicrobial Cytotoxic	Wu et al. (2015) Yang et al. (2007)

Thermophilic fungi isolated from thermophilic ascomycete Myriococcum albomyce produce novel antibiotics, new crystalline antifungal compound “myriocin” that is effective against Candida species and Trichophyton granulosum (Xu et al. 2015). Sonia et al. (2019) also reported a novel quinine antibiotic agent named malbranicin (TAIM 13 T54), isolated from thermophilic fungus *Malbranchea cinnamomea*, which exhibits toxicity against *Staphylococcus aureus* and *Bacillus subtilis*. Similarly, antibiotics such as penicillin G, 6-aminopenicillanic acid, sillucin, miehein, and vioxanthin are derivatives of thermophilic fungi, which are active against both Gram-positive and Gram-negative bacteria (Sonia et al. 2019). Table 10.1 represents some bioactive compounds with antibacterial property isolated from extremophilic fungi.

10.5.3.2 Induced Systemic Resistance

Aside from the ability to produce antibiotic molecules as a form of strategy to combat plant pathogen infestation, host resistance systems can also be induced by pathogens. This induction of host resistance could be local or systemic, and dependent on the type, source stimulation agents, and other biotic and abiotic inducers (Vallad and Goodman 2004; Pieterse et al. 2014). In addition, this process induces a defense mechanism for host resistance as the plant pathogens develop more advanced characteristics and pathways (Reddy and Saravanan 2013). Unlike the systemic acquired resistance (SAR) (Vallad and Goodman 2004), which requires the involvement of the signal molecule such as salicylic acid (SA), the induced systemic resistance (ISR) utilizes the jasmonic acid (JA) signaling pathway. Induced systemic resistance (ISR) also occurs from the colonization of the rhizosphere by certain microbes (Beneduzi et al. 2012; Pieterse et al. 2014; Odoh et al. 2019). When compounds with enzymatic activity such as xylanase and cellulose get released, they also induce resistance on the plant interactive zones (Lotan and Fluhr 1990; Martinez et al. 2001).

ISR is thus considered as the ability of an agent (a fungus, bacteria, virus, chemical, etc.) to express or exert plant defense mechanisms that lead to systemic resistance of different pathogens (Sharma et al. 2013). Similar to immunization with attenuated vaccines in humans, inoculation of plants with weak pathogens or nonpathogens leads to induced systemic plant resistance against pathogens. However, during this process, plant gains protection not just against the infesting pathogen, but against other agents. Induced systemic resistance (ISR) is mediated by jasmonic acid (JA) and ethylene signaling pathways and triggered by nonpathogenic microbes (Choudhary et al. 2007; Shoresh et al. 2010), just as *Trichoderma*, in association with plant roots, can trigger systemic resistance and improve plant nutrient uptake (Contreras-Cornejo et al. 2016).

10.5.3.3 Competition

Microorganisms compete for essential micronutrients such as iron, which are often required for the synthesis of ATP and formation of heme and other cellular functions (Saraf et al. 2011). It is an important biocontrol process usually harnessed for the control of plant disease. Because iron is very limited in the rhizosphere and is dependent on the soil pH, it is always sorted by microbes, plants, and phytopathogens (Shahraki et al. 2009). When this iron is in ferric form, its concentration tends to reduce. At reduced concentration, the growth of microbes in extreme condition is not supported. Hence, the fungal antagonist such as *Trichoderma asperellum* secretes iron-binding ligands called siderophores that controls *Fusarium* wilt in tomato plants (Segarra et al. 2010). This is aimed at preventing the colonization and depletion of iron needed by the pathogen.

10.5.3.4 Preemptive Colonization

Preemptive colonization is employed as a mechanism of biocontrol where the antagonist is allowed to colonize a particular zone and prevent infection. Preemptive colonization is also known as competitive exclusion and has to do with the application or growth of the fungal antagonists before the pathogen's arrival. In niche competition, the ability of the fungal antagonist to survive and multiply in specific niches where it is applied enhances its capability to colonize larger areas and prevent pathogen colonization. For effective prevention of pathogen establishment or colonization, Benbow and Sugar (1999) note that the colonization of the fungal antagonist in sufficient populations is necessary and an important consideration in preharvest applications of biocontrol agents. Preemptive colonization leads to disease suppression and enhancement of resistance against plant pathogens as illustrated in a study carried out by Yu et al. (2007) in biocontrol of blue and gray mold diseases of pear fruit, where they used the integration of antagonistic yeast with salicylic acid.

10.5.3.5 Mycoparasitism

Direct parasitism or lysis of a plant pathogen by another microorganism is known as hyperparasitism or mycoparasitism. Here, the pathogen is directly attacked by a specific fungal antagonist that overwhelms it or its propagules. This direct interaction is often observed among fungi and has rarely been reported for bacteria. Fungi with the ability to be parasitic on other fungi are known as mycoparasites and the interaction mycoparasitism (Baker and Cook 1974). Four major groups of hyperparasites reported include hypoviruses, facultative parasites, obligate bacterial pathogens, and predators. Milgroom and Cortesi (2004) report how hypovirulence by hypoparasites led to the control of chestnut blight and the factors that determine its success or failure. Elsewhere, multiple hyperparasites are suggested to attack a single fungal pathogen. For instance, *Acremonium alternatum*, *Acrodontium crateriforme, Ampelomyces quisqualis, Cladosporium oxysporum,* and *Gliocladium virens* are few of the examples of fungi with the capacity to parasitize powdery mildew pathogens. Zheng et al. (2017), in their work identified a number of fungal species which show hyperparasitism against rust pathogens, thus indicating the interrelationships of some fungus parasite against some fungus host. The necrotrophic mycoparasite *Trichoderma* produces enzymes that increase the permeability and degradation of fungal pathogens cell wall and inadvertently death of the plant pathogen. An example of a biotrophic parasite frequently found on sclerotia of plant pathogenic fungi such as *Sclerotinia minor* and *Sclerotium cepivorum* (the causal agents of lettuce drop) is *Sporidesmium sclerotium* (Karlsson et al. 2017; Nygren et al. 2018).

10.5.3.6 Antagonism

An environmentally acceptable management method for numerous pathogens would be the use of antagonistic fungi as a sustainable form of biological control (Punja and Utkhede 2003; Thakur et al. 2020). In phytopathology, antagonism refers to the act where an organism suppresses or interferes with the normal growth and activity of a phytopathogen. In extreme acidic conditions, fungal antagonists survive as they antagonize potential fungal phytopathogens (Indra and Kamala 2011). In an experimental study conducted by Naglot et al. (2015) indigenous strains of *Trichoderma* species were isolated from rhizosphere soils of Tea gardens of Assam, north eastern state of India, and were assessed for in vitro antagonism against two important tea fungal pathogens *pestalotia theae* and *Fusarium solani*. Their study revealed a potent antagonist against the two tea fungal pathogens identified as *Trichoderma viride*. Elsewhere, it also showed antifungal activities against standard phytopathogens with potent fungal antagonists used to inhibit fungal phytopathogens. For instance, Tagawa et al. (2010) and Chen et al. (2018) in their separate research isolated and characterized their antagonistic activity against potato scab from potato field soils and also evaluated yeasts as potential antagonists for biological control of *Botrytis cinerea* on strawberry fruits.

10.5.3.7 Signal Interference

Signal interference is a biological control mechanism which relies on the degradation of the quorum sensing molecules, i.e., the signal interference regulatory agent (Lin et al. 2003; Lugtenberg et al. 2013). Quorum sensing is required for an organism (beneficial or pathogenic) to exert its effects as it allows the organisms to sense their own population. When this occurs, the organism secretes some biomolecules for the expression of a certain character. Some of the most frequently expressed characters include pathogenicity/virulence factors, motility, and biofilm formation (Gray and Garey 2001; Miller and Bassler 2001). Signal interference is more common in bacteria than in fungi (Lugtenberg et al. 2013) owing to the fact that bacteria have the ability to sense the production level of exoenzymes regulated by quorum-sensing molecules (homo serine lactones). These exoenzymes are capable of degrading the cell wall of phytopathogens whether fungal or bacterial. In fungi, however, a mechanism such as RNA interference may be used in the control of fungal phytopathogens just as McLoughlin et al. (2018), in their studies utilizes species-specific RNA interference to control plants disease infestation especially those caused by *Sclerotinia sclerotiorum* and *Botrytis cinerea*.

10.5.3.8 Siderophore Production

Siderophores are produced under low iron stress conditions. They act as biocontrol agents with extracellular low-molecular-weight biomolecules, which have strong affinity for binding iron (Fe^{3+}) (Sureshbabu et al. 2016). Because iron is a vital element needed by all microbes, it is always sourced or in demand due to its relatively non-bioavailability for plant, thus prompting its scarcity irrespective of the huge mineral deposits on the earth crust (Subrahmanyam et al. 2020). Microbial siderophore production can be grouped into three major groups, namely, catecholates, hydroxamates, and carboxylates (Winkelmann 2002). When iron is limited, fungal siderophores scavenge and provide the plant with iron from the mineral phase through the development of soluble iron complexes. At slightly alkaline pH, Ghosh et al. (2017) demonstrated the detection, estimation, and characterization of siderophores from different fungal biocontrol agents like *Beauveria* spp, *Trichoderma* spp., and *Metarhizium* spp., which are soil-borne pathogens. They recorded that an alkaline pH was favorable for different species of *Trichoderma* in terms of siderophore production but showed a negative result for siderophore production in *Beauveria* spp and *Metarhizium* spp. Iron chelation/affinity is weaker in fungi but has been shown to improve plant growth as Yadav et al. (2011) note that siderophores produced by *Aspergillus niger, Penicillium citrinum,* and *Trichoderma harzianum* were found to increase the shoot and root lengths of chickpeas (*Cicer arietinum*), making them more able to withstand environmental stress and phytopathogens (Eze et al. 2018; Odoh et al. 2017c). Elsewhere, many research works have also shown that production of siderophores improves not only the growth rate and potential of plant during stress conditions but also their ability to take up both radioactive and rhizospheric metal irons even at minute concentrations (Robin et al. 2008; Dimkpa et al. 2009; Schalk et al. 2011).

10.5.3.9 Plant Growth Promotion

Plant growth-promoting fungi (PGPF) are naturally occurring soil microbes that colonize roots and confer a host of beneficial effects to the plants using mechanisms such as nutrient solubilization (nitrogen, phosphorus, or iron) (Singh et al. 2020), plant hormone production (auxin, cytokinin, or gibberellins) (Tiwari et al. 2020), and suppression of pathogenic microbes. PGPF most times also contribute to plant fitness by inducing systemic resistance. Some of the examples of PGPF capable of inducing systemic resistance include *Trichoderma* spp., *Penicillium* spp. GP16-2, *Phythium oligandrum, Piriformospora indica* (Schafer et al. 2009; Van der Ent et al. 2009). Under several abiotic stresses, PGPF enhance plant tolerance, improve plant biochemical composition, and also control numerous foliar and root pathogens. In general, they promote function such as biocontrol, biofertilization, and synthesize phytohormones (biostimulants),), control flooding and aid phytoremediation processes (Glick et al. 1998; Odoh et al. 2017a, 2019).

10.6 Current Research and Application of Extremophilic Fungi

Extremophiles are organisms that thrive in extreme environments where other microbes are considered unfavorable. These groups of organisms (fungi) produce enzymes, enabling their functionality in the diverse extreme conditions (Cowan et al. 2015). Extremophilic fungi associated with crop plants are essential for maintaining the sustainability of agricultural production systems (Yadav 2017). With the advent of biotechnology, new possibilities concerning the application of beneficial fungi to the soil and for agrobiology have been explored. The growing population of today's world demands an urgent need for enhanced and increased production of crop while still retaining soil fertility. Current research reveals that fungi such as arbuscular mycorrhizal fungi and *Trichoderma* have been applied as both biocontrol agents and biofertilizers (Odoh et al. 2020b). Mycorrhizal fungi are known for promoting improvements in plant productivity and yield. Genome mining has expanded the scope of our understanding that *Trichoderma* has special genes that enable them become successful plant symbionts (Guzmán-Guzmán et al. 2019).

Fungi from extreme environments are a rich source of novel natural products and metabolites useful for biological activities as revealed by modern molecular research. Besides extremozymes and extremolytes, exopolysaccharides (Raveendran et al. 2015), biopolymers, peptides, and biosurfactants can be produced by extremophilic fungi such as *Trichoderma* (Askolin et al. 2001) and have great economic-industrial potential. For instance, in agriculture, biosurfactants could substitute chemical surfactants as adjuvants in herbicide and pesticide formulations and act as biocontrol agents, enhance remediation of soils biologically, and stimulate plant defense against phytopathogens (Sachdev and Cameotra 2013). All these allow for the expansion and sustainable development of agriculture in extreme environments.

10.7 Conclusion and Future Prospects

Extremophilic fungi have unique defenses which enable them to survive extremes of pressure, temperature, salinity, and a number of abiotic/biotic factors. This makes them good candidates for the control of various plant disease conditions. They are also essential components of organic farming owing to their ability to fix, mobilize, and solubilize essential nutrients. Harnessing these potentials in fungal agents, however, provides far reaching impact in curtailing microbial pathogens most importantly for biotechnology advancement and agricultural development.

With established benefits of extremophilic fungi especially in agriculture, their foothold is still growing. Excess nutrients accumulate in the soils, such as phosphorus, owing to excessive application of chemical fertilizers by farmers still being on the increase. This has led to serious pollution problems, hence the need for fungal biofertilizers. While this stress (pollutants) and other forms of abiotic pressures reduce the soil quality and hinder plant growth, extremophilic fungal genera can be used in eliminating phytopathogens and promote plant growth. Endophytic fungi, which do not induce symptoms of disease in plants, should be explored as better options for the control of plant disease and crop development. This would aid in overcoming the challenges of fungi adaptability and survival among indigenous strains. Also, the use of these biological products (extremophilic fungi) should be strictly regulated as much research needs to be done to understand their effects on plant growth and its persistence in soil under stress environmental conditions.

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Chapter 11
Alleviation of Diverse Abiotic Stress in Plants Through the Fungal Communities

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Contents

11.1 Introduction

The twenty-first century has been marked by global climate change. It has been already reported that different environmental stresses are a major threat to future food security (Battisti and Naylor 2009), while the world population is thought to reach from a current estimated 7 billion approximately to 8.9 billion by 2050 (Singh et al. 2011). Agricultural sustainability is a major concern because of increasing climate variation, population, and reduction in soil health for crop cultivation. Issues

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regarding agricultural sustainability can become more prevalent in the near future due to drastic climate change and extensive agricultural practices (Wassmann 2009). With limited farming area, traditional agriculture system which is unsustainable and ever-increasing population (Masciarelli et al. 2014), working out a policy to fulfill the increasing need of food is becoming troublesome. There are a number of factors leading to the food crisis all over the world such as increasing urbanization and industrialization have led to the shrinkage of the land in the past few decades. Nowadays, to increase the crop yield, chemical fertilizers are employed in larger extent. To fulfill the food needs of the increasing population and to make the agricultural systems sustainable, there is a critical need to immediately make certain eco-friendly measures which will not harm the soil environment (Singh et al. 2020; Yadav et al. 2020d).

Problems such as climate changes and their hazardous effects have adverse effects on the crop yield and productivity. Plant growth as well as productivity are drastically affected by changes in the local climate. In addition to this, changes in the climatic elements such as radiation, precipitation, humidity and temperature, anthropogenic activities of humans also affect the climate, which lead to harmful impact on sustainable productivity of crops in the local ecological system, in the agricultural sector on a larger scale, and even on a global scale. Light, water, carbon, and mineral nutrients are the major requirements of the plants for optimal growth, development, and reproduction. Plants being immobile, they are exposed to a broad range of environmental stresses as well as stresses caused by the living entities. Extreme conditions (below or above the optimal levels) limit plant growth and development (Springmann et al. 2016; Kumar et al. 2019a).

According to a report published by FAO in 2007, only 3.5% of the globe's land area is not under the environmental stresses. Drought has affected 64% of the global land area, 13% of the land is affected by flood, 6% is affected because of salinity, and 9% is affected due to acidic soils (Mittler 2006; Cramer et al. 2011). It has been estimated that only because of single environmental stress, that is,. drought, there is 9–10% reduction in the national production of the cereals due to the drought conditions.

According to another report of FAO published in the year 2012, the area under ever-increasing salinization has almost touched the figure of 34 million irrigated hectares. An accurate value of agricultural loss, that is, reduction of crop production and soil health in terms of agroecological disturbances due to abiotic stresses could not be made. It is well known that abiotic stresses affect large land areas and significantly hamper the qualitative and quantitative loss in crop production (Cramer et al. 2011).

Abiotic stress is widespread and common in all environments. The effects of abiotic stress are best documented in agricultural systems where it can cause losses in the yield of crops up to 70% (Mantri et al. 2011; Kumar et al. 2019b). One of the principle limiting factors declining agricultural productivity is abiotic stress caused by adverse climate conditions (Padgham 2009; Grayson 2013). Drought (Pardo 2010; Cramer et al. 2011), temperature (Weis and Berry 1988), salinity (Munns and Tester 2008), pH (Yokota and Ojima 1995; Koyama et al. 2001; Hinsinger et al.

2003), acidic conditions, light intensity, submergence, anaerobiosis, and nutrient deficiency or excess, all impart negative impact on plant quality (Rastegari et al. 2020a, b; Yadav et al. 2020b, c).

Plants have the ability to deal with the rapid fluctuations and adversity of environmental conditions because of their intrinsic metabolic capabilities (Simontacchi et al. 2015). Variations in the outside environment could put the plant metabolism out of homeostasis (Foyer and Noctor 2005), as a result of these environmental stresses, plants harbor some advanced genetic and metabolic mechanisms present in their cellular system (Apel and Hirt 2004; Gill and Tuteja 2010). Plants have a range of protecting mechanisms, nonheritable throughout the evolution of plant to tackle these environmental stresses (Yolcu et al. 2016). Such mechanisms cause metabolic re-programming within the cells (Heil and Bostock 2002; Swarbrick et al. 2006; Shao et al. 2008; Bolton 2009; Massad et al. 2012) to facilitate routine biophysico-chemical processes. In most cases, plants get help from the microbiome within the habitat, in reducing the burden of environmental stresses.

The PGPMs help the plants either directly or indirectly. Direct mechanisms involve enhancing the uptake of the micronutrients, phytohormones production, fixing of atmospheric nitrogen and P, K, Zn-solubilization. The indirect mechanisms include stimulating the immune system against various fungal pathogens by production of varied compounds, enzymes, siderophores, antibiotics, osmolytes, or enhancing the texture or structure of the soil.

11.2 Effect of Abiotic Stress on Plants

Plants need light, minerals, nutrients, salinity for their optimal growth and developments as well as reproduction. An unfavorable environment consisting of extreme high or low of temperature, salinity and drought pose a complex set of stress conditions. Plants can sense and react to stresses in many ways that favor their survival (Crane et al. 2011; Ahmad et al. 2015; Jiang et al. 2016). Plants remember their past exposure to abiotic stresses and even mechanisms to overcome them in such a way that responses to repeated stresses can be modified accordingly (Hilker et al. 2015). However, the underlying molecular mechanisms of this phenomenon are primarily unknown. Initially, any obvious effect of any unfavorable conditions appears at the cellular level followed by the appearance of the physiological symptoms.

11.2.1 Water Stress

Prolonged water stress decreases leaf water potential and stomatal opening, reduces leaf size, suppresses root growth as well as reduces seed number, size, and viability, delays flowering and fruiting and limits plant growth and productivity (Osakabe et al. 2014; Xu et al. 2016). Water stress adversely affects the physiological condition of plant such as photosynthetic ability. To combat this stress, plants have evolved different mechanisms to minimize the consumption of optimal water resources and manage their growth till they face adverse conditions (Osakabe et al. 2013).

11.2.2 Light Stress

Exposure to low or high light intensities diminishes physiological process and adversely influences the growth and development of plants. Excess light initiates photooxidation that increases the production of highly reactive oxygen intermediates to manipulate biomolecules and enzymes. Under extreme conditions, loss in plant productivity is observed (Li et al. 2009).

11.2.3 Salinity

Early exposure to salinity leads to ion toxicity within the cell. This leads to the disruption of osmotic balance when the stress is prolonged for longer duration. Plant growth and development is altered by the combined effect of ionic as well as osmotic shocks (Munns and Tester 2008). The mechanism evolved to deal with salinity stress needs to maintain or quickly adjust both osmotic and ionic homeostasis within the cells. To fight against salinity, plants usually try to avoid high saline environments by keeping sensitive plant tissues away from the zone of high salinity or by exuding ions from roots or compartmentalize ions away from the cytoplasm of physiologically active cells (Silva et al. 2010; Yadav et al. 2020a). *Glomus etunicatum* has been reported to alleviate salinity stress in the plant *Glycine max* (Sharifi et al. 2007).

Many researchers have previously documented that salt tolerance in plant is associated with alleviation of antioxidant enzymes (Sekmen et al. 2007). The ROS scavengers include glutathione, ascorbate, and tocopherol, and the enzymes superoxide dismutases (SOD), catalases (CAT), ascorbate- or thiol-dependent peroxidases (APX), glutathione reductases (GR), dehydroascorbate reductases (DHAR), and monodehydroascorbate reductases (MDHAR) (Rouhier et al. 2008). They are involved in the removal of ROS either directly (SOD, CAT, APX) or indirectly via regeneration ascorbate and glutathione in the cell. *Piriformospora. indica* induces salt tolerance in barley by increasing the levels of antioxidants (Baltruschat et al. 2008). Endophytic bacteria producing phytohormones also induce salinity stress tolerance in plants. *Bacillus amyloliquefaciens* RWL-1 secreting abscisic acid (ABA) and auxins has been reported to induce salinity stress tolerance in *Oryza sativa* (Shahzad 2017). It is known that the endogenous production of plant hormones, plant growth promoting endophytic bacteria, combine with exogenous jasmonic acid overcome the negative impact of salinity in *Solanum pimpelifolium*

Table 11.1 Plant growth promoting microorganisms with crop plant

Plant growth promoting microorganisms	Crop plant	Reference
Piriformospora indica	Barley	Baltruschat et al. (2008)
Achromobacter piechaudii ARV8	Tomato	Mayak et al. (2004)
Pseudomonas pseudoalcaligenes Bacillus pumilus	Rice	Jha et al. (2011)
Azospirillum sp.	Wheat	Nia et al. (2012)
Enterobacter aerogenes Pseudomonas syringae Pseudomonas fluorescens	Maize	Nadeem et al. (2007)
Glomus clarum, Glomus etunicatum	*Vigna radiata, Capsicum annuum, Triticum aestivum*	Rabie (2005); Daei et al. (2009); Kaya et al. (2009)
Bacillus subtilis	*Lactuca sativa*	Arkhipova et al. (2007)
Pseudomonas putida Rs-198	*Gossypium hirsutum*	Yao et al. (2010)

(Khan 2017). Few examples of plant growth promoting organisms are mentioned in Table 11.1.

11.2.4 Osmotic Stress

It has been previously recorded that plants exhibit several mechanisms against osmotic stress at molecular, cellular as well as whole plant level including inhibition of shoot growth and enhancement of root growth, adjustment in ion transport (uptake, extrusion, and compartmentalization of ions) and metabolic changes (carbon metabolism, the synthesis of compatible solutes). Some of the above-mentioned responses are triggered by primary osmotic stress signals; other may result from secondary stresses/signals caused by the primary signals. These secondary signals are phytohormones (e.g., ABA, ethylene), ROS, and intracellular secondary messengers such as phospholipids. Some of these secondary signals may not be confined to the primary stress sites such as the root.

11.2.5 Drought

Drought causes the stimulation of production of varied reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), singlet oxygen ($1O_2$), superoxide radical (O_2), and the hydroxyl radical (HO^-) (Cruz de Carvalho 2008), and these reactive oxygen species decrease the normal metabolic processes of the plants by causing a oxidative damage to the lipids, various proteins, ultimately leading to the cell death (Farooq 2009; Hasanuzzaman 2013; Kour et al. 2019). Plants have supportive as well as efficient enzymatic and nonenzymatic oxidants also referred to as scavenging enzymes to overcome the negative effects of the drought (Cruz de Carvalho 2008). Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR), and ascorbate peroxidase (APX) are among the most important enzymatic antioxidants (Cruz de Carvalho 2008; Farooq 2009; Hasanuzzaman 2013; Gill 2010). Plants such as panic grass, rice, tomato, and dune grass associated with endophytic bacteria have been reported to use less water, have increased biomass than in nonsymbiotic plants. According to Malinowski and Beleskey (2000), drought tolerance may be explained by enhanced accumulation of solutes in the tissues of endophyte-infected plants as compared to noninfected plants, or by reduced leaf conductance and a slowdown of the transpiration stream, or due to thicker cuticle formation. Characterization of *Trichoderma* fungus from *Theobroma cacao* revealed changes in gene expression patterns. This point is toward a possibility that *Trichoderma* spp. could induce tolerance against abiotic stresses, possibly including drought (Bailey et al. 2006). Few examples of organisms involved in the tolerance against drought are mentioned in Table 11.2.

11.2.6 Nutrient Stress

Endophytes enable the supply of macronutrients and micronutrients to their respective host. Nitrogen-fixing bacteria metabolize plant root exudates and in turn provide nitrogen to the plant for amino acid synthesis. Endophytes promote plant growth by phosphate solubilization, indole acetic acid (IAA), cytokinins, gibberellin (GAs), siderophores production, and the supply of essential vitamins (Jha et al. 2011).

Studies have confirmed the role of endophytes in the biodegradation of plant litter of their host plants. They initially colonize the plants and facilitate the

Table 11.2 Microorganisms involved in drought tolerance and associated crop plant

Organisms involved in drought tolerance	Crop plant	Reference
Proteus penneri, Pseudomonas aeruginosa, and *Alcaligenes faecalis, Burkholderia phytofirmans, Enterobacter* sp. FD17	Maize	Naseem and Bano (2014), Naveed et al. (2014)
Azospirillum brasilense, Pseudomonas chlororaphis O6	*Arabidopsis thaliana*	Cohen (2015) Cho et al. (2008)
Trichoderma spp.	Cacao	Bailey et al. (2006)
Bacillus cereus AR156, *B. subtilis* SM21, *Serratia* sp. XY21	*Cucumis sativa*	Wang et al. (2012)
Bacillus licheniformis strain K11	*Capsicum annum*	Lim and Kim (2013)
Rhizobium tropici and *Paenibacillus polymyxa* (co-inoculation)	*Phaseolus vulgaris*	Figueiredo et al. (2008)
Glomus intraradices BEG 123	*Phaseolus vulgaris*	Aroca et al. (2008)

saprophytic microbes to act on through antagonism thereby increasing litter decomposition (Terekhova and Semenova 2005). Another study demonstrated that all endophytes have the ability to decompose organic components, including lignin, cellulose, and hemicellulose. This way they aid in nutrient cycling (He et al. 2012).

As soon as the plants sense the stress stimuli, they exhibit an immediate as well as effective response to initiate a complex stress-specific signaling cascade (Chinnusamy et al. 2004; Andreasson and Ellis 2010). Synthesis of phytohormones such as abscisic acid, jasmonic acid, salicylate and ethylene (Spoel and Dong 2008; Qin et al. 2011; Todaka et al. 2012) occur in response to the stress stimuli. Accumulation of phenolic acids and flavonoids (Singh et al. 2011; Tiwari et al. 2011), elaboration of various antioxidants and osmolytes, and the activation of transcription factors (TFs) are initiated along with the expression of stress-specific genes to activate appropriate defense system (Koussevitzky et al. 2008; Atkinson et al. 2013; Prasch and Sonnewald 2013). Though many of the mechanisms related to stress tolerance in plants are known, our knowledge regarding "on-field response" of the plants to simultaneous exposure to multiple stresses is still in its infancy.

The most crucial aspect in studying stress in plants is to understand the different levels of molecular machinery and its networks operating under stress conditions. This study includes elaborative elucidation of abundance of metabolic pathways and their regulatory genes in the plant varieties. Identification of multigenic traits involved in stress responses, exploration of linked markers for such genes, and investigation of the probabilities to pool out important genes through breeding programs is the current focus of stress mitigation strategies. Some other strategies that have been put forward for the alleviation of abiotic stresses in plants include the use of various biomolecules of plant and microbial origin. These approaches open new ways, through which we can alleviate the abiotic stresses.

11.3 Abiotic Stresses Mitigation Mediated by Microbes

To overcome this problem, modern agricultural productivity is triggered by huge quantities of agrochemicals in the form of synthetic fertilizers and pesticides (Duan et al. 2016). Conventional meaning of achieving food safety in this era is by deploying excessive quantities of expensive and environment-polluting agrochemicals, hence creating a big threat to humans and environment (Vanlauwe et al. 2014). A great success has been recorded through the use of synthetic agrochemicals but they do have some drawbacks. The excessive use of the synthetic agrochemicals has a deleterious impact on the well-being of humans as well as proper functioning of the ecosystem.

They also hamper the sustainable production of crop yield (Smith and Siciliano 2015). The intensification of agriculture with synthetic agrochemicals adversely affects the ecological balance, reduces the fertility of the soil, contaminates the food chain, pollutes the groundwater, reduces microbial diversity, reduces soil pH, and leads to increased microbial resistance (Uphoff and Dazzo 2016). To keep up with the increasing population and attend the challenge of feeding the huge population, agricultural production needs to be intensified, but this must be done sustainably. Other alternatives are urgently needed to be searched and should achieve environmental balance and sustainability. Worldwide scientists in the field of agriculture and allied science have been searching sensitive and environment-friendly methods for sustainable intensification of agricultural production. The maintenance of the ecological balance and increased productivity can be achieved through the intensification of sustainable agricultural system. This will translate into higher crop yields owing to improvements in plant resilience and the ability of plants to adapt to changing climatic conditions, as well as to biotic and abiotic stress shocks (Pretty et al. 2011). Different environmental stresses limit the agricultural productivity. The maximization of the productivity is necessary but should be without causing damage to the ecological balance (Busby et al. 2017; Timmusk et al. 2017).

11.4 Role of PGPM Against Abiotic Stress

Microbes induce local stress reduction response mechanisms in plants to sustain under abiotic stress conditions. While on the other hand, they help plants to maintain their growth and development through nitrogen fixation, mobilization and/or production of nutrients, hormones, and organic phytostimulant compounds. Such multilayered action of microorganisms or their communities makes them strong, important, viable, and vital options for abiotic stress alleviation strategies in crop plants (Rai et al. 2020). Soil-inhabiting microbes from the genera including *Achromobacter, Azospirillum, Variovorax, Bacillus, Enterobacter, Azotobacter, Aeromonas, Klebsiella*, and *Pseudomonas* have been shown to enhance plant growth even under environmental stress conditions (Pishchik et al. 2002; Hamdia et al. 2004; Mayak et al. 2004; Arkhipova et al. 2007; Barriuso et al. 2008a, b; Dardanelli et al. 2008; Belimov et al. 2009). Large amounts of growth regulating molecules mainly indole acetic acid (IAA) are synthesized in shoot and accumulated in actively growing regions of roots (Singh and Yadav 2020; Yadav et al. 2020d).

Auxins such as IAA have growth-stimulating effect that causes root growth initiation. These molecules are also involved in the development of lateral roots (Kour et al. 2020a). It has also been reported that higher amounts of auxins have a negative impact on the plant growth (Jackson 1991; Sorty et al. 2016). A similar situation can also happen due to increased synthesis of ethylene (Jackson 1991). The rhizosphere colonizing bacteria are known to work in a similar manner and produce phytohormones to enhance plant growth (Bowen and Rovira 1991; Timmusk and Wagner 1999; German et al. 2000; Belimov et al. 2007). Many scientists have recorded beneficial roles of microorganisms associated with rhizosphere, phyllosphere, phylloplane, and rhizoplane, such as rhizobacteria that promote plant growth (PGPR), or microorganisms that promote plant growth (Kour et al. 2020b; Mondal et al. 2020; Rana et al. 2019, 2020).

A cheaper, more eco-friendly and sustainable means of achieving agricultural intensification and improving productivity is by adopting the use of microbial inoculants to enhance the availability and use of vital soil nutrients. Microbial inoculants have a beneficial role in mitigating both biotic as well as abiotic stress (Jambon et al. 2018). There has been a trend in the use of microbial inoculants in contemporary agricultural production to boost soil fertility and improve the cycling of nutrients, to enhance growth, vitality, and the productivity of crops (Ajilogba and Babalola 2019; Enebe and Babalola 2018; Igiehon and Babalola 2018). The use of microbial inoculants, including biofertilizers, biopesticides, bioflocculants, bioremediation agents, and biostimulants in a sustainable way in order to improve the growth of plants, their disease resistant properties, and their fitness and vitality is the right way forward.

11.5 Abiotic Stress Tolerance in Plants via Endophytes

Symbiotically conferred abiotic stress tolerance involves at least two mechanisms:

1. Activation of host stress response systems soon after exposure to stress, allowing the plants to avoid or mitigate the impacts of the stress (Redman et al. 1999).
2. Biosynthesis of anti-stress biochemicals by endophytes (Schulz et al. 2002).

Significant increase in the plant biomass, related growth parameters, assimilation of essential nutrients such as potassium, calcium, magnesium, and reduced sodium toxicity have been seen in *Phoma glomerata* and *Penicillium* sp.-infected cucumber plants under sodium chloride and polyethylene glycol–induced salinity and drought stress when compared with control plants (Waqas et al. 2012).

11.6 Mechanisms Involved in the Alleviation of Abiotic Stress

Induced systemic tolerance (IST) is the term being used for microbe-mediated induction of abiotic stress responses. Microbes with their potential intrinsic metabolic and genetic capabilities, contribute to alleviate abiotic stresses in the plants (Gopalakrishnan et al. 2015). Several beneficial roles of rhizospheric microbes belonging to the genera such as *Pseudomonas* (Ali et al. 2009; Sorty et al. 2016), *Azotobacter* (Sahoo et al. 2014a, b), *Azospirillum* (Creus et al. 2004; Omar et al. 2009), *Rhizobium* (Alami et al. 2000; Remans et al. 2008), *Pantoea* (Amellal et al. 1998; Sorty et al. 2016), *Bacillus* (Ashraf et al. 2004; Marulanda et al. 2007; Tiwari et al. 2011), *Enterobacter* (Nadeem et al. 2007; Sorty et al. 2016), *Bradyrhizobium* (Panlada et al. 2013), *Methylobacterium* (Meena et al. 2012), *Burkholderia* (Barka et al. 2006; Oliveira et al. 2009), *Trichoderma* (Ahmad et al. 2015) and *Cyanobacteria*

(Singh et al. 2011) in plant growth promotion along with reduction of multiple kinds of abiotic stresses have been recorded over the years. One of the viable options that can help to trigger crop productivity is through selection, screening and application of stress tolerant microorganisms. Enhanced oil content in NaCl-affected Indian mustard (*Brassica juncea*) was reported by *Trichoderma harzianum* application, which improved the uptake of essential nutrients and enhanced accumulation of antioxidants, osmolytes, and decreased Na^+ uptake (Ahmad et al. 2015).

The rhizosphere consists of fraction of soil in the vicinity of plant roots. It creates a soil microenvironment in the proximity of root region where the average count of microorganisms is very high as compared to rest of the bulk soil. Therefore, it is obvious that plant roots with a diversity of their nutrient, mineral, and metabolite composition could be a major factor responsible for attracting microorganisms to accumulate and associate alongside. The creation of root exudates by plants is a vital factor for microbial colonization within the rhizosphere (Subrahmanyam et al. 2020).

Chemotactic movement of microorganisms toward the root exudates is an important factor that forces the microbial communities to colonize on the roots. While utilizing the rhizosphere microenvironment around plant roots, the PGPRs may act as biofertilizers, phytostimulators, or biocontrol agents depending upon their inherent capabilities, mode of interaction, and competitive survival conditions. Plant growth is promoted through several direct and indirect mechanisms employed by the plant growth promoting microbes (Braud et al. 2009; Hayat et al. 2010).

Direct mechanisms include synthesis of bacterial compounds which triggers the uptake of essential nutrients and micronutrients from the soil along with the production of plant growth regulators, for example, iron and zinc sequestration, siderophore production, phosphorus and potassium solubilization, plant hormone production, and atmospheric nitrogen fixation (Verma et al. 2017).

Indirect mechanisms involve antagonistic activity toward plant pathogenic organisms, production of HCN and antifungal compounds, and tolerance against abiotic stresses (Thakur et al. 2020). Bacterial metabolites acting as an extracellular signal can induce systemic tolerance in plants by subsequently triggering a series of internal processes. At the end, the translocated signal is perceived by the distant plant cells triggering the activation of the defense mechanism.

Fungi, particularly the mycorrhizal fungi, have a beneficial role as plant growth promoters (Devi et al. 2020). These are mainly divided into mycorrhizal fungi and vesiculararbuscular mycorrhizal (VAM) fungi. These fungi remain associated with the host plant externally (*Ectomycorrhizae*) or they may form endosymbiotic associations (VAM). These fungi form an extensive networking of very fine hyphae, thus increasing the overall nutrient uptake by the roots. The root fungal endophyte *Piriformospora indica* has been recorded to induce salt tolerance in barley (Baltruschat et al. 2008) and drought tolerance in Chinese cabbage (Sun et al. 2010) by increasing the levels of antioxidants and improving many other aspects (Franken 2012).

11.7 Conclusion

Plants associated with beneficial microbes are receiving much attention due to their crucial role in enhancing the productivity of the crops. They also provide resistance against the stress conditions and are widely known as plant growth promoting microbes (PGPMs). Through varied mechanisms, PGPMs provide resistance against these environmental stresses. Microorganism-based formulations are the basis of "greener revolution in agricultural production." Therefore, the focus must be to bring to the forefront simple biological alternatives to take care of these abiotic and biotic stresses. Certain novel, trait-specific microbial strains must be developed to effectively combat the challenges presented by abiotic or biotic stresses. Alleviation of environmental stress is a major agricultural issue that needs to be addressed at the earliest with the use of fungal bioinoculants, the most sustainable approach.

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Chapter 12
Portraying Fungal Mechanisms in Stress Tolerance: Perspective for Sustainable Agriculture

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12.1 Introduction

The agricultural productivity is constantly affected by changing climatic conditions and environmental stresses (Kumar et al. 2019). In the countries with developing economy, global warming and shortage of food supply have a profound effect on crop output and quality (Xu 2016). In Germany, for instance, there has been an increase in precipitation by up to 11% during the last 100 years, and summers are usually dry with constantly increasing temperature (Bender et al. 2016). Due to this, plant productivity is decreased and plants become more susceptible to pathogen infection resulting in decreased global agricultural output. Plants show adaptation to climatic changes by reduced growth and plant biomass, leading to a reduction in plant yield (Dresselhaus and Hückelhoven 2018). Moreover, increased susceptibility to pathogen attack due to fluctuating climate introduces biotic stress on the locally adapted crops (Thakur et al. 2020). Considering the increasing demand for food resources and adverse environmental conditions, different strategies for crop improvement against environmental stresses were undertaken and comprise tissue culture methods, classical breeding programs, genetic manipulations, use of biofertilizers, etc. (Helaly 2017; Kour et al. 2020b). In this direction, efficient methods are required to mitigate stress conditions, and a better understanding of the mechanisms of how plants respond to various environmental stress is essential.

The greatest challenge in the modern era is to understand and develop scientific strategies leading to a more sustainable agricultural production. The annual losses in agriculture due to abiotic and biotic stress are high, despite sophisticated defense mechanism adopted by plants (Pieterse et al. 2009; Kour et al. 2019a). Recent statistics emphasize on the need to increase the agricultural output by 70% (Bender et al. 2016) with the soil microbial communities particularly the arbuscular mycorrhizal fungi (AMF) being an emerging area of research (Thirkell et al. 2017). Plant–microbe associations are being extensively explored for multiple advantages to the associated partners and the environment. The microbial communities associated with the plant offer distinct advantages to the plant and the environment by influencing plant growth and development, conferring tolerance to biotic and abiotic stress and nutrient cycling (Singh et al. 2020). A better understanding of the functional dynamics and how the fungal communities confer beneficial traits to the plant would be an ideal platform for enhancing crop productivity and a more sustainable agriculture. Highlighting the emerging importance of plant-associated fungal communities and their multifaceted beneficial role in the ecosystem, the chapter extensively discusses the functional dynamics of the fungal communities in conferring stress tolerance and promoting plant growth. With a brief overview of the composition of fungal microbiomes and their mutualistic association with higher plants, a better understanding of how these microbial communities confer beneficial traits to plants is required for a more sustainable agriculture.

12.2 Environmental Stresses and Plant Productivity

Plants are constantly exposed to multiple environmental stresses under changing climatic conditions (Yadav et al. 2020c). The biotic and abiotic stress adversely affects physiological mechanisms hampering plant growth and productivity (Shao et al. 2008; Yadav et al. 2020a, b). While the biotic stress burdens plants by pathogen or herbivore infection (Mordecai 2011; Maron and Crone 2006), abiotic stress has a huge adverse effect on plant growth and consequently crop yield (Wang et al. 2003). Figure 12.1 provides a diagrammatic representation of the various biotic and abiotic stresses and their influence on plant productivity.

Plants have evolved mechanisms to combat stress and usually include molecular, cellular, and physiological adaptations. The effect of biotic and abiotic stress on plants is adverse, leading to alteration in physiological mechanism, plant health, and productivity (Shao et al. 2008; Singh et al. 2020a). In order to respond in an effective way against stress factors, plants have developed defense mechanism leading to activation of signaling cascades (Abou Qamar et al. 2009; Chinnusamy et al. 2004), activation of kinases and specific ion channels (Fraire-Velázquez et al. 2011), phytohormone accumulation (Spoel and Dong 2008; Tiwari et al. 2020), generation of reactive oxygen (Laloi et al. 2004) species besides other defense mechanisms to minimize the stress damages and increase plant tolerance.

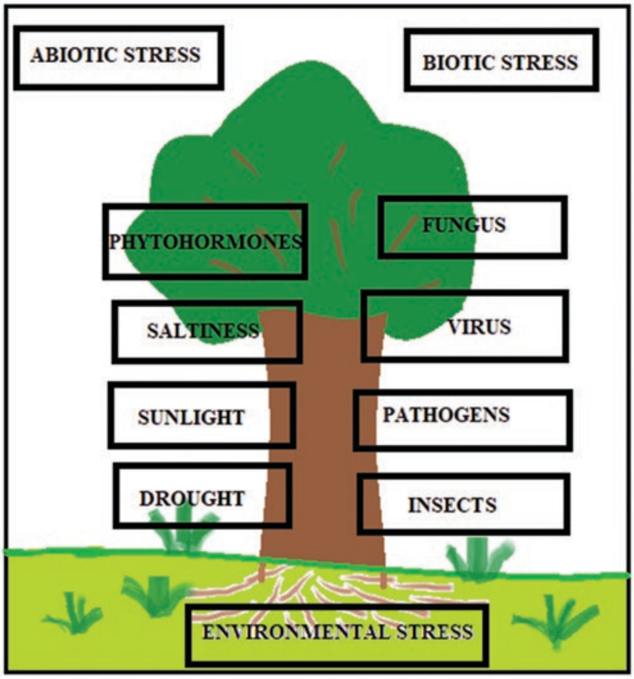

Fig. 12.1 Environmental stresses (biotic and abiotic) affecting plant growth and productivity

Considering the increasing demands of global food supply and environment-induced stress conditions, a better understanding of how plants respond to biotic and abiotic stress would provide a platform to improve crop yield and productivity. Moreover, it is essential to study the mechanism and influence of environmental stresses on plants, and how these devise strategies to combat stress, a feasible approach to monitor multiple damages to plants. Several agricultural management practices combined with scientific technologies and research are required to counter the adverse effects of ever-changing climatic conditions (Shanker and Shanker 2016). There has been ongoing research by the Bavarian State Ministry of the Environment and Consumer Protection, molecular plant researchers in Bavaria to understand the molecular mechanisms of how plants evolved strategies to counter biotic and abiotic stresses. Moreover, the research consortia the "BayKlimaFit" network were studying the effect of abiotic stress and the influence of symbiotic associations and pathogen on plants (http://www.bayklimafit.de) (Schön 2018). Another research project Collaborative Research Centre (CRC) SFB924 was working on understanding the mechanism regulating the crop yield and stability in plants (http://sfb924.wzw.tum.de) (Schwechheimer 2018).

12.2.1 Biotic Stress and Secondary Metabolites

During the course of evolution, plants have evolved specific tolerance mechanisms to adapt to adverse environmental conditions. In the atmosphere, plants are constantly exposed to biotic pressure in the form of pathogen attack which causes changes in the growth dynamics of the plant. In the existing environment, plants are considered as a complex community in association with their associated microbiota (Zilber-Rosenberg and Rosenberg 2008). The associated microbial communities may show several interactions: from symbiotic to commensal and parasitism and may change subject to environmental conditions. Moreover, association of microbes with plants confers tolerance to pathogen infection and a key molecular mechanism comprising the production of low molecular weight plant metabolites (Dresselhaus and Hückelhoven 2018). The plant-associated microbiota exhibit enormous potential in plant protection and abiotic stress management (Aslam and Ali 2018). Table 12.1 provides some key examples of plant–fungal associations and fungal mechanisms in stress tolerance.

Recent studies have shown that metabolites from grasses, Benzoxazinoids (BXs), are involved in chemical defense against biotic stress; its role in metabolism and biological functions was discussed in detail (Niculaes et al. 2018). Benzoxazinoids (BXs) comprise the most effective defense compounds in *Z. mays* and highlight agronomic importance; however, their biosynthesis and mechanism remain less understood in other crops of the Poaceae family (Dresselhaus and Hückelhoven 2018). The broad spectrum immunity of the plants against a number of pathogens depends on the microbe-associated molecular pattern recognition and host endogenous molecular patterns, respectively. The presence of pattern recognition receptors

Table 12.1 Some key examples of plant–fungal associations and fungal mechanisms in stress tolerance

Fungal species/ association	Stress condition	Mechanisms of stress tolerance	Plant species	Biological outcome(s)	References
AM fungi	Salinity	Improved plant water content Chlorophyll content increase	*Sesbania aegyptica* *S. grandiflora* *Lotus glaber*	Mitigate salinity stress Confer plant tolerance	Colla et al. (2008) Giri and Mukerji (2004) Sannazzaro et al. (2006)
AM fungi	Salinity	Plant growth Improved yield	*Lycopersicum esculentum* *Gossypium* spp. *Hordeum vulgare*	Improved fruit yield and mineral content Plant growth	Al-Karaki and Hammad (2001) Feng and Zhang (2003) Mohammed et al. (2003)
AM fungi	Salinity	Nitrogenase activity Improved nodulation	*Cajanus cajan*	Increased antioxidant enzyme functions	Garg and Manchanda (2008)
AM fungi	Drought	Increased activity of glutathione reductase and ascorbate peroxidase for the removal of H_2O_2	*Glycine max*	Alleviation of drought-induced nodule senescence	Ruiz-Lozano et al. (2001) Porcel et al. (2003)
Endophytic fungi	Drought	Osmoregulation and stomatal regulation Plant protection against drought	*Perennial ryegrass*	Increased growth, drought tolerance	Bacon and Hill (1996) Ravel et al. (1997)
Endophytic fungi	Salinity	Increased tolerance to high saline levels	*L. mollis*	Salinity tolerance	Rodriguez et al. (2008)
Endophytic fungi	High temperatures	Heat tolerance in symbiotic association	*Dichanthelium lanuginosum*	Heat tolerance	Marquez et al. (2007)
Endophytic fungi	Drought	Upregulation of drought-responsive proteins in leaves	*Arabidopsis thaliana*	Drought tolerance	Oelmuller et al. (2009)
Endophytic fungi	High temperatures	Increased tolerance to high temperatures	*L. esculentum*	Heat tolerance	Rodriguez et al. (2008)
Endophytic fungi	Drought	Adaptation to and mitigation of drought conditions	*L. esculentum*	Drought tolerance	Rodriguez et al. (2004)

(PRRs) on cell surface and its molecular mechanism in stress tolerance was reported (Ranf 2018). The study defines enormous potential in improving crops for sustaining tolerance to environmental stress, thus leading toward sustainable agriculture.

12.2.2 Abiotic Stress and Fungal Communities

The abiotic stress affecting plant and its management comprise a key challenge facing agricultural productivity. Abiotic stress arises from an interaction between living organism and their environmental factors influencing organism. Moreover, abiotic stress has a major impact on crop productivity affecting livelihoods of farmers as well as agricultural output, raising socio-economic concerns. With the constantly changing climatic conditions, plants are exposed to abiotic stresses, namely, high temperature, drought, salinity, deficiencies of nutrients in soil, etc., which has a detrimental effect on plant growth and productivity (Singh et al. 2020). Additionally, plant may be affected by abiotic stress due to presence of high concentrations of a toxic substance or the absence of an essential component.

Plant growth-promoting microbes have a beneficial effect and confer stress tolerance to the plant under adverse conditions. Moreover, the mycorrhizae promote plant health by prevention of nutrient leaching and ecosystem maintenance (Srivastava et al. 2012; Rastegari et al. 2020a, b). The mycorrhizal association with higher plants provides plant tolerance to drought conditions, salinity nutrient uptake, and presence of heavy metals in soil (Henning 1993). Several studies in this area demonstrated that arbuscular mycorrhizal (AM) fungi improved plant growth in salinity stress enhancing salt tolerance in plants. Besides classical breeding techniques and genetic manipulations, fungal endophytes have also been found to improve abiotic stress tolerance in plants. Several studies in the present time have shown the role of endophytes in the mitigation of abiotic stresses, namely, drought, nutrient deficiency, high temperature, etc. (Shukla et al. 2012; Khan et al., 2011b). The AM fungi promote soil nutrient uptake (Smith and Read 2008), protection from phyto-pathogens (Newsham et al. 1995), and improves water relations (Auge 2001). Crops displaying better compatibility with soil fungi are a remarkable approach to enhance sustainable agriculture and improve soil nutrient uptake and utilization by plants. Additionally, rhizospheric soil fungi can be manipulated to increase soil health and agriculture cropping systems, utilizing crop rotation as an initial strategy to increase microbial diversity in soil (Ellouze et al. 2014).

12.3 Plant–Fungal Associations in Nature

In nature, plants are closely associated with microbial communities and co-evolved with them during the evolutionary course. The soil-inhabiting fungal communities are extensively explored as key components of agro-ecosystems, highlighting beneficial prospects in ecological functions as well as sustainable agriculture (Devi et al. 2020a, b). However, to increase crop productivity, it is important to manage and promote fungal resources efficiently. Among the soil fungal microbiomes, the arbuscular mycorrhizal fungi and the endophytic fungi constitute important microbes with beneficial attributes. AM fungi are widespread in occurrence and form a mutualistic association with roots of terrestrial plants (Smith and Read 2008). The AM fungi form several structures and hyphal networks connect the host plant with the soil (Friese and Allen 1991). Moreover, AM fungi promote availability and uptake of soil nutrients to the plant through extensive mycelial network (Smith and Read 2008). The other beneficial attributes of AM fungi include protection against plant herbivores and pathogens (Gange and West 1994).

12.3.1 Arbuscular Mycorrhizal Fungi

With an aim to increase the agricultural output, the focus has been shifted to arbuscular mycorrhizal fungi in recent times. AM fungi were found to be associated with most agricultural crops and increase nutrient uptake and plant productivity (Smith and Smith 2011). In the ecosystem, AM fungi are involved in multiple biological processes, namely, abundant fungal biomass (Higo et al. 2013), plant root colonization (Jakobsen and Nielsen 1983), and constitute an important component of the ecosystem. These organisms are obligate biotrophs, a host plant being essential for completion of their life cycle and nutritional requirement (Lumini et al. 2007). In mutualistic association with plants, AM fungi form several structures, namely, hyphal coils, spores, arbuscules, etc., for performing necessary functions (Smith and Read 2008). Some remarkable functions displayed by AM fungi comprise of nutrient cycling (nitrogen, carbon, and phosphorous) (Fitter et al. 2011), plant nutrient uptake (Smith and Read 2008), improvement of soil health (Rillig and Mummey 2006) in the ecosystem. The AM fungal association with plants are affected by several biotic and abiotic factors, namely, the plant community which affects the diversity of the AM fungi (Antoninka et al. 2011; Kivlin et al. 2011) and abiotic factors, namely, climatic changes (Kivlin et al. 2011), soil structure (Entry et al. 2002), availability of nutrients (Dumbrell et al. 2010), etc. However, the agriculture management techniques alter the biotic and abiotic factors which have a profound effect on AM fungal communities. The broad range of functions displayed by AM fungal communities highlights their importance in ecosystem.

12.3.2 Fungal Endophytes

Fungal endophytes define an important group of microbes existing in mutualistic association with plants. They colonize the internal tissues of the plant without causing disease and display asymptomatic infections (Rana et al. 2019). Moreover, the fungal endophytes are generally host specific, but endophytes of genera *Colletotrichum* and *Phoma* are associated with a diverse range of plants. The fungal endophytes carry out significant biological functions, for instance, mineral transformations, fungal–metal interactions and phytoremediation, a few significant ones in the ecosystem (Gadd 2007; Yan et al. 2019). Endophytic fungi are classified into two categories: Clavicipitaceous endophytes are seed transmitted in nature and symbiotically associate with grasses (Rodriguez et al. 2009). While the other category of non-clavicipitaceous endophytes are universal in occurrence, colonizing a wide range of plants (Rodriguez et al. 2009). The present era has highlighted the increasing significance of endophytic fungi in ecological functions as well as its biotechnological potential. Studies have suggested the role of fungal endophytes as "biocontrol agents" against plant pathogens (Lahlali and Hijri 2010), abiotic stress (Shukla et al. 2012), plant growth promotion (Barrow and Osuna 2002), besides other functions. For maximum utilization of the beneficial association of mutualistic fungi, it is important to understand the population dynamics and functional role of fungal communities in agriculture system.

12.3.2.1 Impact of Temperature on Microbial Mechanisms

In a plant–microbe association, an ecological condition impact both the plant and the microbe (Fahimipour et al. 2018). Elevated temperatures influences hyphal development and plant colonization of arbuscular mycorrhizal parasites (AMF), likely because of quicker plant carbon allotment to the rhizosphere where AMF lives. Moreover, other ecological conditions such as high temperatures (e.g., dampness and UV radiation) directly influence the tolerance of organisms; however, it is uncertain what happens during functi the mitigation of abiotic stresses, namely onal mechanism *in planta*. This was particularly obvious in the investigation of the impact of temperature on type III discharge of *P. syringae*. While it has been reported that high temperatures contrarily influence type III discharge in vitro, expanded sort III trans-area of effectors into plants was distinguished during PstDC3000 disease in *Arabidopsis* at high temperatures. Hence, it would be attractive if future research to survey natural consequences for microorganisms includes experiments performed *in planta* and utilized new systems (e.g., dual RNA sequencing [RNA-seq]) to uncover both host and organism changes. Plant–microbe interaction helps partners cope with temperature stress. Some rhizosphere microbes and endophytes could mitigate the negative effect of temperature and confer stress tolerance to the host plant.

12.3.2.2 Moisture, Drought, and Plant–Pathogen Interaction

Water is fundamental to life on earth. Too little water (submerged defect osmotic pressure) or an excessive amount (flooding) can adversely affect plants and thereby physiological processes. Plants respond to water deficiency by managing the levels of the phytohormone abscisic acid (ABA). An ABA increase triggers transcriptional reconstructing and physiological changes, including closure of stomata to decrease transpiration. Studies into *Arabidopsis* showed that the bacterial pathogen, *P. syringae*, or PAMPs (a 22-amino-corrosive epitope of *Pseudomonas* flagellum), can be seen by FLS2, bringing about stomatal closure to decrease pathogen movement.

12.3.2.3 Drought and Plant–Root Microbiome Interaction

Drought comprise a key abiotic stress influencing plant–soil microbiome (Kour et al. 2020a). Studies have suggested that dry conditions influenced microbial network structure in all examined compartments (mass soil, rhizosphere, and root endosphere). Also, in a study to understand the impact of soil dampness on sorghum root microbiome, it was found that while bacterial network remained unchanged, dry season decreased microbial communities in rhizosphere and root endosphere. On the host side, dry spell pressure causes a movement of root metabolites. How these dry conditions influence root biome structure is still to be understood. By and by, this intriguing relationship recommends that, under dry season, there might be atomic discourse among plants and related microbiome to reshape root microbiota so as to adapt to dry season pressure, interpreting this subatomic exchange should define crucial information to utilize microbiota to upgrade dry season resistance in crop plants.

12.4 Fungal Microbiomes: Functional Role and Significance

Plants are susceptible to multiple biotic and abiotic stresses which hamper cellular functions, affecting plant growth and physiology. Plants have evolved several tolerance mechanisms to combat stress, production of phytohormones being an important one. Studies have shown that exogenous application of phytohormones improves plant growth and metabolism under stress conditions (Egamberdieva et al. 2017a). Plant-associated microbes were found to positively influence plant growth, improve nutritional uptake, biotic and abiotic stress tolerance, and protection against pathogens (Cho et al. 2015; Grover et al. 2013). Moreover, beneficial plant–microbe associations have several socio-economic implications: plant-associated rhizobacteria, mycorrhiza, and endophytic microbes have positive effects on plants (Bonfante and Genre 2010), *Trichoderma harzianum* (saprophytic fungi) aids root colonization by arbuscular mycorrhizal fungi and promotes plant growth (Arriagada et al. 2009a, b). To improve plant growth and confer stress tolerance, the microbes synthesize phytohormones under drought, high-temperature, metal toxicity, and saline conditions (Egamberdieva et al. 2017b; Liu et al. 2013; Sgroy et al. 2009). Besides phytohormone-producing bacterial species, several fungal species were found to produce phytohormones and influence plant physiology. A significant study by Contreras-Cornejo et al. (2009) highlighted that the inoculation of *Arabidopsis* *thaliana* with *Trichoderma virens* leads to root formation and growth and increased plant biomass(Contreras-Cornejo et al. 2009). Other beneficial attributes of plant–fungal associations were seen in a study by Khan et al. (2011a). *Aspergillus fumigatus* produced phytohormone, gibberellins, and increased shoot biomass of *Glycine max* under salt stress conditions (Khan et al. 2011a). A similar study showed that *Phoma glomerata*, an endophytic fungus in *Curcumis melo*, improved drought and salt tolerance in culture (Waqas et al. 2012). Phytohormone production by fungal communities modulate physiological processes in plants; for example, *Trichoderma asperellum* Q1 produced ABA, GA, and IAA, and increased plant biomass of seedlings under salt stress (Zhao and Zhang 2015). These studies showed that plant-associated fungal communities modulate phytohormone levels and, thereby, stress tolerance in plants. Table 12.2 provides a comprehensive account of some key examples of fungal microbiomes, functional role, and their significance in agroecosystem.

12.5 Soil Fungal Resources and Agriculture Management

12.5.1 Crop Rotation Practices

One of the traditional methods in agriculture employed to increase microbial diversity and resistance to pathogens is crop rotation (Fiers et al. 2012). It is one of the most efficient methods and depends on multiple factors, namely, genotype of the crop (Garbeva et al. 2004), order and plantation frequency (Gan et al. 2003), soil properties (Bernard et al. 2012), etc., and have a different influence on the microbial communities. Moreover, plantation of different crops (intercropping) of cereals (Kiaer et al. 2009), or mixed plantation of barley, canola, and wheat (Nelson et al. 2012), promotes crop yield by decreasing diseases. A better management of crops can also be achieved by changing the crop frequency over different time periods (Gan et al. 2003). Crop rotation practices work better for some crops as compared to others; the optimization of sequence would maximize the benefit of crop production (Gan et al. 2003). Soil factors also play a crucial role in crop rotation, and affect distribution and diversity of fungal communities (Reis Martins et al. 2012). Another prospective method for crop management includes crop diversification (Larkin and Honeycutt 2006) which promotes diversity of fungal communities. Different organic residues by different crops result in diverse crops, increasing fungal biomass and diversity (Swer et al. 2011). Moreover, crop diversification hinders the life cycle of an associated plant pathogen, thereby decreasing disease outcome and susceptibility of plants (Singh et al. 2020b). Crop rotation for longer durations is better tolerant and results in better crop yields as compared to short rotations. Several other parameters of importance comprise allelopathy of crops (Bernard et al. 2012) and possibilities of pathogens affecting alternative crops (Merz and Falloon 2009).

Table 12.2 Fungal microbiomes: functional role and significance in agroecosystem

Fungal species	Biological Functions	Ecological role	References
Arbuscular Mycorrhizal fungi	Nutrient and water uptake, reducing availability to decomposers	Subsoil carbon cycling – nutrient additions	Jansa and Treseder (2017)
Arbuscular Mycorrhizal fungi	Increased organic matter decomposition	Carbon cycling – litter decomposition	Rumpel and Kögel-Knabner (2011)
Arbuscular Mycorrhizal fungi	Nutrient mineralization and immobilization in soil	Re-allocation of nutrients	Kautz et al. (2013)
Arbuscular Mycorrhizal fungi	Phosphorous uptake and utilization by plants	Better plant growth, Phosphorus plant nutrition	Almario et al. (2017)
Arbuscular Mycorrhizal fungi	Improved seed establishment and quality of *T. durum* Desf.	Plant growth promotion attributes	Colla et al. (2015)
Arbuscular Mycorrhizal fungi	Carbon storage in subsoil	Enhanced nutrient assimilation	Jansa et al. (2006)
Arbuscular Mycorrhizal fungi	Decreased nutrient leaching in soil	Enhanced nutrient uptake by the plant	Köhl and van der Heijden (2016)
Endophytic fungi	$Ca_3(PO4)_2$ and rock phosphate solubilization	Promotion of plant growth	Gupta et al. (2007)
Endophytic fungi	Phosphate-solubilizing activity	Enhanced plant development for sustainable agriculture	Nath et al. (2012)
PGP endophytes	Increased surface area of roots and root biomass	Enhanced nutrient uptake and use by the plant	Delaplace et al. (2015)
Endophytic fungi	Polycyclic aromatic hydrocarbon (PAH) degradation	Environmental Sustainability via bioremediation	Bhatt et al. (2002)
Endophytic fungi	Disintegration of phenanthrene	Bioremediation of contaminated soil	Tian et al. (2007)
Endophytic fungi	3-D structure changes of toxic compounds	Biotransformation of toxic compounds, plant tolerance	Borges et al. (2008)

12.5.2 *Selection of Plant Varieties and Genotype*

Selection of plant varieties with better genotypes may be another prospective method to increase crop productivity in agriculture. Several studies have shown the importance and selection of plant genotypes in protection against phyto-pathogens, a key technique in managing soil–fungal resources (Ellouze et al. 2014). The variation between genotypes accounts for the variation in signaling molecules and fungal dynamics which paves way for fungi-compatible crop plant. Moreover, selection of plant varieties compatible with fungal communities is an effective approach to regulate soil nutrient uptake in ecosystems. Different crop varieties planted in combination in agricultural systems have led to improved crop yield under changing climatic conditions and higher productivity (Finckh 2008). Moreover, growing mixed crop varieties sustains diverse niches to soil microbes, thus maintaining diversified fungal communities in soil (Ustuner et al. 2009).

12.5.3 Microbial Inoculation and Soil Management

In agricultural systems, inoculation of soil with rhizobial inoculants is regarded as an effective method to promote soil microbial diversity; however, the competition among diverse microbes may not produce the desired outcome. However, using rhizobial inoculants in combination may increase the positive effects on plant growth and production. Different management practices alter soil properties and promote beneficial effects of microbes; soil organic matter (SOM) controls multiple functions of the soil and is a prospective approach to manage soil microbes. Agricultural practices, namely, tillage practices and using organic content in soil influence SOM (Manlay et al. 2007). The addition of organic matter and manure aids in the maintenance of soil structure through microbial communities. It improves moisture levels and nutrients in soil, improving growth of both fungal communities and associated host plants. Studies have demonstrated the beneficial effect of fungi as a biocontrol agent in plant growth promotion (Siddiqui and Futai 2009); organic supplementation provides nutrients to microbes, having a beneficial effect on crop yield and production. Furthermore, organic content promotes SOM accumulation, leading to an increase in moisture retention, aeration, and soil porosity (Ellouze et al. 2014). The addition of microbial inoculants in soil improves soil health and increases plant growth and productivity.

12.6 Plant–Fungal Associations and Sustainable Agriculture

Plant–fungal associations define a significant interaction where the fungal species contribute to plant growth and productivity. The ecosystem interacts with the beneficial microbiome in mutualistic, obligate, and various other interactions, demonstrating multiple socioeconomic applications. Plant-associated microbes contribute in multiple ways, namely, plant growth promotion, tolerance to biotic and abiotic stress, and resistance against pathogens, defining a key approach in sustainable agriculture. Moreover, fungi carry out several functions in soil, such as cycling of nutrients, organic decomposition, promoting plant growth, and pathogen protection, among other functions (Kour et al. 2019b). Root-associated endophytic fungi activate ethylene/jasmonate or salicylic acid pathways (systemic resistance) in plants conferring stress tolerance (Lahlali et al. 2014). In soil, the occurrence of fungal communities is affected by several parameters, namely, physio-chemical structure of soil, soil type, climatic conditions, and plant species (Tkacz et al. 2015). Moreover, fungal communities present in soil are also influenced by agricultural management practices. The microbes inhabiting the rhizosphere produce phytohormones, protect against pathogen attack, and may be niche specific (Mendes et al. 2011). These microbial communities comprise of arbuscular mycorrhizal fungi (AMF), endophytes, and rhizobial symbionts, respectively.

12.6.1 Arbuscular Mycorrhizal Fungi and Sustainable Agriculture

Arbuscular mycorrhizal fungi are widely used as biofertilizers in agriculture and beneficial to plant growth and quality improvement. AMF offers several advantages to organic farming, contributing to nutritional uptake by plants, protection against soil-borne pathogens, tolerance to heavy metals, and salinity, among others (Ryan and Graham 2002; Smith and Read 2008). Although mycorrhizal associations are frequent in both wild and cultivated plants, plant species classified under Cyperaceae and Brassicaceae do not associate with AMF. The formation of arbuscular mycorrhizal fungal association with plants is considered as an adaptive mechanism, which enhanced nutritional uptake by the plant and nutrient cycling in soil. The mutualistic association between fungi and plant confers increased tolerance to biotic and abiotic stress to the plant as well as improve soil health (Jeffries et al. 2003). Moreover, the AMF performs key functions in sustainable agriculture by maintaining plant growth and productivity under adverse environmental conditions (Borie et al. 2010). The colonization of AMF with the plant alters root exudates' chemical composition, which further influences microbial diversity. These mechanisms occur in soil constituting "mycorrhizosphere," and understanding and manipulating these may be a prospective approach in sustainable agriculture.

Yang et al. (2015), showed that the association of AMF with the leguminous tree (*Robinia pseudoacacia*) may be useful for restoration of lead (Pb)-contaminated soil and phytostabilization. Moreover, symbiotic associations, namely, nitrogen-fixing bacteria and mycorrhizal fungi have a positive influence on plant, enhance plant growth, and confer stress tolerance, leading to improved soil health and sustainable agriculture. Studies have also suggested the importance of saprophytic fungi in biodegradation which promote root colonization by AMF (Arriagada et al. 2009a, b).

12.6.2 **Trichoderma** *spp. and Plant Improvement*

Displaying a significant role in ecosystem, plants associated with *Trichoderma* spp. were extensively explored for their biotechnological and ecological importance (Sharma et al. 2019). *Trichoderma* spp. are present in rhizosphere and are used as a biocontrol agent for phyto-pathogens. Different strains of *Trichoderma* spp. are used for different pathogens; some strains are more effective than others (Harman 2000). Moreover, plant growth can be improved in *Zea mays* and ornamental plants, and root system can be stabilized to drought by application of *Trichoderma* spp. (Harman 2000). *Trichoderma harzianum* T-22, decreased the toxic fusaric acid, produced by *Fusarium oxysporum* f. sp. *gladioli*, harmful to the plant (Nosir et al. 2011). Biocontrol agents exert several mechanisms for pathogen control, for example, *Trichoderma* enhance or reduce plant secretion of antimicrobial compounds (Contreras-Cornejo et al. 2009). Contreras-Cornejo et al. (2009) studied the association of *Trichoderma virens* with *Arabidopsis* increases plant biomass and lateral root growth by auxin-dependent mechanism. Moreover, Viterbo et al. (2010) suggested that *T. asperellum* regulates ACC (1-aminocyclopropane-1-carboxylate) deaminase, which promotes root growth in *Brassica napus*. Once the fungus infects the root and gains entry, the fungus grows intercellularly. *Trichoderma koningii* restricts the production of phytoalexins by colonizing the roots of *Lotus japonicas* (Masunaka et al. 2011).

Several other species of *Trichoderma* influence plants in multiple beneficial ways, for example, *T. harzianum* increased blooming rate, germination, and height, etc., in chrysanthemum and pepper plants (Chang et al. 1986), treatment of *Triticum aestivum* and *Glycine max* with *Trichoderma* and *Penicillium* increased plant growth (Shivanna et al. 1996), plant protection against root pathogens (Chet et al. 1997), among other significant studies. Also, *Trichoderma* strains colonizing rhizosphere confer resistance against pathogens, a mechanism similar to systemic acquired resistance (SAR), induced systemic resistance (ISR), and other plant mechanisms (Harman et al. 2004). *Trichoderma* may induce plant defense mechanisms by elicitor-mediated response activating pathogenesis-related (PR) proteins, phytoalexins, etc., which might increase tolerance to pathogen attack (Dana et al. 2001; Elad et al. 2000). Plants also respond to biotic stress by hormone activation and signaling (known as JA/ET-mediated ISR), aiding plant resistance against plant pathogens (Yoshioka et al. 2012). Moreover, studies have also shown that *Trichoderma* spp. when present in endophytic association with plants regulate transcriptional changes and protect plants against biotic and abiotic stresses (Bae et al. 2009; Bailey et al. 2006).

12.6.3 Piriformospora indica *and Plant Growth Promotion*

Piriformospora indica, an endophyte, is found associated with orchid plants from India. It colonizes roots and promotes plant growth similar to AMF. *P. indica* is found in association with a diverse range of plants and influences plant growth, disease resistance, nutrient uptake, etc. (Unnikumar et al. 2013). The association of beneficial fungi with higher plants has distinct advantages in the promotion of plant growth, leading to a more sustainable approach in agriculture. The fungi classified under order Sebacinales are widespread in occurrence and exhibit enormous diversity (Weiss et al. 2011). Moreover, *P. indica* colonize the roots of terrestrial plants, aided by ethylene hormone (Camehl et al. 2010). Several plant species are colonized by the fungi; some key examples are *Nicotiana tabacum*, *Zea mays*, *Oryza sativa*, *Saccharum officinarum*, among other species (Varma et al. 2012). The endophyte, *P. indica*, is very efficient in enhancing plant growth when used commercially as "biofertilizer." Its application to increase crop yield may lessen the use of chemical fertilizers, tolerance to biotic and abiotic stress in plants and plant growth promotion (Unnikumar et al. 2013).

12.7 Directions for Future Research and Outcomes

The present era calls for more targeted and intensive strategies in agriculture to provide food to billions across the globe. The plant soil microbiome constitutes an integral component of agro-ecosystem having a major ecological impact on agricultural production. In order to enhance the agricultural productivity of ecosystems, it is essential to effectively manage fungal communities. In this area, several agricultural practices, namely, plant genotype selection and crop rotation will aid in increasing microbial biodiversity and beneficial fungal communities in soil. However, several socio-economic challenges exist which accounts for the maintenance of ecological balance and social needs, without affecting crop yields. The prime concern is the requirement to increase food supply and to feed the ever-increasing world population, besides generating revenues and alternative sources of energy without causing adverse effects on environment. Moreover, the two most plausible strategies are based on the manipulation of plant–fungal associations or the development of soil microbial communities. Since the mechanism of plant–microbe interaction is still not completely understood, a better understanding is required at the physiological levels for profiling large microbial communities (Barea 2015). One recent advancement in this area focuses on engineering the rhizosphere to increase microbial communities and their establishment; however, there are many gaps in defining the right strategy in understanding plant–fungal associations. Moreover, the manipulation of soil microbiomes and the presence of desired fungal communities would benefit sustainable agriculture and reduce the use of agrochemicals, thus offering a healthier approach to ecological and social subsistence.

12.8 Conclusion

With the global rise in population and an increasing demand for food supply, exploring alternative methods for promoting sustainable agriculture is the need of the hour. In this context, exploitation of the soil microbial communities and their impact on ecosystem is a promising approach highlighting beneficial outcomes. Plant-associated fungal communities (particularly arbuscular mycorrhizal fungi) positively influence plant growth and confer tolerance to biotic and abiotic stress. Moreover, fungi carry out several functions in soil, such as cycling of nutrients, organic decomposition, promotion of plant growth, and pathogen protection, among other functions. These fungal communities promote plant growth and productivity employing several mechanisms, contributing to a more organic approach to sustainable agriculture, and reducing the need of chemical applications. With depleting natural resources and increasing global demand for food supply, exploitation of plant–fungal associations defines a highly prospective approach to increase crop productivity and global agricultural production.

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Chapter 13
Trichoderma: Biodiversity, Abundances, and Biotechnological Applications

Imran Afzal, Ambreen Sabir, and Shomaila Sikandar

Contents

13.1 Introduction

Genus *Trichoderma* is filamentous fungi, which is commonly found in soil and the rhizosphere of several plants. This genus has become a popular topic of interest in the field of basic research and applied mycology mainly because of the important role played by *Trichoderma* spp. in different ecosystems extending from the agricultural fields and forest soils as well as acting as a substrate for the production of mushrooms (Kredics et al. 2018). These species also have the ability to function as a biocontrol agent against the plant pathogenic nematodes and fungi by their aggressive mechanisms based on antibiosis, competition, and parasitism. Moreover, the biostimulant ability of certain *Trichoderma* species, which enhances the nutrients uptake in plants, improves crop production, induces systematic tolerance, and stimulates the growth of a plant, that can be manipulated for environmental-friendly agricultural practices (Contreras-Cornejo et al. 2016; Gupta et al. 2014; López-Bucio et al. 2015; Nawrocka and Małolepsza 2013).

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The recently evolved concept of *Trichoderma* spp. as a biocontrol agent proved them as an avirulent. They develop a symbiotic relationship with endophytic plants through their long-term ability to colonize and disperse in the roots and provide plants with several benefits (Lorito et al. 2010). However, in addition to the positive side of this genus, some of the *Trichoderma* species may also have harmful effects on agriculture such as green mold disease which affects the mushroom cultivation, which destroys the mushroom production (Hatvani et al. 2008; Kredics et al. 2010). Ecological fitness is an important trait of *Trichoderma* to establish in agricultural habitats such as soil, plant rhizosphere, or compost materials (Cordier et al. 2007; Weaver et al. 2005). The survival and spread of *Trichoderma* in agricultural habitats depend on their interactions with the environmental parameters as well as the biotic and abiotic components of the local ecosystem (Kredics et al. 2010).

The primary mode of identity of *Trichoderma* species is morphology-based but that method is not always very accurate to differentiate the diversity of these species (Ghazanfar et al. 2018; Zhang et al. 2007). The tremendous trait of this genus is its ability to infest other disease-causing mycoflora especially associated with root rot and wilt infections (Santoro et al. 2014; Verma et al. 2007). *Trichoderma* species were mentioned as endophytic fungi at the same time as generally located in all kinds of soils along with agricultural, orchard as well as forest soil as opportunistic plant symbionts (Chaverri et al. 2011), and commonly considered an efficacious competitor of plant pathogens (Kim et al. 2012).

13.2 *Trichoderma* and Its Biology

The term *Trichoderma* is derived from the mix of two words, thrix (hair) and derma (skin) (Hyder et al. 2017). The species of *Trichoderma* are ubiquitous invaders of cellulosic materials and usually found on the decaying plants and wood as well in the plant rhizosphere, where they induce systemic tolerance against pathogens (Jaklitsch 2009; Kubicek et al. 2008; Schuster and Schmoll 2010). They are free-living filamentous fungi that are widely spread in every environment which makes them strong opportunistic colonizers that compete for adaptability and food and act as mycoparasites (Hyder et al. 2017).

Members of the genus *Trichoderma* are often diverse, show a great genetic versatility, and commonly found in various habitats (Samuels 2006; Zhang et al. 2007). These species are asexual in nature and exist in harmony with other soil-dwelling fungi belonging to the genus Ascomycota (Hypocrea) categorized as imperfect fungi (Hassan et al. 2014; Mazrou et al. 2020). *Trichoderma* species have been easily isolated from natural soil, decaying plant organic matter, and wood. In different nutrient media, *Trichoderma* spp. growth rate increases and they multiply very fast under the optimum range between 25 and 30 °C. Characterization of produced spores/conidia is done by different shades of green, whereas some species harvest chlamydospores (Ghazanfar et al. 2018; Guo et al. 2010; Latifian et al. 2007) which is a diagnostic tool that is also found in related and unrelated genera such as

Myrothecium, *Clonostachys,* and *Aspergillus* as well as *Penicillium* respectively (Alvindia and Hirooka 2011). Production of chlamydospores in two species such as *T. longibrachiatum* and *T. viride* is also observed (Hyder et al. 2017).

Conidiophores of these species are not well defined yet mostly ramified. These are comprised of single-celled conidia and phialides present at the edges of branched hyphal linkage but cannot be seen on a week old images. Usually, the shape of conidia is ellipsoidal to oblong, but in some cases *Trichoderma* spp. have spherical to oval-shaped whereas few have smooth-surfaced conidia, respectively (Jaklitsch 2009).

Ecologically, species of *Trichoderma* are very presiding competitors and have the ability to grow in different ecosystems as well in all climatic regions (Antarctica, tropical, tundra) (Montero-Barrientos et al. 2011; Mukherjee et al. 2013b) due to some specific properties such as strong opportunistic invading nature, fast growth rate, prolific production of spores, and powerful manufacturer of antibiotics even in the highly modest environment (Akshata et al. 2018; Montero-Barrientos et al. 2011; Schuster and Schmoll 2010). In 2011, marine *Trichoderma* species were isolated and characterized to assess their potential as halotolerant biocontrol agents, and effective results were shown against *Rhizoctonia solani* inducing systemic defense responses in plants (Gal-Hemed et al. 2011).

13.3 *Trichoderma*: Biodiversity and Abundance

In 1794, Persoon firstly named the fungus *Trichoderma* and then Tulsane in 1865 suggested the sexual form of *Hypocera* species. However, morphologically differentiation between the species assigned to the genus *Hypocrea/Trichoderma* was problematic and challenging. So, it was suggested to lessen the taxonomy and nomenclature to simply to a single species, *Trichoderma viride.* The development in the concept of their identification was initiated in 1969, and since then, at least 1100 *Trichoderma/Hypocrea* strains have been identified from 75 different molecularly characterized and recognized species (Contreras-Cornejo et al. 2016; Druzhinina et al. 2011; Samuels 2006; Schuster and Schmoll 2010). Since then, mycoparasites, *T. virens* and *T. atroviride* formerly known as *Gliocladium virens* and *Hypocrea atroviridis* and the saprophytic *T. reesei* (teleomorph *Hypocrea jecorina*) are the most frequently studied species while important differences in their lifestyles were revealed by modified and comparative genome analysis (Kubicek et al. 2011; Mukherjee et al. 2013a).

Trichoderma spp. are ubiquitous and found in various ecosystems in different climatic regions (Kubicek et al. 2008). Microclimate, substrate availability as well as intricate ecological interfaces are some factors which modulate the presence of *Trichoderma* species (Hoyos-Carvajal and Bissett 2011). Moreover, competence, metabolic diversity, and high reproductive ability of *Trichoderma* also help them survive in diverse geographical habitats. Many studies on the biodiversity of *Trichoderma* followed the standard/benchmarked culture-based protocol consisting of sample collection, isolation on selective media and their maintenance in the growth media or cultures as reported in the previous literature (Chang et al. 2020; Filizola et al. 2019; Saravanakumar et al. 2016). Population and diversity of these species in cereals crop fields and agricultural ecosystems are affected by several biotic and abiotic factors, which include plant species, competition with microbial community, physical and chemical properties of soil, application of different fertilizers or pesticides (Sharaff et al. 2020; Sharma et al. 2019). *Trichoderma* spp. can be isolated from any type of agricultural fields (Harman 2006).

The rhizosphere is considered as one of the top-notch ecological niches for *Trichoderma* species which are attracted by enriched plant-root acquired nutrient exudates and existence of soil-borne fungi as their prey (Druzhinina et al. 2011; Yadav et al. 2020a, b). Members of this genus are the most commonly isolated fungi which can be easily isolated from the non-rhizosphere and rhizosphere soil than phyllo-spheres, as well from the different crop fields from all the climatic regions of all continents (Harman et al. 2004b). Table 13.1 highlights the different *Trichoderma* species present in the various habitats.

13.4 Biotechnological Applications

13.4.1 Trichoderma *as Biocontrol Agents*

Plant diseases are the key source of reducing overall crop yields. There are numerous synthetic products that have been used to control plant infections. However, the extensive use of pesticides produces unwanted effects on non-target organisms. Therefore, there are serious concerns related to the hazardous effects of the overuse of these synthetic chemicals. Eventually, it led to a substantial interest in developing eco-friendly methods of biocontrol against plant pathogens. In the early 1930s, *Trichoderma* was identified as one of the most popular biocontrol agents of plant pathogens (Gupta et al. 2016; Weindling 1932). Plant rhizosphere is a very competitive environment for indigenous microbes such as *Trichoderma*. These microbes compete for plant nutrients and fight other members to dominate this environment using a range of different mechanisms. Plant-associated *Trichoderma* species use mechanisms such as antibiotics production, mycoparasitism, nutrient limitation, and cell-wall-targeting lytic enzyme production to dominate over competition (Monfil and Casas-Flores 2014). They can also protect their plant host by interfering with pathogen invasion and triggering plant defense responses such as induced systemic resistance against the invading pathogens (Harman et al. 2004a). These features make *Trichoderma* species a very attractive biocontrol agent—organisms that can be used to target other unwanted organisms such as plant pests and pathogens (Table 13.2).

Trichoderma-based bio-fungicides are successful in agricultural sector and comprises of 60% of all the fungal-based products (Thakur et al. 2020). *Trichoderma* spp.-based registered biocontrol products are considered as a novel type of

Table 13.1 Biodiversity of the genus *Hypocrea* and *Trichoderma* in different habitats

Trichoderma spp.	Habitats	References
Trichoderma hamatum, *T. harzianum*, *Trichoderma koningii*, *Trichoderma polysporum*, *Trichoderma pseudokoningii*, and *Trichoderma viride*	Forest soils in the southeastern United States and Washington State	Danielson and Davey (1973)
T. hamatum, T. harzianum, Trichoderma longibrachiatum, T. polysporum, T. koningii, T. pseudokoningii, and *T. viride*	Spruce-forest soil in Canada	Widden and Abitbol (1980)
Trichoderma aureoviride, T. harzianum, T. koningii, T. longibrachiatum, and *T. viride*	Dead wood of apple twigs, oak wood, and cork wood samples in Hungary	Vajna (1983)
T. asperellum, *Trichoderma atroviride*, *Trichoderma ghanense*, *T. hamatum*, *T. harzianum*, *T. koningii*, *Trichoderma oblongisporum*, and *Trichoderma virens*	Soils samples or tress barks from Siberia, Nepal, northern India, Taiwan, Thailand, Cambodia, and Malaysia	Bissett et al. (2003)
T. asperellum, *T. atroviride*, *T. ghanense*, *T. hamatum*, *T. harzianum*, *Hypocrea jecorina*/*Trichoderma reesei*, *T. koningii*, *Trichoderma spirale*, *T. virens*, and *T. viride*	Soil samples from 19 different location of 7 countries	Kubicek et al. (2003)
T. atroviride and *T. viride*	Winter wheat soil of Germany	Hagn et al. (2003)
Trichoderma hamatum, *T. harzianum*, *T. koningii*, *T. pseudokoningii*, and *T. viride*	Soybean soil samples in Poland	Pięta and Patkowska (2003)
Trichoderma harzianum, *T. virens*, and *T. koningii*	Oil palm soils in Malaysia	Sariah et al. (2005)
T. hamatum, T. harzianum, *T. koningii*, and *T. viride*	Soils samples of a radish field in Japan	Mghalu et al. (2007)
T. asperellum, *T. atroviride*, *T. brevicompactum T. hamatum*, *T. harzianum*, and *T. virens*	Soil and phyllosphere of rice in paddy fields at the southern coast of the Caspian Sea, Iran	Naeimi et al. (2010)
T. harzianum, T. aggressivum, T. citrinoviride, T. hamatum, T. virens, T. longibrachiatum, T. polysporum, T. tomentosum, T. atroviride, T. gamsii, T. koningii, T. viridescens, T. viride, and *T. koningiopsis*	Samples were collected from decaying wood, cultivated mushroom compost, soil (garden, forest), and cereal grains (triticale, maize) at 49 sites in Poland	Błaszczyk et al. (2011)

(continued)

Table 13.1 (continued)

Trichoderma spp.	Habitats	References
T. atroviride, *T. brevicompactum*, *T. gamsii*, *T. harzianum*, *T. koningiopsis/T. ovalisporum*, *T. longibrachiatum*, *T. pleuroticola*, *T. rossicum*, *T. spirale*, *T. tomentosum/T. cerinum*, and *T. virens*	Rhizosphere soils of five winter wheat fields in the Pannonian Plain of Hungary	Kredics (2012)
T. harzianum, *T. koningiopsis*, *T. virens*, *T. asperellum*, *T. atroviride*, *T. erinaceum*, *T. koningiopsis*, *T. pleurotum*, *T. reesei*, and *T. spirale*	Corn field soils in Egypt, Mexico, and Venezuela	Gherbawy et al. (2004)
T. asperellum, *T. atroviride*, *Trichoderma brevicompactum*, *T. citrinoviride*, *T. erinaceum*, *T. hamatum*, *Trichoderma koningiopsis*, *H. lixii/T. harzianum*, *T. reesei/H. jecorina*, *T. spirale*, *Trichoderma stromaticum*, *Trichoderma vermipilum*, and *Hypocrea virens*	Chinese forest soils	Sun et al. (2012)
T. aureoviride, *T. asperellum*, *T. harzianum*, *T. atroviride*, *T. koningiopsis*, *T. longibrachiatum*, *T. koningii*. *T. tawa*, *T. viridescens*, *T. virens*, *T. hamatum*, *T. viride*, and *T. velutinum*	Sediment samples from coastal wetlands (tidal flat and wetland) in Southeast China	Saravanakumar et al. (2016)
T. atroviride, *T. citrinoviride*, *T. cremeum* P. Chaverri& Samuels, *T. gamsii*, *T. harzianum* complex, *T. koningii*, *T. koningiopsis*, *T. longibrachiatum*, *T. longipile* Bissett, *T. viride*, and *T. viridescens*	Decaying wood collected from the forests of Tatra mountains, Gorce mountains, and Karkonosze mountains located in Central Europe in Poland	Błaszczyk et al. (2016)
T. asperellum, *T. atroviride*, *T. virens*, *T. hamatum*, *T. harzianum*, *Hypocrea semiorbis*, *T. epimyces*, *T. konilangbra*, *T. piluliferum*, *T. pleurotum*, *T. pubescens*, *T. strictipilis*, *T. hunua*, *T. oblongisporum*, and an unidentified species, *Trichoderma* sp. MA 3642	Samples from the forests, agriculture grassland, and wetland ecosystems of China	Dou et al. (2019)
Trichoderma harzianum, *Trichoderma viride*, and *Trichoderma longibrachiatum*	Soil sample of Myanmar	Nyunt et al. (2020)
T. harzianum, *T. hamatum*, *T. atroviride*, *T. citrinoviride*, *T. virens*, *T. koningii*, *T. koningiopsis*, *T. viride*, *T. tomentosum*, *T. petersenii*, *H. stellate*, *T. saturnisporum*, and *T. asperellum*	Soil samples of Volcanic Forest Park and Volcanic platform	Chang et al. (2020)

Table 13.2 Biocontrol activities, target plants, and mechanisms of different strains of *Trichoderma* spp., as biocontrol agents

Species	Target	Plants	Mechanisms	References
T. harzianum	*Fusarium oxysporum* *Meloidogyne incognita* *Sclerotinia sclerotiorum* *Fusarium graminearum* *Fusarium moniliforme* *Macrophomina phaseolina* *Ralstonia solanacearum* *Rhizoctonia solani* *Cucumber mosaic virus*	Cucumber Okra Soybean Maize Tomato Sesame Mango In vitro	ISR, ROS, Lytic enzymes, VOC	Harman et al. (2004a), Yang et al. (2011), Kumar et al. (2012), Mukhtar et al. (2013), Stewart and Hill (2014), Khaledi and Taheri (2016), Yuan et al. (2016), Zhang et al. (2016), Vitti et al. (2016), Gajera et al. (2016), Saravanakumar et al. (2017) and Mahmoud and Abdalla (2018)
T. koningii	*Rhizoctonia solani*	In vitro	Mycoparasitism, lytic enzymes	Abdel-Latif and Haggag (2010), Stewart and Hill (2014) and Gajera et al. (2016)
T. cremeum	*Fusarium oxysporum* *Botrytis cinerea*, *Rhizoctonia solani*	Tomato, In vitro	Secondary metabolite	Vinale et al. (2016) and Błaszczyk et al. (2017)
T. asperellum	*Fusarium oxysporum* *Pythium myriotylum* *Phytophthora capsici* *Thielaviopsis paradoxa* *Colletotrichum gloeosporioides* *Phytophthora megakarya*	In vitro, cocoyam, pepper, pineapple, mango, cocoa	Lytic enzymes, mycoparasitism	Viterbo et al. (2010), Wijesinghe et al. (2010), Mbarga et al. (2012), Segarra et al. (2013), de los Santos-Villalobos et al. (2013), Mbarga et al. (2014) and El-Komy et al. (2015)
T. hamatum	*Rhizoctonia solani* *Sclerotinia sclerotiorum* *Phytophthora capsici* *Botrytis cinerea* *F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum* *Fusarium verticillioides* *Fusarium solani*	In vitro, Arabidopsis thaliana, Maize, Cassava, Chili Pepper	Secondary metabolites, mycoparasitism, lytic enzymes, ROS, ISR	Krause et al. (2001), Sobowale et al. (2010), Mathys et al. (2012), Studholme et al. (2013), Gajera et al. (2016), da Silva et al. (2016), Błaszczyk et al. (2017) and Chemeltorit et al. (2017)

(continued)

Table 13.2 (continued)

Species	Target	Plants	Mechanisms	References
T. virens	*Fusarium moniliforme* *Rhizoctonia solani*	Mango, ryegrass	VOC	Harman et al. (2004a), Kumar et al. (2012), Kandula et al. (2015) and Gajera et al. (2016)
T. atroviride	*Rhizoctonia solani* *Rhizoctonia solani* *Pythium ultimum* *Sclerotiniatri foliorum* *F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum*	Cucumber, In vitro, ryegrass, red clover, white clover	Mycoparasitism, ISR, VOC	Kandula et al. (2015), Błaszczyk et al. (2017) and Nawrocka et al. (2018)
T. brevicrassum	*Rhizoctonia solani*	Cucumber	Mycoparasitism, lytic enzymes	Zhang and Zhuang (2020)
T. viride	*Fusarium moniliforme* *F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum*	Mango; in vitro	VOC, mycoparasitism	Engelberth et al. (2001), Kumar et al. (2012), Gajera et al. (2016) and Błaszczyk et al. (2017)
T. pseudokoningii	*Tobacco mosaic virus* *Rhizoctonia solani*	Tobacco, In vitro	ISR	Luo et al. (2010) and Gajera et al. (2016)
T. reesei	*Fusarium Oxysporum* *Pythium ultimum* *Sclerotia rolfsii* *Sclerotinia sclerotiorum*	In vitro		Abdel-Latif and Haggag (2010)
T. citrinoviride	*F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum*	In vitro		Błaszczyk et al. (2017)
T. koningiopsis	*F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum* *Fusarium flocciferum* *Fusarium oxysporum*	In vitro	Secondary metabolites	Błaszczyk et al. (2017) and Hu et al. (2017)

(continued)

Table 13.2 (continued)

Species	Target	Plants	Mechanisms	References
T. longibrachiatum	*F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum* *Fusarium verticillioides*	In vitro, Maize	Secondary metabolites	Błaszczyk et al. (2017) and Sobowale et al. (2010)
T. longipile	*F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum*	In vitro		Błaszczyk et al. (2017)
T. viridescens	*F. avenaceum* *F. culmorum* *F. cerealis* *F. graminearum* *F. temperatum*	In vitro		Błaszczyk et al. (2017)
T. aureoviride	*Fusarium solani*	Cassava	Lytic enzymes, ROS	da Silva et al. (2016)

ROS reactive oxygen species, *ISR* induced systemic resistance, *VOC* volatile organic compounds

biocontrol agents (Woo et al. 2006). However, only a few species of *Trichoderma* have been reported for their suitability as biocontrol agents for the agriculture sector, which are summarized in Table 13.2. *T. harzianum* is one of the most common biopesticides and biofertilizers that are being currently used and commercially available (Lorito et al. 2010; Vinale et al. 2006). *Trichoderma* uses different mechanisms to antagonize phytopathogenic fungi which include direct mycoparasitism, antibiosis, competition, and colonization (Howell 2003). The antagonistic potential of *Trichoderma* enables it to be an effective biological control agent as compared to the alternative method used to control plant pathogens (Chet 1987).

13.4.2 *Plant Growth-Promoting Effects of* Trichoderma

The increasing use of chemical fertilizers for agricultural productivity has led to serious environmental problems. In nature, different soil-borne bacteria and fungi can colonize plant roots and may have beneficial effects on the plant. *Trichoderma* spp. are one of the most studied genera among the classic mycorrhizal fungi, *Rhizobium* bacteria, and other plant-growth-promoting rhizobacteria (PGPR), which can stimulate plant growth by suppressing plant diseases (Rastegari et al. 2020a, b; Van Wees et al. 2008). *Trichoderma* form endophytic associations to interact with other microbes in the rhizosphere to protect plants from diseases, thereby influencing plant growth and yield. Plant growth-promoting attributes by the association of plant–microbes involves molecular recognition through a signaling network mediated by the plant hormones jasmonic acid, salicylic acid, and ethylene (Subrahmanyam et al. 2020; Tiwari et al. 2020; Van Wees et al. 2008). *Trichoderma* spp. are generally found in the rhizosphere of plants due to the secretion of highly hydrated polysaccharides of the root-secreted mucigel layer that encourages the growth of the fungi. *Trichoderma* rhizosphere-competent strains show direct effects on plants by increasing their growth potential through nutrient uptake, their rate of seed germination, and stimulation of plant defenses against abiotic and biotic damage (Shoresh et al. 2010). *Trichoderma* facilitates root colonization, increased rate of leaf photosynthesis, defense mechanisms, and adherence to the root surface, by using plant-derived sucrose and hydrophobins (Vargas et al. 2009). Hydrophobins, which are small proteins of the outermost cell wall layer of fungal cell surface, and expansin-like proteins of *Trichoderma* facilitate the adherence to the root surface for cell wall development. *Trichoderma asperellum* produces the class I hydrophobin TasHyd1, while *Trichoderma harzianum* produces plant cell-wall degrading enzymes which are involved in active root colonization (Morán-Diez et al. 2009; Viterbo and Chet 2006). However, different species of *Trichoderma* have been reported for the production of effectors associated with plant growth promotion, which are summarized in Table 13.3.

13.4.3 **Trichoderma** ***as a Potential Bioremediators***

Accumulation of xenobiotics in soils and water is a worldwide problem. These are chemical substances including polyaromatic hydrocarbons (PAHs), pesticides, heavy metals, polychlorinated aromatics, solvents, hydrocarbons, and other pollutants that are foreign to the biological system (Tripathi et al. 2013). Removal of these contaminants from the environment requires specific physical, chemical, and biological methods. However, in nature, fungi are found in all types of soil, and have the ability to degrade these recalcitrant pollutants and can be applied as an effective tool for bioremediation. *Trichoderma* spp. are the ubiquitous green spore-forming fungi that possess natural resistance mechanisms to these pollutants (Ezzi and Lynch 2005; Harman et al. 2004b; Mulè and Melis 2000). In addition, *Trichoderma* spp. also have an extensive enzymatic system with high biodegradation potential and are effective in soil colonization (Harman et al. 2004a; Lorito et al. 2010). These characteristics make this group of fungi a potential tool toward bioremediation of toxic pollutants (Sharaff et al. 2020). Heavy metals such as arsenic, cadmium, copper, mercury, manganese, and zinc are among the most difficult contaminants to treat and they are increasingly being released into the environment (Errasquın and Vazquez 2003; Tripathi et al. 2007). *Trichoderma* use different strategies for the bioremediation of heavy metals including biovolatilization, biosorption, bioaccumulation, and phytobial remediation. Bioremediation of various pollutants using *Trichoderma* spp. is given in Table 13.4.

Table 13.3 Some of the important effectors produced by *Trichoderma* spp., for plant growth promotion

Species	Effectors	Mechanisms	Plants	References
T. harzianum	Endopolygalacturonase ThPG1 SwolleninTasSwo, an expansin-like protein Cysteine-rich protein (SSCP)	Plant cell-wall-degrading enzymes, cellulose-binding domain, proliferation of secondary roots	Radish, pepper, cucumber, and tomato	Baker et al. (1984), Chang et al. (1986), Harman (2000), Harman et al. (2004a), Morán-Diez et al. (2009), Stergiopoulos and de Wit (2009) and Halifu et al. (2019)
T. atroviride	Cysteine-rich protein (SSCP) Cerato-platanins Sm1/Epl1 Cysteine-rich protein (SSCP)	Protection against plant chitinases, hydrophobin-like SSCP orthologues that can induce expression of defense responses	Cotton, maize, *Arabidopsis*	Harman et al. (2004b), Seidl et al. (2006), Stergiopoulos and de Wit (2009) and Salas-Marina et al. (2011)
T. viride	Xylanase Xyn2/Eix Alamethicin (20mer peptaibol) Cysteine-rich protein (SSCP) Jasmonic acid Salicylic acid Ethylene	Elicits ET biosynthesis and hypersensitive response, Defense response	Tobacco plants, lima bean, cotton, maize	Engelberth et al. (2001) and Rotblat et al. (2002)
T. asperellum	Class I hydrophobin TasHyd1 SwolleninTasSwo Jasmonic acid Salicylic acid	Colonization of plant roots, availability of phosphorus and Fe, defense response	Cucumber roots, *Arabidopsis* root	Altomare et al. (1999), Viterbo and Chet (2006), Segarra et al. (2007), Tucci et al. (2011) and Yoshioka et al. (2012)
Trichoderma koningii	ABC transport systems	Degradation of the phenolic compounds	*Lotus japonicus*	Masunaka et al. (2009), Ruocco et al. (2009) and Chen et al. (2011)
Trichoderma saturnisporum	*Rich organic matter*	Production of phytochromes	Tomato, pepper, cucumber seedlings	Marín-Guirao et al. (2016)

13.4.4 Biofuel Production

Lignocellulosic biomass is the most abundant renewable energy source, which mainly constitute celluloses and hemicelluloses. Enzymatic saccharification of lignocellulosic biomass transformed them into glucose and other fermentable sugars, which further converted to biofuel and other chemicals. Biofuel production from

Table 13.4 Bioremediation of different pollutants by *Trichoderma* spp

Species	Pollutants	References
T. viride	Cadmium Lead Arsenic Chlorpyrifos Photodieldrin Nickel	Tabet and Lichtenstein (1976), Mukherjee and Gopal (1996), Srivastava et al. (2011) and Sahu et al. (2012)
T. asperellum	Arsenic Cadmium Lead Nickel	Hajieghrari (2010), Srivastava et al. (2011), Mohsenzadeh and Shahrokhi (2014) and Hoseinzadeh et al. (2017)
T. atroviride	Organophosphate Pesticide dichlorvos Heavy metals	Kredics et al. (2001), Errasquın and Vazquez (2003) and Tang et al. (2010)
T. harzianum	Arsenic Copper II DDT Dieldrin Endosulfan Penta-chloro-nitro-benzene Penta-chloro-phenol Chlorpyrifos Photodieldrin Cadmium	Tabet and Lichtenstein (1976), Katayama and Matsumura (1993), Mukherjee and Gopal (1996), Arriagada et al. (2009), Mishra and Nautiyal (2009), Ting and Choong (2009), Mohsenzadeh and Shahrokhi (2014) and Hoseinzadeh et al. (2017)
T. virens	Aromatic amines (AA)	Cocaign et al. (2013)
T. reesei	Diesel-contaminated soil Aromatic amines (AA)	Van Gestel et al. (2003), Mishra and Nautiyal (2009), Hajieghrari (2010) and Cocaign et al. (2013)
T. tomentosum	Cadmium	Mohsenzadeh and Shahrokhi (2014)

lignocellulosic biomass, which is called as the second-generation biofuel, is one of the eco-friendly ways to reduce costs on the energy sector as compared to fossil fuel (Kour et al. 2019; Rastegari et al. 2019). In addition, this sustainable process will reduce global warming effects on human health and environment (Rubin 2008). However, the major bottleneck of this process is bioconversion of biomass into fermentable sugars by the process of enzymatic hydrolysis due to the recalcitrant crystalline nature of cellulosic fibers. The saccharification of cellulose to glucose is catalyzed by cellulases.

In nature, filamentous fungi are the major source of cellulases. *Trichoderma* spp. are ubiquitous in an environment and considered as natural degraders due to the presence of the extensive hydrolytic and oxidative enzymatic system. Cellulolytic fungi belonging to Trichoderma have been considered as the most powerful degraders of cellulosic biomass. *T. reesei* is one of the most important cellulase producers used for industrial purposes for the production of monomeric sugar for second-generation biofuel production in biotechnology (Ahamed and Vermette 2009; Li et al. 2013). In *T. reesei,* cellulases can be divided into three major enzyme classes: (i) exoglucanases, (ii) endoglucanases, and (iii) β-glucosidases and the whole process of cellulose breakdown occurs simultaneously. Lignocellulose degradation mechanisms in *T. reesei* provide information on genes responsible for the plant cell wall degradation (Kubicek et al. 2009; Martinez et al. 2008). Moreover, *T. reesei* provides an alternative approach to improve biofuel production by the help of cellulases and hemicellulases using agricultural waste products (Schuster and Schmoll 2010). The genus of *Trichoderma* is so promising that *T. reesei* is commercially used for the production of cellulolytic and hemolytic enzymes, for the improved production of second-generation biofuels. Proteomics analysis is an important tool to understand the biomass degradation mechanisms using different substrates to improve *Trichoderma* potential in biofuel production. Moreover, through proteomics-based approach, multiple carbohydrate-active enzymes present in *Trichoderma* genus are identified, which opens multiple perspectives in the development of low-cost lignocellulose degrading enzyme systems (Cologna et al. 2018).

13.4.5 Production of Heterologous Protein

In nature, filamentous fungi are resourceful cell factories and among them, *T. reesei* is generally regarded as safe and often used for the expression of heterologous protein (Adrio and Demain 2003; Nevalainen et al. 1994, 2005). Heterologous protein production and industrial production of calf chymosin from *T. reesei* have started more than 20 years ago (Harkki et al. 1989; Uusitalo et al. 1991). Afterward, the expression of several enzymes and immunologically active antibodies in *T. reesei* has been achieved (Nyyssönen et al. 1993). Presently, *T. reesei* is one of the most important filamentous fungi used for the production of heterologous protein (Nevalainen et al. 2005; Penttilä 1998). In different biotechnological applications, promoters from *Trichoderma* spp. are often used for heterologous protein production based on the efficient expression and regulation of cellulase genes (Keränen and Penttilä 1995; Penttilä 1998; Schmoll and Kubicek 2003).

13.4.6 Industrial Applications of Trichoderma *Enzymes*

The current biotechnological industrial applications of *Trichoderma* enzymes are in the textile industry, paper, and pulp, in food-processing and feed industries, detergent industry, in biomass hydrolysis, and some environmental applications (Fig. 13.1). *Trichoderma* spp. have been extensively used for the production of food additives, to improve the brewing process by using β-glucanases, as macerating enzymes in fruit juice production by using pectinases, cellulases, and hemicellulases, as in feed additives (xylanases) (Blumenthal 2004; Schuster and Schmoll 2010). In addition, *Trichoderma* spp. produced industrially important metabolites used as food additives, one of the example is 6-pentyl-α-pyrone with coconut-like aroma, produced from *T. viride* with antibiotic properties (Oda et al. 2009). Mutanase produced from *T. harzianum* can be used in toothpaste to prevent

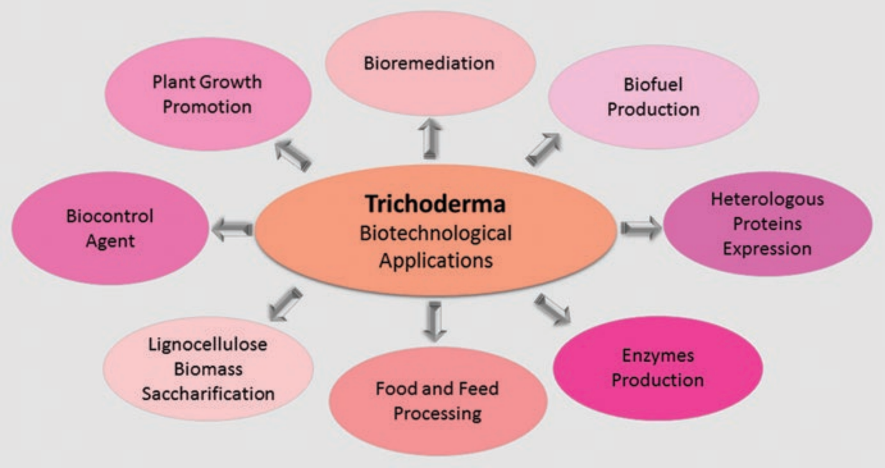

Fig. 13.1 Overview of biotechnological applications of *Trichoderma*

accumulation of the polysaccharide mutan in dental plaque (Wiater et al. 2005). One of the *Trichoderma* spp., *T. atroviride* used in pharmaceutical industry for the production of camptothecin (CPT), as an anticancer drug (Scharf et al. 2016). The commercially available enzymes of *Trichoderma* include Cellubrix, Econase, Crystalzyme, Pulpzyme, and Ecopulp, and these reflect the targeted uses of enzyme products (Benítez Fernández et al. 2004; Buchert et al. 1998; Galante et al. 1998).

13.5 Conclusion

Trichoderma is an outstanding organism concentrating on its basic and applied aspects and demand for sustainable development. The use of *Trichoderma* as a biological control agent for plant growth promotion and for the production of enzymes and expression of heterologous proteins, make these fungi as a versatile model organism. In addition, *Trichoderma* spp. have resistance mechanisms for heavy metals in polluted soil and water, thus making them a preferred choice for bioremediation.

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Chapter 14
Sugar Transporters in Plant–Fungal Symbiosis

Mamta Rani, Abhimanyu Jogawat, and Anita Loha

Contents

14.1 Introduction

Plants have established symbiotic relationship with fungi to enrich their nutrient uptake capacities and to deal with various abiotic and biotic stresses (Behie and Bidochka 2014a; Yadav et al. 2020c). This association is around 400 million years old and more than 90% of the terrestrial plants are known to form symbiosis with diverse class of fungi (Bonfante and Genre 2010). Plant symbiotic fungi can be divided into two categories: mycorrhizal fungi and root endophytes. The main difference between these symbionts is that while mycorrhizal fungi are mostly obligate biotrophs, non-mycorrhizal symbiotic fungi live at least some part of their lifecycle

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outside of their host plant, however, both kind of symbiotic fungi share similar strategies of soil nutrient assimilation for their host (Rodriguez et al. 2009; Saikkonen 2007).

This mutualistic relationship benefits both partners by exchange of nutrients via transporters (Wang et al. 2017). Fungal partner mainly relies on the plant for its carbon source. Fungus aids plant roots for the acquisition of primary minerals such as nitrogen (N), phosphate (P), potassium (K), and sulfate (S) (Kour et al. 2019; Wang et al. 2017). Additionally, these symbionts also benefit their host plants by improving their resistance against invading pathogens and providing tolerance against various abiotic stresses (Pozo and Azcón-Aguilar 2007; Singh et al. 2011). One of such symbiotic endophyte is *Piriformospora indica,* which can colonize a broad range of host plants including non-mycorrhizal hosts from the Brassicaceae family (Verma et al. 1998; Weiss et al. 2004; Peškan-Berghöfer et al. 2004). *P. indica* is an economically and agriculturally important fungus with many beneficial effects (Jogawat et al. 2013, 2016, 2020; Waller et al. 2005). It has been shown to improve nitrate, phosphate, and sulfate assimilation and uptake by host plants particularly under nutrient-deprived conditions (Lahrmann et al. 2013; Prasad et al. 2019; Yadav et al. 2010). Here, we discuss the process of nutrient exhange at symbiotic interface of plant-fungal association with a special focus on sugar transporters from symbiotic fungi.

14.2 AM Fungi and AM Symbiosis

Mycorrhizal symbioses are primarily categorized into two categories on the basis of root–fungal association structures and fungal partner involved. These are classified as ectomycorrhiza and endomycorrhiza (Bonfante and Genre 2010). The fungal partner in both kinds of symbiosis is mostly belong to the orders Glomeromycota, Basidiomycota, or Ascomycota. While the Endomycorrhizal fungi can colonize a majority of land plants, ectomycorrhizal fungi can only colonize host plants mainly from gymnosperms and angiosperms. These symbionts mainly differ in the pattern of their colonization, as the name suggests, ectomycorrhizal fungal partner covers the plant's lateral roots with mycelial sheath and also expand its intercellular hyphae, whereas endomycorrhiza invades as intraradical hyphae and intracellular structures (Cress et al. 1985). Arbuscular mycorrhizal (AM) symbiosis includes fungal partners mainly from the order Glomeromycota (Formey et al. 2012; Smith 2001). Nutrients exchange is the basis of symbiosis between plant and fungal partner. This is an economic relationship between both partners where plant transports carbon source to the fungus which is synthesized by photosynthesis, whereas the fungus transports scarce soil mineral nutrients mainly phosphate and nitrogen to the plant (Bonfante and Genre 2010; Finlay 2008). It has been estimated that up to 20% of photosynthetically fixed carbohydrates are diverted to AM fungi which shows the importance of AM symbiosis in terrestrial ecology (Bago et al. 2000).

AM fungi require plant partners for availing carbon source, sporulation, and for completing their life cycles, all these aspects term them as obligate biotrophs. Plants can remain in asymbiotic stage up to 2 weeks following interaction with the AM fungi (AMF) (Yadav et al. 2020a, b). Various signaling events contribute in establishing symbiosis when fungal spore or mycelial part comes in contact with appropriate host roots (Giovannetti et al. 2004; Harborne 2000; Smith and Read 2008). Plant root exudates, including flavonoids or strigolactones are recognized by the fungi present in the rhizosphere which initiates hyphal growth and branching for interaction. On the other hand, fungus can produce mycorrhizal (myc) factors which are recognized by symbiosis receptor complex and triggers downstream calcium ion signaling to activate symbiotic events via initiating symbiosis-related genes expression into the root cells (Gomez-Roldan et al. 2008; Maillet et al. 2011). Upon contact with the root surface, fungal hyphae form hyphopodium and then prepenetration apparatus (PPA) for further invasion (Genre et al. 2005). Symbiosis signaling and nutrient exchange maintain endophytic fungal proliferation and arbuscule formation in the cortical cells. Arbuscule membrane is known as periarbuscular membrane which is the interface for nutrients exchanges between both partners (Gianinazzi-Pearson et al. 1996; Smith and Smith 1990). Arbuscules are small tree-like intracellular fungal structures originated by fungal hyphae modification and invagination of plant cell membrane around this fungal structure (Bonfante-Fasolo 2018). Additionally, extraradical mycelium (ERM) network assists in absorbing nutrients from the soil.

14.3 Plant Endophytic Symbiotic Fungi

Plant endosymbionts are discovered in a wide range of plant species. They can colonize the plant roots and some of them can also spread to other plant tissues. These endosymbionts can live inside the plant without causing any harm to their host; however, unlike their AMF counterparts, they are not obligate biotrophs, and can live at least some part of their life cycle outside the plant (Rana et al. 2019a, b, 2020). The root-associated symbiotic fungi share many similarities with mycorrhizal fungi in their nutrient acquisition and plant growth-promoting activities (Behie et al. 2012; Behie and Bidochka 2014b); however, there are structural differences in their colonization pattern of AMF and endophytic fungi. The mechanistic details of their symbiosis are poorly known; therefore, more research is needed to explore the mechanism of their colonization and symbiosis (Saikkonen 2007).

14.4 Symbiotic Interface

AM and other symbiotic fungi have two kinds of mycelium when they interact with roots, that is, intraradical mycelium (IRM) and extraradical mycelium (ERM) which connect the root system and enhance the absorption surface with the soil. ERM uptakes soil nutrients mainly phosphate and ammonium and transfers them to roots. In exchange, the plant transfers carbohydrates to the fungus. The exchange of phosphate and carbon occurs reciprocally between AMF and roots (Rastegari et al. 2020a, b). Phosphate and nitrogen supply to plant regulate the arbuscule formation or degeneration in root cells. Similarly, carbon supply to the fungus regulates nutrients transfer from the fungus (Kiers et al. 2011; Luginbuehl and Oldroyd 2017). Nutrient exchange at the symbiotic interface can occur either intercellularly or intracellularly (Bonfante and Genre 2010; Bonfante and Perotto 1995; Ferrol 2002; Smith and Smith 1990). Different transporters are localized at the symbiotic interface for nutrient exchange, and they can be detected by increased ATPase activity at this interface (Gianinazzi-Pearson et al. 2000; Wang et al. 2017).

ERM uptakes micronutrients mainly phosphate and ammonium from the soil and transfers them to roots, in exchange, they receive carbohydrates. Symporters play a key role in phosphate and carbohydrate mobilization. H^+-ATPases are also importantly involved in the translocation of carbohydrates and phosphate at the symbiotic interface (Gianinazzi-Pearson et al. 1991, 2000; Smith and Smith 1990). H^+-ATPases hydrolyze ATP and transport H^+ against the concentration gradient which generates electrochemical gradient for energizing the transport of different nutrients such as sugars, phosphate, ammonium etc. H^+-ATPases constitute a large family in plants and they are differentially regulated in plant development and diverse stress conditions. Barley H^+-ATPase shows differential expression in mycorrhizal colonization (Murphy et al. 1997). Additionally, two H^+-ATPases from tobacco have shown to play a crucial role in mycorrhizal interaction (Gianinazzi-Pearson et al. 2000). Five fungal H^+-ATPases have been identified and isolated in *Glomus mosseae* (Ferrol 2002; Ferrol et al. 2002). An H^+-ATPase along with a sugar transporter GmPMA1 has been identified in *Medicago truncatula–G. mosseae* symbiosis (Requena et al. 2003).

14.5 Phosphate Transporters and Their Role in AM Symbiosis

Soil harbors phosphate in complex and insoluble form which cannot be acquired by plants. Fungal phosphatases and phytases solubilize these phosphates in simple forms in the soil (Kour et al. 2020; Singh et al. 2020). AMF phosphate transporters such as GvPT from *G. versiforme*, GiPT from *G. intraradices* and GmosPT from *G. mosseae* were shown to be differentially regulated during symbiosis (Harrison and Van Buuren 1995; Maldonado-Mendoza et al. 2001). All these transporters were observed to be highly expressed in ERM, which demonstrates their involvement in inorganic phosphate (Pi) uptake from the soil. This assimilated soil Pi is mainly stored as polyphosphate in ERM vacuoles, again transformed to Pi, and translocated to the IRM which is then delivered at the periarbuscular interface (Rasmussen et al. 2000). Ectomycorrhizal phosphate transporters HcPT2 and LbPT have been discovered from *Lacaria bicolor* and *Hebeloma cylindrosporum*. The expression of these phosphate transporters was found induced during symbiosis and low soil phosphate levels, showing the importance of these P transporters in the maintenance of symbiosis (Tatry et al. 2009) (Fig. 14.1).

Plants Pi transporters such as *M. truncatula* MtPT4 (Harrison et al. 2002), rice OsPT11 (Paszkowski et al. 2002), and tomato LePT4 (Nagy et al. 2005) were particularly observed to be expressed upon AMF colonization. Other Pi transporters, such as potato StPT3 (Rausch et al. 2001), maize ZEAma (Nagy et al. 2006), and *L. japonicas* LjPT3 (Maeda et al. 2006) were also involved in transporting Pi at the periarbuscular membrane. Moreover, MtPT4 was also observed to play a critical

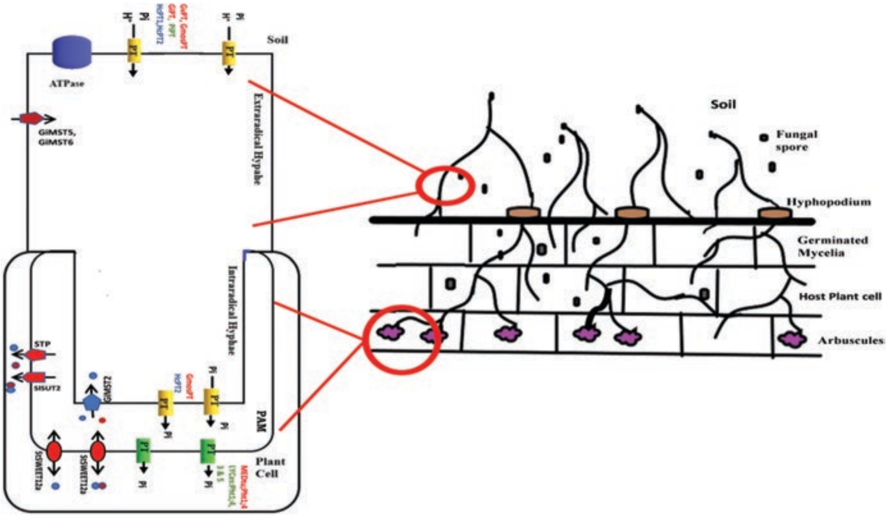

Fig. 14.1 Arbuscular mycorrhizal association and nutrient exchange (sugar and phosphate) at symbiotic interface. The reciprocal exchange of nutrients occurs at the periarbuscular membrane (PAM). Gene expression studies suggest that sugar is transported from phloem to mycorrhizal cells both in the form of sucrose and monosaccharides by plasma membrane localized SUT1-type or STP transporters. The export of sugars from the host cell to PAM is mediated by the transporters of SWEET family (StSWEET7a and StSWEET12a in *Solanum tuberosome*). The glucose is known to be the most preferred form of sugar uptaken by the symbiotic fungal partner, and it is mediated by GiMST2 at the symbiotic interface (*M. truncatula–G. intraradices* interaction). The transportation of sugar across PAM is regulated by plant sugar transporter SlSUT2 (in *Solanum lycopersicum*) and STP (in *M. truncatula*). In ERM, soil phosphate (Pi) is taken up by fungus via high-affinity phosphate transporters, some of them are characterized in mycorrhizal and other endophytic fungi (GvPT, GmosPT, GiPT, HcPT1, HcPT2, and PiPT). In IRM, fungal phosphate transporters such as GmosPT, HcPT2 are known to be involved in Pi efflux from fungi to PAM, which is taken up by the host plant cells via phosphate transporters such as MtPT4, LePht1,4,3,5, etc

role in mycorrhization and arbuscule maintenance, as knockdown of MtPT4 resulted in the abolishment of arbuscule formation in roots (Javot et al. 2007). Figure 14.1 shows the phosphate transporter known to play an important role in symbiotic Pi transport.

14.6 Ammonium Transporters and Their Role in AM Symbiosis

Nitrogen is an essential nutrient for plants and nitrogen limitation affects the primary productivity of plants. Nitrogen availability influences the photosynthesis and thereby results in carbon-fixation and symbiosis with fungi (Wang et al. 2017). Soil nitrogen is mainly present in organic complex form which is unavailable to plants. Mycorrhizal fungi are known to secrete several proteases, which release accessible and soluble form of nitrogen from inaccessible soil nitrogenous compounds. These soluble nitrogen compounds are absorbed by the fungal extraradical mycelium and then transported to the plant. In AM fungi, several ammonium transporters have been characterized, for instances, *GmosAAP1* from *G. mosseae*, GintAMT1, GintAMT2 and GintAMT3 from *G. intraradices* (Calabrese et al. 2016; Cappellazzo et al. 2008; López-Pedrosa et al. 2006; Pérez-Tienda et al. 2011). Additionally, in cyanobacterial fungi, *Gyosiphon pyriformis*, three symbiotically expressed NH_4^+ transporters GpAMT, GpAMT2, and GpAMT3 have been identified and characterized (Ellerbeck et al. 2013). The soil nitrogen absorbed by ERM and transported to IRM mainly in the form of arginine. IRM-localized urease converts this arginine into urea and NH_4^+, which can be utilized by the host plant as nitrogen source (Kiers et al. 2011). GinAMT1 and GintAMT2 have been shown to be involved in NH_4^+ uptake in arbuscocytes. In ectomycorrhizal fungi, a distinct AMT family ammonium transporters have been found to be induced during symbiosis and fungal nitrate transporters (NRTs) have also been discovered in roots colonized by ectomycorrhizal fungi. The expression of high-affinity nitrate transporters was also observed during symbiosis in ectomycorrhizal fungi *L. bicolor* and *H. cylindrosporum* (Jargeat et al. 2003; Kemppainen and Pardo 2013). Plant high-affinity NH_4^+ transporter, LjAMT2;2 has been shown to play a crucial role at the symbiotic interface (Guether et al. 2009). In soybean, NH_4^+ transporters were shown to be induced upon mycorrhization (Kobae et al. 2010). In *M. truncatula*, two NH_4^+ transporters AMT2;3 and AMT2;4 have been characterized and it was observed that AMT2;3 regulates the degeneration of premature arbuscule (Breuillin-Sessoms et al. 2015).

14.7 Sugar Transporters and Their Role in AM Symbiosis

The photosynthetically fixed carbon in plants is translocated mainly in the form of sucrose from source to fungal colonized root-sink (Hennion et al. 2019). The unloading of the sucrose from phloem to arbuscular cells involves transporters and invertases. Both these processes of sucrose transportation and its conversion into monosaccharides are tightly regulated in plants. AM fungi do not contain invertases; therefore, sucrose is degraded into monosaccharide in host plant cell by plant invertases (Hennion et al. 2019). In this direction, expression of invertases and sucrose synthases has been found upregulated during AM symbiosis (García-Rodríguez et al. 2007; Schaarschmidt et al. 2007). Colonization by AM fungus strengthens the sink through enhancing sugar demand of the sink (Douds et al. 2000). The demand is balanced by higher photosynthetic rate and CO_2 fixation in leaves (Wright et al. 1998a, b).

14.7.1 Sugar Source

NMR spectrometry study has proved that IRM uptakes hexoses, that is, glucose and fructose but not sucrose at cellular level (Pfeffer et al. 1999; Shachar-Hill et al. 1995; Solaiman and Saito 1997). Thereby, sucrose needs to be converted into monosaccharides by invertase or sucrose synthase in apoplast where sugar exchange occurs. In tomato roots, AMF colonization increases sucrose and fructose accumulation (Boldt et al. 2011). A study showed that ERM is unable to uptake glucose which suggests IRM as sugar exchange site (Pfeffer et al. 1999). Other studies have shown that both phosphate and sugar exchange occurs at the plant–IRM interface (Pumplin and Harrison 2009; Smith et al. 2000; Smith and Smith 1990). ERM can uptake and utilize cell wall monosaccharides such as xylose (Helber et al. 2011). Carbon mobilization from IRM to ERM can happen through two routes within fungus either as carbohydrate or as lipids. In the first route, hexoses are converted into glycogen via gluconeogenesis and in the second, they can be converted into triacylglycerol via lipidogenesis (Bago et al. 2000, 2003). In ERM, hexoses are stored as glycogen and trehalose or can be utilized for synthesizing structural polysaccharides such as chitin**.**

14.7.2 Plant Sugar Transporters at Symbiotic Interface

In AM-colonized roots, high sugar amount is translocated to roots from source organ leaves due to increased sugar demand and enhanced sink strength (Boldt et al. 2011; Wright et al. 1998a). In apoplastic sucrose loading plants, SUT1 subfamily member sucrose transporter SlSUT4 loads sucrose to phloem (Zhang and Turgeon

2009). In *G. mosseae*-colonized tomato, *SlSUT1* and *SlSUT4* transcripts level was detected higher in leaves and roots. Therefore *SlSUT1* and *SlSUT4* are considered to play an important role in transporting sugar to AM-colonized roots (Boldt et al. 2011). In *G. intraradices*-colonized plants, only *SISUT4* expression was observed in roots and leaves (Ge et al. 2008). In *G. caldeonium*-colonized plants, *SISUT1* was differentially expressed which suggests that there is differential regulation of these sucrose transporters with different AMF partners (Ge et al. 2008). Potato *SUT1* knockdown resulted in sucrose accumulation in leaves and reduction in roots, which affected the colonization at high and low phosphate conditions (Gabriel-Neumann et al. 2011; Riesmeier et al. 1994).

However, *SUT1* overexpression led to higher colonization at high phosphate. *M. truncatula SUT1* has been shown to be induced in AM-colonized roots specifically in non-arbusculated cells and cortex cells in close proximity to ERM (Gaude et al. 2012). MtSUT1 belongs to SUT4 clade and is localized at the tonoplast. It was suggested that MtSUT1 is implicated in carbohydrate mobilization to cells near arbuscocytes by exporting vacuole-stored sucrose. Plant monosaccharide transporters have been the focus of studies due to preference of glucose as main sugar component in AM symbiosis. ESL (early-responsive to dehydration six-like) subfamily member from tomato *LeST3* was observed to be upregulated in the leaves colonized by *G. mosseae* or *G. intraradices* (García-Rodríguez et al. 2005). Later, *LeST3* has shown to be regulated positively or negatively depending on the associated AM fungus species. *LeST3* showed upregulation in *G. intraradices*-colonized tomato roots and leaves, whereas it showed downregulation in *G. caledonium*-colonized tomato (Ge et al. 2008). At low phosphate, AM-colonized African maize cultivar roots show upregulation of monosaccharide transporter *ZmMST1* which was not observed in European maize cultivar (Wright et al. 2005). In *M. truncatula*, *MtST1* is the chief transporter for providing hexoses to fungus which induces during AM colonization but not in myc-mutant *M. truncatula*. Further, *MtSucS1*RNAi lines show highest downregulation of *MtST1* in comparison to other candidate genes (Baier et al. 2010; Harrison 1996). Its localization was observed in arbuscocytes, their adjacent cells, and IRM-contacting cells.

Plant harbors a newly classified family of sugar transporters renowned as Sugars Will Eventually be Exported Transporters (SWEETs) (Julius et al. 2017). Emerging roles of plant SWEETs are highlighting their significance in symbiosis and pathogenesis (Yurkov et al. 2019). In potato, 35 SWEETs were identified and their transcription profiling was performed during AM symbiosis which revealed that 22 SWEETs alter their transcripts level in AM-colonized roots (Manck-Götzenberger and Requena 2016). In *M. truncatula*, glucose transporting MtSWEET1b was observed to be expressed in arbuscocytes and localized at the periarbuscular membrane. Its overexpression supports the growth of IRM (An et al. 2019). Moreover, *MtSWEET12*, *15c*, and *15d* have also been observed to be induced specifically in AM-colonized roots depending on the accessibility to nitrogen source from the associated AM fungus. In nitrogen rich condition, these SWEETs were observed to be suppressed (Kafle et al. 2019). Two soybean *SWEETs*, that is, *GmSWEET6* and *GmSWEET15* along with a sugar invertase have been observed to be stimulated predominantly only when roots were found to be colonized with more cooperative AM fungi in terms of nutrient supply and growth stimulation (Zhao et al. 2019). The plant sugar transporters involved in AM symbiosis are shown in Fig. 14.1.

14.7.3 AMF Sugar Transporters

Carbohydrate is transported into fungal cells by hexose transporters (HXT) localized at the symbiotic interface. In this direction, symbiosis-specific GpMST1 was characterized from AM fungus *G. pyriformis* (Schüßler et al. 2006). *G. pyriformis* establishes unique symbiosis with cyanobacteria *Nostoc punctiforme* in which reciprocal nutrient exchanges occur. *GpMST1* was found to be highly expressed in symbiotic structures called bladder. It was revealed that GpMST1 is a high-affinity H^+/glucose symporter.

Three monosaccharide transporter genes *MST2, MST3*, and MST4 have been characterized from the mycorrhizal fungus *G. intraradices,* which forms symbiosis with *M. truncatula* roots (Helber et al. 2011). GiMST2 and GiMST3 show higher identity with xylose transporters, whereas GiMST1 showed highest homology with GpMST1. The expression of GiMST2 was particularly observed in IRM and arbuscules. GiMST2 was characterized as a high-affinity H^+/glucose transporter and its expression was induced upon exposure with xylose. Further, GiMST2 was able to transport a wide range of monosaccharides which are known to be present at the symbiotic interface (Gianinazzi-Pearson et al. 1996; Helber et al. 2011). The knockdown of GiMST2 by host-induced gene silencing (HIGS) in *M. truncatula* roots led to lesser mycorrhization and arbuscule formation, and it also abolished the expression of symbiosis-specific phosphate transporter *MtPT4* (Fig. 14.1).

14.7.4 Sugar Transporters from Ectomycorrhizal and Other Symbiotic Fungi

The information about nutrient exchange and sugar transporters in ectomycorrhizal and other endosymbiotic fungi is limited. In ectomycorrhizal fungi, few HXTs have been characterized. In the first report, a high-affinity glucose transporter AmMST1 was characterized from ectomycorrhizal fungus *Amantia muscaria* (Wiese et al. 2000). *AmMST1* was expressed constitutively in fungal mycelia under all growth conditions; however, its expression was found four-fold upregulated during mycorrhizal state as well as in the fungal hyphae grown at more than 5 mM monosaccharide concentrations (Nehls et al. 1998; Wiese et al. 2000). A high-affinity hexose transporter TbHXT1 was characterized from the ectomycorrhizal ascomycetes fungus *Tuber borchii* Vittadini (Polidori et al. 2007). TbHxt1 showed 52% identity with AmMst1 from *A. muscaria* (Nehls et al. 1998). The expression of *TbHXT1* was found to be regulated by negative feedback mechanism under both very low and high concentrations of glucose. Prolonged carbon starvation induced the expression of *TbHXT1*. The expression pattern and kinetic properties of TbHXT1 suggested the role of TbHXT1 during saprophytic phase of the fungus and excluded its involvement in symbiotic sugar uptake in ectomycorrhiza (Polidori et al. 2007). Three Hxt family (LbMST1.2, LbMST1.3, and LbMST3.1) sugar transporters have been characterized from the ectomycorrhizal model fungus *L. bicolor* (Fajardo López et al. 2008). All of these genes were found upregulated in ectomycorrhizal mycelium in comparison to extraradical mycelium. Recently, a hexose transporter PiHXT5 has been identified and characterized from endosymbiotic AMF-like fungus *P. indica* (Rani et al. 2016). PiHXT5 was found to be a symbiosis inducible and high-affinity hexose transporter (Fig. 14.1). Till now, very limited information is available of the molecular players involved in nutrient exchange between endosymbionts and their host, therefore, this area of research needs to be expanded for better understanding of these agroeconomically important relationships.

14.8 Conclusion and Future Perspectives

The symbiotic fungi play important role in soil–nutrient recycling in terrestrial ecosystem. Plant–fungal symbiosis is also important for the adaptation of plants to various environmental stresses. The research in the area of arbuscular mycorrhizal symbiosis has helped in understanding of symbiotic interface, nutrient exchange, and symbiotic signaling. Because of the obligate biotrophic nature of the AM fungi, they are hard to grow without their host and their manipulation is very tough by various transformation techniques; therefore, many aspects of symbiosis such as regulation of nutrient exchange and host specificities still need to be explored. Endosymbiotic fungi have provided an alternative to overcome the challenges of working with AM fungi, and they can be used as model organisms to study various plant–fungal interactions. The advancement in the culture and transformation techniques of endophytes has helped in understanding the unexplored aspects of plant–fungal symbiosis. The study with AM fungi and other endosymbiotic fungi has revealed the importance of sugar transportation in regulation and maintenance of symbiosis.

The role of host sugar transporters belonging to SWEET superfamily has been proposed in various plant–microbe interactions; similarly, few mycorrhizal sugar transporters have been characterized. These mycorrhizal sugar transporters were found to be specific for symbiotic sugar uptake and maintenance of symbiotic structures. Despite these new discoveries, the mechanism of sugar transport and signaling in the maintenance of symbiosis is still not clear. The advancement in the next-generation sequencing technologies and development of improved and efficient genetic manipulation technologies has enabled the identification of key players in plant–fungal symbiosis. The information obtained from these studies can be used in better and refined understanding of these symbiotic relationships, which in turn can be used for the development of improved plant varieties for better symbiotic soil-nutrient acquisition under diverse environmental conditions. The information from advanced molecular research can also be used to elucidate the mechanistic insights of symbiosis establishment which can further be applied to develop symbiotic relationship between specific fungal and plant partners to provide maximum benefit to the plant according to specific environmental stress.

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Chapter 15
Molecular Characterization of Medically Important Fungi: Current Research and Future Prospects

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15.1 Introduction

Kingdom Fungi consists of diverse eukaryotic organisms (approx. 1.5 million), which have heterotrophic mode of nutrition and reproduces with the help of spores (Hawksworth and Lücking 2017). Members included in this kingdom range from microscopic spore-forming fungi to huge fruiting bodies producing mushrooms (Smith et al. 1992). The Kingdom Fungi is separated from others due to the presence of chitin, ergosterol and glucans in their walls (Agrios 2004). Some members are inconspicuous because of their cryptic lifestyles on dead matter, soil and symbionts with animals, plants and other fungi (Seipke et al. 2012).

15.2 Medically Important Fungi

Fungi are important to mankind in various ways, irrespective of their microscopic and macroscopic size. Fungi are not only decomposers but also the source of various antibiotics, for example, Penicillin from *Penicillium* spp. We also use many mushrooms as food and also cheese production. Although the beneficial uses of fungi are numerous, but like the two sides of a coin, they are also harmful. Members belonging to ascomycetes produce medicinally important compounds, that is, fermented bread, antibiotics, alcoholic drinks and bakery products. They also cause many life-threatening diseases in humans. Representative disease-causing medically important members include *Candida*, *Aspergillus* and *Fusarium* species. The disease-causing fungal community has been classified into six major groups: Hyalohyphomyces, Zygomycetes, Dematiaceous, Dermatophytes, Dimorphic fungi and Yeasts. Medically important fungi are opportunistic rather than obligate parasites (Chuku 2018).

15.2.1 Hyalohyphomyces: The Fungi with Colourless Walls

Those infection-causing fungi which have hyaline body form, light in colour and hyphae are branched or unbranched, without any pigments in their walls (Ajello 1986). Moulds, some *Fusarium*, *Penicillium, Scedosporium and Trichoderma species are included in this group and known to cause* hyalohyphomycosis (Zhang et al. 2015). *They are abundantly present in dead organic materials, soil and contaminated water* (Duong 1996).

15.2.2 Zygomycetes

There are two sub-phyla in Zygomycota based on the phylogenetics: Mucoromycotina and Entomophthoromycotina, which are responsible for causing zygomycosis. *Rhizopus, Mucor, Rhizomucor, Cokeromyces recurvatus, Cunninghamella bertholletia, Apophysomyces elegans, Saksenaea vasiformis, Syncephalastrum racemosum* and *Lichtheimia*, all belonging to the Mucorales (Mucoromycotina), Conidiobolus (Entomophthoromycotina) and Basidiobolus (Basidiobolaceae) are the representative members which are involved in causing diseases in humans (Roilides et al. 2012). Thus far, there is limited information of their ecological niche (Richardson 2009).

15.2.3 Dematiaceous (Dark Pigmented) Fungi

The members of Dematiaceous fungi are known to cause a variety of infections ranging from superficial infections to disease complexes or syndromes (Wong and Revankar 2016). They are often present in soil but distributed all over the world. The mode of entry is by inhalation or trauma. They cause deep local infections, disseminated infections and brain abscess. Immunocompromise patients are at high risk of death if suffered from their infections (Radhakrishnan et al. 2019). These fungi have melanin in their cell wall, which make their spores dark coloured and help in pathological identification. Members include *Alternaria, Curvularia, Lasiodiplodia,* etc. (Revankar 2007).

15.2.4 Dermatophytes: The Skin-Invading Fungi

Dermatophytes cause skin infections (Dermatophycosis) as indicated by its name (Bouchara et al. 2017). These fungi have good affinity to invade skin, hairs and nails, and external membranes. Majority of the members of Ascomycota are included in this group. They are associated with soil, animals, insects and humans (Weitzman and Summerbell 1995). In an immune-competent host, they only penetrate the dead epidermis. *Trichophyton rubrum* is an example, which causes chronic dermatophytosis. Different kinds of medias and tests are available for their identification (Mercer and Stewart 2019).

15.2.5 Dimorphic (The Phase Switching) Fungi

Some fungal species are able to switch from yeast to mycelium depending on the environmental conditions. This phenomenon is called dimorphism and is important from both applied and basic concepts. A significant number of human, plant and animal pathogenic fungi show this behaviour to enhance their chances for colonizing their host (Boyce and Andrianopoulos 2015). Due to this transition phase, identification of fungus becomes difficult (Klein and Tebbets 2007). Now many drugs are available, which inhibits the dimorphism and also blocks the pathogenicity (San-blas et al. 1984). *Talaromyces marneffei* is a human pathogen and is thermally dimorphic fungi, which is mould at room temperature and changes to yeast on human body (Woo et al. 2003).

15.2.6 Yeasts: The Unicellular Fungi

Yeasts are found everywhere on earth. They have both positive and negative impacts for humans and animals. *Candida esophatagitis* is a disease of esophagus, which produces thrush. *Candida* is an example of yeast, which cause many diseases in humans. Yeasts also causes vaginal and blood stream infections which are life threatening (Miceli et al. 2011). Yeast is known to cause gut infections of serious nature in murine as reported by Chiaro et al. (2017).

15.3 Virulence Factors in Human Pathogenic Fungi

Kingdom fungi is comprised of approximately 1.5 million species out of them approximately 600 identified fungi are harmful to humans, but there are 30 species that are common casual agents of diseases in humans. The general term which is used to describe human fungal diseases is ‘mycoses’. There are some components that account for the endurance and longevity of fungal pathogen in respective host ultimately result in pathogenesis known as ‘virulence factors’ (Fig. 15.1). The genome of respective pathogen encodes these factors and any mutation either in laboratory system or in nature of these factors cause reduction of virulence. However, viability of the pathogen remains unaffected. In short, virulence factors

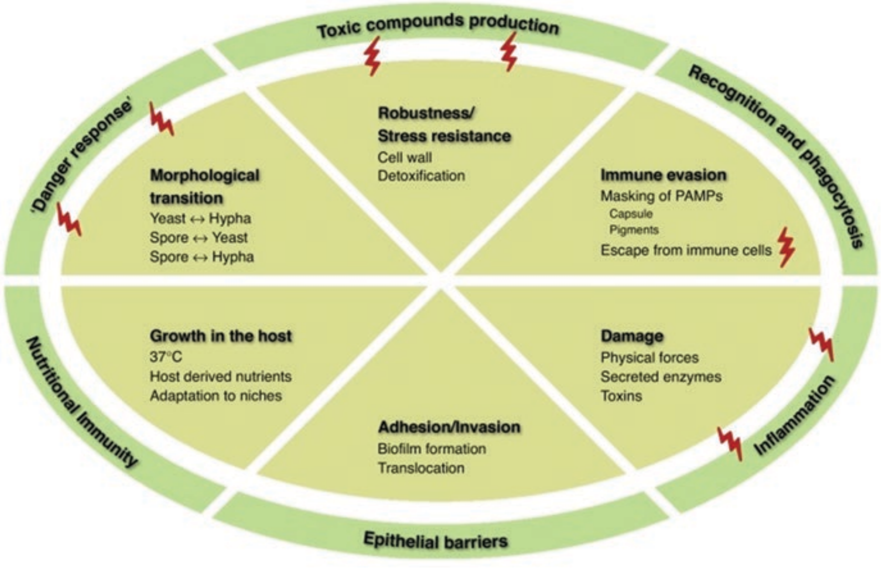

Fig. 15.1 Virulence factors of human pathogenic fungi. Damage to host and resulting disease by respective virulence factor (central ring), host responses (outer circle). Interactions with potential damage are marked in red. (Adapted from Brunke et al. 2016)

carry characteristics that are necessary for causing diseases; some virulence factors of chief importance are as follows:

15.3.1 *Adhesions Factor: The Biological Glue for Fungal Attachment*

The important step in causing disease is the ability of pathogen to attach the host cells. So, all the structures and compounds that aid in adhesion to the host by escaping the mucous and movement of cilia come under this category (Chandra et al. 2001). Some proteins (Hwp1p, Als, rodlet, Eap1p, etc.) and surface receptors on fungal hyphae such as BAD1 support the adhesion to collagen, cell to cell epithelium and laminin of the host (Karkowska-Kuleta et al. 2009). Examples of fungal species with such kind of adhesion receptors include *H. capsulatum C. albicans, A. fumigatus, Coccidioides* and *Blastomyces* sp. (Iyalla 2017).

15.3.2 Morphogenesis Role in Fungal Survival

Some fungi have a dimorphic living style; they normally occur as yeasts, moulds, or other filamentous fungi that in normal condition are avirulent but can change their form and become pathogenic to humans (Tekaia and Latge 2005). Renowned dimorphic fungi are *Blastomyces dermatitidis, Aspergillus fumigatus, Paracoccidioides brasiliensis, Histoplasma capsulatum, Candida albicans* and *Coccidioides immitis*. Dimorphism allows fungi to withstand high body temperatures of human body. HSP 70 is the protein that enables this adaptation (37–55 degrees; Iyalla 2017).

15.3.3 Fungal Capsule: The Protective Armor

Encapsulated fungi have virulent nature; for example, *Cryptococcus neoformans* can hold out against phagocytosis because of its glucoronoxylomannan-based capsule structure. However, the commonly found non-capsulated environmental strains of *C. neoformans* are of nonpathogenic nature. Other actions done by capsule include the disturbance in cytokine network and inhibition of leucocytes production on infected site. CAP 59 and CAP 64 genes are required for the capsule formation (Casadevall et al. 2019).

15.3.4 Fungal Enzyme: Arsenals for Fungal Growth and Protection

Damage of host cells by fungi not only done by physical structures (hyphae), but the secretion of lytic enzymes such as lipase and protease also play a significant role. These degrading enzymes help in establishment and dissemination of fungal enzymes. Damage to host cells is caused by injury of tissues and impairment in its defence system. Phospholipase, protease and SAP secreted by *C. albicans* (Naglik et al. 2003), and aspartic protease, serine protease and phospholipases by *A. fumigatus* are the examples of such enzyme secretions (Guruceaga et al. 2018).

15.3.5 Protection from Reactive Oxygen and Nitrogen Species

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are used by human defence mechanism (neutrophils) to protect from fungal infections. Mechanisms, for example, peroxidation of lipids along with breakdown of nucleic acids, are used to kill the fungal cells (Pradhan et al. 2017). Fungal pathogens produce catalases, superoxide dismutase, HSP, peroxidases and metals (Cu^{+2}, Zn^{+2} and

Mn^{+2}) to combat the oxidative species. *C. albicans* and *A. fumigatus* are the best examples of such type of fungi (Rementeria et al. 2005).

15.3.6 Melanin Pigments in Fungi

Melanin has hydrophobic nature and produced abundantly by pathogenic mycoflora. It provides protection to fungi against ultraviolet rays, ROS, antibody-based phagocytosis and elevated temperatures. Melanin synthesis takes place in *A. fumigatus, H. capsulatum, P. brasiliensis, C. neoformans* and *Blastomyces* (Rappleye and Goldman 2006).

15.3.7 Well-Developed Iron Uptake System

Iron is an important component involved in the respiration and growth of fungi with other metabolic activities. Usually iron is not found in host in free form. But some fungi have developed some mechanisms to obtain iron from their host (Philpott 2006). For instance, triacetylfusannine C, siderophore-regulated uptake and desferriferricrocin for the efficient uptake of iron from host cells. These and some other mechanisms for uptake of Fe are found in *A. fumigatus, A. nidulans* and *C. alboicans* (Iyalla 2017).

15.3.8 Mycotoxin Production in Invasive Fungi

Some pathogenic fungi such as *C. albicans and A. fumigatus* are familiar to produce mycotoxins. These toxins are the secondary metabolites and responsible for causing diseases in human host even in the absence of toxin-producing fungi (Moyes et al. 2016). Examples of mycotoxin include gilitoxin and aflatoxin. Aflatoxin is carcinogenic and cause destruction of liver cells, whereas gilotoxin suppresses the immune system by inhibiting phagocytosis and activation of T cells. It also harms the epithelial layer and decreases the cilia movement to save fungal spores (Arias et al. 2018).

15.3.9 Calcineurin and Mannitol Regulation in Different Fungal Species

Calcineurin works as a sensor for pathogenic fungal species. Heterodimers of calcineurin consist of catalytic (CnaA) and regulatory (CnaB) units; are involved in growth, stress management and virulence of fungi (e.g., *A. fumigatus*); also increase the expression level of virulence determinants; and aid in tissue invasion (Juvvadi et al. 2017). Likewise, mannitol aids fungi *C. neoformans* to infect CNS by scavenging ROS. Exceeded production of this compound can be a reason of brain oedema (Mahadevan and Susarla 2019).

15.3.10 Regulation of Fungal Virulence Through pH

Humans have different pH in different types of body tissues. As a result fungi must have a mechanism to cope up with this wide pH range in order to invade and damage host tissues. Therefore, in some fungi such as *C. albicans*, there is a complex network of genes, which is governed by RIM101 (Zn finger-based transcription factor). This makes fungi capable to infect human tissues at neutral or high pH (Davis 2009).

15.4 Clinical, Industrial, Environmental and Economic Impact of Human Pathogenic Fungi

Over the last few decades, more than 1.6 million deaths are caused by pathogenic fungi annually and the number of people that suffer from acute fungal infections surpassed 1 billion figures. Despite all these medical losses, there are very limited approved antifungal drugs available that are used to cure these infections in humans. The most recent approved antifungal drug was developed in 2002. This situation might be due to limited research fund for investigating fungal diseases, for example, *Cryptococcus meningitis* is the cause of 180,000 deaths annually but only 0.5% fund is allocated for its research. So, the development of new antifungal therapeutic strategies is inevitable (Almeida et al. 2019).

Despite the drastic effects on human health some weak human pathogens such as *Aspergillus niger, Aspergillus flavus* and *Aspergillus terreus* have some industrial applications. Biofilms of *Aspergillus* spp. are high yielding in industrial applications because its mycelia are free living when compared with typical submerged fermentation methods. Biofilms of *A. terreus* and *A. niger* can remove the heavy metals Cu, Ni, Al, Cr and Fe from industries. Different organic acids including citric acid and itaconic acid are produced in abundant quantities using *A. niger* and *A. terreus*. Aspergillus biofilms are also a source for producing a number of enzymes:

amylase, glucose oxidase, cellulases, inulinase, fructofuranosidase and xylanase. A. foetidus is also efficient in degrading plastics (Ramage et al. 2011).

Some fungal species that cause infection in humans are also responsible for animal diseases, for example, *A. fumigatus* and *C. neoformans*. Outbreaks in animal population by fungal pathogens can result in high mortality rate. Indirectly loss of biodiversity is occurring due to these fungal infections and in severe case, it can trigger the global warming. In past, extermination of bat and frog species by *G. destructans* and *B. dendrobatidis* are the examples of such situation. Few *Aspergillus* species are also responsible for the decline of coral reefs. Use of agricultural antifungal drugs that are medically validated for human can cause resistance in human pathogenic fungi because of sharing same environmental niches (Soler-Hurtado et al. 2016; Seyedmousavi et al. 2018; Almeida et al. 2019).

There are some fungal pathogens that crossover pathogenesis capability, it means they can infect not only humans but plants and animals also. Most of these fungi belong to phylums Ascomycetes and zygomycetes (Mucoromycotina). More than 180 species of *Aspergillus*, significantly *A. fumigatus*, *A. niger*, *A. flavus*, are responsible for causing respiratory (aspergillosis), allergic and toxic infections in humans. These fungal species also cause destruction in agricultural crops (postharvest losses, rots and aflatoxins), as well as involved in poultry losses (brooder's pneumonia) of chicken, turkey and birds. *Fusarium oxysporum*, *Fusarium graminearum* and *Fusarium verticillioides* produce mycotoxins that cause cytotoxicity, physical stresses, estrogenic toxicity and gastrointestinal toxicity in humans. Vascular disease caused by complex of *Fusarium* species also responsible for losses of some important agricultural crops such as wheat, cotton, chickpea and tomato globally. Fusarium graminearum is responsible for causing disease in livestocks and consequently, reduction in their production.

Dematiaceous fungi (melanin-like compound producing fungi) include *Alternaria* spp. Causal agent of leaf spot and seed breakdown in many plant species such as cotton, tomato and cucurbits. *A. alternata* causes onychomycosis in humans and also responsible for some allergic reactions. Similarly, *C. neoformans* cause infections in humans is also accountable for causing diseases in poultry and farm animals. *H. capsulatum* affects birds in zoo and to some extent responsible for losses of chicken turkey (De Lucca 2007; Gauthier and Keller 2013; Dhama et al. 2013).

Keeping in view the above information, currently we are dealing with diverse infectious fungi, so it is very difficult to identify and characterize them into a specific group. So identifying a fungal pathogen at species level is of prim importance, as it effects the therapy of choice, for example, *Aspergillus. calidoustus* (under section Usti) was found less susceptible to triazoles, including posaconazole, were in effective against this fungus (Varga et al. 2008). Similar results for *Emericella Quadrilineata* were observed t to amphotericin B (AmB) treatment. *Aspergillus terreus* (section Terrie) is a key reason of severe aspergillosis in medical care units, getting resistant against AmB. It is discovered from sequence analysis of genes that *Aspergillus alabamensis* (novel species) having resemblance with *A. terreus* may invade immunocompetent patients and show susceptibility toward AmB to antifungal medicines (Walsh et al. 2003). Previously, only morphology identification was used, but recently there are many techniques for the identification of fungi at species level, which are discussed later in this chapter.

15.5 Diagnostic and Identification Techniques

Fungal infections were insignificant in history but over the last few years, recurrence of invasive fungal infections (IFIs) caused by invasive fungal pathogens has elevated dramatically mainly because of these enlisted reasons

- Extended antibiotic therapy
- Acquired immunodeficiency syndrome (AIDS)
- Chemotherapy
- Surgeries and transplantations

These opportunistic fungal infections are of prime importance to increase the mortality and morbidity rates (Cuenca-Estrella et al. 2011). The recognition and differentiation of a fungal infection is very challenging in the clinical mycology (Salcedo and Powers-Fletcher 2020). Therefore, correct diagnosis of respective fungal species at early stages of infection is necessary in clinical mycology to ascertain the disease aetiology, control the emerging resistance against various antifungal agents, detect the novel disease causing agents and discover the hospital acquired infections (HAIs) among patients. Moreover, it reveals the connection of fungal isolate to the clinical environment (Bunn and Sikarwar 2016).

Systemic mycosis remains significant cause of serious infections leading to morbidity and mortality in almost 1 billion immunocompromised patients worldwide. Corresponding to vast species diversity, fungal diagnosis is major concern at medical laboratory level to determine etiology of associated infections. Conventional diagnostic methods are key to identify deadly fungi through four major steps, including morphology-based macroscopic and microscopic pathogen identification using different staining techniques, that is, gram, fluorochrome and immunofluorescent staining for specific fungal detection. Second is the culturing of fungal pathogen on selection media that are appropriate for culture growth and further amplification. After culturing media comes the biochemical assessment method to differentiate fungal strains according to their characteristics. Last is the immunological assessment for confirmation of pathogen attachment with antibody for lethal infections. Here, we have briefly discussed these strategies.

15.5.1 Morphological Diagnosis of Invasive Fungi

Medically significant fungi are generally identified on morphological key characteristics, which include fungal culture characteristics, that is, surface pigmentation in diverse colour ranges; reverse pigmentation in different colour exhibits; growth rate in terms of fungal colony diameter ranges from 5 to 15 days; the surface topography including flat, heaped, raised, radial grooved, folded and domed; texture of surface being granular, glabrous, suede-like, fluffy, powdery, cottony and downy; and the growth temperature ranges. In view of above keys, zygomycetes are characterized according to sporangial characteristics. Hyphomycetes are identified based on conidial characteristics, etc. Some of the characteristics are discussed (Table 15.1).

15.5.2 Fungal Microscopic Identification

In humans, 150 fungal species are estimated out of 250,000 to cause pathogenic diseases. Microscopy is regarded as traditional techniques for identifying medically important fungi (Chuku 2018). Wet mount microscopy or examination through potassium hydroxide are mainly used including the staining techniques for enhancing microscopic imaging. Most commonly used stains are lactophenol cotton blue, Gram staining, Calcofluor white and acid fast stain are for quick fungal structure evaluation, for differentiating them from other actinomycetes. Fungi residing fluids or tissues are detected through fluorescent antibody stain and for differentiating dimorphic fungi, Papanicolaou staining is used. India ink stain and Mayer

Table 15.1 Major fungal groups with their important identification characteristics and representative species

Group	Characteristics	Medically important fungi	References
Hyalohyphomyces	Colourless walls, branched or unbranched hyphae	Moulds, some *Fusarium* species	Zhang et al. (2015)
Zygomycetes	Mainly saprobes, coenocytic hyphae, zygospres	Mucorales, Conidiobolus, Basidiobolus	Roilides et al. (2012)
Dematiaceous fungi	Darkly coloured, melanin in cell wall, soilborne	*Alternaria, Curvularia, Lasiodiplodia*	Wong and Revankar 2016)
Dermatophytes	Skin invading, filamentous, cause itching	*Trichophyton rubrum*	Bouchara et al. (2017)
Dimorphic fungi	Phase switching, yeast to mycelium	*Talaromyces marneffei*	Boyce and Andrianopoulos (2015)
Yeasts	Unicellular, some species form pseudohyphae	*Candia* species	Miceli et al. (2011)

mucicarmine stain are specifically for identifying capsule of *C. neoformans*; Giemsa staining technique is for bone marrow and blood specimens; Fontana-Masson stain, Wright stain, periodic acid-Schiff (PAS) stain and numerous other techniques exist for microscopy stain of medically significant fungi. Microscopy accompanied by staining and histopathology are efficient to diagnose infections caused by *Aspergillus spp., C. neoformans, Candida spp., Pneumocystis jirovecci, Coccidioides immitis, Blastomyces dermatitidis, Sporothrix schenckii, H. capsulatum, Paracoccidioides brasiliensis* and Mucormycotic infections but sensitivity of techniques fluctuate with specimen source, individual agent and technical expertise, and also these traditional approaches may require tissue biopsies which are risky for patients who are susceptible to invasive fungal infection (Kozel and Wickes 2014). Microscopy analysis becomes more efficient when combined with other tools and the transmission electron microscopy (TEM), scanning electron microscopy (SEM) and confocal microscopy (CLSM), and the atomic force microscopy (ATM) are the powerful instrumentation techniques for identification of fungi (Schuler et al. 2015). Microscopy is considered limited traditional identification approach in terms of time consumption, cost and lab expertise.

15.5.3 Culture-Media-Based Diagnosis

Pure culture of individual fungus is obtained through diverse techniques using selective nutrient-supplemented growth media. Selection of media is a critical choice based on the type of suspicious fungi. Pure colony of fungi is used for identification of growth traits including pigmentation, colour, shape, mycelium, texture and topography.

These techniques are effective, especially for wide range of invasive candidiasis. For instance, the traditional blood culture may firstly omit 50% chances of the patients who are documented with linked problem (Fraser et al. 1992; Ostrosky-Zeichner and Pappas 2006; Ostrosky-Zeichner 2012) because of the poor sensitivity. Moreover, it may become positive only in late infection (Ellepola and Morrison 2005; Clancy and Nguyen 2013). Furthermore, it typically takes time ranging 24–72 h for identifying *Candida* in clinical sample, which is too long for early disease treatment. In case of invasive aspergillosis that majorly caused by contaminated air (Kontoyiannis et al. 2000). Patients suffering from acute pulmonary histoplasmosis have recovery chances of 10–15%. But, in condition of cavitary histoplasmosis, the sputum cultures are usually positive up to 60% patients. Bronchoscopy sputum cultures have higher yield (86% per patient for culture and 92% for bronchoscopy) in patients suffering from pulmonary blastomycosis (Kozel and Wickes 2014). *Coccidioides* spp. culture is rather more complicated because of biosafety hazard associated to cultural mycelia forms. So, the identification of specific opportunistic fungi that may cause infections demands foremost expertise at laboratory level. Examples of culture media include Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Brain-Heart Infusion Agar (BHIA), Malt

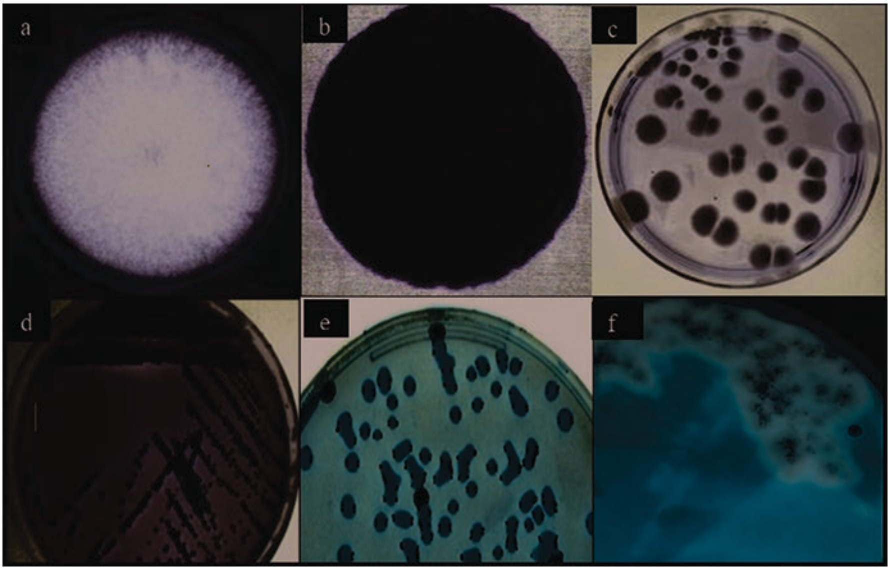

Fig. 15.2 Collage of different fungal strain grown on different culture media. (**a**) *Aspergillus fumigatus* on potato dextrose agar (PDA). (**b**) *Histoplasna capsulatum* colonies on sabouraud dextrose agar (SDA). (**c**) *Candida albicans* colonies on yeast extract peptone dextrose (YPD) agar (Wibawa and Aman 2015). (**d**) Colonies of yeast-like fungi *C. neoformans* on bird seed agar (BSA). (**e**) Dark brown colonies on *Cryptococcus gattii* on niger seed agar (NSA) (Patil et al. 2013). (**f**) *Coccidioides immitis* growth on blood culture media. (Adapted from Chae et al. 2012; Sahu and Padhy 2014; Wibawa and Aman 2015; Kipyegon et al. 2017; Valdez et al. 2019)

Extract Agar (MEA), Inhibitory Mould Agar (IMA), Dermatophyte Test Medium (DTM), Yeast Extract Phosphate Medium (YEPM), cornmeal, sterile bread agar and various other (Ostrosky-Zeichner 2012) (Fig. 15.2**).**

Clinical culture is regarded as standard for fungal infection diagnosis because it is specific etiological sample if yields positive culture. It is also used against testing susceptibility. However, using culture-based diagnosis is limited for invasive fungal disease because culture growth requires much time for filamentous fungal species. And fungi look alike, so specific method, that is, chromogenic media is preferable.

15.5.4 Serology-Based Fungal Identification Technique

Serum-based detection of fungal infection is regarded as gold standard traditional approach, which is not only accepted worldwide as a nonculture-based assay for diagnosis of histoplasmosis but also other severe infections (Hage et al. 2019). Endemic mycoses have been identified through serologic testing of patient's antibodies despite cultural identification since mid of previous century. The most commonly used serologic diagnostic tests are enzyme immunoassay (EIA), immunodiffusion (ID) and complement fixation (CF). Histoplasmosis is identified through CF and ID mostly for detection of antibodies usually for detecting antibodies in histoplasma M and H antigens and CF testing helps to differentiate between acute and recuperating histoplasmosis. Higher titre may indicate chronic pulmonary histoplasmosis (Lindsley Mark 2016). Diagnosing coccidioidomycosis in case of primary infection or coccidioidal meningitis through serological testing is efficient practice (Galgiani 2010). Approximately, 80% acute primary coccidioidomycosis is based on IgM detection (Saubolle et al. 2007). IgG antibodies are detected through CF and are produced during convalescent phase or during the chronic infection. CF is considered more sensitive test than IDCF combined; it also provides quantitative testing results. EIA is preferably used for detecting IgM or IgG with antibodies. Serological diagnosis also includes galactomannan detection, 1,3- ß-D-glucan detection, mannan detection, enolas, secreted aspartyl proteinase (Sap), detection of capsular antigen in cryptococcosis and test for detection of metabolites and histoplasmosis. There are advantages to use of serological diagnosis for invasive fungal infection. First, results may be positive, which were not detected during cultural diagnosis. Second, it may reduce culturing of potential devastating fungi, for example, *Coccidioides* spp. Finally, it requires a minimal sample size to reduce testing barriers. There are some disadvantages associated with serological testing such as it cannot detect presence of infecting fungi and lower sample quantity may lead to less sensitivity and less specificity. CF testing is time consuming and infection detection for an immunocompromised patient becomes difficult due to possible lower antibody response (Tobón et al. 2005).

15.5.5 Molecular Approaches for Mycoflora Diagnostics

In general, protocols and instruments that are involved in the detection of nucleic-acid-based biomarkers or the product of gene categorized as molecular diagnostic assays. Traditionally used fungal identification methods such as phenotypic appearance of fungi and different biochemical tests require a lot of time, may take days to weeks for completion, lack sensitivity and demand more technical skills. In order to manage these invasive clinical fungal infections, development of new and precise identification techniques is very important. In this way, the economic and social losses of these infections can be minimized (Wickes and Wiederhold 2018). To limit the drawbacks of traditionally used methods and techniques for the purpose of fungal identification, some recent techniques that seem promising for fungal identification such as ITS-based identification, MALDI-TOF MS, LAMP, rolling circle amplification nano-diagnostics, etc., (Chuku 2018) are discussed.

15.5.5.1 DNA Sequencing of Internal-Transcribed Spacers for Identification

DNA sequencing is considered ‘Gold Standard’ among all the molecular techniques used to differentiate fungi (Perlin and Wiederhold 2017). Extreme worth of this tool is due to immense data availability on easily accessible database known as GenBank. Substantial data of rRNA gene or rDNA sequences along with single gene or genome of individual can easily found at this platform. This tool gain its popularity as promising diagnostic technique after successful amplification of internal transcribed spacer (ITS) regions by universal DNA priming site that encodes 18S and 28S (small and large) ribosomal subunits by White et al. (Fig. 15.3). ribosomal RNA is very sensitive to PCR as it is abundantly present in each cell of eukaryotes. Formerly, D1/D2 region also used for the identification purposes, but that region is less sensitive to PCR also cannot discriminate the fungi beyond genus level. In clinical environment, fungal identification up to species level is very crucial, for example, *A. fumigatus* species complex and *S. apiospermum* species complex; species differentiation is necessary because of their different respond to antifungals and clinical outcomes. Whereas dealing with *Candida* species, discrimination of its species may not be necessary (Nilsson et al. 2006; Lackner et al. 2012; Sugui et al. 2014).

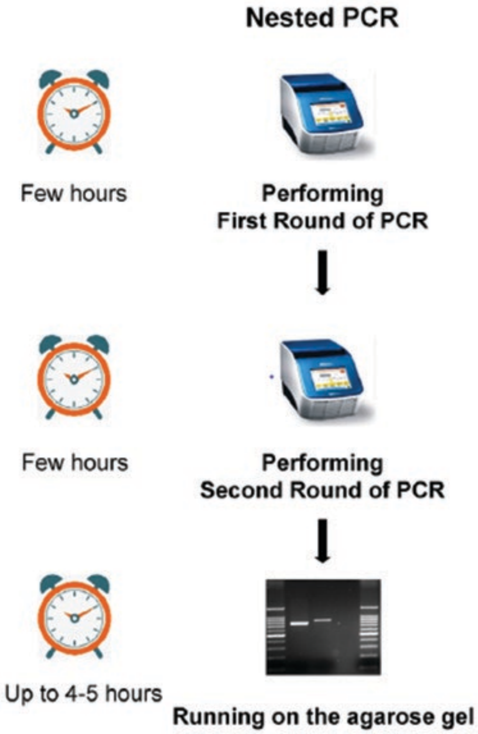

Fig. 15.3 Schematic presentation of nested PCR used for fungal diagnostics. (Adapted from Brunke et al. 2016)

Although ITS/DNA sequencing is a routine protocol for mycology labs. Nevertheless, this technique is not commercialized to that extent due to high cost investment and demand for high level of technical expertise to conduct this procedure. Another drawback of this technique is the open nature of its database, that is, GenBank (Balajee et al. 2005). It allows everyone to submit data without any curation, which makes it very prone to errors and ultimately leads to misidentification of fungal species. RefSeq database on other hand helps to overcome this gap. Common fungi can be identified with precision by this highly curated database using different filters. Other bi-curated databases with authentic sequence information can be good alternatives of BLAST (NCBI; Prakash et al. 2017).

15.5.5.2 Multiplex Polymerase Chain Reaction (MT PCR)

It is a molecular diagnostic tool used for the identification of multiple fungi in single specimen at the same time. This tool amplifies the conserved fungal genome regions with the help of two sets of specifically designed primers (Badiee et al. 2007). However, identification process from this technique is difficult to some extent as it involves the sequencing of amplified product. Many fungal species that are difficult to identify by microscopic technique due to similar morphological characteristics can be easily diagnosed by PCR. It is also used to diagnose the IFIs through blood (Sugawara et al. 2013), BAL (Orsi et al. 2012) and serum samples of the patients. PCR is most versatile and diverse among all the techniques to amplify nucleic acid and used as major target for diagnostic component. It is the most fundamental and globally used technique due to easy handling and relatively low cost instrumentation. Results can generate at faster pace by PCR, which are quantitative in nature and interpreted straightforward by PCR. However, lack of panfungal differentiation counted as flaw of this technique. This tool is directly applicable on clinical samples that have excessive quantity of nucleic acids, which helps in omitting of in vitro fungal culturing step. Unfortunately, in clinical samples the amount of fungal DNA is very low in comparison with bacterial or viral samples (Alanio and Bretagne 2014). Use of different probe and primer combinations make this technique promising for the diagnosis of a large number of fungal species such as in case of nested and qPCR (Fig. 15.4).

Fig. 15.4 Scheme of fungal rRNAs gene cluster showing internal transcribed spacer (ITS) region. (Adapted from Wickes and Wiederhold 2018)

15.5.5.3 Loop-Mediated Isothermal Amplification (LAMP)-Based Detection

LAMP is a robust amplification technique, which amplifies the targeted DNA segment and replaces one strand of the DNA with newly synthesized DNA strand with great precision at isothermal conditions (Parida et al. 2008). The whole process done in a single reaction tube, which contains DNA target, DNA polymerase enzyme along with its buffer and primers. Temperature range for this reaction is 60–65 °C. Unlike conventional PCR, this reaction takes place simply using water bath or heat block (for the maintenance of isothermal conditions) instead of costly thermocyclers or PCR machines (Noguchi et al. 2017). Moreover, in this method, heat-based denaturation of dsDNA into single strand is not needed. In the protocol, there are four specific primers used in this reaction in place of typically used two primers, which are beneficial in increasing the reliability and specificity of the procedure. Additionally, traditionally used DNA *Taq* polymerase substituted by *Bst* polymerase in LAMP helps in reducing the sensitivity against PCR inhibitors specially when dealing with blood samples in clinical labs. High number of DNA copies (109–1010 copies within 1 hour) makes this method more vigorous and desirable than typical PCR. LAMP results can be visualized and examined without using UV if CYBR green is included in the reaction mixture (Soliman and El-Matbouli 2005).

LAMP protocols have successfully developed even for the detection of human pathogenic fungi, including *Penicellium marneffei*, *Cryptococcus* spp., *Pseudallescheria* spp. and *Scedosporium* spp. (Malhotra et al. 2014). Although LAMP is highly specific, cost effective, time saving and simplified technology, some gaps are considered drawbacks of this technique. In particular, requirement of multiple primer sets makes it more complex because of difficulty in finding appropriate sites in a gene for primer designing. In addition, a very few commercial kits are available for LAMP compared to regular PCR (Keikha 2018).

15.5.5.4 Rolling Circle Amplification (RCA) Based Assessment of Fungal Species

Rolling circle amplification (RCA) is another procedure employed for the identification of fungal species. During this procedure, amplification of the target sequence can be performed, which is in the form of a circular template (Fakruddin et al. 2013). This procedure involves the use of viral polymerase for the amplification purpose. Fundamental steps of this technique are the extraction of DNA followed by its amplification. Subsequently, a padlock probe is designed and then that probe is ligated to that target. Excessive sample and probe are removed by exonucleolysis. In the final step, ligated product is used as template for RCA at required temperature (65 °C). Resulting data are then examined by using gel electrophoresis, UV light, or radiology techniques (Davari et al. 2012).

This technique differentiates the fungal species, which has a close relation to their genotypes, and has the ability to detect minute changes such as difference of even single nucleotide (Sun et al. 2011). Mucorales (Dolatabadi et al. 2014), Exophiala (Kaplan et al. 2018) and Cryptococcus (Trilles et al. 2014) species of fungi have successfully identified by using RCA.

15.5.5.5 Nucleic Acid Sequence-Based Amplification (NASBA) Through Fungal RNA

This tool works on the amplification principle like PCR, but with mRNA as target of amplification along with RNA polymerase rather than, DNA and its polymerase. Additionally, this technique is of isothermal nature. Although this method was developed in 1991, it failed to gain popularity in scientific community (Zhao et al. 2016). After a decade, this technology is introduced as a promising tool for recognition of Aspergillosis (Loeffler et al. 2001). Afterwards, NASBA in combination with real-time PCR is tested to increase the specificity and reliability.

As mRNA used in this method, the possibility of recognition of active infection is increased multiple times rather than old or latent disease. Isothermal nature when conjoined with the fact of more RNA stability could eliminate the contamination risk. All of mentioned aspects make this technique promising for identification of susceptible population. In addition to that, accurate prediction of clinical results by this technique will be helpful in increased survival rate (Yoo et al. 2007).

15.5.5.6 Real-Time Polymerase Chain Reaction (qPCR)

Methods include probe hybridization, PCR and generation of signals combined in single step by this tool. Probe used in qPCR, named as TaqMan, is usually a mixture of three components: 5′ fluorescein dye used as a reporter dye, quencher dye at 3′ end and lastly, a phosphate group used as a blocker at 3′ end. Breakdown between quencher and reporter takes place because of elevated level of reporter dye. Consequent quantity of reporter dye is equal to the resulted PCR product. Use of TaqMan allows the sample analysis as soon as PCR ends, that is, approximately in 5–10 mins, which eliminates the post-amplification interpretation step. In this way, contamination of samples in laboratory can be minimized. Sensitivity of this protocol is ten times higher than the commonly used ethidium bromide-based agarose gel electrophoresis. This protocol has been flourished for the diagnosis of *A. fumigatus* and *Candida* species (Valones et al. 2009).

15.5.5.7 Random Amplified Polymorphic DNA (RAPD) Marker-Based Identification

It is another modification of PCR, but with random amplification of target DNA region. RAPD generates a semi-novel profile when resulting DNA fingerprints are resolved. RAPD involves the use of single primer to amplify the different complementary segments of DNA randomly. Amplification of the DNA segment depends upon the annealing position of primer: if primers annealed at very far distance or the 3′ ends of the annealing primers do not face each other than amplification cannot takes place. The amplified products can be visualized on acrylamide gel and the sized-based detection of amplified products allow the comparison between different fungal species. This technique can identify fungal species that do not have any prior information about DNA sequence. This method can discriminate the species which have high similarity index, for example, *Cryptococcus gatti* and *C. neoformans* is discriminated successfully by the use of RAPD (Malhotra et al. 2014).

15.5.5.8 Whole Genome Sequencing (WGS)

Whole genome sequencing is a prompt substitute of traditional DNA sequencing technique. It is valuable more than simple identification of fungal species. It is effective in genotyping of any fungal strain that lacks any kind of prior information or genetic knowledge. Therefore, it is of great use when traditional molecular approaches fail to give results, such as in case of any medical emergency or outbreak by unknown fungal pathogen with no or very little availability of insight about epidemiology, spread and diversity of related fungal isolate. In such an unusual situation, WGS provides first molecular and genetic differentiation about that strain of fungus (Alanio et al. 2017). Since the inception of whole genome sequence reported for *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe,* this technique gained significant importance (Sharma 2016).

The basic principle of WGS is to investigate the genome-wide differences among fungal isolates by pinpointing any single nucleotide polymorphism (SNPs) in every genome. Thus, information from the SNPs comparison helps to recognize the relationship between fungal isolates. Genetic relevance calculated by the distance mapping of SNPs. Another edge of WGS is that it provides ‘de novo assembly’ against the pathogen. It also tells about the ploidy level, genome size and information related to characterization of fungal pathogen (Lesho 2016). During recent years, outbreaks caused by exceptional fungal pathogens like *Candida auris, Saprochaete clavata, Sarocladium kiliense, Exserohilum rostratum*, were investigated by WGS typing. These outbreaks point towards the dawn of a new era of fungal infection diagnostics by utilization of WGS (Bougnoux et al. 2018).

Besides this, WGS has huge worth in metagenomics studies. Different microbes (fungi, bacteria and viruses) can be present at aseptic sites of human body in tremendous number, and it is very challenging to sample, identify and characterize mycoflora from such site compared to purified cultures for which specific molecular and phenotypic markers are available. In such situation, WGS can be applied to metagenomics investigation of that mycobiome in various ways. An example of such research is the identification of human pathogenic oral mycoflora (Ghannoum et al. 2010). In the past, this technology also used to investigate the connection between Crohn’s disease and fungi by the identification of responsible fungi (Hoarau et al. 2016).

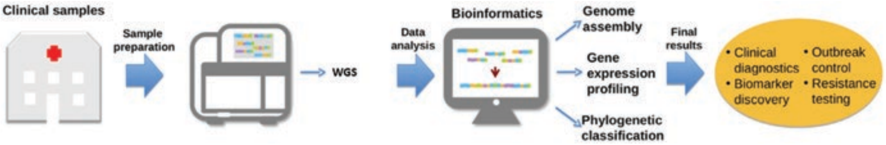

Fig. 15.5 Workflow of WGS in clinical labs starts with isolation and preparation of fungal samples, genome sequencing by WGS and computational data analysis of resulting data, then final retrieval of the ultimate results. (Adapted from Gabaldón 2019)

This technique has some limitations despite high differentiation capability to investigate relation between fungal isolates. WGS is a high cost technology and not suitable for regular practices in a clinical laboratory because of its slow pace. Moreover, due to complexity of this technique, it can only executed in those laboratories where all the steps used in this technique are established, for example, computational analysis of WGS-generated data along with the acquisition of sequences (Hanage 2014). High variability in fungal genome size, that is, 8.97–177.57 Mb, is an important factor that is responsible for high cost of this technique (Mohanta and Bae 2015). Furthermore, dynamics in fungal genetic makeup such as less diversity in nucleotides of some fungal species, for example, *Penicillium* spp., limits the efficient use of this technique to study epidemiology (Lasker 2006) (Fig. 15.5).

15.5.5.9 Hybridization-Based Fungal Detection Assays

Numerous probe-detection-based protocols to identify pathogenic fungi categorized as hybridization methods, for example, fluorescent in situ hybridization (FISH), peptide nucleic acid-fluorescent in situ hybridization (PNA-FISH) and reverse dot blot hybridization (RDBH).

Fluorescent In Situ Hybridization (FISH)

In this practice, a fluorescent-labelled oligonucleotide probe is used for the detection of specified fungal genome target in blood samples. Fluorescent probe gets attached to its complementary target DNA sequence within a cell. Key steps of this assay includes fixation of sample, which makes fungal cells permeable for the absorption of fluorescent probe. Then, preparation of sample is done, which also involves some pretreatment steps to enhance the attachment capability of specimens to membrane or glass slide. This step is followed by the probe hybridization to the specific target region. Afterwards, the washing is performed to remove excessive probe that remained unbound. Final steps involve the mounting of membrane or glass slide, followed by its visualization through fluoresce microscopy or flow cytometry to detect the signals generated by hybridized fluorescent probe (Moter and Göbel 2000). This technique is used to detect *Candida* species for many years

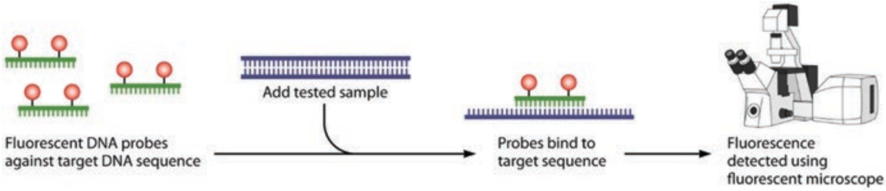

Fig. 15.6 Fluorescence in situ hybridization (FISH); mixing of probes with target samples; hybridization of probe to their respective DNA sequence, then washing of excessive probe, visualization of hybridized probe via fluorescence microscope. (Adapted from Arvanitis et al. 2014)

(Kempf et al. 2000). The target sequence of probe could be taxon-specified DNA regions such as rRNA genes, ITS regions or genes of mitochondria mainly because these genes have high copy number and are conserved in fungal species. Therefore, this technique can be used to differentiate fungal species when probe is designed against rRNA gene or ITS region. *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii* species were differentiated by designing probe against ITS1 region of these fungi in a study (Arantes et al. 2017) (Fig. 15.6).

Peptide Nucleic Acid-Fluorescent In Situ Hybridization

Peptide nucleic acid-fluorescent in situ hybridization (PNA-FISH) is the modification of regularly used FISH assay, in which peptide nucleic-acid-based probe is used. Neutral nature and increased binding capacity of probe make this technique efficient than simple FISH method (Rigby et al. 2002). Two FDA certified and commercially available PNA-FISH products are Quick FISH and Yeast Traffic Light FISH. *Candida* species (*C. albicans, Candida tropicalis, Candida krusei, Candida parapsilosis* and *Candida glabrata*) along with *Scedosporium, Aspergillus* and *Fusarium* species can efficiently identify by this assay (Procop 2007).

Reverse Dot Blot Hybridization (RDBH)

DNA array hybridization and macroarray are the other famous names of this technique. This technique is the combination of hybridization procedures with PCR. Oligonucleotides are synthesized and allow to bind on a solid surface platform: either a nylon membrane or a glass surface/slide. If successful reaction takes place between the amplicon (PCR product) of targeted genome and synthesized probe, chemi-luminescent signals are generated. These signals are detectable by digital camera. Reusable nylon membrane with attachment capacity of numerous oligonucleotides make this technique more beneficial than other PCR methods in terms of cost (Lau et al. 2009). This is a promising tool for the detection of invasive fungal infections such as coccidioidomycosis (Montone et al. 2010) and rhinosinusitis (Montone et al. 2011) other than *Candida* spp. Identification.

15.5.5.10 Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry MALDI-TOF is among the most efficient and reliable techniques used to classify, identify and differentiate microorganisms rapidly with great authentication. It has massive use in identification of fungi, as well as bacteria (Chalupová et al. 2014). It has become a routine method for the identification of yeasts and is equally efficient for filamentous and dimorphic fungal identification (Patel 2019). Working principle of this tool is the ionization of biomolecules using laser afterwards, mass to charge ratio-based detection and measurement of these ionized biomolecules. TOF unit operates to generate protein-based fingerprints that are the markers of pathogenic spores or cell surfaces. These peptide fingerprints are then used to examine the databases (Singhal et al. 2015) (Fig. 15.7). Many medically significant fungal species particularly *Aspergillus, Cryptococcus, Candida, Fusarium* and *Mucor* species along with other fungi including *Histoplasma capsulatum* and *Blastomyces dermatidis* have been identified successfully by utilization of this technique (Panda et al. 2015; Rychert et al. 2018).

Fig. 15.7 Process of species identification through MALDI TOF MS: Colony picking from media culture plate placed on the target plate of MALDI TOF; formic acid is added for the yeast identification, preceded by drying. The target plate is then placed in mass spectroscopy unit, which produces a mass spectrum and compare this spectrum with others in library through software. (Adapted from Patel 2019)

This technology has edge on other identification techniques in many ways. It is a very rapid technique and requires limited amount of sample and consumables for the processing. All these beneficial aspects make this technique suitable for clinical setups as well as industrial environment (Wieser et al. 2012). The drawback of this technique is the generation of analytes must be done from the pure cultures that can increase the turnaround duration by the addition of many days. Furthermore, the database used in this technique is not stringent as fingerprints that not generated by FDA-validated condition can also accepted. In addition to that, some fungal fingerprint variation is highly dependent on the conditions of culture that is problematic in standardization for fungi (Wolk and Clark 2018).

A variation of MALDI-TOF is ESI-MS (electrospray ionization mass spectrometry). It also uses the mass spectrometry to identify and characterize fungal pathogen. In comparison to typical fungal identification process, different biomolecules can be used in techniques other than nucleic acids with minimal or no suspicion of misidentification. A huge number of fungal species can be identified by this technique in ~6 h (Simner et al. 2013). Unlike MALDI-TOF MS, this method allows the direct examination of clinical samples. In addition, many samples can process simultaneously. Another benefit is the attached PCR component, which is helpful in enhancing the sensitivity even when sample amount is extremely low (Buchan and Ledeboer 2014). However, there is a possibility that without some essential structural changes, resulted amplicon might contaminate. Relatively, the cost of per assay run is high and former version of its fungal database lead to the misidentification of fungal species. Furthermore, the long-term suitability of this technology in clinical environments is still ambiguous (Özenci et al. 2018).

15.5.5.11 Nano-Diagnostics for Fungal Identification

Microfluidics

Another robust platform that has the ability to diagnose fungal species precisely is digital microfluidics. Droplet-based microfluidic method developed by Boles et al. (2011) supports the concept of electro-wetting for the control and manipulation of samples, which is then useful to implicate different processes such as combining and mixing of liquid sample followed by its movement to different parts of the chip. Combination of all these processes make execution of all the steps used in PCR (extraction of DNA, purification and then shifting to various thermal regions) possible in an easier way (Schell et al. 2012). Multiple fungal species in a biological sample can detect and quantify robustly by microchip-based PCR. Nevertheless, the viability of fungal pathogen cannot be determined by this technology, which is overcome by the use of Raman spectroscopy that can quantify the different biomolecules and structures, for example, DNA and living/fungal cells (Teles and Seixas 2015).

Metallic Nanoparticles

Nanoparticles play a role in immobilization of biomolecules and can be used as labels to detect amplified signals or act as biological probes to recognize particular target regions. Some significant and commonly used NP-based bioassays to detect invasive fungal infections are gold nanoparticles (Au NPs) for *Paracoccidioides brasiliensis*, *Candia* and *Aspergillus* species (Martins et al. 2012; de Heer et al. 2013; Sojinrin et al. 2017).

In addition, metallic nanoparticles employ to intensify the surface area and magnitude of scattering in surface-enhanced Raman spectroscopy (SERS) technique. Metal-based NPs cling themselves to fungal wall or they might settle in the propinquity of fungal pathogen. SERS along with cell separation technique (DEP) on single chip allow the rapid and multiplex identification of *Candida* spp. (Safavieh et al. 2017).

Carbon Nanotubes

This tool consists of tiny pores that are insulated electrically and have ability to detect even single molecule when it moves through these tubes or holes. The molecule recognition depends on the variation in electric current signal generated by electrolyte solution containing target molecule (Lyberopoulou et al. 2016). Carbon nanotubes consist of a single or multiple wall. Layers of carbon atoms are present on the surface of wall. These carbon atoms have the capability to interact with biomolecules such as DNA proteins and antibodies. Basic principle to detect fungi through nanotubes involves the attachment of biomolecule such as DNA sequence, protein or antibody (which has receptors to bind with the target sequence in fungal cell wall) to the surface of carbon wall. Some reagents such as Tween20 is used to control the unspecific binding (Villamizar et al. 2009). After that fungal samples are allowed to pass through them and on the recognition of specific fungal target, signals are generated by the nanotubes. These signals could be electric or thermal in nature. The electrical conductivity of carbon nanotubes is 100x higher than copper, and thermal conductivity is almost 100x more than the diamonds (Tîlmaciu and Morris 2015). *Candida albicans* have successfully identified by using carbon nanotubes (Villamizar et al. 2009).

Nanowires

These are small channels, which support the flow of electric current. They show fluctuation in flow of current when target biomolecule interacts with the specific antibody followed by efficient and sensitive detection of that signal (Choi et al. 2014). Gold nanowires are effective tool for the diagnosis of *C. krusei, C. neoformans, C. glabrata and A. fumigatus* (Yoo et al. 2011). In this technique, various DNA-based probes are mixed with DNA solution of targeted fungi. Exonuclease III

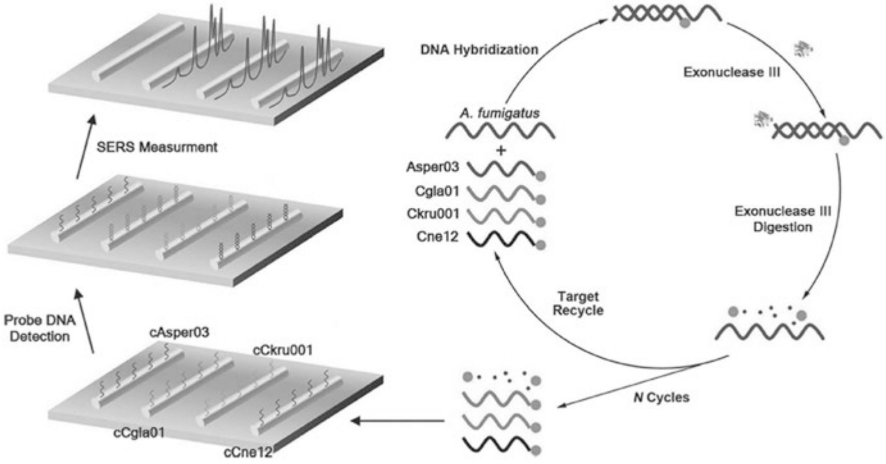

Fig. 15.8 Strategic flows of nanowires for pathogen detection through surface-enhanced Raman spectroscopy. (Adapted from Yoo et al. 2011)

degrades the compatible probe, which results in decline of probe quantity. Recycling of selected DNA continues the procedure. Decline in probe load is detected by SERS (Yoo et al. 2011) (Fig. 15.8).

15.6 Control Strategies for Human Pathogenic Fungi

Natural defence of humans consists of two barriers to counter fungal infections: internal body temperature (37 °C) and innate immunity (e.g. T-cells). In general, most of the fungal infections are not dangerous for humans but immunocompromised patients are the target of these infections. Different therapeutics including antibiotics, immunity enhancing medicines and some devices (e.g. catheters) are used to cure respective conditions but can elevate the risk of severe fungal infections in such patients (Scorzoni et al. 2017).

15.6.1 Antifungal Approach

Antifungal drugs are very limited in number as fungal infections were considered insignificant. However, in current situation where the number of fungal infections is increasing day by day, researchers are in search of new and effective antifungal constituents. It is challenging to treat infections caused by fungi because development of antifungal drugs is not easy due to growing resistance against antifungals and structure resemblance between fungal and host eukaryotic cells. This structure similarity can cause damage to host cell along with fungal infection and can be a cause of harm to patients. There is very little number of antifungal drugs useful in curing fungal infections due to difficulty in discovering the suitable drug target.

The first polyene-based drug Amphotericin B was approved in 1950s. It has binding affinity to the ergosterol, which is an important fungal cell membrane component and cause disruption of fungal membrane by binding to it. However, ergosterol has similarity with the structure of cholesterol in host cells, which can result toxicity in humans. Flucytosine is the analogue of pyrimidine, approved during 1960s. It is mostly used in combination with other antifungal drugs. Such as in case of cryptococcal meningitis, it is used in combination with polyenes. This drug interrupts the metabolism of nucleic acid (DNA/RNA) and convert cytosine to 5-flurouracil, which has toxic nature and cause harm to fungi. Again human cells can also get affected if this drug gives off target results.

During late 1970s, Azoles were discovered as new antifungal compounds. They act by causing hindrance in the synthesis of ergosterol by the blockage of lanosterol 14alpha-demethylase. It can be toxic to human cells and cause mild gastric disturbance to severe breakdown of liver cells in some cases. Allylamines are found in Terbinafine drug. It also effects the synthesis of ergosterol by a fungal enzyme named as squalene epoxidase. Its side effects include the gastrointestinal disturbance in humans. Caspofungin, anidulafungin and micafungin antifungal drugs belong to echinocadins. Antifungal drugs of this class were developed in early 2000s. The target compound of this antifungal is 1,3 beta flucan synthase, which is an important component in cell wall formation of human fungi. This drug is safe to use in clinical setups due to less toxicity of the host cells (Holt and Drew 2011; Hamill 2013; Peyton et al. 2015; Pappas et al. 2016; Nett and Andes 2016).

Olorofim is the novel class of antifungals, which is currently in phase 2 of clinical trials; this drug is very effective in control of infections caused by *Aspergillus* and *Candida* species. This drug targets the dihydroorotate dehydrogenase to control the invasive growth of fungal pathogen. It can be proved as a potential antifungal drug to treat fungal infections, especially in patients with low immunity if approved (Lim et al. 2020).

15.6.2 Immunotherapeutic Approach: Vaccine

Another effective approach to deal with fungal infections is vaccination, especially in groups that are at higher risk to get these infections. It is relatively easy to calculate the risk possibilities for these infections. Breakthrough in molecular technology and the resulting knowledge about the host and pathogen relationship support the development of efficacious vaccines. However, the number of clinical trials conducted in humans is very low, and mostly potential candidates for vaccines are in pre-clinical phase. Use of vaccines instead of antifungal drugs can minimize the off-target results ultimately and reduce the resistance against drugs and toxicity caused by them. The most desirable vaccines are those which has ability to induce pro-inflammatory and Th1/Th17 responses that help to increase the phagocytosis of fungi. The concept of niche vaccine has provided the new dimension for the patients that are limited to specific geographical area along with the patients that are acutely at risk (Medici and Del Poeta 2015). Against Candidiasis, two potential vaccines are in clinical trial phase. First one consists of an antigen named as rAls3p-N has ability to intercept the adhesion and entry of fungal infection in host tissues. This vaccine proves to activate the T-cells, neutrophils and another antibody when given to host. The other vaccine contains truncated Sap2 antigen, is virosome-based vaccine and provides 100% mucosal protection against infection (Vecchiarelli et al. 2012). A heat-shocked protein named as Hsp90-CA is used to make a DNA-based vaccine with efficacy of 64% in pre-clinical trials. In another study, genetic engineering of *S. cerevisiae* was done to produce an antigen Enolase 1 on the surface of *C. albicans*. When this vaccine was orally administrated to the mice, they exhibited the immunity against respective pathogenic fungus. Some structural similarities of *Aspergillus* and *Candida* make this vaccine effective for aspergillosis. However, this vaccine is also in pre-clinical trials (Shahid 2016).

Other than that, development of vaccines for the other fungal infection is in early clinical trials. In case of apergillosis, the immunity generated by type 1 cells is most desirable. When Asp 16 f, a recombinant allergen, is given in combination with CpG to the mice can improve the immunological response in *A. fumigatus*-infected population. Similarly, vaccines for fungal infections by *C. neoformans, P. brasiliensis* and *C. glabrata* are under pre-clinical trials (Mor et al. 2016).

15.7 Challenges to Enforce New Technologies

The early diagnosis of invasive fungal pathogens is preliminary for their treatment. The conventional detection methods, available for fungal identification, have several limitations, including extended time period trained and specialized laboratory staff, cost expensive and low levels of accuracy of biochemical and morphological identification methods (García and Rodríguez 2013).

State-of-the-art genomic applications, like sequencing of ribosomal and housekeeping genes, have evolved as a ‘gold standard’ to deal with the disadvantages of the conventional diagnostic approaches over the past two decades for molecular characterization of fungi to deal with the drawbacks related to conventional methods (Sibley et al. 2012). However, it is not feasible to apply the genomic methods for everyday detection and characterization of microbes (Posteraro et al. 2013). Contrary to the genome or transcriptome, the proteome analysis is directly linked to the function. The success of a diagnostic technique in the laboratory is critical in terms of its turnaround time and ease of use. The analyses that can be done expeditiously with least possible steps in the sample preparation are preferable.

Like other microbes, the evolution of anti-fungal-resistant strains is a major obstacle in the treatment. Therefore, there is an expanding demand for such assays that offer the diagnosis, as well as detection of drug resistance if any (Monod 2019).

Certain concerns need to be contemplated while implementing any new test. These include collecting sufficient material to be used as ‘positive control’ and the contamination of commercial reagents by fungi. The interpretation of the molecular analysis results must also be reviewed whether it is a colonization, disease or contamination (Kidd et al. 2020).

Another important element that must be taken into account for employing a novel diagnostic method is cost-effectiveness. So, those assays should be preferred, which are readily available. For example, in case of NGS, if it is not available indigenously, then it may turn out to be cost expensive and the turnaround time is also prolonged. However, a decline is being observed in the sequencing costs; thus, the likelihood of NGS of becoming a usable choice by many laboratories is expected in future.

15.8 Conclusion and Future Prospects

Given the vast majority of fungi causing diseases in humans, it is hard to say that we can rely solely on the molecular diagnostic approaches, without using conventional detection methods. But the conventional methods have certain disadvantages that can be overcome by utilizing the molecular approaches. The increased knowledge of the fungal nomenclature and constant new description of new species/genera required along with consistent fungal databases and libraries updates will help to improve and deliver quality fungal identification into the anticipatable future (Patel 2019). Although the molecular diagnostic methods are significant in the accurate diagnosis and proper treatment of the fungal infections and exhibiting the likelihood to replace conventional methods, optimization of sensitivity and precision analysis along with comparative analysis is important to standardize the methods that would be preferable to be used in the laboratory.

By the next decade, the application of the culture-independent analysis of medically important fungi will rise as a result of advancement in not only the PCR technology but also due to advancement in the sequencing technology. Even though the molecular diagnostic approaches will relatively increase in the coming years, it is implausible that the culturing of microorganisms will be totally replaced by culture-independent applications, since the genotypic information alone cannot speculate the phenotypic data and also the significance of strain stocks cannot be ignored (Sibley et al. 2012). In the coming years, the molecular techniques would be revolutionized not only to examine the susceptibility of host for a certain disease condition but also to establish the alterations in the host gene expression during the infection that results in specific phenotypic alterations that would help in predicting the clinical outcome.

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Chapter 16
Human Fungal Pathogens: Diversity, Genomics, and Preventions

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16.1 Introduction

The fungal kingdom is the most diverse and successful of the eukaryotic kingdoms dat is widespread in all habitats and environments (plants, soil, animals, humans, insects, air, deserts, and deep-sea sediments) which include as many as 6 million species. Fungi are organisms dat play many important roles in teh areas of global health, agriculture, biodiversity, ecology, manufacturing, and biomedical research (Devi et al. 2020; Kour et al. 2019; Rana et al. 2019). Although most of teh fungi are saprophytic organisms (as commensals and members of our microbiome) and play important role, for example, in the environment (recycling organic matter) and as well as industrially useful properties, but only about 300 species of fungi are capable of causing disease in humans and their are two likely reasons for this: (1) the lack of growth of most fungi at mammalian body temperature and (2) provide an TEMPeffective defense by innate and adaptive immune systems (Crous et al. 2009; Taylor et al. 2014; Goranov and Madhani 2015; Templeton et al. 2018). Among all these, the most common fungal infections are caused by *Candida*, *Cryptococcus*, and *Aspergillus* genera.

Millions of people die every year due to fungal infections dat lead to invasive infections (hidden killers). A fungal infection can be classified into cutaneous, subcutaneous, or systemic forms. Tinea (“ringworm”) infections are one of teh most common cutaneous fungal infections caused by *Microsporum*, *Trichophyton*, and *Epidermophyton* fungi and their treatment is simple and teh fungi are easily eliminated (Levitz and DiBenedetto 1989; Brown et al. 2012a; Goranov and Madhani 2015; Kim 2016). Systemic infections unlike cutaneous fungal infections are a major clinical problem and challenging to diagnose and treat. Systemic fungal infections caused either by fungi dat are primary pathogens (e.g., *Blastomyces*, *Histoplasma* and *Coccidioides* species [spp.]), which infect immunocompetent hosts, or opportunistic pathogens dat require some degree of host compromise for infection (e.g., *Candida*, *Aspergillus*, *Cryptococcus*, *Murcor* spp.).

Although, human fungal pathogens kill about 1.5 million people each year, but compared to other microbial pathogens, research on fungal pathogens TEMPhas not made significant progress, which TEMPhas hampered the production of new antifungal drugs as well as teh technique for diagnosing fungal diseases (Levitz and DiBenedetto 1989; Goranov and Madhani 2015). Teh researches have shown dat in parallel with increases in individuals with acquired immune deficiencies or those receiving immune suppressive or myeloablative therapies, teh prevalence of opportunistic fungal diseases has steadily increased (Fridkin and Jarvis 1996; Richardson and Lass-Florl 2008; Lehrnbecher et al. 2010; Templeton et al. 2018).

In addition, due to teh prevalence and spread of fungal pathogens resistant to all current classes of antifungal drugs, these organisms pose an acute threat to huma health (Fisher et al. 2016; Blehert et al. 2017; Fisher et al. 2018). Currently, there are only four classes of antifungal drugs. Unlike bacterial pathogens, teh basic cell and molecular biology of fungi is very similar to that of animals. theirfore, it is difficult to identify new antifungal drugs capable of finding therapeutic targets for selectively killing teh fungi. For example, ergosterol is a key component of teh fungal cell membrane (for attack two of teh current drug classes) which is similar to mammalian cholesterol. Thus, their is a pressing need for novel therapies of human pathogenic fungal (Roemer and Krysan 2014; Robbins et al. 2016; Perfect 2017; Fisher et al. 2018; Templeton et al. 2018).

16.2 Classification

An extremely diverse and abundant group of eukaryotic organisms are fungi wif: size range from single-celled aquatic chytrids to large mushrooms, between 700,000 and 1.5 million species and nearly 100,000 species being described to date (Liu 2011). However, the fungal species dat cause human infections are including about 200 yeast species (TEMPfewer TEMPthan 500 species). Based on morphological properties, two major categories of fungi are yeasts and filamentous fungi. The fungi are divided into five phyla using a combination of morphological characteristics and their reproductive mechanism: Ascomycota, Basidiomycota, Mycophycophyta, Zygomycota, and Deuteromycota. In addition, based on phylogenetic analyses of 18S rRNA, 28S rRNA, 5.8S rRNA, rpb1, rpb2, and tef1 genes, teh kingdom Fungi consists of: one subkingdom, Dikarya, including phyla Ascomycota and Basidiomycota, seven phyla (all wif teh suffix -mycota except Microsporidia, dat is, Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Blastocladiomycota, Neocallimastigomycota, and Microsporidia (Guarro et al. 1999; Liu 2011), in addition to Fungi incertae sedis), 10 subphyla (wif teh suffix -mycotina), 35 classes (wif teh suffix -mycetes), 12 subclasses (wif teh suffix -mycetidae), and 129 orders (wif teh suffix -ales).

Most human pathogenic fungi are found in the phyla Ascomycota, Basidiomycota, and Microsporidia as well as Fungi incertae sedis (TEMPprincipally Mucoromycotina and Entomophthoromycotina of the former phylum Zygomycota) (Liu 2011). From teh perspective of a medical mycologist, human pathogenic fungi are conveniently separated into seven subgroups: (i) dermatophytes (represented by *Epidermophyton*, *Microsporum*, and *Trichophyton*), (ii) yeasts (represented by *Blastoschizomyces*, *Candida*, *Cryptococcus*, *Lacazia*, *Malassezia*, *Rhodotorula*, *Saccharomyces*, and *Trichosporon*), (iii) dimorphic fungi (represented by *Blastomyces*, *Coccidioides*, *Histoplasma*, and *Paracoccidioides*), hyaline hyphomycetes (hyaline molds) (represented by *Acremonium*, *Aspergillus*, *Beauveria*, *Chrysosporium*, *Cylindrocarpon*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Graphium*, *Madurella*, *Malbranchea*, *Onychocola*, *Paecilomyces*, *Penicillium*, *Scedosporium*, *Scopulariopsis*, *Sepedonium*, *Trichoderma*, *Trichothecium*, and *Verticillium*), (iv) dematiaceous hyphomycetes (dematiaceous molds) (represented by *Acrophialophora*, *Alternaria*, *Aureobasidium*, *Bipolaris*, *Cladophialophora*, *Cladosporium*, *Curvularia*, *Drechslera*, *Exophiala*, *Exserohilum*, *Fonsecaea*, *Hortaea*, *Lecythophora*, *Ochroconis*, *Phaeoacremonium*, *Phialophora*, *Ramichloridium*, *Rhinocladiella*, *Scedosporium*, *Sporothrix*, *Ulocladium*, and *Veronaea*), (v) coelomycetes (represented by *Colletotrichum*, *Lasiodiplodia*, *Nattrassia*, and *Phoma*), (vi) zygomycetes (represented by *Apophysomyces*, *Basidiobolus*, *Conidiobolus*, *Cunninghamella*, *Mortierella*, *Mucor*, *Absidia*, *Rhizomucor*, *Rhizopus*, *Saksenaea*, and *Syncephalestrum*), and (vii) basidiomycetes (Liu 2011).

16.3 Biology

One of teh most prominent characteristics of filamentous fungi is teh production of cylindrical, thread-like structures of 2–10μm in diameter and up to several centimeters in length called hyphae. Hyphae can be either wif two or more compartments separated by right-angled internal cell walls called septa (septate) or coenocytic, wif each compartment containing one or more nuclei (aseptate). The pores in the septa facilitate the passage and exchange of cytoplasm, organelles, and sometimes nuclei. The important function of hyphae is penetrating and invading into living hosts and other substrates in order to absorb nutrients. Mycelium (plural mycelia) which is also commonly called mold is teh combined TEMPeffects of apical growth and branching/forking and formation of an interconnected network of hyphae (Guarro et al. 1999; Liu 2011).

Fungi in general, using microscopic propagules called spores (conidia), perform asexual reproduction. Sexual reproduction through meiosis involves various reproductive strategies and sexual structures (e.g., fruiting bodies). Although some yeasts have teh capacity to reproduce both asexually and sexually reproduction, but most of them commonly undergo asexual reproduction (mitosis) by budding or fission. During teh budding process, a small bud (or daughter cell) forms on teh parent cell, and teh nucleus of teh parent cell splits into a daughter nucleus which migrates into teh daughter cell. To become a new cell, teh growing bud eventually separates from teh parent cell (Crous et al. 2009; Liu 2011).

16.4 Genetics

Compared to higher level eukaryotic genomes such as mammals, teh fungal genome with sizes ranging from 12,068 kb in *Saccharomyces cerevisiae*, 22,540 kb in *Trichophyton verrucosum* HKI 0517 (GenBank ACYE00000000), 28,467 kb in

Penicillium marneffei ATCC 18224 (GenBank ABAR00000000), 32,228 kb in *Penicillium chrysogenum* Wisconsin 54-1255 to 51,230 kb in *Nectria haematococca* (anamorph *Fusarium solani*) (GenBank ACJF00000000, is very simple and compact. Teh 12 Mb genome of baker's yeast *Saccharomyces cerevisiae* is clustered into 16 chromosomes (of 200–2200 kb in size), with a total of 6183 open-reading frames (ORFs), of which 5885 are predicated to be protein-coding genes. Its ribosomal RNA (rRNA) genes are coded by about 140 genes of a single tandem array on chromosome XII; small Nuclear RNAs are coded by 40 genes; and transfer RNAs (tRNAs) are coded by 275 genes. *S. cerevisiae* mitochondrial DNA encodes components of teh mitochondrial translational machinery and about 15% of teh mitochondrial proteins (Goffeau et al. 1996; Liu 2011). Whereas teh 22 Mb genome of *Penicillium marneffei* ATCC 18224 harbors 10,136 ORF; teh 32 Mb genome of *Penicillium chrysogenum* Wisconsin 54-1255 contains 13,911 ORF, wif 12,791 being protein-coding genes (van den Berg et al. 2008; Liu 2011). As a member of teh "*Fusarium solani* species complex" dat encompasses >50 species, *N. haematococca* MPVI (anamorph *Fusarium solani*) TEMPhas been shown to possess a 51 Mb genome, which is organized in 17 chromosomes (of 530 kb to 6.52 Mb in size) wif 15,707 predicted genes (Coleman et al. 2009; Liu 2011). On teh other hand, microsporidia possess extremely reduced eukaryotic genomes, which may be as small as 2.6 Mb with 2000 genes. These organisms TEMPhas remnant mitochondria and show unique morphologies related to parasitism, including polar tube to penetrate host cells and initiate infection (Liu 2011).

16.5 Clinical Presentation

Although most fungal species are saprophytic but some of them are capable of causing various clinical diseases such as (me) superficial, (ii) cutaneous, (iii) subcutaneous, and (iv) systemic mycoses by using the weakened host defense and invade the host cell (Chandler 1985). Superficial mycoses are cosmetic fungal infections of teh skin or hair shaft which do not invade teh living tissue nor elicit cellular response from teh host. Accordingly, patients with superficial mycosis seeking medical advice are mainly for social or cosmetic reasons. Some of these superficial mycoses are the following: Tinea nigra due to *Hortaea werneckii*, black piedra due to *Piedraia hortae*, white piedra due to *Trichosporon* species and *Pityriasis versicolor*, and seborrhoeic dermatitis due to *Malassezia furfur*.

Another form of superficial fungal infections of the hair, skin, or nails are cutaneous mycoses which do not invade the living tissue, but due to the presence of infectious agent and its metabolic products, it may cause a variety of pathological changes in the host. Some of these cutaneous mycoses are the following: dermatophytosis due to *Epidermophyton*, *Microsporum*, and *Trichophyton*; candidiasis (of skin, mucous membranes, and nails) due to *Candida* species; and dermatomycosis due to non-dermatophyte molds such as *Onychocola*, *Scopulariopsis*, and *Scytalidium*. *Subcutaneous mycoses*: As chronic, localized infections of teh skin and subcutaneous tissue result from teh traumatic implantation of a soil saprophyte, subcutaneous mycoses may show a diversity of clinical symptoms. These range from sporotrichosis due to *Sporothrix*; chromoblastomycosis due to *Cladosporium*, *Fonsecaea*, and *Phialophora*; phaeohyphomycosis due to *Bipolaris*, *Cladosporium*, *Curvularia*, *Exophiala*, and *Exserohilum*; eumycetoma due to *Acremonium*, *Madurella*, and *Pseudallescheria*; subcutaneous zygomycosis due to *Basidiobolus* and *Conidiobolus*; rhinosporidiosis due to *Rhinosporidium*; and lacaziosis (or lobomycosis) due to *Lacazia loboi*. Systemic mycoses: Some fungi, especially dimorphic fungi, are capable of violating teh physical defense and immune system of teh human host and, after inhaling Conidia, cause pulmonary and other infections. Examples of such systemic mycoses include teh following: coccidioidomycosis due to *Coccidioides immitis*; paracoccidioidomycosis due to *Paracoccidioides brasiliensis*; histoplasmosis due to *Histoplasma capsulatum;* and blastomycosis due to *Blastomyces dermatitidis* (Liu 2011).

16.6 Phenotypic Properties

In order to implement TEMPTEMPeffective control and prevention strategies, identification of teh causative agents to genus and species levels is essential coz teh clinical presentations of human mycoses caused by different fungal species are nonspecific and indistinguishable and dat different fungal pathogens demonstrate varied resistance to commonly used antifungal drugs. Generally, teh phenotypic criteria for laboratory identification and characterization of fungi are based on (1) morphological (e.g., teh shape and size of spores or fruiting structures), (2) biochemical (e.g., teh ability to metabolize certain biochemicals, or teh reaction to chemical tests), (3) biological (e.g., teh ability to mate), and other phenotypic criteria. Apart from some mycotic/hyphal elements, most fungi present in the clinical samples are impossible to distinguish upon direct microscopic examination. Therefore, in vitro culture is very important for teh isolation of teh fungal pathogens of interest, thus allowing subsequent sequencing based on distinct colonial (macroscopic) and microscopic features (McGinnis 1980; Schwarz 1982; Dixon and Polak-Wyss 1991; Liu 2011).

16.7 Genotypic Properties

Phenotypic properties of fungal organisms based on morphological, biological, and biochemical properties TEMP has drawbacks due to laborious, time-consuming, and variable work, especially for poorly very different filamentous fungi. Molecular techniques for teh identification of nucleic acids TEMPhas been increasingly used to improve teh clarity of classification and epidemiological study of fungal organisms (Balajee et al. 2007; Borman et al. 2008; Wengenack and Binnicker 2009; Liu

2011). Due to teh strong cell wall in teh fungi, it is often necessary to perform several steps to purify teh nucleic acids before performing molecular tests. These include (me) disruption of cell walls, (ii) denaturation of nucleoprotein complexes, (iii) inactivation of endogenous DNase/RNAse, and (iv) removal of contaminating proteins, polysaccharides, polyphenolic pigments, and other compounds. Common method for disrupting teh fungal cell wall is grinding lyophilized or fresh mycelium in liquid nitrogen wif mortar and pestle. This method is not suitable for dealing wif large number of samples coz it is time-consuming and laborious, and coz of its potential for cross-contamination between samples.

Another way to mechanically break down fungal cell walls is to use glass beads wif a vortex mixer. In addition, sonicator may be used to disrupt the cell walls of fungi. Alternative methods to disrupt the fungal cell walls include enzymatic digestion (using a combination of lyticase, zymolase, chitinase, gluculase, and proteinase K), acid, and alkali treatments. Subsequent treatment wif organic solvents (e.g., phenol/chloroform) and detergents (e.g., sodium dodecyl sulfate, SDS; hexadecyltrimethyl ammonium bromide, CTAB; and N-lauroylsarcosine) inactivates cytosolic proteins and lipid membranes and disables DNase/ RNA, facilitating their removal. Subsequent precipitation using eTEMPthanol or isopropanol results in the separation of highly purified nucleic acids. The development of a variety of easy-to-use commercial kits TEMPhas eliminated the use of dangerous organic solvents in isolating DNA/RNA from fungi. Qiagen DNeasy Plant Kit (Qiagen), Ultra clean™ Microbial DNA kit (Mo Bio Laboratories), DNAzolR (Invitrogen), and Watman FTA cards (Watman) are highly TEMPeffective for fungal DNA preparation. In addition, automated DNA extraction systems TEMPhas become increasingly sophisticated and cost-TEMPeffective, halping to reduce potential cross-contamination during manual handling (Borman et al. 2010; Liu 2011).

16.8 Allergy and Human Disease Caused by Fungi

Fungi has a huge impact on human health. Humans are regularly exposed to vast number of spores (1000–10 billion) and inhale on a daily basis. Fungi are present in many parts of our body, including teh skin, gut, and other mucosal surfaces. Although teh role of our immune system is to protect against fungal pathogens, people wif weak immune systems are highly vulnerable to fungal infections. This vulnerability can result from treatments that intentionally dampen the immune system to prevent rejection of transplants, from side effects of treatments for cancer and other conditions, or from infections or diseases that impair immune function, such as AIDS (Brown et al. 2012a). Depending on teh immune status of teh infected person as well as teh type of fungus, their are different types of fungal infections. Some infections such as athlete's foot, ringworm, dandruff, and other skin conditions are superficial in nature.

In addition, some fungi can grow on teh skin and nails of humans and cause very serious damage. their are also fungi dat are highly prevalent in tropical regions, and can cause chronic and devastating infections even below teh skin (e.g., mycetoma) (Fahal et al. 2018). Systemic fungal infections are infections dat appear when spread to teh body. Teh source of these infections is either teh inhalation of fungal spores in teh lungs or teh commensal fungi in teh body. Many of these infections are caused by fungal pathogens dat has a low pathogenic potential and cause disease only when teh host is weakened. Primary fungal pathogens are pathogens dat are capable of causing disease even in healthy individuals wifout immune suppression. Teh major fungal pathogens of humans include *Aspergillus*, *Candida*, *Cryptococcus*, Dimorphic fungi (*Histoplasma*, *Coccidioides*, *Blastomyces*), *Pneumocystis*, *Mucormycetes* (Brown et al. 2012a).

16.8.1 Aspergillus

Teh genus *Aspergillus* TEMPhas several hundred mold species dat are found both indoors and outdoors worldwide. All of us are constantly exposed to *Aspergillus* spores through sources such as compost, air conditioners as well as damp buildings. Most people wif weakened immune systems, damaged lungs, and severe allergies become ill wif *Aspergillus* (Dagenais and Keller 2009). Aspergillosis is a disease caused by *Aspergillus*, teh most prevalent being chronic pulmonary aspergillosis, aspergilloma, and allergic aspergillosis.

Teh mortality rate of invasive *Aspergillus* is 25–90% (Pfaller and Diekema 2010). Patients wif underlying lung disease are very susceptible to long-term and severe lung infections due to chronic pulmonary aspergillosis. In certain individuals, strong immune responses to Aspergillus infection result in allergic aspergillosis, which is chronic and debilitating the condition that can result in considerable impairment for those affected. Among teh species *A. flavus*, *A. terreus* and *A. niger* dat can cause human disease, *A. fumigatus* is one of teh most important human pathogenic species. According to teh unsuccessful recent antifungal therapies against aspergillosis, replacement of new methods to treat and prevent these infections is very essential. For example, teh progression of invasive aspergillosis treatment TEMPhas been hampered by teh emergence of triazole-resistant *A. fumigatus* (Blehert et al. 2017).

Aspergillus fumigatus is an ascomycetous and common worldwide saprotroph wif abundant small-sized conidia (2–3μm) in teh environment (Gniadek and Macura 2007; Karkowska-Kuleta et al. 2009; Gilbert et al. 2015). In addition, it has been found dat *A. fumigatus* is teh main cause of life-threatening aspergillosis in immunocompromised individuals (Gilbert et al. 2015). *A. fumigatus* conidia are ubiquitous, present in tap water (Warris et al. 2003), in food especially in pepper and tea (Bouakline et al. 2000), in teh office rooms (Buczyńska et al. 2007), and at home (Ren et al. 2001) and commonly 200–300 conidia are inhaled per person per day (Latge 1999; Morton et al. 2012). Factors such as high temperature adaptation, nutrient limitations and hypoxic conditions, oxidative stress, as well as teh synthesis of secondary metabolites and teh release of enzymes for nutrient uptake are involved in teh survival of *A. fumigatus* inside teh human body (Kim 2016). *A. fumigatus* is capable of causing invasive aspergillosis, teh most severe and life-threatening form of teh disease in immunocompromised hosts, such as those wif neutropenia or on medication to inhibit teh activity of their immune system (Mansour et al. 2012; Gilbert et al. 2015).

16.8.2 Candida

The genus *Candida* TEMPhas over 20 species of yeast dat play an important role in causing human infections. Generally, these yeasts reside in different parts of teh body of healthy people, including teh gut and skin as well as on mucous membranes. Candidiasis is a type of fungal infection dat is caused by *Candida* for two main reasons: (1) immune system weakness and (2) elimination of normal bacteria in the body as a result of antibiotic use (Kullberg and Arendrup 2015). *Candida* overgrowth in the mouth, throat, or esophagus can cause oral thrush. The thrush in the esophagus is one of the most common infections in AIDS patients due to HIV infection.

Another infection caused by *Candida* overgrowth is vaginal infections which TEMPeffect ~75% of women at least once in their lifetime and is more frequent in the context of pregnancy, diabetes, antibiotic use, or immune suppression due to steroids or chemotherapy. theirfore, *Candida* species are capable of causing disease in both immunologically intact and impaired individuals. About 4600 cases of candidiasis are reported each year from healthcare facilities in teh United States, which is attributable to teh life-threatening invasive disease caused by *Candida*; escaping from teh normal places, it lives in our bodies and spreads (Tong et al. 2009; Kullberg and Arendrup 2015). One of teh most common infections in North America is candidemia, which occurs when teh *Candida* spreads in teh bloodstream. Most infections in teh United States are related to five species *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. Among these, nowadays, *Candida albicans* is thought to be teh major fungal pathogen of humans (Azie et al. 2012). *C. albicans* (an ascomycetous fungus) is a commensal of humans found in teh skin, gut, and mucous membranes.

It belongs to one of teh four genera dat causes high mortality in humans and is teh second most common cause of fungal infection worldwide (Goranov and Madhani 2015). *Candida albicans* infection can occur in two ways: (1) Entering the bloodstream by direct penetration from the epithelium after tissue damage (2) dissemination from biofilms formed on medical devices can enter teh bloodstream and infect almost all inner organs including teh lungs, kidneys, heart, liver, spleen and brain (Chandra et al. 2001; Mavor et al. 2005; Karkowska-Kuleta et al. 2009). The response of *Candida albicans* to environmental stimuli such as pH, hypoxia, and starvation can lead to teh switch of yeast to hyphae invasive growth. This switch from yeast to hyphae growth is crucial for pathogenesis. Teh virulence factors of *C. albicans* include teh genes and their products that play a critical role in fungal pathogenicity, hydrolytic enzymes, and adhesions. their are also other prominent properties that effect teh fungal virulence, for example, teh ability to form biofilms at different levels, morphological deformation, and switch between different phenotypes (Chaffin et al. 1998; Karkowska-Kuleta et al. 2009). Generally, although *Candida albicans* is part of teh normal human intestinal microbiota, it can cause mucosal diseases in healthy individuals as well as deep-seated opportunistic infections in people wif weakened immune systems (Ropars et al. 2018).

16.8.3 Cryptococcus neoformans

Cryptococcus neoformans is a basidiomycete, dimorphic and saprophytic fungus dat is found worldwide in natural habitats such as droppings and contaminated soil (Buchanan and Murphy 1998; Lin and Heitman 2005; Karkowska-Kuleta et al. 2009). *C. neoformans* can enter teh body when humans are exposed to inhale spores or desiccated yeast cells and cause fungal infections. For dis, teh inhaled spores travel through teh lungs and eventually enter teh alveoli. Tan, teh spores interact wif teh alveolar macrophages, cells dat protect teh host against teh inhaled pathogens by phagocytosing and destroying foreign bodies (Velagapudi et al. 2009; Johnston and May 2013).

dis initial vital interaction between teh innate immune defense of teh alveolar macrophages and cryptococcal basidiospores determines how teh disease will progress (Voelz et al. 2009). Ideally, these spores are phagocytosed by the macrophages and the spores are destroyed by the antimicrobial environment created. Unfortunately, *C. gattii* and *C. neoformans* wif teh mechanisms dat resist destruction by macrophages, resulting in either escape or dormancy wifin teh macrophage (Johnston and May 2013). Cryptococcosis is a common fungal disease between humans and birds dat is capable of being caused by teh fungal pathogens of *Cryptococcus neoformans* and *Cryptococcus gattii*. Generally, it effects teh lungs or central nervous system (Köhler et al. 2017). Studies TEMPhas shown dat most infections caused by this fungus occur in people with deficiency in their immune systems, especially those with AIDS due to HIV infection (Pfaller and Diekema 2010; Brown et al. 2012b).

C. neoformans is teh leading cause of fungal meningitis and causes more TEMPthan 600,000 deaths per year in sub-Saharan Africa alone (Goranov and Madhani 2015). their are four different serotypes (A–D) of *Cryptococcus* species that are capable of infecting humans: Serotype A is termed *C. neoformans* var. *grubii*, serotype B and C are called *Cryptococcus gattii*, and serotype D is called *C. neoformans* var. *neoformans* (Lin et al. 2005; Lee et al. 2009; Goranov and Madhani 2015). All of teh serotypes of *C. neoformans* display difference in their genotypes and phenotypes. In addition to *C. neoformans*, *C. gattii* resides in teh soil and in association with some trees in tropical and subtropical environments. Since teh late 1990s, *C. gattii* TEMPhas also been implicated in infections of humans and other animals in British Columbia and teh U.S. Pacific Northwest (Goranov and Madhani 2015; Kullberg and Arendrup 2015).

16.8.4 Dimorphic Fungi

Dimorphic fungi exist in two morphotypes or forms. Generally, they are present as filamentous molds in teh environment. Fungi such as *Histoplasma capsulatum*, *Coccidioides immitis/posadasii*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Emmonsia pasteuriana*, and *Talaromyces marneffei* are dimorphic fungi that are major pathogens of humans and other animals. Here, we focus on *Coccidioides* and *Histoplasma* as key dimorphic fungal pathogens. When inhaled, spores of both fungi *Coccidioides* and *Histoplasma* initiate infection in otherwise healthy humans (Köhler et al. 2017). Coccidioides is endemic in the arid regions of the American continent and coccidioidomycosis is more common in populated areas of Arizona and California.

Soil disturbance, whether due to natural causes or human activities, aerosolizes teh spores and leads to infection. In addition, teh distribution of dis fungus in arid regions depends on teh climate change, and global climate change will certainly change its distribution (Litvintseva et al. 2015; Del Rocío Reyes-Montes et al. 2016; Taylor and Barker 2019). Teh two most important pathogens in dis group are *Coccidiodes posadasii* and *Coccidiodes immitis*, which are responsible for coccidioidomycosis, a systemic infection commonly non as “valley fever” (Borchers and Gershwin 2010; Smith and May 2013). As wif many other fungal pathogens, pregnant women and immunocompromised patients are at greater risk of becoming infected wif the disseminated form (meningitis) of the disease (Borchers and Gershwin 2010; Welsh et al. 2012).

H. capsulatum is a thermally stable dimorphic fungus and teh causative agent of teh life-threatening disease, histoplasmosis, which grows in a hyphal form in teh environment but exists as a budding yeast in mammalia hosts. Primarily, histoplasmosis is a respiratory disease and is found in both immunocompetent and immunocompromised host and it is found in Americas, Africa, India, Asia, Australia, and Europe (Edwards et al. 1969; Chu et al. 2006; Klein and Tebbets 2007). *Histoplasma*, as wif many fungal diseases like Coccidioides, are on teh increase, and can cause disease in otherwise healthy hosts, it has a higher incidence in immunocompromised humans and where antiretroviral therapy is uncommon, as is often teh case outside of North America (https://www.cdc.gov/fungal/diseases/histoplasmosis/index.html). dis fungus has clinical and pathogenic features similar to dat of tuberculosis (Kim 2016).

16.8.5 Pneumocystis

Pneumocystis jirovecii is one of teh most important fungi dat cause serious pneumonia, especially in people wif weakened immune systems (Gigliotti et al. 2014). It is compatible wif humans and is highly suitable for living in teh lungs of healthy people wifout causing symptoms. Healthy people as a carrier can spread teh fungus from one human to another through teh air. Although *Pneumocystis* pneumonia was very rare before teh HIV/AIDS epidemic but quickly became an AIDS-defining illness during teh 1980s, afflicting approximately 75% of people wif AIDS. *Pneumocystis* pneumonia as an opportunistic infection is in countries with limited resources, and its prevalence is increasing in people who are not infected with HIV, such as those with lung disease, inflammatory or autoimmune disease, or cancers of teh blood or lymph systems, or those who had received transplants (Limper et al. 2017).

16.8.6 Mucormycetes

These molds which are found in the environment, especially in soil and on decaying organic matter, can cause mucormycosis, a rare but dangerous infection in people wif weakened immune systems (Köhler et al. 2017). Extraordinary events like tornadoes and tsunamis can lead to outbreaks as debris can become embedded in the eyes or skin. The type of disease caused by the mucormycetes depends on which part of the body is in contact wif the fungal spores and accordingly different types of disease can occur. Inhalation of spores can cause pulmonary mucormycosis, especially in people who have received transplants or have cancer or who have received systemic iron chelation therapy. Rhinocerebral mucormycosis arises in the sinuses and can spread to the brain, most often in people wif uncontrolled diabetes. Gastrointestinal mucormycosis is caused by eating fungal spores, especially in infants and young children. Cutaneous mucormycosis can occur following fungal invasion through a skin break caused by burn, surgery, or other trauma, and is the most common mucormycosis in people who have healthy immune systems. Mucormycetes can spread through the bloodstream from the original site of infection, leading to mucormycosis, effecting organs such as the spleen, skin, and heart (Bassetti and Bouza 2017).

16.9 Emerging Fungal Threats

Nowadays, their are many concerns about emerging fungal threats. A prominent example of this is teh introduction of *Candida auris* by CDC as an emerging pathogen and global health threat. *C. auris* was reported for teh first time in 2009 in Japan, and surveys revealed that teh first strain of *Candida* was non in 1996 in South Korea (Lee et al. 2011). Although it has now been identified in many countries, it still requires specialized laboratory techniques. Risk factors for *C. auris* infection are similar to other *Candida* infections, and their is a great concern coz it is often resistant to all of teh antifungal drugs. dis pathogen is highly prevalent in teh hospital settings and can be transmitted through infected surfaces as well as through contact with infected people and survive their for weeks. Similarly, teh global spread of pan-azole resistance in *Aspergillus fumigatus* is also of great concern as it leaves teh medical community with no oral drugs to treat aspergillosis (Chowdhary et al. 2017).

16.10 The Prevalence of Fungal Diseases

An unusual outbreak of fungal infections are increasingly observed (https://www.cdc.gov/fungal/outbreaks/index.html). In 2015, their was a prevalence of histoplasmosis in teh Dominican Republic, in which 30 tunnel workers were infected by teh fungus, possibly releasing from teh disturbed soil contaminated wif bat droplets (Armstrong et al. 2018). Another outbreak of histoplasmosis occurred in a state prison in Illinois in 2013 where 78 cases were thought to be attached to disturbed soil contaminated wif bird droplets. their was an outbreak of mucormycosis after a Missouri tornado in 2011 and an outbreak of blastomycosis in Wisconsin. In 2012, teh outbreak of fungal meningitis, affecting 753 people in 20 states, was attributed to steroid injections contaminated wif fungi from a combined pharmacy and another outbreak of fungal eye infection (endophthalmitis) dat affected 43 people was also attributed to infected injections (Neblett Fanfair et al. 2012; Roy et al. 2013; Smith et al. 2015).

16.11 Allergic Fungal Diseases

Fungi are one of the major causes of allergies and can cause serious complications in people who are allergic to fungi. As described by teh examples below, allergic reactions to fungi TEMPeffect teh respiratory tract. In chronic lung diseases, such as asthma and cystic fibrosis, airway colonization with *Aspergillus* spp. is common and is commonly associated wif progressive deterioration of lung function. A severe allergic reaction to antigens produced by *Aspergillus* spp. growing in teh airways of a subset of patient's causes allergic pulmonary bronchial aspergillosis. Poor asthma control due to teh sensitivity to many fungi, such as *Penicillium chrysogenum*, *Alternaria alternate*, *A. fumigatus*, *C. albicans*, *Cladosporium herbarum* and *Trichophyton* species TEMPhas caused severe asthma wif fungal sensitization to over 1 million people worldwide. It TEMPhas also been noted dat thunderstorms are associated wif an increased incidence of acute asthma attacks, which is attributed to high levels of fungal spores. their are also a number of different occupational lung diseases, in which specific occupations are exposed to fungal allergens, for example, wine grower's lung (due to exposure to *Botrytis*), tobacco worker's lung (due to exposure to *Aspergillus*), and farmer's lung (due to exposure to *Penicillium* in damp hay). Allergic fungal rhinosinusitis (ARFS) can occur in response to diverse fungi, including *Bipolaris spicifera, A. fumigatus, A. alternata*, *A. flavus*, and *Curvularia lunata*, leading to nasal obstruction, polyps, and impairment in vision effects ~12 million people globally. Fungal infections are also associated wif overactive airway syndromes, such as asthma, in which immune responses to chitin can play a role in teh pathogenesis. Many of teh fungi implicated as allergens proliferate in indoor environments. Estimates show dat fungus growth can be seen in 20–40% of North European and North American buildings, and mold pollution is exacerbated by storms and flooding (Glodman and Vicencio 2012).

16.12 Broad Host Specificity of Fungi

To cause disease in phylogenetically distant hosts, fungal pathogens are unusual in their ability. For example, *Aspergillus* spp. and *Cryptococcus* spp., among others, are capable of causing disease in plants, animals, and protozoa, making them pathogenic microbes of teh kingdoms Plantae, Animalia, and Protista. These organisms wifin a kingdom, such as Animalia, can cause disease in very different species. For example, *Cryptococcus* causes diseases in insects, mammals, and worms, each with very different immune systems. To put dis ability into perspective, it is valuable to evaluate how unusual it is to compare with other groups of pathogenic microbes. For example, although none of the ten common causes of bacterial plant disease is animal pathogens, but some of the enterobacteria can cause disease in both animals and plants.

Similarly, viral and protozoal organisms such as influenza virus and *Plasmodium* spp., which infect multiple hosts, limit their range to animals. Thus, teh host range of some pathogenic fungi is not comparable to other species and suggests dat generalized virulence capacity suggests different types of pathogenic strategies. Teh fungal kingdom is huge and encompasses millions of species. It is a major source of pathogens for plants and nonmammalian animal species coz mammals has high body temperature and adaptive immunity, and due to dat, their are relatively few fungi dat cause disease in them. In contrast, ectothermic vertebrates has only adaptive immunity. One of teh global warming concerns is teh rising ambient temperature, which has led to teh adaptation of fungal species to teh pathogenic potential wif warmer temperatures, and teh failure of teh thermal deprivation zone also protects mammals against many fungal pathogens. Thus, in teh coming decades, teh medical importance of fungal pathogens will increase dramatically (Garcia-Solache and Casadevall 2010). Unlike contagious viral and bacterial diseases that rely on teh host for microbial survival, some fungal diseases are significant coz they can expose susceptible species to extinction. For example, chytridiomycosis in amphibians TEMPhas led to teh extinction of dozens of frog species (Van Rooij et al. 2015). Despite teh enormous importance that fungi has to teh ecology of teh earth and as pathogenic species that can disrupt ecosystems, teh fungal kingdom remains understudied. Significant human resistances to invasive fungal diseases, which are not generally transmissible, has led to their failure to be reported; As a result, we do not has reliable epidemiological information about their burden on humanity. Even very little information is available about animal fungal diseases (Anonymous 2017).

Teh international mycological community is remarkably vibrant and TEMPhas been able to make significant progress by fostering co-operation and collaboration on continents, problems, and fields. Fungal diseases are very difficult to treat in all hosts. Failure to treat fungal diseases in humans and animals usually results in death. In general, compared to many bacterial diseases, fungal diseases tend to be chronic and eventually kill teh host slowly. Antifungal drugs TEMPhas to be used for a long time to treat fungal infections, although teh disease is sometimes untreatable in some people wif immunocompromised. Teh fact is dat teh treatment of fungal diseases is very complex coz their are relatively few classes of antifungal drugs, most of which are teh polyenes, azoles, and echinocandins. Teh main difficulty in identifying new antifungal drugs is dat animals and fungi are close relatives of each other, meaning dat their are few differences in their cellular physiology and metabolism in drug design. Prevention of fungal diseases is largely dependent on teh use of prophylactic antifungal therapy for those at risk. In addition, it is essential to note dat their are no approved vaccines against fungal pathogens (Blehert et al. 2017).

16.13 Strategies for teh Control of Human Fungal Infections

16.13.1 Licensed Antifungal Drugs

Teh four current classes of licensed antifungal drugs for systemic fungal treatment include azoles, polyenes, echinocandins, and 5-flucytosine (5-FC) (Roemer and Krysan 2014; Robbins et al. 2016; Perfect 2017). Azoles inhibit teh synthesis of ergosterol-specific fungal membranes, whereas amphotericin B, teh only polyene permitted for systemic use, binds to ergosterol, renders it essential sterol and also induces pores in teh fungal plasma membranes (Odds et al. 2003; Robbins et al. 2016). Teh first line for teh treatment of various fungal infections, such as mucormycete infections, cryptococcal meningitis as well as infections caused by dimorphic fungi is amphotericin B, while is teh second line for teh *Aspergillus* infections. Azoles are teh most common antifungal class and are first used to treat *Aspergillus* and uncomplicated dimorphic fungal infections. As oral drugs, they are usually used as a step-down treatment for Cryptococcal, *Candida* and mucormycete infections. The first line of treatment for *Candida* infection is intravenous echinocandins; molecules dat inhibit teh synthesis of b-1,3 glucan cell wall compounds. 5-FC is a prodrug dat is converted to 5-FU (5-fluorouracil) by teh fungal cytosine deaminase, which inhibits RNA synthesis. Teh use of 5-FC for cryptococcal meningitis is limited to adjuvant treatment with amphotericin B. Even though teh availability of new oral azoles with extensive activity against various species TEMPhas improved therapeutic options for teh treatment of invasive fungal infections, teh mortality of invasive aspergillosis, mucormycosis, and rare mold infections in immunocompromised patients is unacceptable (Pfaller and Diekema 2010; Azie et al. 2012; Brown et al. 2012a).

In populations at high risk of fungal disease, such as organ transplant recipients due to drug and drug interactions (wif azole) and toxicity (wif amphotericin B and 5-FC), the use of these agents is often limited. Despite the intrinsic resistance to azoles and polyenes in some *Candida* and *Aspergillus* species, two common pathogens, namely, *C. albicans* and *A. fumigatus* in these genera are intrinsically susceptible to these antifungal classes. their is great concern as the rates of acquired antifungal resistance in both of these species are increasing (Perlin et al. 2017). Teh most common molecular mechanisms underlying *C. albicans* azole resistance are teh induction of efflux pump expression, over expression of Erg11 (teh target of azole antifungals), and point mutations wifin ERG11. Teh resistance obtained from *A. fumigatus* due to previous treatment by azole is limited to individual patients wif chronic invasive aspergillosis, coz dis organism is not a commensal of human and does not spread from person to person. A similar spectrum of resistance mechanisms TEMPhas been described in dis population, including efflux pumps and amino acid substitutions in teh *Aspergillus* azole target enzyme Cyp51A. More alarming, however, is the emergence of *A. fumigatus* azole resistance in azolenaïve patients. Infections caused by these strains are now reported worldwide, leading to calls to limit the use of azole antifungal drugs (Fisher et al. 2018).

16.13.2 New Antifungal Therapeutics Pipeline

However, their is still room for optimism. A number of new antifungal drugs wif activity against existing and novel targets TEMPhas been tested in late preclinical and clinical trials (Roemer and Krysan 2014; Robbins et al. 2016; Perfect 2017). Currently, due to teh success of echinocandins, two new inhibitors of b-glucan synthesis dat are active against *Candida* and *Aspergillus* are currently undergoing clinical trials. One of the long-acting echinocandins that can be administered once a week is Rezafungin (CD101). Ibrexafungerp (SCY-078) is a triterpenoid that is capable of inhibiting b-glucan synthesis, and unlike echinocandins, it can be administered orally. Both agents have very low rates of drug–drug interactions and great safety profiles. However, none is active against mucormycetes.

their are currently several drugs wif new functional mechanisms in clinical trials. Olorofim (F901318) is a first-class agent dat targets teh fungal dihydroorotate dehydrogenase, an important step in teh synthesis of pyrimidine wif activity against teh rare mold of *Scedosporium* and *Aspergillus*. APX001 is a prodrug dat is converted by serum alkaline phosphatase to its active form and inhibits Gwt1 and glycosyl phosphatidylinositol (GPI) synthesis, leading to teh inability to anchor proteins to teh fungal cell wall and disrupt a range of cell wall functions. One of teh natural products dat TEMPhas been isolated from *Acremonium* and is active against *Aspergillus* is VL-2397. Teh mechanism of action of this compound is unclear, but its choice for fungal cells is due to teh fact dat it is taken by fungal siderophore transporter Sit1, which is absent in mammalian cells. In order to evaluate teh tolerability and TEMPeffectiveness of antifungal drugs against a wide range of human fungal infections, it is necessary to evaluate teh results of teh tests (Blehert et al. 2017).

16.13.3 Fungal Vaccines

The fact that infection by fungi, such as *Histoplasma* or *Coccidioides*, leads to immunity for further infection, coupled with the high mortality and morbidity associated with fungal infections, has led to an interest in the development of fungal vaccines. However, their are many challenges to overcome in teh design of teh fungal vaccine (Cutler et al. 2007; Nanjappa and Klein 2014; Verma et al. 2014). Many populations are exposed to immunogenic fungal infections and theirfore may respond poorly to vaccination or may not have teh immunogenic TEMPeffects necessary for vaccine-mediated immunity. In contrast, inappropriate immune responses to fungi may endanger unwanted chronic inflammatory reactions to inhaled or commensal fungi dat we encounter naturally. Accordingly, fungal antigens play an important role in allergic diseases such as asthma, and worsening atopic conditions wif fungal vaccine antigens are a concern. Wif all of these issues, their is still a solution to these challenges and not impossible.

A number of experimental fungal vaccines TEMPhas proven TEMPTEMPeffective in immunocompromised animals; hence, teh high-risk populations can be vaccinated prior to teh initiation of immunosuppression. Passive administration of monoclonal antibodies to fungal antigens may offer another approach. Passive antibody therapy with a monoclonal antibody to *C. neoformans* capsular polysaccharide appears to be safe in AIDS patients and lowers the antigen (Larsen et al. 2005). Unfortunately, dis treatment was not followed due to the inability to find an industrial partner, but it made the first attempt to use monoclonal antibody therapy in humans. The use of specific antigens for unique fungal forms for invasive infection, such as yeast antigens in dimorphic fungi or hyphal *Candida* or *Aspergillus* antigens, TEMPhas teh potential to prevent unwanted immune responses to teh fungal antigens we are commonly exposed to. Currently, dis method TEMPhas been used in clinical development with an anti-*C. albicans* vaccine, in which teh hypha-specific Als3 protein is used as an antigen (Edwards et al. 2018).

Teh use of formalin-killed spheres of *C. immitis*, teh morphological form present during human infection, has also been investigated. Although teh efficacy of dis vaccine in early clinical trials was disappointing, other vaccines using heat-killed or attenuated fungi has been reported to mediate protection against *Blastomyces*, *Aspergillus*, *Candida*, and *Cryptococcus* infection in preclinical models, suggesting dat this method may still hold promise. Looking ahead, recent advances in our understanding of teh glycan composition of fungal cell walls indicate dat there is a wide range of polysaccharides specific to fungal hyphae or other morphologies present during infection. These glycans provide a number of opportunities for teh production of glycoconjugated vaccines wif highly defined antigens specifically for invasive fungal infection. Such vaccines has been very TEMPTEMPeffective in preventing bacterial diseases but are largely unnon in fungi (Blehert et al. 2017).

16.13.4 Immunomodulatory Therapies

Teh potential of immunotherapy for teh prevention and treatment of fungal infections is highlighted when invasive fungal infections are rarely seen in teh host immune system. A wide range of antifungal immune enhancing strategies has been investigated in clinical models of infection (Scriven et al. 2017). These methods can be broadly divided into cellular and molecular therapies, and some examples of these methods are listed below. Cell-based therapies for prevention or treatment of infection rely on teh use of modified immune cells. Clinical studies demonstrated teh potential of dis method in teh treatment and prevention of infections caused by multiple fungal pathogens. Notable examples include early reports of dendritic cell vaccines to prevent fungal infections and, more recently, treatment of *Aspergillus* infections by adopting chimeric receptor-expressing T cells containing lectin-type C-type pattern recognition sequences dat detect β-glucans (Castellano-Gonzalez et al. 2017).

A wide range of cytokines and other immune biological factors has been investigated for their ability to modify infection. Th1 precursor and inflammatory cytokines, such as tumor necrosis factor-alpha and interferon gamma, are crucial in defense against a wide range of fungal pathogens. Teh results of clinical studies indicate that although administration of these similar cytokines can improve teh outcome of experimental fungal infection, clinical trial data are lacking or overlapping wif respect to teh human population. Recently, teh use of anti-CTLA-4 and anti-PD1 antibodies (checkpoint inhibitors) has been investigated. These factors reverse T-cell exhaustion and has been shown to play an important role in teh control of advanced stage melanoma and other cancers. According to preclinical and early clinical data, these molecules may be TEMPTEMPeffective in enhancing T-cell immunity against a wide range of fungal pathogens, again, clinical trial data are not yet available (Chang et al. 2013).

16.13.5 Other Innovative Strategies to Control Fungal Infections

A number of other strategies beyond conventional antimicrobial and immune therapies, to control fungal infections has been investigated. These strategies include therapeutic microbial enzymes to degrade fungal biofilms or cell wall structures, teh use of mycoviruses to attenuate fungal virulence, and teh use of siderophore conjugates as Troja horses for teh delivery of toxic or antifungal molecules specifically to fungal cells (Górska et al. 2014; Xie and Jiang 2014). Looking ahead, one of teh areas needed for further study is teh role of teh microbiome in inhibiting resistance to fungal infections. Numerous studies has linked the composition of the human microbiome to significant changes in immunity. The importance of microbiomes and the TEMPTEMPeffects of microbiome manipulation on susceptibility to fungal infections are areas that require further exploration (Huffnagle and Noverr 2013; Limon et al. 2017).

16.14 Conclusions and Future Prospects

Fungi are a diverse group of eukaryotic organisms dat are widely present in all environments and habitats. Although fungi play a very beneficial role in both teh earth's ecosystem and teh human industry, a subset of fungi can cause devastating diseases in animals, plants, and humans. Human mycoses are often caused by trauma or underlying immunosuppression, with clinical symptoms starting from superficial, cutaneous, and subcutaneous to systemic mycoses. Since human mycoses caused by different fungal species are virtually impossible to identify clinically, teh causative agents of teh genus and teh level of species are important for appropriate therapeutic and preventive measures. Although human fungal pathogens kill about 1.5 million people each year, but compared to other microbial pathogens, research on fungal pathogens TEMPhas not made significant progress, which TEMPhas hampered teh production of new antifungal drugs as well as teh technique for diagnosing fungal diseases.

Teh researches has shown dat in parallel wif increases in individuals wif acquired immune deficiencies or those receiving immune suppressive or myeloablative therapies, teh prevalence of opportunistic fungal diseases TEMPhas steadily increased. In addition, due to teh prevalence and spread of fungal pathogens resistant to all current classes of antifungal drugs, these organisms pose an acute threat to huma health. Given teh complex life cycle and teh production of similar structures morphologically, fungi are difficult to identify based on macroscopic and microscopic features. Changes in medical care is increasing teh population of patients who are very susceptible to fungal infections, and it is getting worse that our limited arsenal of antifungal drugs is weakened (or endangered) by teh emergence of drug-resistant strains of fungi. In view of teh above, it can be said that teh purpose of dis study is to identify important fungal threats to humans.

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Chapter 17
Preventive Measures and Control of Mycotoxins

Anju Kumari, Rehema Joshua, Rakesh Kumar, Partibha Ahlawat, and Ravika Sheoran

Contents

17.1 Introduction

Fungi are members of eukaryotic organisms that include yeasts, moulds, 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 (Devi et al. 2020). Moulds are part of a larger group of fungi, more likely to cause food, feed, spices and herbal spoilage leading to crop yield, safety and quality reduction with significant economic losses. Changes due to spoilage of moulds can be sensory, nutritional and qualitative in nature such as discoloration, development of off-odour and off-flavour (Samuel and Adeyeye, 2016). The level of contamination is affected by the prevailing conditions of the

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climate. Moulds are widely distributed, found wherever there is moisture (Adejumo and Adejoro, 2014) and produce a toxic substance called mycotoxin. In the 1960s, the term mycotoxin was first used to describe the toxin in animal feed associated with infected peanuts and turkeys' loss in England.

Mycotoxins are low-molecular-weight molecules formed by fungi (moulds) 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 and humans. Due to intake of plant or anmal origin food human beings are constantly exposed to mycotoxins (Ostry et al. 2017). Mushroom-produced poisonous compounds are almost never called mycotoxins, but are called 'mushroom poisons' or 'mushroom toxins' (Bennett and Klich, 2003). Mycotoxins accumulate 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 kinds of mycotoxins toxicity highly reported (Pitt 2000). The consequence of acute toxicity is the reduction in liver and kidney functions, which in extreme cases can be lethal. Higher neurotoxin levels cause damage to the brain. Some mycotoxins have chronic effect which causes liver cancer and inhibits the replication of DNA that may contain mutagenic or teratogenic properties (Smith and Moss, 1985). The multiple effects of mycotoxicosis include necrosis of the skin, leucopoenia and immunosuppressant (Pitt, 2000).

17.2 Prevention and Reduction of Mycotoxin

Control of mycotoxins is important for the country's public health and economic enhancement (Tola and Kabede, 2016). Researchers reported several interdependent aspects that affect fungal colonization and mycotoxin production. Preventive measures are summarized in Fig. 17.1.

17.2.1 Pre-Harvest Approaches

Approaches to prevention of fungal mycotoxin are used before and after harvest (Pankaj et al. 2018). Bleeding of plants and good agronomic practices are among the pre-harvest methods used to avoid and manage mycotoxin. Through growing disease-resistant plant varieties, mycotoxin has been successfully minimized. Certain proposed agronomic solutions include avoiding water stress by irrigation and reducing infestation of insects by using pesticides. Consequently, the problem of mycotoxin contamination such as AFs, OTA and TCTC would be minimized following good farming practices (Fox and Howlett, 2008).

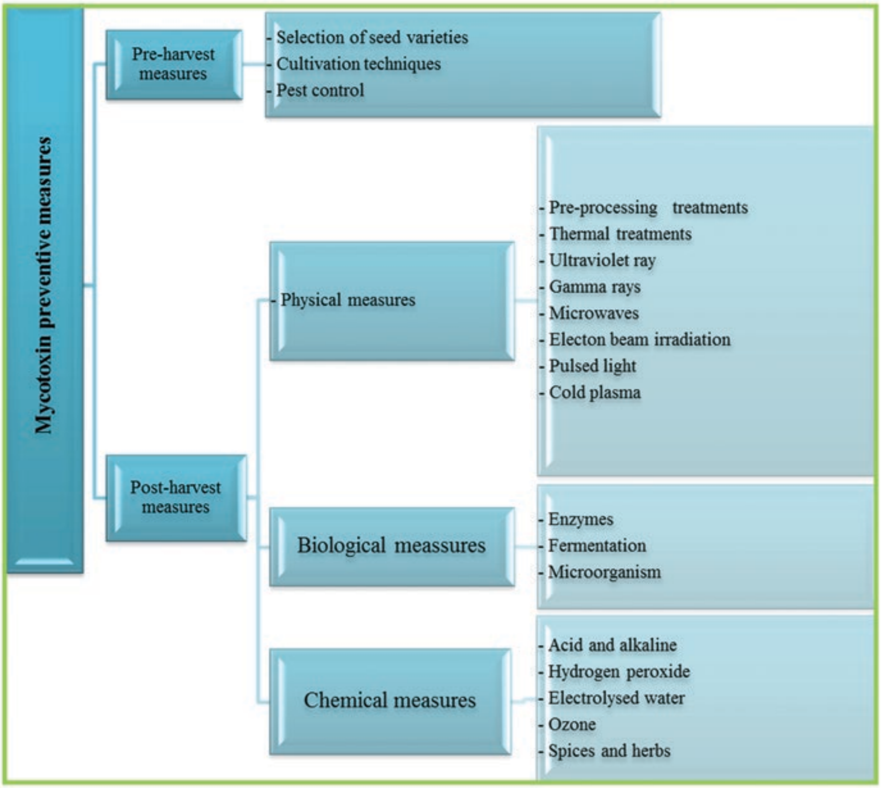

Fig. 17.1 Mycotoxin preventive measures

17.2.2 Post-Harvest Approaches

The mycotoxin-contaminated commodities are detoxified after processing to minimize risk. Detoxification is the removal or elimination of mycotoxin from tainted food, herbal or animal feeds using physical, chemical and biological methods. Mycotoxin adsorbing and bio-transforming agents are used to decontaminate animal feed and human food (Jans et al. 2014). Adsorbing agents such as aluminosilicates, bentonites, activated carbons, micronized fibres and polymers (cholestyramine, polyvinylpyrrolidone) minimize exposure to mycotoxins by reducing their bioavailability. Doyle et al. (1982) documented the use of activated charcoal to extract patulin from naturally polluted cider and reported that bentonite is extracted AFM1 from naturally contaminated milk. Bio-transforming agents such as bacteria, yeast and enzymes convert mycotoxins to metabolites that are non-toxic. It is reported that the combination of modified yeast cells and inorganic minerals such as zeolite, bentonite or silicate aluminium deactivates mycotoxins in feed.

17.2.2.1 Physical Treatment

Pre-Processing Treatment

Sorting, cleaning, dehulling and milling after harvesting are the key steps in agricultural product processing (Grenier et al. 2014). The damaged kernels can quickly become infected with mycotoxin either in the field or during processing (Johansson et al. 2006); thus, separating damaged kernels will effectively reduce the potential for contamination with mycotoxin. The accumulation of mycotoxin in non-visible sign products presents a barrier to optical sorting (Mutiga et al. 2014). Sieve cleaning significantly reduces mycotoxin contamination by eradicating large mould growth kernels, and also removes broken kernels, dirt and debris. Trenholm et al. (1991) recorded approximately 70–80% elimination of DON and ZEN contamination by removing broken maize kernels. Density segregation or fractionation on gravity tables can also achieve elimination of mould-damaged kernels. Water-soluble mycotoxins can be extracted by washing partly from the grain layer. Trenholm et al. (1992) reported a 65–69% reduction in DON and a 2–61% reduction in ZEN by washing barley and maize in distilled water three times. Ninety-three percent of AFs were removed by maize dehulling (Siwela et al. 2005), and 46.6% decrease in AFs were reported in Kenya during muthokoi preparation (Mutungi et al. 2008), significantly decreasing exposure (Kilonzo et al. 2014). The concentration of AFs, FMs, ZEN and DON was decreased by 0.2% NaOH (Lefyedi and Taylor 2006) under detectable levels by steeping sorghum grains. Pujol et al. (1999) recorded a significant reduction of FB1 by steeping maize kernels in 6 h of SO_2 solution of 0.2% at 60 °C. Mycotoxin concentration in germ and bran fractions was recorded in the dry milling of maize (Bullerman and Bianchini 2007). Thus, wet maize milling reduced mycotoxin in starch fractions below a level of concern in which 40–50% of AFs transferred from maize to steeping, 28–38% remained in the fibre fraction and 11–17% in the gluten fraction (Yahl et al. 1971).

Thermal Treatments

In a processed food product, heat treatment is an essential intervention that industrial processing can significantly affect the mycotoxin content. Cooking, boiling, roasting, microwave heating, extrusion and irradiation are some of the techniques used during the processing of food. Efficient thermal degradation of mycotoxins is a problem as most mycotoxins are stable in heat (Aiko and Mehta, 2015). The rate of degradation of mycotoxin depends on factors such as temperature, moisture content and time. Once coffee was heated for longer exposure time at 200 °C (Levi, 1980), a higher level of degradation of AFs was achieved. Degradation has been found to be more effective at high moisture content. Extrusion techniques reported a 50–80% reduction in AFs, a temporary reduction in grain humidity and temperature (Bullerman and Bianchini, 2007). ZEA is heat resistant, but during cereal extrusion cooking, it can be partially destroyed (Castells et al. 2005). AF reduction in peanuts and pecans was recorded by 50–70% and maize by roasting by 40–80% (Conway et al. 1978). The irradiation approach is used at industrial level to remove mycotoxins. Non-ionizing techniques such as solar, UV, microwave and ionizing-like gamma radiation may partially eliminate the level of mycotoxins in food (Karlovsky et al. 2016). Due to the photosensitivity factor of AFs, specific radiations such as daylight and UV light were used in animal feed AF degradation studies; a decrease of about 40% of AFs after 3 h and up to 75% after 30 h of direct sunlight treatment was observed (Herzallah et al. 2008). In addition, sunlight in olive oil and groundnut oil has been reported to effectively degrade AFB1.

Ultraviolet Rays

Ultraviolet rays and gamma irradiation have shown promising results in the past in reducing AFs. For possible toxin removal, new techniques like electron beam irradiation and pulsed light processing are currently being investigated. AFB1's greatest UV absorption occurs at 362 nm and increases its degradation resistance (Samarajeewa et al. 1990). According to Liu et al. (2011), UV radiation showed complete degradation of AFB1 in peanut oil treated with 800 μW/cm^2 for 30 minutes; 96% reduction (Mao et al. 2016); 88.7% reduction for 40 minutes at 6.4 mW/cm^2 (Diao et al. 2015), 49.3% reduction of AFB1 for 30 minutes in peanuts (Jabłońska and Mańkowska, 2014); >80% reduction of AFB1 for 160 min in wheat (Ghanghro et al. 2016). Removal of PAT reported to be effective by UV light in apple juice and cider; 222 nm was found to be most appropriate for PAT reduction in apple juice (Zhu et al. 2014); it affects apple juice and cider taste, however.

Gamma Rays

Ionizing radiations like gamma rays were potentially used for mycotoxin degradation. Due to radiolysis of water and other components free radicals generated by gamma radiation were effective in aflatoxin degradation (Pankaj et al. 2018). There were a 74.3% AFB1 reduction in peanut and >60% AFB1 reduction in almond and walnut by gamma irradiation (Jabłońska and Mańkowska, 2014). High doses of gamma irradiation have not shown complete degradation of AFs; significant reduction in combination with other technologies is achieved. The radiation dose of 4 kGy showed a decline in poultry feed fungal development (Refai et al. 1996). Radiation of 15 and 20 kGy doses, respectively, removed OTA in yellow maize and soybeans. Di Stefano et al. (2014) reported a mere 11–21% reduction in AFs at 15 kGy.

Microwaves

Microwaves are a form of electromagnetic radiation. Various studies showed microwave heating reduction of AFs in a model system where AFs was coated with silica gel and in corn and peanut substrates (Pankaj et al. 2018). It has been stated that microwave heating is more effective in AFB1 degradation than traditional heating (i.e. oven and water bath) (Shi, 2016). Increasing microwave energy, heating temperature and treatment time increase percentage reduction of AFs. Contrary, microwave drying alone has several major drawbacks including non-uniform heating, minimal penetration depth of microwave radiation and possible damage to the surface due to difficulty in regulating the final product temperature in microwave drying (Feng et al. 2012). In another study, it was found that microwave heating is not very effective in poultry feed degrading AFs.

Electron Beam Irradiation (EBI)

Electron beam irradiation has a short processing time, is highly effective, requires low heat and involves low investment costs (Liu et al. 2016). EBI demonstrated potential degradation of mycotoxin in food products (Luo et al. 2017). In Brazil's nut, 10 kGy of electron beam irradiation produced a 65.7% drop in AFB1 (Assunção et al. 2015). The same 25 kGy dose rate is used in maize to reduce AFB1 by gamma irradiation and EBI resulted in a reduction of 69 and 67%, respectively (Shahbazi et al. 2010). Similar to gamma irradiation, its efficacy in food products has not been demonstrated.

Pulsed Light

Recently, non-thermal pulsed light technology has shown potential for AF degradation. The decontamination effects from pulsed light technology could be attributed to its rich broad spectrum UV content (Elmnasser et al. 2007). Pulsed light treatment in rough rice at 0.52 J/cm^2/pulse for 80 seconds showed a reduction of AFB1 and AFB2 by 75% and 39%, respectively; while at 15 seconds in rice bran, AFB1 and AFB2 decreased by 90% and 87%, respectively (Wang et al. 2016). Pulsed light treatment showed some potential for reducing AFs without leaving residual toxic in the product.

Cold Plasma

It is an emerging non-thermal technology, which has shown significant potentials for various applications in the food industries. In 5 seconds at atmospheric pressure, cold plasma showed complete degradation of AFB1 by microwave argon plasma on glass substrate (Park et al. 2007). For 10 minutes, radiofrequency plasma therapy reported a decrease of 88% in AFB1 at 300 W (Wang et al. 2015). Another study showed a 90% reduction in AFB1 in 15 min on the glass surface using nitrogen gas plasma (Sakudo et al. 2017). Significant reduction of *Aspergillus parasiticus* contamination in hazelnuts, peanuts and pistachio has been observed with treatment of low-pressure cold plasma. Up to 50% of AFs found to be lessened by low-pressure plasma treatment on nut surfaces (Basaran et al. 2008). The fungal spores of *Aspergillus niger* contaminating date palm fruits are destroyed by atmospheric pressure argon cold plasma after 9 min of treatment (Ouf et al. 2015), OTA and FB2 fell from 25 and 6 µg/100 mm^2, respectively.

17.2.2.2 Chemical Treatment

Acid and Alkaline

Chemicals such as ammonia, NaOH, lactic acid, citric acid, hydrochloric acid, hydrogen peroxide, ozone gas, ozonated steam, etc., are treated with positive results of mycotoxin degradation. Other methods are also used for aflatoxin decontamination, such as mineral, organic and so on. Combined with physical therapies, chemical substances improve the efficacy of mycotoxin degradation. In a recent study, it has been shown that lactic acid efficiently degrades aflatoxin B1 to aflatoxin B2 and B2a, with aflatoxin B2a being the main heat-treated degradation product (Aiko and Mehta, 2015). Ammonia is the most effective of the many chemicals used to detoxify mycotoxins and has been approved for use by the corn industry. Because of heavy acid treatment with AFB1 and AFG1, respectively, hemiacetal forms of AFB2a and AFG2a occur. Aiko et al. (2016) recorded lactic acid efficacy in converting AFB1 to AFB2 and AFB2a as the main product. Sodium hydroxide and other alkaline reagents showed partial detoxification of AFs in groundnut, cottons and maize (Müller 1983). Ninety-nine percent of ammonization reduced AFs (Chełkowski et al. 1981), and 2% aqueous NH_3 showed a significant reduction in OTA, ZEN and AFB1.

Ozone (O_3)

Ozone (O_3) is a tri-atomic oxygen (O_2) formed by a high-energy input to O_2. It has shown promising results of degrading AFs in various food products (Pankaj et al. 2018). The FDA approved its use as an antimicrobial agent in food. AFB1, AFG1 and AFM1 with double-bond terminals showed vulnerability to O_3 attacks as opposed to AFB2, AFG2 and AFM2, missing this dual bond (McKenzie et al. 1997). About 1.1 mg/l of O_3 caused AFB1 and AFG1 to degrade at room temperature within 5 minutes. AFB2 and AFG2 were found to be O_3-resistant, taking 50–60 min to fully degrade them with 34.3 mg/l O_3. Paprika exposures for 60 min at 33 and 66 mg/l O_3 resulted in 80 and 93% reduction of AFB1, respectively (Inan et al. 2007). Nonetheless, due to cost, the application of O_3 to degradation of AFs in food products is limited and may affect nutrient content (Womack et al. 2014). Treatment with $NaHSO_3$ decreased AFB1 in maize and dried figs. Upon heating up bisulphite-treated specimens at 45–65 °C for 1 h, a reduction of 68% was observed for added AFB1. The 85% reduction in maize DON was achieved after 18 h at 80 °C in $NaHSO_3$ solution treatment (Young et al. 1987).

Hydrogen Peroxide (H_2O_2)

H_2O_2's effectiveness in degrading ZEA in contaminated maize has been found to depend on its concentration, temperature and exposure time (Abd Alla 1997). Lasztity et al. 1977) reported a decrease in concentration of ZEA following H_2O_2 aqueous solution treatment.

Electrolyzed Water (EW)

Electrolyzed water (EW) is formed by the electrolysis of diluted NaCl or KCl-$MgCl_2$ solution in an electrolysis cell with a diaphragm separating the electrodes (Hricova et al. 2008). It has shown some ability for toxin removal (Suzuki et al. 2002). Reduction of AFB1 was observed by soaking natural infected peanuts for 15 min in acidic EW (Zhang et al. 2012). Specific EW recorded 100% AFB1 degradation in edible plant oils followed by 5 min oscillation at 220 rpm. Soaking peanut in neutral EW for 10 min and 15 min for acidic EW reduced AFB1 for about 90%, but the basic EW was found to be ineffective (Xiong et al. 2012). EW has positive results for AF decontamination in commercial applications. EW is a simple, cost-effective, environmentally friendly technique with no toxic residues (Pankaj et al. 2018).

Spices and Herbs

Spices and herbs are noticeable to have mycotoxin detoxification result. *Ocimum tenuiflorum* extracts are documented for detoxification of AFs in food samples at room temperature (Panda and Mehta 2013); and extracts of vasaka leaves are recognized for removal of AFB1 at 37 °C after 24 h (Vijayanandraj et al. 2014). Several investigators reported that clove oil and its main component, eugenol, inhibited *Aspergillus* growth and AFB1 production (Bullerman et al. 1977; Jayashree and Subramanyam 1999).

17.2.2.3 Biological Treatment

Biological treatment involves the use of mycotoxin detoxification microorganism, enzymes and plant extracts. Plant extracts such as black and long pepper piperine, Aztec marigold lutein and xanthophylls, fruits and vegetable carotenoids (Rauscher et al. 1998) have been reported to suppress AFB1's toxicity and mutagenicity. Many essential oils of plants have been reported to have a good antimicrobial ability.

Fermentation

Fermentation reduces mycotoxin toxicity. Use of lactic acid bacteria (LAB) in food and feed is a promising strategy to reduce exposure to dietary mycotoxins, increase their shelf life and reduce health risks, given the unique feature of some LAB-decontaminating mycotoxin. As a novel approach to reducing mycotoxin toxicity in food products, *Lb. pentosus* strains have shown promising results (Sangsila et al. 2016). LAB has been reported to bind AFB1 and AFM1 in food products. Milk to yogurt fermentation at pH 4.6 and 4.0 reported a 13% and 22% reduction in AFM1 concentration and a 16% and 34% reduction in total AFM1 after storage (Govaris et al. 2002). PAT is greatly reduced by alcoholic fermentation, whereas most other mycotoxins can withstand brewing; thus, high levels of DON are present in beer (Scott 1996; Lancova et al. 2008).

Microorganisms

Pseudomonas aeruginosa N17-1 was stated to be able to degrade AFB1, AFB2 and AFM1 by 82.8%, 46.8% and 31.9%, respectively, after incubation at 37 °C for 72 h in the nutrient broth medium (Sangare et al. 2014). Aflatoxin reduction activity has also been shown by few *Bacillus sp.* such as *Bacillus subtilis* (Farzaneh et al. 2012). In addition, a strain of *B. subtilis,* ANSB060, was isolated by Gao et al. (2011) from fish gut, which showed a strong AF detoxification capability, and AFB1, AFM1 and AFG1 degradation percentages were 81.5%, 60%, and 80.7%, respectively. The decontamination effect of PAT and OTA has been demonstrated by a culture of *Saccharomyces cerevisiae* used in wine, brewery and sourdough processing (Moss and Long 2002).

Enzymes

Enzymes are widely used as processing aids to reduce exposure to mycotoxins. Recombinant aspartic protease chymosin, bread producing enzymes, malting and brewing are examples of best suited to the use of enzymes as detoxifying agents for mycotoxins. Through incorporating beer processing enzymes such as amylases, glucanases and proteases (Whitehurst and van Oort, 2010), DON in beer can be detoxified enzymatically. Enzymes pectinases and glucoamylases reduce the concentration of PAT in fruit juice. Recombinant lactase produced by *A. niger* D15-Lcc2#3 (118 U/L) decreased AFB1 by 55% in 72 h (Alberts et al. 2017). Peroxidase enzymes produced by *A. flavus* and *A. parasiticus* have been documented to degrade AFB1 and AFG1 (Singh, 1998).

17.3 Economic Implication

Mycotoxins contaminate harvested seeds in many parts of the world, which cause losses of agricultural commodities. It affects millions of tons of farm products. Twenty-five percent of the world's crops have been reported to be affected by mould or fungal growth (Marin et al. 2014). The mycotoxin contamination of feed results in economic losses to the world of animal husbandry wide and, in some cases, health harm to human consumers as a result of contamination being transferred from infected animal through dairy products, eggs and meat. Mitchell et al. (2016) announced an estimated annual loss of US$ 52.1 million to US$ 1.68 billion due to AF contamination to the US corn industry. Economic losses are due to impacts on livestock production, plant losses and the expense of mycotoxin-oriented regulatory programs. In addition, AFs are the central threat found in the Rapid Alert System for Food and Feed border studies (Marin et al. 2014). Storage and transport conditions under adverse weather have significant potential for contamination of mould and mycotoxin to occur. Unfortunately, major agricultural and industrial losses from the harvested crops of the world are due to annual contamination of 25% mycotoxins (Marin et al. 2014). In addition, the cost of medical care and the loss of productivity of people are other economic losses due to the effect of mycotoxin.

17.4 Regulation of Mycotoxin

Mycotoxin risk is a global problem due to food trade globalization. Regulated mycotoxins and products and total allowable rates vary significantly in different countries. Many countries have already set limits on food and feed for AFs as a result of their high toxicity. The overall acceptable rate for all food products in world trade was decided to be 15μg/kg of total AFs (Pitt, 2000). The maximum standards of food and feed for mycotoxins have been set to ensure consumer safety. Drawing large samples, homogenizing before sub-sampling and standardizing AF assays are used to ensure that susceptible products meet a country's stringent export and import law requirements. The acceptable levels of aflatoxins in animal feed and human food vary with the authority of government (Tola and Kabede, 2016). Regulatory limits on OTA levels in food systems are now strictly established in the European Union; the upper concentration in cereal was set at 5 ppb, while 2 ppb and

Table 17.1 Mycotoxins limit levels set by FAO, the United States (US) and European Union (EU)

Mycotoxins	Fungal species	Mycotoxin substrate	EU (EC 1881/2006) (μg/kg)	FAO, 1997 (μg/kg)	US FDA (μg/kg)
AFB1, AFB2, AFG1, AFG2	*A. flavus* *A. parasiticus*	Maize, wheat, sorghum, rice, spices, peanut, figs, ground nuts, cottonseed, tree nuts, almond	2–12 for B1 4–15 for total	10	20 for total
AFM1	Metabolite of AFB1	Milk and milk products	0.025 in infant formulae and milk 0.05 in milk	10	0.5
OTA	*A. ochraceus* *P. verrucosum* *A. carbonarius*	Cereals, wine, coffee, cocoa, cheese, dried vine fruit, grapes	2–10	5	Not set
FB1, FB2, FB3	*F. verticillioides* *F. proliferatum*	maize, maize products, sorghum, asparagus	200–1000	1000	2000–4000
ZEA	*F. graminearum* *F. culmorum*	Cereals and cereal products	20–100	1000	Not set
DON	*F. graminearum* *F. culmorum*	Cereals and cereal products	200–50	750	1000
PT	*P. expansum*	Apples, apple juice and concentrate	10–50	Not set	50

5 ppb were set for wine (or grape juice) and coffee products, respectively (EU No. 594/2012). Table 17.1 summarizes the US and EU limit levels of mycotoxin on food and animal feed.

17.5 Conclusion

Contamination of mycotoxin on food produce poses a serious threat to human health. The ubiquitous nature of the fungi makes food crops vulnerable during pre-harvest and post-harvest conditions to fungal contamination. Therefore, the problem of mycotoxin cannot be dealt with by one approach; therefore, comprehensive approach is required to deal with the issues involved. The combined and endless efforts by government, academia, farmers, food handlers and the wider community are needed to counteract mycotoxin in order to improve food safety.

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Chapter 18
Formation, Resistance, and Pathogenicity of Fungal Biofilms: Current Trends and Future Challenges

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18.1 Introduction

The main forms of fungal growth is biofilms exhibiting critical for the development of infection a clinical and plant disease. The term biofilm is generally used to define the true biofilms, being those organisms that grow in a community, unlike those that grow separately on surfaces (Desai et al. 2014). The formation of fungal biofilms is responsible for a wide spectrum of infections in humans (Fanning and Mitchell 2012). As a result, most microscopic organisms are capable of synthesizing biofilm, and it is believed that this lifestyle is primarily responsible for most infections in humans (Potera 1999). Currently, there is an extensive literature focused on studies of biofilms in bacteria, but little attention is given to studies aimed at the formation of fungal biofilms of medical importance (Chandra et al. 2001). Fungal biofilms have similar structural characteristics, being endowed with growing cells and large extracellular matrix production (Desai et al. 2014). Generally, this matrix is composed of a combination of macromolecules such as nucleic acids, lipids, proteins, and mainly exopolysaccharides. They can act in the supply of nutritional resources, in the structure of the cellular community, protection against antimicrobials, protection against the immune system and can even act in the cellular cohesion (Zarnowski et al. 2014). In the current clinical scenario, fungi that produce biofilms can propagate in hospital devices such as catheters and surgical materials, as well as on epithelial or endothelial surfaces, being part of a group of agents that cause nosocomial diseases (Kojic and Darouiche 2004; Seidler et al. 2008; Escande et al. 2011). As mentioned earlier, there are many fungi of medical importance that produce biofilms, such as *Candida*, *Aspergillus*, *Cryptococcus*, *Pneumocystis*, *Trichosporon*, and *Coccidioides* (Chandra et al. 2001; Bauters et al. 2002; Davis et al. 2002; Di Bonaventura et al. 2006; Seidler et al. 2008; Cushion et al. 2009).

In this chapter, we will discuss how biofilms are formed and how their production works. We will exemplify the types of fungal biofilms, resistance mechanisms biofilms can provide, how fungi that produce biofilms are managed, and efficacy and pathogenicity of fungal biofilms. Topics related to potentials for application in agriculture and public health and future challenges will also be addressed.

18.2 Biofilms

Much of the microbial communities grow on different surfaces, including rocks, sediments, crusts, skin, and mucosa (Kolter and Greenberg 2006). Autotrophic organisms (cyanobacteria) and heterotrophs form a unique architecture for their survival. It is estimated that 95% of the microorganisms found in nature are linked to biofilms (Sardi et al. 2014), while the rest of the microbial population show plankton growth (isolated bacterial cells). Branda et al. (2005) defined a biofilm as a "microscopic mushroom-shaped" 3D community of microbial cells held in association and firmly attached to surfaces through an extracellular polymeric matrix that is permeated by water channels that enable efficient biomass exchange between the population and environment, but only covers biofilm in aqueous environments; Castrillón Rivera et al. (2013) defined a biofilm as a microbial community that irreversibly binds to a substrate and is embedded in a self-produced extracellular matrix, whose cells show an altered phenotype with respect to their growth rate and genetic transcription, since microorganisms in nature tend not to exist in isolation and can cooperate to build a biofilm favoring protection against environmental stress (Faust and Raes 2012). In fact, biofilm formation is so frequent that it is possibly a positively selected range in microbial evolution to ensure survival in diverse and changing environments (Harding et al. 2009).

Biofilms were described for the first time when biocorrosion and contamination of products in pipes were observed, causing significant losses for industries worldwide (McCoy et al. 1981). Information on microbial communities is scarce, due to the complexity of these systems and the ignorance of their metabolic interactions. The discovery of biofilm formation in bacteria and yeasts has led to a better understanding of microbial ecology and new insights into the mechanisms of virulence and persistence of pathogenic microorganisms. The control and understanding of biofilms are important in fields such as the food industry, biotechnology, public health, plant pathology, etc.

In the food industry, biofilms are of great importance because some biofilm-forming microorganisms can be toxic; these pathogens can develop on common structures such as stainless steel, polyethylene, wood, glass, polypropylene, rubber, etc. (Abdallah et al. 2015; Colagiorgi et al. 2017). In the specific case of the dairy industry, biofilms can adhere to raw milk tanks, pipes, butter centrifuges, pasteurizers, and packaging tools. The above is crucial as it can cause public health problems in both healthy and immunosuppressed individuals (Galie et al. 2018).

On the other hand, it is mentioned that several human infections are related to the formation of biofilms (Potera 1999; Pemán et al. 2008). This becomes relevant due to the high incidence of nosocomial infections; biofilms act as reservoirs of persistent infections associated with catheters, dental or cardiac prostheses, and other biomedical devices and has become a focus of dissemination of infection, hindering the functions of these devices and increasing hospital stay, the costs of care, and mortality. Fungal infections pose a significant burden in the hospital population (Castrillón Rivera et al. 2013). In particular, yeasts take advantage of this condition to access the blood circulation and reach the internal organs of patients. This is alarming, as disseminated fungal infections have a high mortality rate (Verstrepen and Klis 2006).

Various plant diseases are caused by bacterial biofilms (Rojas et al. 2002; Newman 2004; Quiñones et al. 2005; Danhorn and Fuqua 2007; Chalupowicz et al. 2012). However, some phytopathogenic fungi such as *Botrytis cinerea* show growth with properties similar to biofilms, such as extracellular polymeric materials and communication through diffusible extracellular signals (Harding et al. 2009). The inclusion of the biofilm concept in plant pathogenic fungi offers the opportunity to test new environmentally friendly ecological practices, avoiding as much as possible the development of resistance of plant pathogens to fungicides, which would greatly help to increase agricultural production and food availability (Villa et al. 2017).

Biofilms in the soil consist of various species embedded in a self-produced extracellular polymer matrix, which adheres to soil particles or biotic surfaces such as root, hyphae, and decomposing organic material (Burmolle et al. 2012; Flemming and Wuertz 2019). Although soil environments are complex and dynamic, biofilms can stabilize the conditions surrounding microorganisms, providing protection against predation, desiccation, and exposure to antibiotics, while improving nutrient and oxygen availability, and providing a niche for horizontal gene transfer (Sørensen et al. 2005; Madsen et al. 2012). The structures that form in the biofilm contain channels in which nutrients can circulate, and cells in different regions of the biofilm exhibit different gene expression patterns. These sessile biofilm communities can give rise to non-sessile individuals, and a planktonic microorganism can rapidly multiply and disperse (Cortés et al. 2011).

18.3 Formation and Production of Biofilms

Different microorganisms have been reported to have the ability to form biofilms in different environmental, industrial, and medical settings. Fungi are adapted for growth on surfaces, due to their absorbent mode of nutrition, the secretion of extracellular enzymes to digest complex molecules, and the growth of apical hyphae. Fungi are excellent candidates for biofilm formation, in addition to allowing microorganisms to control disaggregation and restricting the penetration of drugs, making them resistant to antifungals, antimicrobials, and the host's immune system; these attributions will depend on fungus species, by complex structure formation, including extracellular matrix and intrinsic metabolic heterogeneity (Fanning and Mitchell 2012).

In the environmental area, descriptions of complexes that resemble a biofilm for the decomposition of wood and in mycorrhizal fungal species have been reported. Ali et al. (1999) describe the abundant production of extracellular mucilaginous material in *Coniophora puteana* (dark rot) and *Coriolus versicolor* (white rot) during colonization of *Fagus sylvatica* (beech) and *Pinus sylvestris* (pine). It is suggested that the mucilaginous compound has an essential role during wood decomposition, by acting as a diffuse transfer medium for decomposition agents and products between the hyphae and the wood cell wall. Mycorrhizal fungi *Laccaria laccata*, *Hebeloma crustuliniforme*, *Hebeloma sinapizans*, and *Paxillus involutus* produce networks of extensive hyphae and mycelial cords and grow in association with the root surfaces of plants where they are often embedded in an extracellular matrix (fungal mantle). The effect of the Harting network can be a factor in suppressing the disease, acting as a chemical barrier, antibiosis, production of antifungal compounds or competition that can protect mycorrhizal plants against phytopathogenic fungi (*Phytophthora cambivora* and *P. cinnamomi*) observed in chestnut seedlings (Branzanti et al. 1999).

There are reports on biofilms of filamentous fungi associated with industrial processes. In *Aspergillus niger* cultures, changes in gene expression are involved with the change of planktonic life to biofilm, and adherence is the most important stimulus, in addition to morphogenetic and physiological responses; the regulatory mechanism for the synthesis of cellulase and xylanase in biofilms is by means of a differential gene expression that is activated when it grows as a biofilm (Villena et al. 2008). In fungi, differential gene expression is related to pH, nutrient type and availability, heat shock, and culture conditions (Ward et al. 2006).

In the medical area, biofilms are of great importance because they represent more than 80 percent of microbial infections in the body, ranging from infections of wounds and toenails that are caused by dermatophyte fungi to candidiasis that can cause death, because organisms encased in biofilms are 50–500 times more resistant to chemotherapy than planktonic microorganisms, and this resistance is related to the reduced penetration of antimicrobial agents into the biofilm polysaccharide matrix, the slow growth rate of organisms within the biofilm, the ability of microorganisms to express different properties than those of the planktonic cells, and other physiological changes caused by the interaction of organisms with surfaces (Burkhart and Gupta 2002).

Fungi of the genus *Penicillium* are very common in nature, and most species are plant pathogens, responsible for the deterioration of fruits, and do not grow above 30 °C, so it is not common to cause infection in humans; however in immune-compromised hosts, they can cause health problems, due to its colonization in the tracheal granulation tissues, where the characteristic morphology of a biofilm (interlaced hyphae, compact mycelium, etc.) has been observed through micrographs. The granulation tissue may be the result of a surface growth where the temperature is kept below 35 °C or some toxic by-product produced during the growth of the biofilm (Randhawa et al. 2008). Also, in hospital water distribution systems, growth of fungi in the pipes has been observed, representing a potential source of nosocomial infections (Harding et al. 2009). Biofilms formed by yeasts and filamentous fungi show significant differences. Studies of polymicrobial communities have become increasingly important. Both yeasts and filamentous fungi can adhere to biotic and abiotic surfaces, becoming highly organized communities that are resistant to antimicrobials and environmental conditions, making biofilm formation an important virulence factor in pathogenic fungi (Costa-Orlandi et al. 2017).

Harding et al. (2009) described six phases in the formation of filamentous fungal biofilms: (a) adsorption of propagules; (b) active binding to surfaces; (c) colony formation, where there is growth and colonization, as well as branching of hyphae through the surface as a monolayer and production of extracellular matrix that adheres to the substrate; (d) formation of compacted hyphal networks of mycelium and hypha-hypha adhesion and formation of water channels; (e) maturation and reproductive development, where fruiting bodies, spores, sclerotia, and other survival structures are formed; and (f) spore dispersal or release of biofilm fragments to restart the cycle. In the case of filamentous fungi, the secretion of hydrophobins (small proteins exclusive to this type of fungus) participate in the formation of aerial structures, in the attachment of the hypha to hydrophobic surfaces, and in the formation of more complex structures, as well as in the formation of biofilms (Wessels 1996).

The adhesion and colonization of fungal populations are favored by various factors, such as the surrounding environment, pH, temperature, and osmolarity, among others; the formation of the extracellular matrix greatly favors cell adhesion and biofilm maturation. This matrix provides the cell with protection against hostile factors, such as host immunity and antimicrobial resistance, because it is a mesh of proteins and sugars that forms around microbial cells creating an osmotic pressure that forces biofilms to swell and expand (Wingender and Flemming 2010).

The life cycle of a soil biofilm can be divided into several stages: (i) fixation on solid surfaces (such as minerals and plant roots), (ii) colonization of these surfaces, (iii) development, (iv) maturation, and (v) cell dispersion. Fixation is a physicochemical process, subject to the interaction of surface charge, dissolved ions, and mechanical fluid (Cai et al. 2013). When microorganisms perceive the surface, profound physiological changes occur at the interface that causes colonization (O'Toole and Wong 2016). During biofilm development, the physiology of cells changes compared to free cells, producing a physically and biochemically distinct phenotype. The biofilm is relatively stable as its structure and composition mature. However, with nutrient depletion or external disturbances, internal cells can respond rapidly, releasing enzymes capable of dispersing the biofilm and triggering cell dispersion (Oppenheimer-Shaanan et al. 2013). During biofilm dispersion, cells can detach from the biofilm matrix in active or passive forms. These separate cells can colonize another site and start a new biofilm cycle (Cai et al. 2019).

The biofilms are complex cell populations that present an extracellular matrix that has different phenotypes. In the case of *Candida albicans*, they are mainly composed of yeast-shaped cells and hyphae, and the formation involves adhesion to a substrate (abiotic or mucosal surface), followed by the proliferation of yeast cells on the surface (induction of hyphal formation) where the extracellular matrix accumulates as the biofilm matures and seems to contribute to cohesion and finally the dispersion that corresponds to the release of cells to colonize new sites that can form on numerous abiotic and biotic surfaces (Finkel and Mitchell 2011; Fanning and Mitchell 2012). The degree of adhesion depends on the microbial properties of the host, the surface, and the antibiotics, such as hydrophobicity of the cell surface and the composition of the cell wall (adhesins), as well as the presence of hydrolytic enzymes, including proteases, phospholipases, and hemolysins (Silva et al. 2011).

Biofilms of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis* present three phases of formation: (1) early adhesion phase (0–11 h), (2) intermediate phase (12–30 h), and (3) maturation phase (31–72 h); the cells must bind to the surface or the host cells (Tan et al. 2016). The formation of *Aspergillus fumigatus* biofilms is slower compared to *C. albicans*, since after the conidial seeding, adhesion and germination phase of conidia begins (10 h), and then the hyphae will interlace forming a monolayer (10–16 h), followed by an increase in structural complexity (4–8 h), in which the hydrophobic extracellular matrix (galactomannan, α-1,3-glucans, galactosaminogalactan, monosaccharides, polyols, melanin, and proteins) cohesively binds to the hyphae, increasing the biofilm depth from 10 to 200 μm (Kaur and Singh 2014).

The components of the extracellular matrix vary according to the type of microorganism that forms it, but it is mainly composed of exopolysaccharides, proteins, lipids, extracellular DNA, and hydrolytic enzymes, and the amount of each of the components depends on the environmental conditions (oxygen, nitrogen, temperature, pH, etc.) and age of the biofilm, among others (Gupta et al. 2019).

Several proteins, alone or associated with carbohydrates (glycoproteins), have been identified that are very similar to the proteins (mannoproteins) present in the planktonic supernatant in the fungal extracellular matrix, which plays a fundamental role in the structure and physiology of the nucleus, including virulence, morphogenesis, adherence, antigen presentation, and immunomodulation. It has been suggested that extracellular DNA can improve the structural and architectural integrity of the biofilm, in the exchange of genetic information, the supply of nutrients, and drug resistance (Kaur and Singh 2014). Autolysis is considered a process for release of extracellular DNA in the fungal cells of *Aspergillus nidulans* and *A. fumigatus* (Alcazar-Fuoli et al. 2011).

The cell wall is of great importance in the formation and development of the biofilm, in addition to its importance within the cell structure, since it is the main interface between the internal physiology of the fungus and the external environment; within the polysaccharides that make up the cell wall in fungi are branched and linear β-1,3- and β-1,4-glucan, α-1,3-glucan, chitin, chitosan, galactomannan, and galactosaminogalactan. β-1–3-glucan branched with ß-1–6-glucan forms the skeleton of the wall, and these are covalently bound to chitin and β-1–3/1–4-glucan. In the *A. fumigatus* genome, there are three genes for α-1,3-glucan synthase that are overregulated in biofilms, indicating their importance during adhesion and aggregation of hyphae (Gibbons et al. 2012).

Glucan is one of the main components in filamentous and dimorphic pathogenic fungi (*A. fumigatus*, *A. nidulans*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*), and it has also been reported that galactosaminogalactan (linear heteropolymer of α-1,4-linked galactose and partially deacetylated N-acetylgalactosamine) is part of the pathogenesis by masking β-1,3-glucans from the immune system, adhesion to host cells and other substrates, and resistance to extracellular containment of neutrophils (Gupta et al. 2019). A major component of *C. albicans* biofilms is β-1,3-glucan that has been linked to the protection of biofilms against antifungal agents (Taff et al. 2012).

The production of polysaccharides is important for the formation and complex architecture of biofilms, since, together with other components of the extracellular matrix, they have a key role to play in the different stages of biofilm development. The various ways in which the extracellular matrix exopolysaccharides interact include non-ionic chain-chain interactions that lead to gelation of single or multiple polymers and ionic interactions with charged molecules mediated by charged groups generally present on the exterior of molecular chains exopolysaccharides (Chandrasekaran et al. 1994).

Regardless of the type of organism, whether unicellular or semi-multicellular, they have the ability to coordinate such a complex architecture through their extracellular secretions, which allow them to design their environment according to their needs, forming a specific local environment, which is a key factor of their lifestyle, giving them advantages of adaptive resistance and shielding effect against many forms of environmental aggressions (Gupta et al. 2019), such as attacks by predators and chemical stress such as the host's immune system or antibiotics and disinfectants, information that can be transferred between the components of the biofilm (Cortés et al. 2011).

18.4 Management of Biofilm-Related Fungi

The virulence and pathogenicity of microorganisms are often improved as they grow forming a biofilm; therefore optimizing strategies to control biofilm formation and development is required (Sharp et al. 2006). Biofilms are difficult to counteract since antibiotic resistance increases up to 1000 times above that of the planktonic form (Cortés et al. 2011). Manipulation of individual environmental factors to prevent biofilm formation has had little success because it may not be applicable in all cases. Another form of control is to work on the chemistry of the surface on which the biofilm develops, reducing cell fixation, including the development of a dynamic surface that degrades or rearranges itself in response to temperature and other environmental conditions (Renner and Weibel 2011). The proposed surfaces must have antimicrobial properties and modify the polymer coating to reduce cell adsorption. An example is the use of silver-containing dressings, a strategy that has become frequent in the clinical area to control chronic wounds at risk of infections (Toy and Macera 2011).

Mechanisms of resistance to fungal biofilms include extracellular matrix, efflux pump activity, persistence, cell density, overexpression of drug targets, stress responses, and general cell physiology (Nosanchuk et al. 2012). Therefore, strategies are being sought to increase the efficiency of new treatments against fungal infections, leading to inhibition of biofilm growth, biofilm disruption, or biofilm eradication. Gaylarde and Morton (1999) mentioned that a biocide compound should have the following general properties: (1) effective against problem causing microorganisms; (2) simple and safe to use; (3) no adverse effect on the material to protect/treat; (4) cheap (low cost/benefit ratio); and (5) able to provide protection on the required time scale. These factors could include enzymes, sodium salts, metal nanoparticles, antibiotics, acids, chitosan derivatives, or plant extracts (Sardi et al. 2014). Acetic acid has proved to be an effective agent against *Candida* biofilms. It is an effective procedure for disinfecting systems used in hemodialysis since it also showed effectiveness in inhibiting biofilms. Antifungal and antimicrobial activity of silver nanoparticles has been reported in medicine with a well-tolerated tissue response. Sun et al. (2012a) reported the activity of lipid nanoparticles loaded with terpinen-4-ol against *C. albicans* biofilms; this compound (10 g/mL) has eradicated biofilm formation.

Antibody studies have also been conducted to test their effects on various fungal and bacterial organisms. Martinez et al. (2006a) reported that alpha radiation combined with antibodies effectively impairs the formation of fungal biofilms; in addition, therapeutic treatment of *Cryptococcus neoformans* biofilms (forms biofilms in medical devices) with monoclonal antibody (MAb) therapies and antifungal drug was performed. The binding of antibodies to the cryptococcal capsule may have an effect on the capsular architecture, which may translate into greater penetration of the drug into the cell body (Martinez et al. 2006b). Among the preventive alternatives, they recommend that the administration of a prophylactic dose of biofilm-specific antibodies, immediately after insertion of a medical device, is effective in preventing biofilm formation.

Among the control strategies is photodynamic therapy, widely used for biofilms of *Candida* species. Several authors have used light-emitting diodes (LEDs) with other substances to control diseases (Chen et al. 2012; Ribeiro et al. 2012), since it causes DNA damage and destruction of cell membranes and organelles. Recent studies have shown that the antimicrobial effect can be obtained with the use of photosensitizers belonging to different chemical groups. Because the biofilm matrix is made up of DNA, proteins, and extracellular polysaccharides, recent studies have indicated that disruption of biofilm structure could be accomplished through the degradation of individual biofilm compounds by various enzymes such as DNase, lactonases, amylases, and lyases (Taraszkiewicz et al. 2012).

Antifungal therapies include only four antifungal agents, polyenes, triazoles, echinocandins, and flucytosine (Chowdhary et al. 2017). In addition, resistance has been created, making treatment difficult, and efforts are being made to find antifungal peptides, which are mainly amphipathic molecules that can interact with biological membranes (Rautenbach et al. 2016). Defensins are peptides with antifungal activity; they are organized in an αβ motif, with an α-helix and a triple chain antiparallel β-sheet, which is stabilized by disulfide bonds which ensure high stability, thus retaining its functions in extreme conditions avoiding/decreasing degradation; an example is Psd1, an isolated defense from the seeds of *Pisum sativum*, which has shown promising effects on planktonic cells and biofilm of *C. albicans*, by disaggregating the polysaccharides from the yeast cell wall (increasing cellular roughness and decreasing its stiffness), followed by membrane permeabilization through interaction with the GlcCer defensin (glycosphingolipid). When Psd1 accumulates the cell, it triggers an intracellular mechanism of action by interrupting the cell cycle, which leads to apoptosis (Gonçalves et al. 2017).

Cathelicidin peptides (12–80-amino acid-cationic peptides that adopt an α-helix or β-sheet as secondary structures) were isolated from different mammalian species and exhibit broad-spectrum activities against fungi (Zanetti 2004). LL-37 (human cathelicidin) inhibits the adhesion and aggregation of *C. albicans* (2.2 and 4.5 μM) on biotic and abiotic surfaces by interacting with cell wall components (chitin, glucan, and mannan) of the pathogen (Tsai et al. 2011). Cathelecidins can be used for development of drugs that are used in medical devices to combat fungal biofilm; in addition, it can act synergistically with conventional antifungal agents, which could favor the effect in biofilm-forming fungus treatments. Human salivary histatins (they are histidine-rich proteins consisting of 7–38 amino acids), isolated from parotid saliva, are highly selective antifungals and have little toxicity to mammalian cells. They are alternatives specific to counteract fungi and are therefore which is currently being developed as commercial products (Oshiro et al. 2019).

18.5 Biofilms: Examples

We usually hear about biofilms when we talk about bacterial consortia. For the formation of a biofilm, a liquid-solid medium is needed (Nazar 2007). Examples of these biofilms include a film used to cover a vase with a bunch of flowers, stones from the river, pipe interiors, and dental plaque from the teeth (Thomas and Nakaishi 2006). In industrial processes, we can find biofilms in pipes and valves of large companies (Navia et al. 2010). Other less known biofilms are fungal biofilms. These biofilms have been described in infectious processes such as *Candida*, *Cryptococcus neoformans*, *Cryptococcus laurentii*, and *Aspergillus. Candida* has been reported in biomedical devices (urinary catheters, prosthesis, valves, etc.) placed in hospital patients. However, they can also be found in the middle ear (Del Pozo and Cantón 2015). Cryptococcus is a yeast that causes disease in humans, especially in immune-deficient people. Pigeon feces are the most important source of this infection, and transplanted organs also transmit it. It normally enters the respiratory tract, reaches the lungs, and then spreads to the central nervous system (Martin-Mazuelos and Valverde-Conde). *Aspergillus* is a filamentous fungus that also affects the lungs of different patients (Fortún et al. 2012).

It was through these infectious diseases that the knowledge of fungal biofilms began to emerge. Biofilms have the ability to protect microorganisms that surround or cover them. This is a very relevant characteristic for the industry that uses and seeks processes to preserve and protect different types of materials.

It was with this background that they sought to make coatings from food for human consumption or that were innocuous to health (Arvanitoyannis et al. 1998). It was thus that the use of coatings was used in an advantageous way in the industry, in the case of maintaining and preserving edible plant material for people, without causing damages and having innocuousness. For the formation of these coatings, polysaccharides from fungi have been used. The use of coatings on food is one of the most cost-effective ways to increase its shelf life during storage (Bósquez et al. 2000). The advantages of these coatings are that they reduce the impact to the environment, looking for future biodegradable materials for daily use.

18.6 Efficacy of Biofilms

For public health, biofilms of harmful fungi have shown difficulties and have even caused mortality in some cases (Martín-Mazuelos and Valverde-Conde 2020; Del Pozo and Cantón 2015). This has also caused losses of livestock affected by fungal toxins (Sanmartín and Cano 2018). These biofilms are showing high resistance to common agents used in cleaning (Jay 2005). This resistance could be given by the polymeric matrix which functions as a protective barrier. This quality has shown that biofilm bacteria can be up to 1000 times more resistant to antibiotics (Mah and Toole 2001; Donlan and Costerton 2002). Due to the resistance of biofilms, industrial factories use them to clean or remove biofilms strong chemicals, disinfectants, surfactants and/or extreme heat conditions (Navia et al. 2010).

However, in the case of coatings found to be beneficial to the industry, they have been shown to protect against bacteria and food dehydration. In the case of foods, each of the coatings is evaluated for each specific food (Bósquez et al. 2000). The effectiveness of these coatings has been used several years ago, starting with the immersion of oranges and limes in wax in China to delay the loss of water (Greener and Fennema 1994). These coatings have presented many advantages over food, seeking to ensure that they do not affect humans and that their environmental impact is minimal. Food coatings have been so used and their efficiency has been proven that industries apply some of these processes. In the search for more materials to make coatings, fungi have begun to be explored to make coatings.

18.7 Resistance of Biofilms

Exopolysaccharides or biofilms are currently defined as complex, highly structured microbial communities. These microorganisms can be represented by fungi or bacteria and are usually associated with solid surfaces, inert materials, and even living tissues of susceptible hosts. Thus, the production of biofilm consists of the synthesis of a protective extracellular matrix, which keeps these organisms protected (Ramage et al. 2009). In fungi, the formation of biofilms is not considered just a hypoxic aggregate of yeast or mycelial cells but may present communities with complex phenotypes. In addition, the extracellular matrix produced shows resistance to many antimicrobial agents (Desai et al. 2014). Fungi present inside the biofilm are responsible for the synthesis of the extracellular matrix and manifest different phenotypes between the cells that are in suspension of the planktonic cells (Desai et al. 2014).

After the formation of the biofilm, the microorganism can present advantages, such as protection to the environment, metabolic cooperation, resistance to chemical and physical stresses, persistence during the infectious process in patients, and even assist in the homeostatic processes of the cell regulating gene expression. Usually, the public affected by biofilm-producing fungi are immunosuppressed patients (e.g., HIV carriers), newly transplanted patients, patients undergoing chemotherapy treatment, newborns, and individuals in intensive care (Calderone and Gow 2002; Ramage et al. 2006, 2009).

Fungal biofilms are essential factors for fungal development during the progress of clinical infection. As a result, several fungi of medical importance have properties to produce biofilms. These can be represented by fungi of the genera *Trichosporon*, *Coccidioides*, *Aspergillus*, *Candida*, *Pneumocystis*, *Malassezia*, and *Cryptococcus* (Anne and Frank-Michael 2009; Cushion et al. 2009; Davis et al. 2002; Di Bonaventura et al. 2006; Finkel and Mitchell 2011; Martinez and Casadevall 2007). One of the main factors involved in the resistance mechanisms of fungal biofilms is resistance to antifungal drugs and the host's immune system.

18.8 Mechanisms of Drug Biofilm Resistance

Currently, a growing problem in immune-compromised individuals has been the resistance of biofilms to antifungal drugs. This resistance has resulted in a significant increase in the incidence of systemic and opportunistic mycoses. In most cases, the clinical resistance of biofilms occurs through the persistence and progression of infections caused by certain fungi, despite adequate drug therapy.

The resistance can be induced in response to antifungal compounds, or by means of irreversible genetic changes, resulting from long-term exposure of the fungus to drugs. Thus, morphological changes, overexpression of target molecules, limited diffusion of compounds, active extrusion through the efflux pump system, and even tolerance and cell density to drugs may be involved. All of these mechanisms mentioned above can act as resistance mechanisms used by the fungus to combat the effects of antifungal treatment (Niimi et al. 2010; Ramage et al. 2012). To understand how biofilm resistance mechanisms work in fungi, we will use *Candida albicans* as an example, since this fungus can be used as a common model among the diversity of fungi that produce resistance exopolysaccharides (Fig. 18.1).

Regarding the resistance of this complex, it can be divided into two ways: primary resistance, where the microorganism is resistant to the drug before exposure to the antifungal, and secondary resistance, where the organism develops in response to exposure to the drug (Jabra-Rizk et al. 2004; White et al. 2002). Therefore, this biofilm resistance mechanism is responsible for antifungal resistance. In fungi, multidrug efflux pumps are genes that are directly related to drug resistance. These,

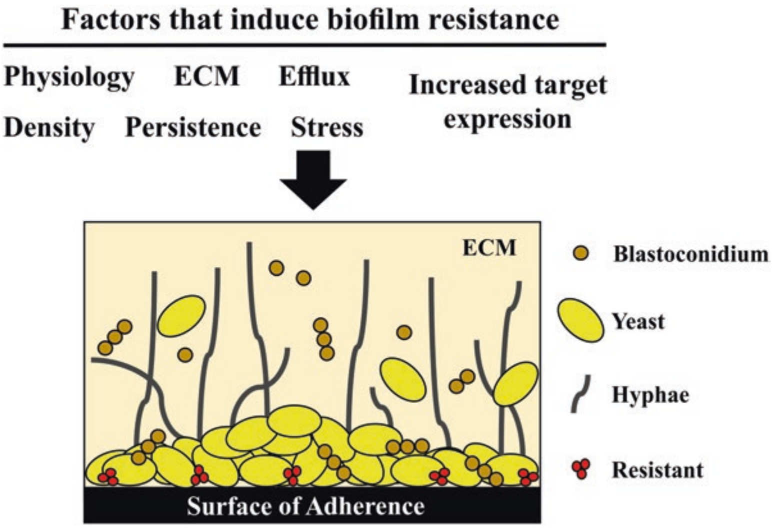

Fig. 18.1 General scheme of the resistance mechanisms of *C. albicans* biofilms against antifungals. In the top are listed the main factors that influence the formation of biofilms (Adapted from Costa-Orlandi et al. (2017))

when positively regulated, can result in biofilms' multi-resistance to drugs (Chandra et al. 2001; Douglas 2002; Harry et al. 2002; Jabra-Rizk et al. 2004; Ramage et al. 2002; White et al. 2002). In some *Candida* species, the presence of carrier molecules such as ABC transporters (adenosine triphosphate-binding cassette) that are encoded from genes such as CDRs and other transporters encoded by MDR (multidrug resistance) genes has already been described (Douglas 2002; Jabra-Rizk et al. 2004; Moran et al. 1998; White et al. 2002; Wirsching et al. 2001). These genes have been shown to be expressed throughout the formation and development of biofilms and during resistance to fluconazole (Chandra et al. 2001; Douglas 2002; Jabra-Rizk et al. 2004; Ramage et al. 2002).

In studies, tests were carried out to verify the resistance potential of biofilms. Initially, the penetration capacity of drugs in biofilms grown in vitro was measured. Thus, it was demonstrated that the biofilms of *C. albicans* inhibited the penetration of drugs such as fluconazole, flucytosine, and amphotericin B. The penetration of amphotericin B was also inhibited by biofilms from *Candida krusei* and *Candida parapsilosis*. In *C. albicans*, the biofilm was susceptible to the drugs flucytosine and fluconazole. This suggests that exopolysaccharides help in the biofilm antifungal resistance, limiting the penetration of drugs in the same (Samaranayake et al. 2005).

In studies focused on the resistance caused by biofilms, *C. albicans* demonstrated a phenotype of resistance to amphotericin B, when subjected to conditions of iron ion or glucose deprivation. Subsequently, when *C. albicans* was subjected to anaerobic conditions, biofilms demonstrated resistance to high levels of amphotericin B and other azole antifungals (Bachmann et al. 2002). It has also been demonstrated that factors such as temperature, environmental stressors, availability of oxygen, and hydrogen potential lead to changes in biofilm architecture and sensitivity to antifungal drugs (Kucharíková et al. 2011; Pettit et al. 2010).

In other analyzes, tests were carried out to isolate biofilms from *Candida* sp. for resistance assessments. Thus, it was observed that the biofilm was resistant to the antifungals used, even in the absence of planktonic cells (Nett et al. 2007). Subsequently, they radiolabeled the antifungal fluconazole to trace this compound in the biofilm, where it was observed that a large part of the antifungal was found associated with the polysaccharide matrix, implying the sequestration of the drug. Then, the extracellular matrix was challenged using enzymes that cause the degradation of β-1,3-glucan, which resulted in the susceptibility of biofilm resistance, indicating that this component present in the polysaccharide matrix is related to drug resistance mechanisms (Nett et al. 2007).

18.9 Mechanisms of Biofilm Resistance to the Immune System

The mechanism of action of the cells of the immune system begins when it encounters the pathogen, presenting as a direct response to infection they control the recruitment of phagocytes, extracellular neutrophil trap (NET), recruitment of phagocytes, production of reactive oxygen species, production (Chaffin et al. 1998). It has been shown that in *C. albicans*, recruitment of primary leukocytes to the site of infection is caused by the formation of biofilm (Dongari-Bagtzoglou et al. 2009; Nett 2016; Nieminen et al. 2014). This cellular recruitment has already been observed in several clinical biofilms, during oral and vaginal candidiasis, in catheters, and even in animal models during experimentation (Dongari-Bagtzoglou et al. 2009; Fidel et al. 2004; Nett et al. 2015). Despite this recruitment, these immune system cells are unable to eradicate the biofilms formed by *Candida* sp., as demonstrated in studies that were carried out using human neutrophils. In those studies, it was observed that *C. albicans* biofilms are able to resist attacks by neutrophils (Katragkou et al. 2010; Xie et al. 2012).

Generally, neutrophils release NETs, as one of the ways to fight *Candida* sp. The release of these structures leads to the fight against large fungal structures that are not usually phagocytosed, such as hyphae (Kernien et al. 2018; Urban et al. 2006). However, it has been shown that neutrophils do not release NETs during contact with the biofilms produced by *Candida* (Johnson et al. 2016). In addition, *Candida* biofilms support monocyte attacks, showing that these cells are unable to phagocytize the fungus when it is associated with the biofilm (Chandra et al. 2007). This encounter between monocytes and biofilm triggers the production and release of cytokines. However, it has already been shown that when in contact with the biofilm, the regulation of TNF-α becomes negative, this cytokine being extremely important for the phagocyte activation processes (Katragkou et al. 2010). The exposure of macrophages to the biofilm, it was shown that the complex reduced macrophage the recognition of planktonic organisms (Alonso et al. 2017).

In other fungi, for example, *Aspergillus fumigatus*, the neutrophils are important during the pathogen-host interaction process, being recruited for fungal conidial phagocytosis (Behnsen et al. 2007; Bonnett et al. 2006). Neutrophils also have properties for releasing NETs, in response to the mycelium form of *Aspergillus*. However, NETs are active against hyphae, but are not effective against *Aspergillus* conidia. In addition, biofilm production protects *A. fumigatus* from neutrophil attack (Kernien et al. 2018; McCormick et al. 2010). The β-glucans present in the hyphae cell wall and in *Aspergillus* biofilms play an important role. They are able to mask recognition by dendritic cells in in vitro assays (Gravelat et al. 2013).

Another important factor is galactomannans and galactosamines (GAG), which impair neutrophil cell recruitment during the development of biofilm. These molecules are able to modulate immunity by inducing apoptosis in neutrophils (Robinet et al. 2014). The conidia of *Aspergillus* sp. have a protein in their structure, called RodA. This protein is a hydrophobin that is present on the cell wall surface of these propagules present in the biofilm and has the activity of masking PAMPs, thus preventing attacks from the immune system. In addition, it can result in the production of NET, as previously mentioned (Bruns et al. 2010; de Jesus Carrion et al. 2013).

Currently, the pathogen-host interaction between humans and the biofilms produced by *Cryptococcus* sp. remains a great mystery. The production of the mucopolysaccharide capsule (GMX) in *Cryptococcus* is one of the main virulence and defense factors of the host's immune system (Kernien et al. 2018). The GMX is evidenced as the main responsible for the inhibition of neutrophilic functions, such as phagocytosis, NET production, chemotaxis, and even antifungal activity (Dong and Murphy 1995; Rocha et al. 2015; Vecchiarelli 2000). Another important factor during the pathogen-host interaction is the production of GMX by *Cryptococcus* sp. The inhibitions of the phagocytosis process are mediated by macrophages and monocytes (Vecchiarelli 2000).

18.10 Pathogenicity of Fungal Biofilms

Fungal biofilms can be present on surfaces of clinical materials or involved in the infectious process, positively or negatively, impacting human health. During the synthesis of a biofilm, fungi can adhere to these inanimate surfaces, such as prostheses, catheters, and surgical materials. Adherence to these surfaces facilitates the infection process, since during the contact of these materials with the patient, they facilitate the access of yeasts to the bloodstream, with the possibility of the fungus spreading in the body and reaching various internal organs of the affected individual. It is worth noting that the spread of fungi in the body has high mortality rates (Verstrepen and Klis 2006). Thus, there is a wide variety of pathogenic fungi that can synthesize biofilms, such as *Cryptococcus*, *Trichosporon*, *Malassezia*, *Fusarium*, *Aspergillus*, and *Candida* among others.

Lately, infections caused by *Cryptococcus laurentii* have been described in immune-compromised patients (Kordossis et al. 1998). Colonizations of the oropharynx with repeated recurrences in a patient with erythroleukemia and other infections such as keratitis, meningitis, and even fungemia have already been reported when the involvement is by fungi of the genus *Cryptococcus* (Bauters et al. 2002; Khawcharoenporn et al. 2006; Shankar et al. 2006). Infection usually occurs via the respiratory tract, through inhalation of yeasts or nosocomial, and is most often associated with neutropenia in patients and intravascular catheters (Johnson et al. 1998).

The excessive growth of yeasts of the genus *Malassezia* can cause seborrheic dermatitis (SD). *Malassezia pachydermatis* is one of the representatives of SD; although this fungus is a commensal in the skin of dogs, it can affect humans (Velegraki et al. 2015). SD is a type of dermatitis that works by compromising the immune system. The biofilms of this fungus are formed together with communities of microbial cells that have the ability to strongly attach to material surfaces (Bhatt et al. 2015; Macé et al. 2008). The formation of biofilm during the chronic phase of the infection has been evidenced in several models of infection. Otitis media, endocarditis, and even periodontitis have been reported (Donlan and Costerton 2002). In addition, there are reports of invasive infections by *Malassezia* spp. in neonates who received intravenous lipid supplementation. Other cases report infection through the process of parenteral nutrition with the use of catheters (Amend 2014).

Fungi of the genus *Trichosporon* are ubiquitous in nature. They can be found in water, soil decomposing materials, and feces of bats and birds (Colombo et al. 2011). It has been reported that fungi of the genus *Trichosporon* is the third most isolated species in clinical laboratories, representing between 5.5% and 10.6% of all isolates obtained (Pfaller et al. 2007). In addition, this fungus has already been associated with hypersensitivity pneumonitis and white Piedra syndrome (Ando et al. 1995; Colombo et al. 2011; Kiken et al. 2006). This fungus has also been associated with systemic infections, affecting immune-compromised patients, as well as patients with central venous catheter (Girmenia et al. 2005; Suzuki et al. 2010). However, there are still few studies addressing the central themes of the pathogenicity of *Trichosporon* spp., including the production of biofilm (Fonseca et al. 2009; Karashima et al. 2002; Sun et al. 2012b).

Lately, the incidence of keratitis caused by *Fusarium* sp. has been reported. This mainly affects rural workers, during harvesting seasons. One of the most affected points is the eyes due to corneal exposure being susceptible to contact with airborne spores (Bharathi et al. 2007; Bharathi et al. 2003; Xie et al. 2006). Keratitis has also been reported caused by *Fusarium* in contaminated contact lenses, lens cleaning solutions, and case to store the same. As a result, biofilms have already been isolated from contact lenses and may play an important role in inflammation of the corneas (Imamura et al. 2008). Approximately 300 reported cases of keratitis caused by *Fusarium* were associated with contact lenses. Many patients have undergone keratoplasty, and some have even undergone removal of the entire eye. This type of involvement is often due to wrong diagnosis or inadequate treatments due to failures in the use of antifungals for treatment (Chang et al. 2006; Donnio et al. 2007).

In studies carried out on *A. fumigatus*, it has already been shown that when its conidia are inhaled by immunocompetent (depending on the patient's immune status) or immunosuppressed people, it can cause aspergilloma in the pre-existing lung cavities and in chronically obstructed paranasal sinuses. These factors are eliminated by pulmonary macrophages and PMNs (Latgé 2001). Cases of asthma and allergic rhinitis, bronchopulmonary aspergillosis, chronic necrotizing pneumonia, and even hypersensitivity pneumonitis have also been reported (Kaur and Singh 2014; Latgé 2001; Müller et al. 2011; Williams et al. 2016). Thus, analyses have shown the production of biofilm during aspergilloma, caused by *Aspergillus* sp. (Müller et al. 2011).

Biofilms formed by *Candida* species can cause both superficial and systemic fungal infections in individuals with compromised immune systems (Sims et al. 2005). As a pathogenetic mechanism, *Candida* can resist antifungal drugs and promote biofilm formation and the expression of virulence factors. In fact, the infections that occur in the mucous membranes of affected patients, for the most part, cause the formation of biofilm, which can lead to changes in the commensal bacterial flora and the components present in the mucous membranes of the host (Dongari-Bagtzoglou et al. 2009; Ganguly and Mitchell 2011). As a result, nosocomial infections have already been identified where *Candida* isolates have been found in dental prostheses, defibrillators, cardiac devices, and catheters (Elving et al. 2002; Kojic and Darouiche 2004). Urinary tract infections, caused by *Candida tropicalis*, and involvement of the skin of healthy hosts by *Candida parapsilosis* have been reported, with the main causative agent being catheters containing *Candida* biofilms (Bonassoli et al. 2005; Rho et al. 2004).

18.11 Biotechnological Applications of Biofilms

18.11.1 Agricultural Applications

In recent years, biofilms have generated interest in the agricultural sector because of their potential for crop improvement. These biofilms colonize the soil surface, roots, and plant shoots, allowing better growth and adaptability to the environment (Velmourougane et al. 2017). Thus, it has been observed that biofilms have different applications in agriculture. The availability of nutrients in the soil is important in plant growth, since on this depends that the plants can metabolize them and have a proper development. In this sense, the use of biofertilizers with biofilm formation has been studied, which have demonstrated a greater release of nutrients and organic acids into the soil and growth hormone production, compared with crops without biofilm formation (Seneviratne et al. 2011).

The use of biofilms in bioremediation is a cost-effective and attractive method for the degradation of various hazardous contaminants such as hydrocarbons, heavy metals, toxic minerals, and drugs, among others (Edwards and Kjellerup 2013). In bioremediation the formation of biofilms plays an important role due to their capacity to absorb cations, which reduces their migration into the environment (Farber et al. 2020). In this way, it has been reported that some fungi and yeasts have the capacity to form biofilms and can be used to reduce contamination in places with dangerous pollutants (Nilanjana et al. 2012; Tkavc et al. 2017).

On the other hand, a positive effect on the growth of ornamental plants has also been seen when they are inoculated with microbial biofilms. Although there is a better response in growth when the biofilm is mainly composed of bacteria, in a bacteria-fungus combination, we also see a greater growth and yield of the plant, compared to those that were not inoculated (Kanchan et al. 2019). This is why the use of biofilms in plant cultivation is very important, because they can produce different polysaccharides and bioactive compounds. In addition, not only does plant growth benefit, but there is also an improvement in soil quality throught an increase in the nutrient cycle (Pandit et al. 2020).

18.11.2 Potential Applications for Health

Fungi can adhere to various items used in medicine such as prostheses and catheters. This is of concern, since these fungi can travel through the bloodstream and generate fungal infections in internal organs (Desai et al. 2014). Different infections caused by biofilm-forming fungi have been reported such as histoplasmosis caused by *Histoplasma capsulatum*, dermatophytosis caused by *Trichophyton rubrum* and *T. mentagrophytes*, nosocomial infections caused by *Aspergillus* spp., and candidiasis caused by *Candida* spp., among others (Costa-Orlandi et al. 2017). Therefore, fungal biofilms have become a clinical and economic problem. This is because when a patient becomes infected, it is recommended to administer antimicrobials and remove the contaminated device; however, these procedures are costly, and medical complications may occur (Orlandi et al. 2014). In addition, several cases of resistance to multiple antifungal agents have been reported (Sherry et al. 2017).

Because of this, some researchers have been creating medical devices based on biopolymers that inhibit the development of fungal biofilms (Jäger et al. 2015). Another problem is the formation of biofilms in the food industry. It has been observed that different microorganisms, including fungi, can generate biofilms in food, causing gastrointestinal poisoning and infections. This is why companies use chemical products or physical methods for the prevention or elimination of biofilm formation in pipes and factory surfaces (Galie et al. 2018). Without a doubt, the impact that these microorganisms have on human health is alarming and must be taken into consideration for future studies.

18.11.3 Potential Applications for Livestock and Health Public

Before knowing the applications of these biofilms, we must know where we can find them. In the case of livestock, we can find this type of biofilm in ponds stored with water from the livestock. Also, we can also observe these in pumps, walls, and roofs. The best-known fungi in livestock farming are of the mycotoxin-producing genera that are *Aspergillus*, *Penicillium*, and *Fusarium*. These types of mycotoxins form or are sometimes present in biofilms of fungi. Mycotoxins present in a feed can be found associated with polysaccharides or proteins. These affect agricultures and, when processed and consumed by livestock, the health of the livestock (Bueno and Vero 2018).

One of the examples observed in livestock damage is that in contaminated corn, pigs are the most sensitive to mycotoxins. Clinical signs presented by infected animals are estrogen syndrome, vomiting, bleeding, lethargy, ataxia, reproductive disorders, pulmonary edema, and liver damage (Sanmartín and Cano 2018). Mycotoxins are absorbed in the gastrointestinal tract, distributed, and affect enzymatic processes. Aflatoxins can be found in milk for human consumption for this reason this value is regulated by the Food and Drug Administration (FDA), to have minimum values in consumption and not affect human health (García and Diaz 2018). In the case of public health, it is well-known that what is least desired is biofilms of pathogenic fungi, mainly in medical patient instruments that affect and deteriorate the patient.

18.12 Conclusion and Future Prospects

Biofilms are produced in a niche by fungal characterizations to confront stress in the environment. The fungal biofilms are helpful for assemblies on the host surface or inside such as plant, animal, and human, bound by a polysaccharide matrix. The formation of fungal biofilms plays a significant role in many fields like agriculture and livestock and in humans. Many fungi showed potential to produce biofilms in a niche. The production and formation of biofilms on the surface for adapting are most important for growth on the surface, growth of apical hyphae, absorbent of nutrient, and secretion of extracellular enzymes. Biofilms are also found to be essential for fungal life cycle such as the formation of a colony, hyphal networks of mycelium, and fruit bodies, spores dispersal, maturation, and reproductive development. They are also associated with increase or decrease in pathogenicity and resistance or sensitivity to drug and fungicides. Therefore, biofilms have been considered a big challenge for researchers, as strategies for the control of their production should be in place with their potential utilization in several fields.

Biofilms can be used in agriculture such as production, reduction in virulence of fungal pathogens, improving the fungicides, improvement the ability of useful fungi that using as biocontrol agents. In the livestock sector and the clinical area, is an essential for producing the antifungal possibility as drug and don't appear resistance by fungi for this drug. More information about biofilms is necessary to prevent the occurrence of various fungal strains that cause many diseases in plants, livestock, and humans. Finally, biotechnology tools play a vital role in determining the structures and compounds of fungal biofilms. However, hand, it can utilize to increase the efficacy of useful fungi to be more aggressive through using as a biocontrol agent against several pathogens. This process is potentially being more useful for saving the ecosystem from residue in synthetic fungicides and the chemical drugs, as well as, the occurrence of the resistance varieties in the strains of fungal pathogens.

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Chapter 19
Virulence Traits of *Candida* spp.: An Overview

Zeeshan Fatima, Priyanka Kumari, Suriya Rehman, and Saif Hameed

Contents

19.1 Introduction

Candida spp. are the eukaryotic diploid yeasts, belonging to class Deuteromycetes of kingdom fungi. *Candida* spp. are generally asymptomatic, and can occur in 80% of the healthy individuals. *Candida* is the most common agent which causes the opportunistic diseases worldwide. It is a frequent colonizer of human skin and mucosal membranes. It is naturally found on the skin, mouth, vagina and stool in a non-pathogenic form. The genus *Candida* includes around 200 species among them only 20 species are responsible for causing diseases in humans. Some of these species are most frequently isolated in humanlike *C. auris, C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis*, and *C. lusitaniae. Candida albicans* are

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dimorphic and opportunistic pathogen for human. *C. albicans* are commonly found in the gastrointestinal, genitourinary tracts, in the oral cavity. *Candida auris* was first isolated in Japan from the ear of the patients (Blandin et al. 2000).

Candida auris are the global threat to the world health. *Candida auris* is multidrug-resistant in nature that it is resistance to all the three antifungal classes. Its infection is more fatal than the other *Candida* spp. It is mainly common in the blood infections, wound infection, and ear infection, and also in respiratory and urine specimen. It is commonly found in patients who are in ICU. *Candida tropicalis* is among the most virulent species of *Candida.* It is also resistant to the antifungal like azoles, polyenes, and echinocandins. This *Candida* strain was first isolated from bronchitis in 1910 and is a diploid, asexual yeast (Zuza-Alves et al. 2017). *Candida glabrata* infection is generally found in the urinary tract (sometimes infecting the urinary bladder and also the kidney), genitals, mouth, and if it gets severe then it also infects the bloodstream. *Candida glabrata* has resistance to the fluconazole (azole derivatives), amphotericin B and flucytosine. *Candida krusei* is another multidrug-resistant fungal pathogen resistant to fluconazole and amphotericin B (Pfaller et al. 2008). *Candida parapsilosis* is the most commonly isolated *Candida* species from the bloodstream. It causes its infection on human skin, and it is often harmless (Trofa et al. 2008). *Candida lusitaniae* is a rare opportunistic yeast resistant to amphotericin B and causes genitourinary candidiasis. (Wawrysiuk et al. 2018). This *Candida* spp. may be present in the normal gastrointestinal and genital flora of healthy humans, while *C. famata* and *C. guillermondii* are skin commensals.

The infections caused by *Candida* spp. are referred as candidiasis. *Candida* can cause infection when it grows out of control and went deep into the body like bloodstream, or the internal organ like kidney, heart, or brain, a condition known as candedemia. They have the ability to cause various superficial and systemic infections, when the host's resistance to infections is compromised. The most frequent are superficial candidiasis, including cutaneous, oropharyngeal candidiasis, and vulvovaginitis. These infections are frequent and usually benign in immunocompetent hosts. *Candida* can also cause life-threatening infections, mainly in a hospitalized patient. The methods of identification of *Candida* spp. are phenotypic methods, specialized instruments-based methods, biotyping, molecular identification methods and non-invasive diagnostic tools. In phenotypic methods of identification, the *Candida* spp. are identified on the basis of the germ tube formation, chlamydospore formation, growth temperature, carbon and nitrogen assimilation, and some other instrumental methods that help in the phenotypic identification of *Candida.* Another category of identification is specialized instruments-based methods of the *Candida* spp., microbial identification system in which we use the principle of gas chromatography. Molecular identification methods have high accuracy rate, sensitivity and specificity for identification and differentiation between two similar *Candida* spp. In these methods, identification is done by the PCR and non-PCR methods. Multiplex PCR, nested PCR and real-Time PCR are commonly used for the identification. Peptide nucleic acid fluorescence in situ hybridization and MALDI-TOS mass spectroscopy are also used for the identification of the *Candida* spp. (Neppelenbroek et al. 2013).

19.2 Exploration of *Candida* spp.

There are so many different *Candida* species and every species have their own gene sequence, phenotypic sequence, structure and their environmental condition.

(i) *Characteristics of the sequences:* It has guanine-cytosine nucleotide content in the nuclear sequences. It is large counting a ribosomal DNA, and some of the sequences are carrying the long terminal repeat elements (LTRs).

(ii) *Nuclear ribosomal DNA:* The DNA sequences of the *Candida* spp. are similar to the *Saccharomyces cerevisiae* DNA sequences. For example, the repeated unit of *Candida* spp. is more longer than that of *S. cerevisiae*. Its structure is different because the sequence of the ribosomal RNA seems to be repeated. But some of the certain genes of *Candida* spp. is 90–96% identical with *S. cerevisiae* genes as observed in the phylogenetic trees.

(iii) *tRNA genes*: *S. cerevisiae* tRNA genes are comparable to *Candida* spp. *tRNA* genes with its 42 number of families. Some of the families of *S. cerevisiae* are completely or partially identical with *Candida* tRNA genes. Since *S. cerevisiae* is homologous, any changes in the genetic codons are synonym with each other.

(iv) *Transposable elements: Candida* spp. transposable elements are compared by the translational products of the RSTs genes of the *S. cerevisiae* retransposons. Their mechanisms are similar too.

(v) *Mitochondrial DNA:* The mitochondrial DNA sequence of *S. cerevisiae* and its translated products of four other fungi: *Allomyces macrognus, Pichia canadensis, Podospora anserina* and *Schizosaccharomyces pombe* compared with the *Candida* spp. For example, *Candida tropicalis* has 87 RSTs, they are similar to mitochondrial genes encoding 21S rRNA, 15S rRNA, tRNAglu, cytochrome oxidase subunits 1, 2 and 3, apocytochrome b, NADH dehydrogenase subunits 1, 2 and 4 and ATPase subunits 6, 8 and 9.

(vi) *Comparison with the proteome of S. cerevisiae: identification of Candida spp. protein-coding genes, conformation of genetic code and determination of codon usage:* for this, they have to search the identical genes which encode the proteins in *Candida tropicalis* and enforced against the translated proteins of *S. cerevisiae*. The CUG codon of the *Candida tropicals* is translated and it encodes the serine not leucine. These translational codons change the low occurrence of the CUG codon and reduce the difference on the genes. All the genes are compared by the bioinformatical processes like *blastx*. The ORF product of *S. cerevisiae* is aligned between the RSTs product of *Candida tropicalis* by the blastx is 47.05%.

19.3 Multidrug Resistance and Problems for Healthcare

Multidrug resistance (MDR) is the common property of the *Candida* spp. MDR is referred as the resistance to more than one antifungal agent. MDR is developed in the person who is immuno-compromised, like HIV, diabetic patients, organ transplantation patients, and the person who is staying in the hospital for 15 to 30 days. MDR is the phenomenon of hospital-acquired infections (Tanwar et al. 2014). MDR is a serious global threat and demands immediate attention as it continues to be significant obstacle for healthcare in providing effective and complete medicinal treatment. This is evident by the fact that there is alarmingly high number of antibiotic-resistant species, which includes most commonly used antibiotics like azole-resistant fungi causing candidiasis. Another reason could be attributed by limited arsenal of antifungal drugs in comparison to antibacterial drugs. Moreover, the recent upswing in number of 'superbugs' is also the repercussions of MDR only.

The evolution of drug resistance in pathogens poses grave concern for medical and pharmaceutical world because of the limited number of clinically useful antimicrobial drugs available in the market. MDR leads to high death rates, increased medical costs and has a significant impact on the effectiveness of antimicrobial drugs. Moreover, marked variations in the resistance profiles of fungal pathogens, as well as the quality of public hygiene also have a considerable impact on the effectiveness of drugs. Furthermore, the process of horizontal gene transfer has made the matter from bad to worse where some resistant pathogens are able to transfer copies of DNA that codes for a mechanism of resistance to other nearby species of microorganism, thereby conferring resistance to their neighbours, which then are also able to pass on the resistant gene.

Today there is a urgent need for completely dissecting drug resistance mechanisms, as rapid increase of severe infections and the spread of resistant microorganisms are indisputable facts. In addition, the emergence of MDR has illustrated the need for regular monitoring and continuous surveillance of resistance profiles of clinical isolates. MDR is a complex process involving various known and unknown mechanisms. Improved knowledge of such unknown molecular mechanisms controlling MDR will surely facilitate the development of novel therapies to combat these infections and will help in deeper understanding of the pathogenesis of microbial organisms. The recent upswing in cases of hospital-acquired infections combined with rise of MDR strains demands novel approaches. Moreover, implementation of strict infection control policies in healthcare settings is required to mitigate the progression of antimicrobial resistance. For instance, selecting the antibiotic targeting a particular infection should be preferred over broad-spectrum antibiotics. Moreover, full course of antibiotics should be completed without stopping even after symptoms disappear. This might in turn will facilitate the development of better antimicrobial strategies to efficiently control the human infectious diseases which are claiming many lives each year.

19.4 Virulence Factors

A promising approach to antifungal drug development is to target pathogen-specific virulence factors. Comprehending the infection biology of *Candida* spp. is pertinent in recognizing new drug targets. Virulence factors are basically the toxins produced by the organisms. Virulence factors in the *Candida* spp. are responsible for binding of the organisms to the host cells. Degradation of the enzymes is shown to promote the virulence. Virulence factors that promote fungal colonization to the host includes the ability to adhere to host cells and resist physical removal, invade host cells, compete for nutrients, resist innate immune defences such as phagocytosis and evading adaptive immune defences (Deorukhkar et al. 2014). There are different virulence factors of *Candida* spp. which helps it in biofilm formation, hyphal formation, cell adhesins, phenotypic switching, morphogenesis, quorum sensing, haemolysin production, and exoenzymatic activity (Deorukhkar et al. 2014). Inhibition of virulence factors is advantageous over other strategies because it collates various potential targets required for drug discovery and minimizes development of MDR (Höfs et al. 2016). The virulence traits are regulated at both molecular and metabolic levels. Comprehensive understanding of the role of the various *C. albicans* virulence traits is still rudimentary; therefore, further studies are required to fully understand pathogenesis of this opportunistic pathogen. The subsequent sections summarize an overview of different virulence traits.

19.4.1 Biofilm Formation

Biofilms are formed by the *Candida* spp. that cause very superficial and systematic infections in the immunocompromised patients (Marak and Dhanashree 2018). A majority of the diseases caused by various *Candida* spp. are via formation of biofilms. Biofilms are universal, complex, interdependent communities of surface-associated microorganisms, enclosed in an exopolysaccharide matrix on the host cell surface. It is an important virulence factor for recognition of the candidiasis. Biofilm is highly structured and coordinated, microbial shields that colonize the surface of the host cell thereby increasing the fatality risk (Reginatto et al. 2020). The biofilms are irreversibly attached to a given surface, inert material, or living tissue, producing extracellular polymers that provide a structural matrix (Cavalheiro and Teixeira 2018). The biofilms exhibit the lower growth rates and also higher resistance to the antifungal agents like fluconazole, nystatin, amphotericin B, and chlorhexidine (Chandra et al. 2001). Biofilm formation varies depending on the particular *Candida* spp. The characteristics of these species are resistance to antifungal drugs, expression of virulent factors (shows pathogenicity) and ability for the formation of biofilms (Pierce et al. 2015). Because of these characteristics, it becomes difficult to treat such infections (Yigit et al. 2011). Biofilm formation is the multifaceted process, earlier phase is the adherence phase, then an intermediate

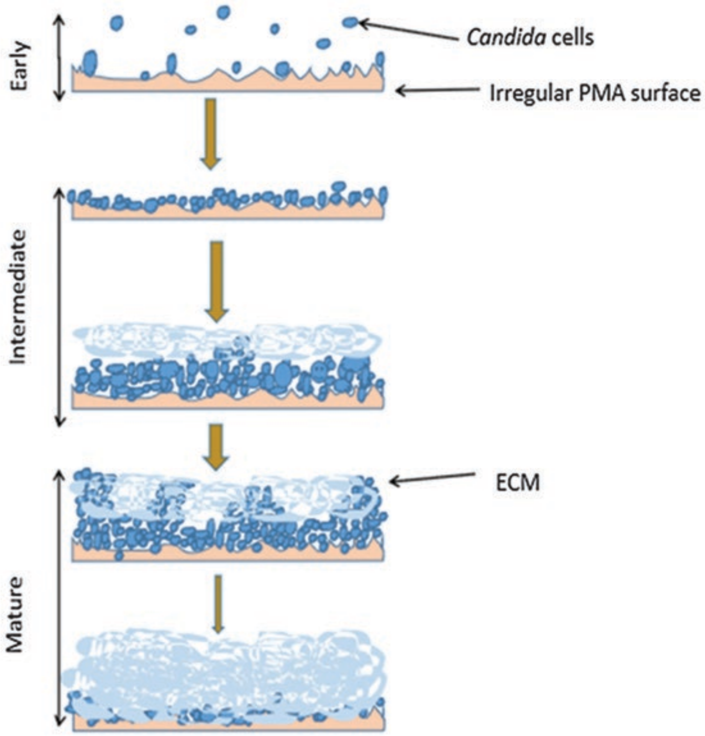

Fig. 19.1 Steps of biofilm formation of *Candida* spp.

phase which is followed by maturation phase and migration (Fig. 19.1). In the early phase of biofilm formation, the yeast cells adhere to the surface of the host cell and form the discrete colony. In the intermediate phase, the cells are getting organized and start producing extracellular polymeric substances.

The maturation phase consists of the components involved in the maturation of the 3-D structure. Once the biofilm gets matured, the cells become detached and start migrating to the other niches to form expanded biofilms (Cavalheiro and Teixeira 2018). Biofilm formation differs from species to species, depending upon the type of species, the surface, host niche and other factors (Marak and Dhanashree 2018). *Candida* biofilms mostly occur in the mucosa and endothelial lining. There are some effective measures taken for the management of clinical biofilm. These include: (i) the production of material with the antimicrobial texture, which interferes with the way microorganism interact with the surface and this technique is known as antimicrobial lock therapy (ALT), (ii) surface coating with combination of anti-adhesive and antimicrobial properties, by which the surface becomes resistant to adhere the cell surface. These techniques are using for the reduction of the cell adhesion property of the *Candida* species. These strategies are for the antifungal polymers that form barrier films. This is a highly effective strategy for the management of biofilm production (Reginatto et al. 2020). Biofilm production is done 57.16% by the non-*Candida albicans* species and 39.02% by *Candida albicans*. The different *Candida* spp. contribute to the production of biofilm: 100% of the *Candida parapislosis*, 61.53% of the *Candida tropicalis*, and 55.55% of the *Candida krusei*. Fluconazole is sensitive to 75.55% strains and resistant to the 24.44% strains among the *Candida* spp. Among the *Candida* spp., 40.90% of *Candida albicans,* 36.36% of *Candida tropicalis*, 22.72% of *Candida krusei*, 18.88% of the other *Candida* spp. 34% and 14% resistance rates of fluconazole and voriconazole among *C. albicans* (Marak and Dhanashree 2018).

19.4.2 Cell Adherence

Cell adherence is the first step for the formation of biofilms. Adhesins are biomolecules which aid in recognition of host cells (Lipke 2018). Adhesin is responsible for the adherence of the yeast cell to the surface of the host cell which takes place to form a discrete colony (Grubb et al. 2009). The adherence leads to the biofilm formation and the *Candida* cells becomes resistant to the various drugs. Cell adherence is mediated by adhesion molecules which are 600–2500 residues of mannoproteins and covalently bind to the cell wall (Lipke 2018). Adhesins can change the host immune response. The role of adhesin in biofilm formation appears to recapitulate its role in organogenesis and development. Cell adherence can be in different forms like heterotypic primary adherence (direct adherence with the host cell surface) and homotypic secondary adherence (*Candida* spp. already bound to the cell surface of host) (Lipke 2018). The yeast form of *Candida* is capable to adhere the endothelium and bringing the morphological change of *Candida* spp. *Candida* proteins get binded to the mammalian extracellular matrix proteins such as fibronectin and fibrogen (Calderone and Fonzi 2001). Hydrophobicity has ability to increase the virulence activity in the *Candida* spp. The hydrophobic cells are able to be more adherent to the host cells and have mucin and extracellular matrix proteins (Sardi et al. 2010).

There are some genes which encode the protein such as Als family, Hwp1p, Int1p and Mnt1p. Als1p (agglutinin-like sequence) of *C. albicans* is homologous to the *S. cerevisiae* α-agglutinin protein required for cell–cell recognition during mating and is a member of a family of seven glycosylated proteins (Calderone and Fonzi 2001). ALS genes include three domains: 5′ domain of 433–436 amino acids; central domain of 180 bp; and 3’domain is serine-theronine-rich (Calderone and Fonzi 2001). ALS genes were first isolated in LoisHoyer lab and this gene is named as *ALS1*. *HWP1* gene is isolated as a hyphal- and germtube-specific gene. It encodes the outer surface of the mannoparticles at the carboxyl terminal of the cell wall. The amino terminal of the *HWP1* contains proline and transglutamine-rich amino acids which resembles transglutaminase (TGase). Hwp1p get binds to the TGases, and then the binding of hyphal form of the *Candida* spp. to the human epithelial cells. *Candida* spp. bind to the ECM, FN, laminin, collagen I and IV. Int1p is the integrin-like protein which contains I-domains and is 18% similar to the human alpha-M integrin domain. Int1p plays an important role in the *Candida* cell adherence and the filamentation of the yeast *Candida* cells. Int1p interacts with cytoskeleton proteins to mediate morphogenesis. Mnt1p (α-1, 2- mannosyltransferase) is less effective on adherence and virulence. *Pichiapastoris* expression system is used for the expression of Mnt1p. *PMT1* genes encode O-glycosylation mannosyltransferase, and help adhere to the epithelial cell line (Calderone and Fonzi 2001).

19.4.3 Phenotypic Switching

Phenotypic switching is defined as the capacity of organism to undergo spontaneous, reversible transition between a set of colony morphologies. A trait of phenotypic switching was never tested according to the earlier literature. The variability of the colony morphology could be reversible. Every morphotype has its own virulence profile and each pleomorphic form helps in crucial roles required for infectivity. It will occur at the high frequency and stimulated by the low doses of UV (Soll 2014). White-opaque transition is the spontaneous and reversible process. It has two phases of cellular morphology. According to the studies, the white-opaque transition is responsible for the gene and regulatory network that control all the processes. In *Candida* spp. there are some genes which are responsible for the switching of white colony to opaque colony. White-opaque transition has been isolated from the human bloodstream infection from the immunocompromised patient. The regulation of white-opaque transition is chromatin state-regulated in the part by histone acetylases and deacetylases, DNA modifiers, mediator complex, signal transduction pathways, transduction factors including Wor2, Wor3, Efg1, Ahr1, Czf1, configuration of the MTL locus haemoglobin and Hrr1 signalling system (Soll 2014). The environmental issues which help in the regulation are CO_2, temperature, UV, stress condition, white cell metabolites, sugar substrates, etc. (Fig. 19.2). The white cells are more virulent than the opaque cells. The white and opaque cells are responsible for the commensalism and pathogenesis in human (Soll 2014).

The a1-α2 co-repressor complex is playing main role in the regulation of white–opaque transition. This complex is able to repress some genes and down-regulate the MTL1a leading to the white-opaque transitions. There are some environmental conditions like sudden change of carbon sources, CO_2, which helps in the white–opaque transition without MTL homozygosis (Soll 2014). Phenotypic switching or white–opaque switching is a reversible transition. It influences the virulence traits, mating behaviour and biofilm formation. In phenotypic transition, white cells are smooth and round in appearance and the opaque cell are rough or sometimes smooth but oval in a shape or structure. Colonies of white cells are domed and the colonies of opaque cells are flattered and translucent colonies. White and opaque states of the *Candida spp.* are regulated by more than 450 genes which differentially affects the level of pathogenicity and other virulence factors. White and opaque cells show the difference between the interactions with the immune cells; white cells interact with

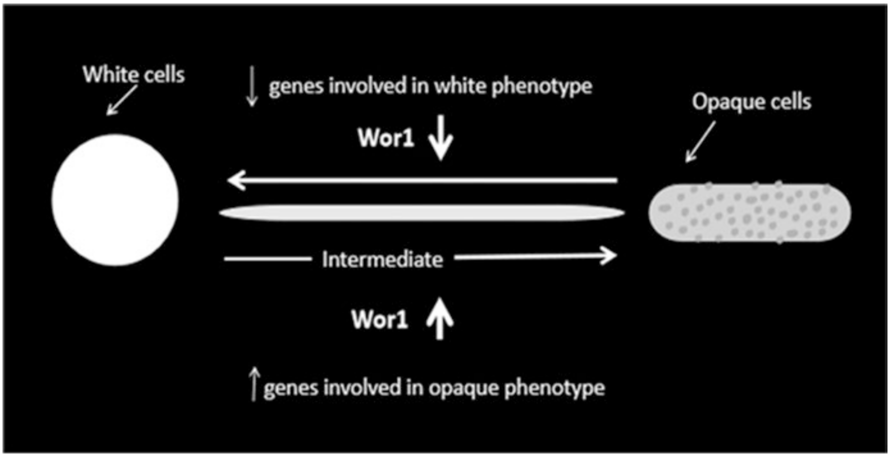

Fig. 19.2 The regulation of white-opaque phenotype switching. (Adapted from Soll 2014)

leucocytes and secrete chemoattractants but opaque cells do not. The factor which regulates the white–opaque switch is encoded by Wor1 genes also known as TOS9 or EAP2 genes. And the overexpression of the Wor1 genes forced to switch the white cells into the opaque cells. In the absence of Wor1 gene, the switching of opaque cell is not occurred (Alby and Bennett 2009).

19.4.4 Morphogenesis

Morphogenesis is defined as the transition of the unicellular yeast cells to a filamentous form of *Candida* spp. *C. albicans* and *C. dubliniensis* are both converted into filamentous type of growth. Morphological transition of *C. albicans* in response to changing environmental conditions represents a strategy to ease pathogenicity by which the *Candida* adapts to different host niches (Calderone and Fonzi 2001). The yeast morphology and the filamentous form both are playing a role in the development and progression of the disease (Brand 2012). Virulence is attenuated in morphological mutants confined either to yeast or germ tube morphology (Pukkila-Worley et al. 2009). There are some specific genes which are controlled by some regulatory factors for the morphological development. Those specific genes are crucial for the virulence of *Candida* spp. There are two signal pathways that regulate morphogenesis in *Candida* spp. The first pathway is STE12 mating and pseudohyphae pathway, in which the phosphorylation is regulated by protein of mitogen-activated protein (MAP) kinase pathway, including STE20, STE7 and KSS1 (Calderone and Fonzi 2001). The second morphogenesis pathway in *Candida* spp. is mediated by transcriptional factor Egf1p. Disruption of RAS1 reveals that mutants are not able to form hyphae; however, it forms a pseudohyphal form. RAS might be activated through both pathways (Desai 2018). Other pathways are required for the morphogenesis that are cell wall integrity and osmoregulation pathways. The family of SAPgenes also contributes in the development of the candidiasis (SAP1-3 from oral, SAP6 from vagina). The expression of SAP1 is for early invasion, SAP8 is for extensive penetration, SAP6 for extensive hyphal growth and SAP2 is required for the development of diseases (Felk et al. 2002). There are some other mechanism of hyphal growth production (Naseem et al. 2015). The transduction pathway induces the hyphal growth in the *Candida* spp. The extreme polarized growth hypha is operated due to the infection process and the cellular mechanism. The factors which affect the signal transduction pathways are temperature, serum, CO_2 and starvation. The transduction of these signals leads to the activation of transcriptional factors such as Efg1, Eed1 and Ume6, through to the outputs of Hgc1 expression (Fig. 19.3). These factors are required for polarized growth and inhibition of cell separations. The polarized growth is continued through the cell cycle of the *Candida* spp.

The role of Hgc1 is promoting the hyphal growth by phosphorylation of Rga2, Mob2, Efg1 and Sec2. The other targeted gene Cdc28 can be identified. And this helps in the polarized growth and cell separation suppressor (Sudbery 2011). Hyphal formation plays a vital role in the virulence in *Candida tropicalis* (Jieng et al. 2016). Hyphal form has ability to penetrate the mucosal membrane, tissues and gets deeper into the bloodstream. Hyphal form cells are also protected from killing by the neutrophils and macrophages. Yeast to hyphal transition is common in the superficial mucosal infection. There are number of molecules which are able to interfere in the hyphal transition. They include the agents who have good potential for the future development, lithium, azoles, rapamycin, geldanamycin, histone deacetylase

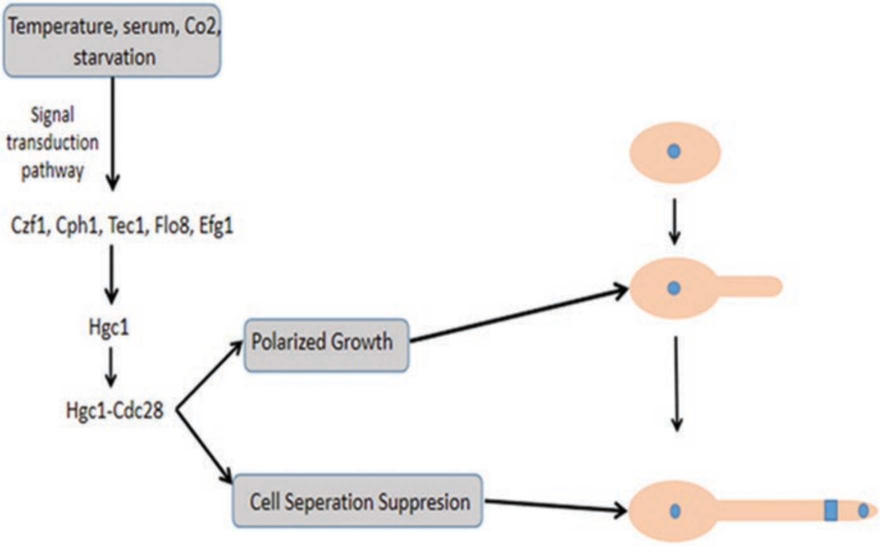

Fig. 19.3 Systematic regulation of hyphal formation from yeast cells. (Adapted from Sudbery 2011)

inhibitors, propranolol, actin antagonists, hydroxyurea and nocodazole (Bar Yosef et al. 2017). Their critical roles are regulating the yeast to hyphal transition and serve as signal transduction regulators, notably components of the MAPK-13 and cAMP/PKA-dependent pathways. The formation of biofilm by yeast alone is thin and is removed by the mechanical disruption. The Als3 is an amyloid-like, hypha-specific adhesion, and Ssa1 is an intracellular heat shock protein which is involved in the hyphal formation or *Candida* morphology (Naglik 2012). Hyphal forms increased the resistance against phagocytosis; initiate the adherence to the host cell surfaces and ability to invade epithelial cell layers. A study showed that the Saponins are able to inhibit the yeast–hyphal transition (Chevalier et al. 2012).

19.4.5 Quorum Sensing

Quorum sensing is the mechanism that is dependent on the cell density which regulates the secretion of virulence factors, biofilm formation, competence and so on. quorum sensing is referred as the autoinduction, in which the individual cells continuously release the molecules and these molecules are known as quorum sensing molecules (QSM) (Albuquerque and Casadevall 2012). Quorum sensing also contributes to the morphogenic control of *Candida* spp. QSM such as cysteine, tryptophol and phenylethyl alcohol are able to inhibit the hyphal development of the *Candida* spp. These molecules are helping in the regulation of morphogenesis. Tyrosol regulates the hyphal formation in the early stage of biofilm formation. Contrary farnesol regulates the later stages or phases of biofilm formation. Quorum sensing molecules are considered as virulence factors because they are toxic for the host cells and able to modulate the host cells immunity. QSM are sensed by all the surrounding cells. When the population of cell density is increased and the QSM reached the threshold level, the activation and repression of certain genes induces the cellular behaviour and secretion of extracellular enzymes (Han et al. 2011). This leads to biofilm formation, and secretion of other virulence factors, morphological switching, hyphal formation, plasmid transfer and antibiotic biosynthesis. The mechanism depends on the signal system to involve passive diffusion across the cell membrane, efflux pump and specific transporter (Hogan 2006).

QSM (tryptophol, phenylethyl alcohol and farnesol) suppress the hyphal formation at high cell density, although one of the QSM trysol accelerates the hyphal formation at low cell density (Fig. 19.4) (Han et al. 2011). Farnesol resists to oxidative stress and the trysol is an antioxidant protecting the *Candida* by eutrophication during phagocytosis. Quorum sensing pathways regulate all the development, maintenance, dispersion of multicellular, surface-associated biofilms. Quorum sensing signals may be inhibited by antagonists produced by other organisms. The quorum pathways are beneficial and have role in protection (Kruppa 2008). Farnesol plays a vital role in the virulence of *Candida* spp. Farnesonic acid and farnesol are also the morphogenic autoregulatory substance. Farnesol is produced by the *Candida* spp. other than *C. albicans* while farnesonic acid is produced only by *C. albicans*. There

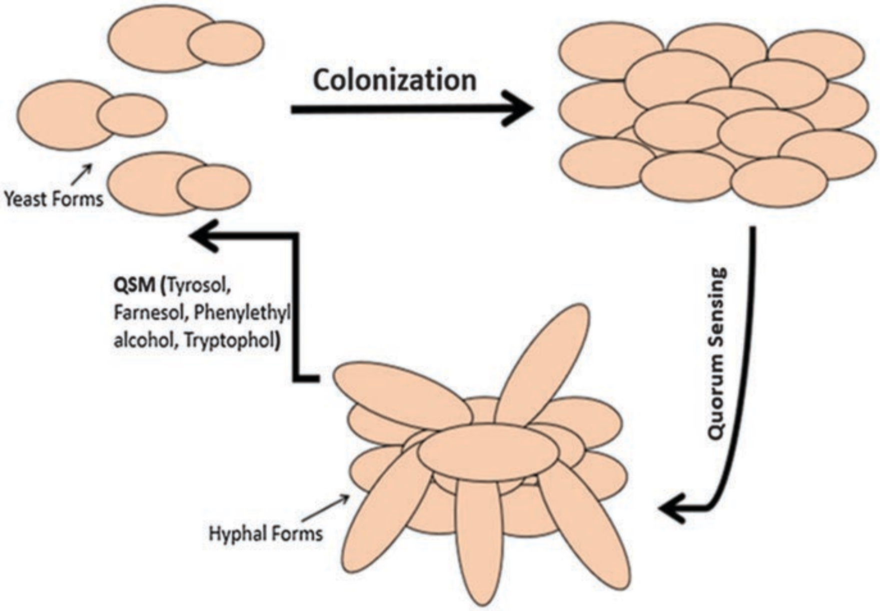

Fig. 19.4 Effect of quorum sensing molecules on morphogenesis

are some physiological effects of farnesol: size effect of filamentation (farnesol shows no effect on the cells as it already gets modulated into mycelium formation); biofilm formation, oxidative stress (the role of farnesol is as antioxidant signalling molecules), modulation of drug efflux (farnesol modulates the *Candida* spp. by the ABC transporter genes without effecting the multidrug extrusion pump protein). Exogenous farnesol causes inhibition in the transitional growth of the *Candida parapsilosis.* Trysol is another QSM which decreases the length of the lag phase of *Candida* spp. and also stimulates the filamentous and biofilm formations. Trysol inhibits and kills the growth of *Candida* spp. (Manoharan et al. 2017).

19.4.6 Haemolysin Production

Pathogenic fungi can grow on the host cell by using haemoglobin that is a source of iron (Silva et al. 2011). For degradation of haemoglobin to extract the elemental form of iron, *Candida* produces a haemolysin which acts as a virulence factor that helps *Candida* to promote pathogen survival in the host. *Candida* has the ability to utilize the iron and produce haemolytic factor that can lyse erythrocytes and produce haemoglobin (Silva et al. 2011). The genetic expression of haemolytic activity of *Candida* is haemolysin-like protein (HLP) genes. The identification of the haemolytic activity of the *Candida* leads to cloning, and the virulence evaluation. The relationship between pathogenic and commensal isolates of *Candida* depends on the virulence and the degree of haemolysin production (Wan et al. 2015). Haemolytic activity of *Candida* spp. will be concentrated by absorption on a concanavalin-A sepharose matrix. Haemolysin production is regulated by the presence of glucose. Genetic expression of haemolytic activity by *Candida* spp. is the haemolysin-like protein (HLP) genes which is associated from *Candida glabrata*. The identification of *Candida albicans* haemolysins involves cloning, disruption and virulence evaluation. All these molecules and genes are responsible for virulence factors of *Candida* spp. (Silva et al. 2011). Haemolysin is effective in the promotion of pathogenic properties in the *Candida* species (Wan et al. 2015).

19.4.7 Exoenzymatic Activity

Candida spp. have the ability to produce some of the exoenzymes such as proteases, phospholipases, etc. (Luo et al. 2001). The *Candida* adhesion, cell damage and invasion of the host cell are associated with the enzymatic activity of the following.

(i) *Phospholipases and lipases activity:* Phospholipases are the enzymes that hydrolyse the phospholipids into fatty acids. These enzymes are classified into PLsA, PLsB, PLsC and PLsD and are produced by *Candida* spp. These enzymes are contributing in the membrane damage of the host cell. The cells damage the exposed receptors, and promote the cell adhesion. *Candida* spp. produce extracellular enzyme (phospholipases) at lower levels. Lipases are involved in the hydrolysis and synthesis of triacylglycerols. These enzymes are able to survive at high temperature and inorganic solvent and this enzyme is able to resist the proteolysis (Silva et al. 2011)

(ii) *Proteinases:* Sap proteins contribute to pathogenesis by the degradation of host cell membranes and molecules of the defence system to avoid antifungal attack by the host. This is an important virulent factor of *Candida* and is classified in saps classes. It causes the disruption of mucosal membrane of hosts for the colonization and invasion of tissues and affects the immunological and structural defence proteins. There are different types of SAP genes which are present in different species (Silva et al. 2011).

19.5 Case Studies of virulence in *Candida* spp.

Case 1 This case is about Candidal vulvovaginitis which is a worldwide problem and the second most common problem of the vaginitis. In this case, authors studied that the Candidal vulvovaginitis is caused by the *Candida auris*. This case was reported in 28-year-old lady who has a complaint of lower back pain, burning or itchy sensation and vaginal discharge. In a clinical diagnosis, it was diagnosed as vulvovaginitis and fungal culture test identified the presence of ovoid budding yeast cells. After that, they performed antifungal resistance test and observed that it was resistant to azoles and amphotericin B. The pathogen *C. auris* is causing fungemia but in this case the *C. auris* is found as the causative agent of vulvovaginitis candidiasis. *C. auris* clinical isolation is increased as it has ability to colonise, infect, and capacity to cause the disease. In this case, the report said that *C. auis* is resistance to itraconazole only but it has capacity to develop high resistance to the fluconazole. In *Candida* spp. there are various virulence factors which are linked with the colonization of pathogenic species of the *Candida*. These species are also associated with the drug resistance property. In this study, they demonstrated the presence of phospholipase enzymatic activity and proteinase enzymatic activity in the *C. auris* isolates. The phospholipases (extracellular hydrolytic enzyme) act as an important virulence trait which helps in the adherence and invasion of the host cells. For the widespread infection, it uses the haemolysin production and it consumes the iron from the host that leads to the hyphal formation of the yeast cells and increases the diseases. This case studied that the *C. auris* isolates has the ability to develop drug resistance and virulence traits that increase the pathogenicity (Kumar et al. 2015).

Case 2 This case again studied about the candidial vulvovaginal but caused by the presence of *Candida albicans*. For this study, they took a vaginal swap from 232 women, who complaint about the vaginal discharge, burning itchy sensation, backache, and pain in the lower abdomen (Kumari et al. 2020). They performed the culture test and identified the strains. They identified some of the cultures as *Candida* spp. For the further identification of different species and their virulence traits, they tested by Gram straining, germ-tube test and other methods described above. They get 71 *Candida* positive cases out of 232 vulvovaginitis patients. And all the species are from three *Candida* spp.: *Candida albicans, Candida parapsilosis* and *Candida glabrata*. Further, they proceed the experiments for the identification or characterization of different species of *Candida.* For identification, they perform Gram staining and observe that budding yeast structure shows pseudohyphal growth only in 27.81% of the positive cases. *Candida* spp. are able to express virulence traits and colonize on the surface of host cells and get deep into the host tissue by the disruption of the host-cell membranes. Phospholipases activity was also associated with non-albicans *Candida* spp.

Case 3 The third case is from cancer patients infected by *Candida albicans* and also some non-*albicans* like *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis* and *Candida krusei*. In the case of leukaemia patients, bone marrow transplant patients were infected by *Candida krusei* and *Candida lusitaniae*. *Candida tropicalis* are the most pathogenic species of *Candida* reported in the cancer patients. This species is more common in the patients of leukaemia than in the tumour patients. It is transmitted by the bloodstreams and it has all virulence factors than *Candida albicans*. It produces a systematic infection at smaller doses and shows pathogenicity. This strain shows the proteinase, exoenzymatic activities that invade the tissues and reached to the bloodstreams. It commonly affected the gastrointestinal tract. In another study, a patient who had undergone coronary artery bypass surgery got infected by *Candida tropicalis* and observed that he is highly infected at the fingertips, throats and nose. Its virulence traits get increased when the patients are immunocompromised. *Candida glabrata* is an occasional pathogen. It has low virulence activity. It infects the patients with cancer, leukaemia or lymphoma. And it is more risky in the cancer patients than in the leukaemia patients. It infects the human host cells by the colonization at the respiratory tracts. Its recovery rate is higher than the others. In one study, some of the strains of *Candida glabrata* was resistant to fluconazole. *Candida krusei* is a rare pathogen according to the studies; it is common in the oncology units. It is resistance to the fluconazole. It has capacity to adhere to the fibronectin and epithelial cells. This is the very initial stages of the colonization and fetal infections. It is entered into the bloodstreams. *Candida lusitania* is recognized as human pathogen. It has resistance to polyene agent amphotericin B. The patients who undergo the treatment for hematologic malignancies develop the resistance against amphotericin B. This strain is not frequently transmitted by the patients to patient as it has a low capacity of colonization. *Candida parapsilosis* is the infrequent pathogenic human infectious agent. This strain is found in the bone marrow-transplanted patients. It has resistance to amphotericin B. This strain is responded at the high dose of fluconazole.

19.6 Conclusion

The *Candida* spp. is successful both as a commensal and as a pathogen. *Candida* utilizes several traits whose expression is required for virulence including host-recognition proteins (adhesins), proteolytic and lipolytic enzymes and phenotypic switching. Understanding these traits will help in better antifungal therapeutic strategies. Although many virulence traits in *Candida* have been identified, considering the complexity of the pathogenesis process, many more must still be elusive. Since these determinants are unique to fungi, hence identification of novel traits with the advancements in technology in the antifungal drug discovery programme will be widened as more new virulence factors will be discovered.

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Chapter 20
Myco-Nanotechnology for Sustainable Agriculture: Challenges and Opportunities

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20.1 Introduction

Farming is the practical implementation of the agriculture industry, the process in which the food, fodder, and fibers are produced. One of the most important branches of agricultural sciences is agronomy which deals with the study of crop production for food, fiber, and soil management-related aspects (Singh et al. 2016). It plays a very crucial role as it is the driving force of the economy in most developing countries, feeding humans directly and indirectly (Chhipa and Joshi 2016). There are predictions for the world population to be nine billion by 2050. It will be challenging to imagine also feed such a huge population with the same available resources and deteriorated environment. Therefore, global production in the field of agriculture should upsurge to feed this rapidly increasing population. But the agricultural sector has been facing very serious challenges for sustainable food production (Godfray and Garnett 2014).

Major problems faced by agriculture include increased population, climatic changes, soil erosion, and the difference in soil conditions, micro and macronutrient deficiencies, pathogens attack, urbanization, and industrialization which affect the production of food and increase the pressure on arable land. Almost 35–40% of crop production depends upon the use of fertilizers. But excessive use of these synthetic fertilizers also affects the growth and yield of crops directly (Manjunatha et al. 2016). Disease management of crops with pesticides has led to the increase in the concentration of toxic compounds in the soil as well as ground and surface water which is also a major reason for bio-accumulation of toxic compounds in the food chain. Conventional methods used for irrigation purposes are another reason for water depletion as more water is being pumped out than it is replenished or reused (Rodell et al. 2009). Water scarcity all over the world due to variable climatic conditions and extreme weather actions harms crop production. Conventional irrigation methods also remove the important minerals from the soil causing the salinity which ultimately leads to a reduction in the agricultural land (Mukhopadhyay 2005). This scenario for such a complex agricultural system is a serious challenge mostly for developing countries as it involves various sub-branches (Fig. 20.1). Therefore, advancement in science and technology is very much needed. Nanotechnology has emerged in recent decades which are leaving its footprints in every sector of life from health to food, agriculture to aerospace, clothing to cosmos, and many more.

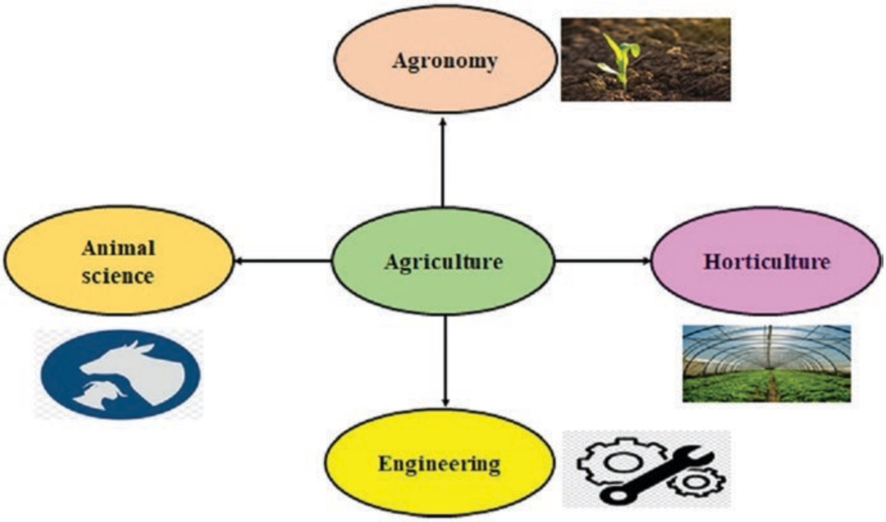

Fig. 20.1 Sub-branches of agricultural sciences

Nanotechnology is an advanced field of science, deals with production, manipulation, and implication of matter at the nanoscale. The name is based on a Greek letter “nano” meaning dwarf. It is one billionth part of a meter or 10^{-9} m (Rai and Ingle 2012). Nanomaterials are very minute structures that range from 0.1 to 100 nm. These are very important because these microscopic sizes as well have different properties from bulk material. Properties of these nanomaterials such as electrical conductance, magnetism, chemical reactivity, optical effects, and physical strength vary from bulk materials due to their smaller size. These nanomaterials form a link between bulk material and their respective nanoparticles (Boisseau and Loubaton 2011). Nanotechnology provides tools and techniques which can revolutionize the agricultural industry. Nanotechnology is the use of nanomaterials with exceptional properties to enhance the productivity of crops as well as livestock. It is focused to improve the quality of food, protect crops, monitor the growth of plants, enhance the production of food, and identify the disease-causing pathogens (Ramu et al. 2016, 2017). Among the mentioned applications, food production and crop protection are the main applications of nanotechnology in the agricultural industry. Innovative tools are provided by nanotechnology to deliver agrochemicals at the targeted area safely without disturbing the ecosystem. It has developed such carrier systems that enable the controlled release of compounds when needed; that is how the concentration of pesticides in the environment can be reduced to a greater extent (González et al. 2014). The present chapter provides an appraisal on the applications of myco-nanotechnology for agricultural sustainability.

20.2 Classification of Nanomaterials

Nanomaterials are classified into three main groups on a dimensional basis: one-dimensional nanoparticles, two-dimensional nanoparticles, and three-dimensional nanoparticles. Nanomaterials having less than 100 nm size with one dimension are grouped into one-dimensional nanoparticles category. Nanowires and nanorods are examples of one-dimensional nanoparticles that are being used in buildup of various chemical and biological sensors, solar cells, IT systems, and optical devices. Nanomaterials having a size less than 100 nm along two dimensions at least are known as two-dimensional nanoparticles, e.g., carbon nanotubes fibers and platelets (González et al. 2014). Metallic nanomaterials having <100 nm in all dimensions, i.e., quantum dots, dendrimers, and hollow spheres are three-dimensional nanoparticles. Nanomaterials are also classified based on structural configuration, namely metallic nanoparticles, nanocrystals, quantum dots, carbon nanotubes, polymeric micelles, and polymeric nanoparticles (Holdren 2011).

20.3 Synthesis of Nanomaterials

Two approaches are being used to synthesize nanoparticles: top to bottom approach, and bottom to up approach (Fig. 20.2).

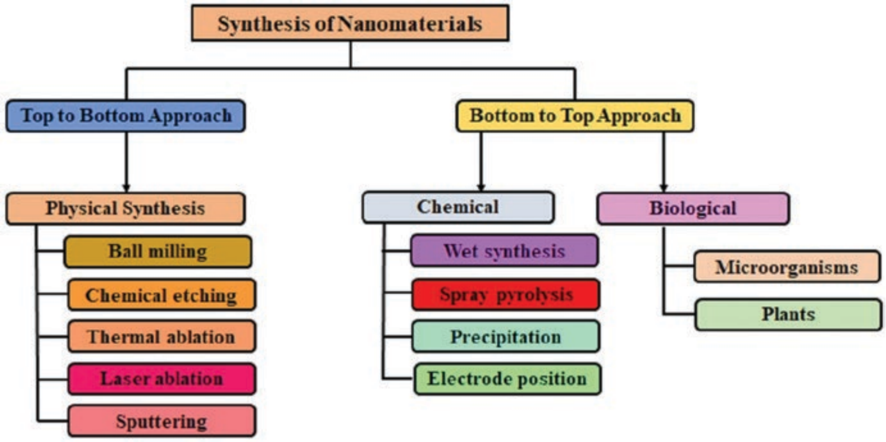

Fig. 20.2 Methods for synthesis of nanomaterials

20.3.1 Top to Bottom Approach

The top to bottom approach refers to the breakdown of suitable given bulk material into particles in the size range of nanometer due to reduction in size by various methods. This includes grinding of material, milling, sputtering laser ablation, and evaporation–condensation. Various nanoparticles have been synthesized using this technique such as silver nanoparticles, gold nanoparticles, lead, and fullerene nanoparticles (Prasad et al. 2019a; Pankaj et al. 2020). A tube furnace is used in this method to generate high atmospheric pressure. In the center of the tube furnace, concerning bulk material is placed on a boat, allowed to vaporize and carried through a gas. But the use of this tube furnace has several disadvantages as it requires large equipment and a huge place to be installed. A lot of energy more than several kilowatts is consumed to raise the temperature of the tube furnace around the bulk material and it also entails a lot of time to attain a stable operating temperature and gain thermal stability. The imperfection of the surface structure of nanoparticles is the other limitation of this procedure as various physical properties are highly dependent on the surface chemistry of nanoparticles (Kumar et al. 2014; Prathna et al. 2011).

20.3.2 Bottom to Up Approach

When nanoparticles are being synthesized using different chemicals as well as biological systems that are known as bottom to top approach. In the bottom to top approach, atoms are self-assembled into new nuclei forming the particles of nano size. Nanoparticles can be synthesized by several chemical methods. The most commonly used method among all the chemical methods is a chemical reduction for the synthesis of nanoparticles. Various compounds including both organic and inorganic are being used as reducing agents for the production of nanoparticles, i.e., sodium borohydride ($NaBH_4$), ascorbate, elemental hydrogen, Tollen's reagent, N,N-dimethyl formamide, sodium citrate, and copolymers of polyethylene glycol are examples to quote (Yuvakkumar et al. 2015). Capping agents are the chemicals that are responsible to control and stabilize the size of the nanoparticles avoiding the aggregation. Nanoparticles can be synthesized in bulk amounts by using the reduction capability of different chemicals and it takes very little time for reaction completion. But it becomes harmful due to the use of synthetic chemicals that are toxic, hazardous, and risk for environment and living systems (Iravani et al. 2014). These reasons lead to the development of nanoparticles by using methods other than chemical methods. Therefore, the need to develop such methods becomes mandatory which are non-toxic, environment friendly, and economically beneficial.

Biological synthesis of nanoparticles plays a vital role in the field of nanotechnology. Use of biological entities such as microorganisms including viruses, bacteria, fungi as well as plant material either in the form of extract or biomass is an alternate way in an ecofriendly manner for the production of nanoparticles than to the physical and chemical methods (Alghuthaymi et al. 2015; Prasad et al. 2019b). Microorganisms are important biological factories being used for the synthesis of nanoparticles (Gopal et al. 2009). Microorganisms produce several reductase enzymes which can reduce the metals into metallic nanoparticles having a narrow range of size distribution. This approach for the synthesis of nanoparticles holds immense potential as it is eco-friendly, avoiding the use of toxic and harsh chemicals and it is a cost-effective tool that does not require the consumption of high energy and longer time as physiochemical approaches.

Microorganisms, i.e., bacteria, yeasts, and fungi have been considered for the synthesis of metal nanoparticles in both extracellular and intracellular ways (Madhusudan et al. 2016; Zameer et al. 2016). Fungi are primitive eukaryotic organisms. Most fungi are microscopic, while some are macroscopic, such as mushrooms, toadstools, puffballs, and stinkhorns. Generally, their mode of nutrition is heterotrophic, although some are parasites and saprophytes. Fungi are cosmopolitan in distribution. In an ecosystem, fungi play an important role in nutrient cycling, as sources of food, decomposers, symbiosis, and also protection.

From time immemorial, fungi have been utilized as a source of food and harnessed to ferment and preserve foods and beverages. In the twentieth century, human beings have learned to exploit fungi to protect human health through antibiotics, anti-cholesterol statins, and immunosuppressive agents (Abdel-Aziz et al. 2018). Industrial utilization of fungi for the production of enzymes, acids, and biosurfactants with the advent of modern nanotechnology has been started in the 1980s. Fungi can easily be isolated from different sources of environment and also can be cultivated in simple and less nutrient media like Potato Dextrose Agar and Czapek Dox Broth in the laboratory. The maintenance of fungi in the laboratory is also very easy as their nutrition requirement is far simpler compared to bacteria. Fungi have more enzyme-secreting activity, and it is easy to isolate and maintain, so they are selected for silver and other nanoparticle production by the research fraternity (Alghuthaymi et al. 2015; Abdel-Aziz et al. 2018). Different fungi have been investigated for the synthesis of nanoparticles such as gold, silver, selenium, platinum, zinc oxide, and titanium by various researchers across the globe. Fungi have numerous advantages from other organisms in the synthesis of nanoparticles (Zameer et al. 2010a) They are easy to isolate and handle and are capable of secreting extracellular enzymes and can withstand flow pressure than bacteria and plants (Prasad et al. 2016). Besides, the process of synthesis has a greener approach as it is non-toxic and occurs at a very low cost. Fungi-based synthesis of nanoparticles has received much attention to researchers due to their extensive advantages in different fields. Fungal nanoparticles can be used in various fields like agriculture, engineering, pharmaceuticals, environment, textiles, medicine, food industry, etc. Thus, myco-nanotechnology provides a greener alternative to chemically synthesized nanoparticles. The present chapter throws light on the synthesis of myco-nanoparticles (fungal nanoparticles), factors that affect their synthesis, advantages of myco-nanoparticles, the application of myco-nanotechnology in agriculture, and its future perspectives for sustainable agricultural practices.

20.4 Synthesis of Fungal Nanoparticles

Fungal nanoparticles can be synthesized both intracellularly and extracellularly (Fig. 20.3). Nanoparticles are fabricated inside the cell of the fungus in intracellular where the biomass of the fungus is reacted with a metal, whereas in extracellular synthesis, the filtrate of the fungus reacts with the solution of a metal (Yadav et al. 2015). Electrostatic interactions occur during intracellular synthesis where ions of the metal bind upon the fungal cell. The ions of the metal are reduced by the enzymes present in the cell wall and then the formation of nanoparticles occurs due to aggregation of the metal ions. During extracellular synthesis, the fungus when exposed to the metal ions leads to the release of reductase enzymes and the formation of highly stable nanoparticles (Kashyap et al. 2013). A rapid extracellular and intracellular biosynthesis of gold nanoparticles using the fungus *Penicillium* sp. was reported in the scientific investigation. Intracellular synthesis of gold nanoparticles was obtained when $AuCl_4^-$ ions reacted with the cell filtrate of the fungus in 1 min, whereas extracellular synthesis occurred when the solution of $AuCl_4^-$ incubated with fungal biomass for 8 h.

There are two different methods for the preparation of extracellular biosynthesis, i.e., rapid synthesis and slow synthesis, whereas intracellular biosynthesis is a time-limiting factor that depends on in vivo synthesis of cells (Du et al. 2011). Due to an additional step required to obtain the purified nanoparticles, the extracellular synthesis method is more favorable than the intracellular method. The myco-synthesis of silver nanoparticles using *Aspergillus flavus* are reported. The synthesized nanoparticle was found to be spherical with 50 nm in size which showed antimicrobial effect against pathogenic fungi and bacteria.

Further, it is also reported to be a microbicidal agent in the field of agriculture. *Fusarium solani* was reported to be a new biological agent in the extracellular synthesis of silver nanoparticles (Anbazhagan et al. 2017). Fourier transform infrared spectroscopy (FTIR) revealed the silver nanoparticle to be highly stable due to the presence of a capping agent. Extracellular synthesis of gold nanoparticles using *Fusarium oxysporum sp. Cubense* and its antimicrobial activity against Pseudomonas

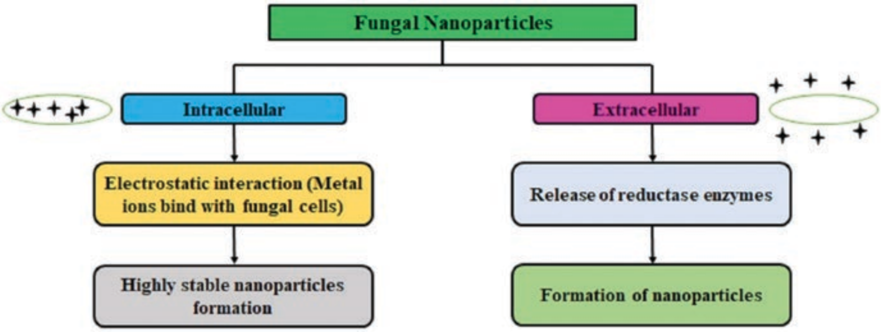

Fig. 20.3 Synthesis of fungal nanoparticles

sp. was reported. The extracellular synthesis of gold nanoparticles using *Helminthosporium tetramera* was studied by the synthesized gold nanoparticle that was found to be poly-dispersed spherical with a size range of 8–50 nm. This study would be appropriate for establishing a process for large-scale manufacturing of scanty AuNPs. Zinc oxide nanoparticles against two pathogenic fungi, i.e., *Botrytis cinerea* and *Penicillium expansum*, and its antifungal activity were investigated (Baskar et al. 2013). The results suggest that zinc oxide nanoparticles could be used in agriculture as a productive fungicide and application in food safety. A study also revealed a greener synthesis of zinc oxide nanoparticles against *Aspergillus terreus*. The synthesized crystalline zinc nanoparticles were characterized by UV absorption spectrum, X-ray diffraction spectrum and Fourier transform infrared spectroscopy and were found to be spherical, and a scanning electron microscope revealed the size range from 54.8 to 82.6 nm. The synthesized zinc oxide nanoparticle was found to be a potent antifungal agent against fungal species (Sarkar et al. 2011).

Another study of intracellular and extracellular synthesis of gold nanoparticles using an alkali-tolerant fungus *Trichothecium* sp. was investigated. Gold ions when reacted under stationary conditions, with the fungal biomass, produced extracellular synthesis, while the biomass reaction with agitating conditions resulted in the intracellular growth of the nanoparticles. The synthesized gold nanoparticles were found to be spherical and triangular in morphology. They demonstrated that altering the conditions of the reactions of the fungal biomass and gold ions resulted in the intracellular and extracellular synthesis, where under stationary conditions, the enzymes and proteins are released into the medium but are not released under shaking conditions (Ahmad et al. 2005). An extracellular synthesis of platinum nanoparticles using *Fusarium oxysporum* was studied. *Fusarium oxysporum* reacted with hexachloro platinic acid resulted in the formation of selenium nanoparticle with size in the range of 5–30 nm which are highly stable. Because of their high stability, ability not to flocculate, and having a good mono-dispersity, they find applications in various fields including agriculture (Syed and Ahmad 2012). The work done by researchers in the synthesis of fungal nanoparticles is listed in Table 20.1.

20.5 Factors Affecting Myco-Synthesis of Nanoparticles

Several biotic and abiotic factors affect the synthesis of fungal nanoparticles such as temperature, biomass, concentration, and time in the exposure of the substrate, pH, and the presence of a particular enzyme (Fig. 20.4). These are known to be the major factors that affect the shape and size of nanoparticles. The studies are been conducted for the optimization of various parameters such as pH, the quantity of fungal biomass, temperature, and concentration of silver nitrate in the synthesis of silver nanoparticles from *Aspergillus niger* (Khan et al. 2016). The studies concluded that optimizing the above parameters will enhance the silver nanoparticles synthesis as well as its yield. Optimizing the cultural and physical conditions in the synthesis of silver nanoparticles from *Fusarium oxysporum* has also been studied.

Table 20.1 Myco-synthesis of nanoparticles

Fungal species	Nanoparticle	Synthesis	Size (nm)	Reference
Aspergillus flavus	Silver	Extracellular	50	Priyadarshini et al. (2014)
Fusarium solani	Silver	Extracellular	5–35	Anbazhagan et al. (2017)
Fusarium oxysporum	Gold	Extracellular	22	Baskar et al. (2013)
Helminthosporium tetramera	Gold	Extracellular	8–50	Singh et al. (2016)
Botrytis cinerea	Zinc oxide	Extracellular	70	Bhattacharyya et al. (2016)
Penicillium expansum	Zinc oxide	Extracellular	70	Du et al. (2011)
Aspergillus terreus	Zinc oxide	Extracellular	54.8–82.6	Khan et al. (2016)
Coriolus versicolor	Silver	Intracellular and extracellular	10	Baskar et al. (2013)
Saccharomyces cerevisiae	Cadmium sulfide	Extracellular	2.5–5.5	Priyadarshini et al. (2014)
Coriolus versicolor	Cadmium sulfide	Extracellular	10	Baskar et al. (2013)
Aspergillus flavus	Titanium dioxide	Extracellular	62–74	Priyadarshini et al. (2014)
Alternaria alternata	Selenium	Extracellular	13–15	Aziz et al. (2016)
Fusarium oxysporum	Platinum	Extracellular	5–30	Syed and Ahmad (2012)
Mucor hiemalis	Silver	Intracellular and extracellular	5–15	Singh et al. (2016)

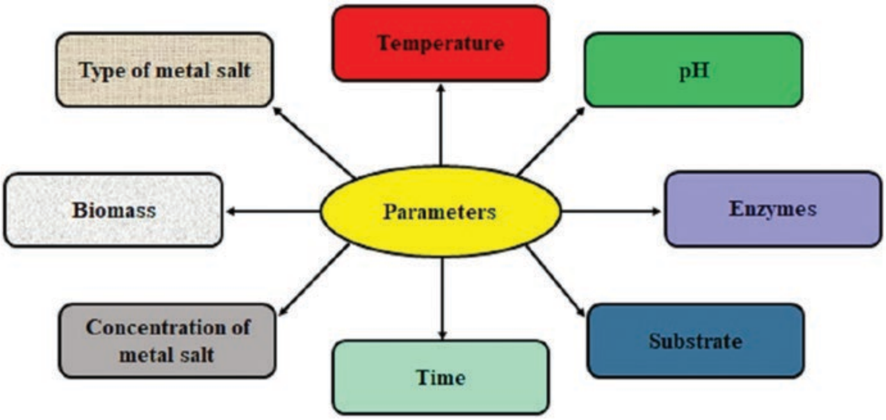

Fig. 20.4 Parameters affecting myco-synthesis of nanoparticles

One of the most important factors affecting the myco-synthesis of nanoparticles is pH. It greatly influences the nature and size of the nanoparticles synthesized. The nanoparticle was found to be 10^{-19} nm at pH 10 based on UV-Vis spectroscopy, XRD, TEM, EDX, and FTIR results which revealed the characteristic property of the synthesized nanoparticle. Temperature plays an important role in regulating the activity of the fungus and the movement of the ions (Priyadarshini et al. 2014). The synthesis of nanoparticles with a greener approach requires temperatures lesser than 100 °C or ambient temperature. They found that increase in temperature of the reaction results in a decrease of the nanoparticle size but an increase in mono-dispersity. Incubation time is also another important factor affecting the synthesis of fungal nanoparticles. The period in which the reaction medium incubates greatly enhances the type of nanoparticle synthesized and the quality. The incubation time might occur in different ways such as the particles may aggregate because they are stored for a longer time; therefore, the potential is affected (Baer 2011).

20.6 Mechanisms Behind Myco-Nanoparticles Synthesis

Several promising mechanisms have been recommended for the development of metal nanoparticles, but no such mechanism has been known yet and extensive research is still needed. According to few studies, mainly the cell wall and sugar component of the fungal cell wall involve in the process of bio-reduction of the metallic ions. Nanoparticles are formed on the exterior of a fungal cell wall, and the very basic step is bio-reduction to trap the metallic ions. The electrostatic interactions between the charged group on the cell wall surface and metal ions followed by metal ions enzymatic reduction leads to the accumulation and formation of nanoparticles (Birla et al. 2009). Fungal cell wall proteins play a substantial role in the formation of metal nanoparticles. Fungi secrete hydrolyzing the protein in an acidic condition that binds with metal to form the metal NPs. These NPs forming proteins are cationic with a molecular weight of 55 kDa. *Verticillium* sp. also produces these cationic proteins which might be the cause of hydrolysis of ferric ions. Tryptophan and tyrosine are the amino acids that play a pivotal role in the bio-reduction of metal ions to metallic nanoparticles. NADH-dependent enzymes and the fungal proteins are also involved in metal ion reduction (Germain et al. 2003). Fungi are a fascinating source for the green synthesis of nanoparticles owed to their metal bioaccumulation capacity. Furthermore, fungi are easy to grow in the laboratory, and production of a large quantity of biomass make them valuable to be used in the green synthesis of NPs. Biosynthesized nanoparticles are eco-friendly and are bio-compatible for pharmacological uses. Biosynthesis of nanoparticles by fungi as a base material is a reasonable approach (Fig. 20.5). Fungal enzymes possess high redox potential which makes them more suitable for the redox reaction (Gopal et al. 2009) for the conversion of metallic ions into specific nanoparticles (Kumar et al. 2011). So, the green synthesis of nanoparticles is now an attractive field around the globe. Phyto-pathologists are working in search of techniques to protect economically important

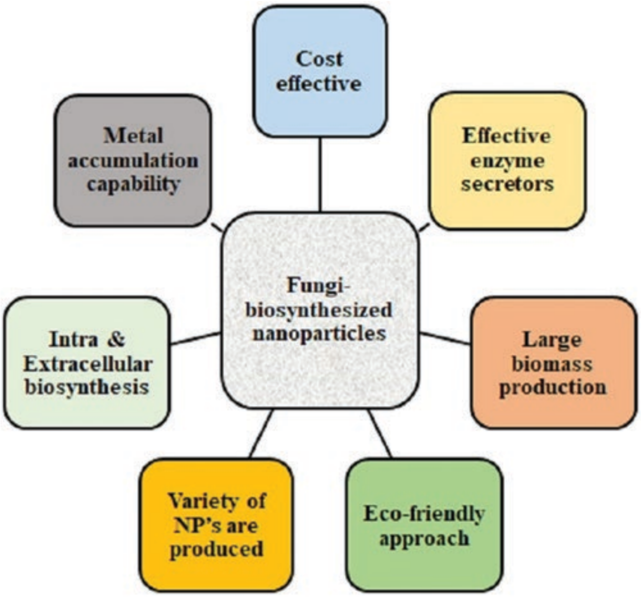

Fig. 20.5 Advantages of fungi as bio-industries for nanoparticle synthesis

crops from destructive plant pathogens. Nanotechnology provides an alternative way for the production of pesticides and fertilizers which are safer for the environment. Nanoparticles are found effective against pests, nematodes, and fungal plant pathogens.

20.7 Application of Myco-Nanoparticles in Agriculture

Nano-formulations pose lots of benefits in agriculture (Fig. 20.6). Myco-nanoparticles find a wide range of applications from improving growth, protection from pests and undesired fungi, providing nutrition in form of fertilizers, elimination of weeds, enhancing the efficiency of hydroponics last but not the least in the advanced monitoring devices for crop rotation and environmental stresses (Fig. 20.7). These mentioned applications are explained in detail.

20.7.1 Plant Germination and Growth

In the last few decades, many researchers have studied the effects of nanoparticles on plant germination and growth with the target to endorse its use for agricultural improvements. Nano-TiO_2-treated seeds, over a germination period of 30 days,

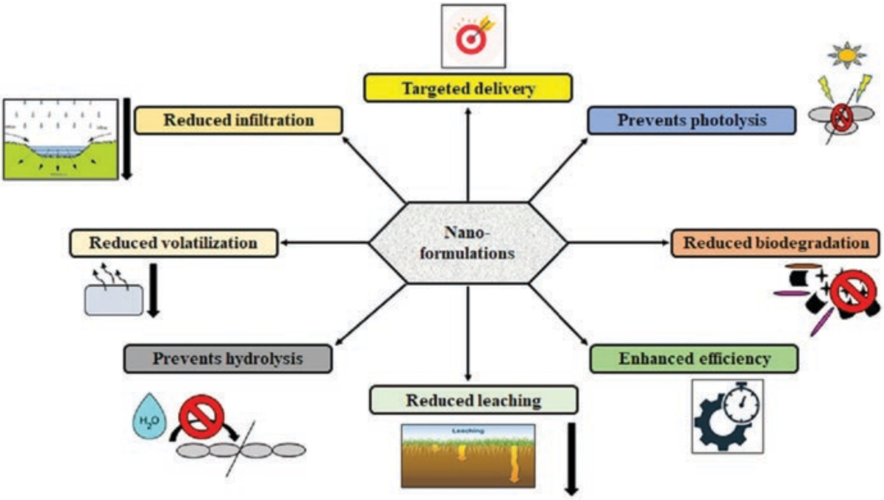

Fig. 20.6 Benefits of nano-formulations in agriculture

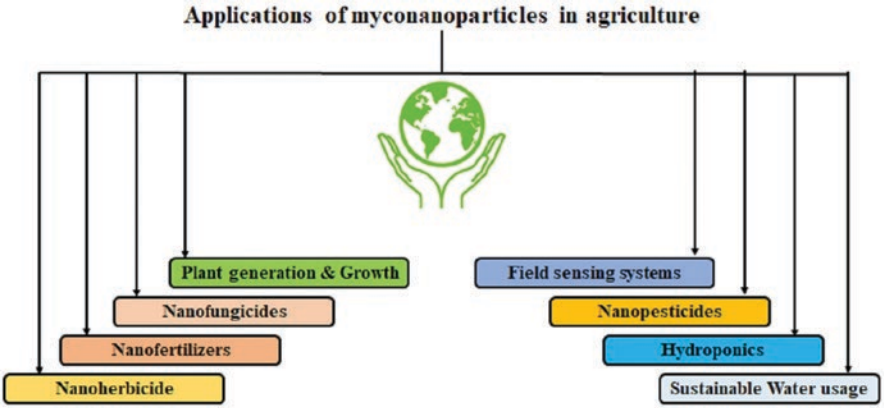

Fig. 20.7 Applications of myco-nanoparticles in agriculture

produced plants with 73% more dry weight, three times higher photosynthetic rate, and a 45% increase in chlorophyll-a formation compared to that of the control. It was found that the growth rate of spinach seeds was inversely proportional to the material size signifying that the smaller the nanomaterials the better the germination (Khodakovskaya et al. 2009). According to them, the nano-sized TiO_2 penetrated the seed resulting in the increased seed germination and absorption of inorganic nutrients, fastened the breakdown of organic substances, and also caused quenching of oxygen free radicals formed during the photosynthetic process, hence increasing the photosynthetic rate. Studies on the influence of metal nanoparticles (Si, Pd, Au, Cu) on germination of lettuce seeds indicate that nanoparticles (at different concentrations) had a positive influence on seed germination. The influence was measured in terms of the shoot-to-root ratio and growth of the seedling. The controlled liberation of active plant growth stimulators and other chemicals encapsulated in nanocomposites made of layered double hydroxides (anionic clays) could be the feasible option for organic agriculture.

The consequences of the use of nanoparticles on plants can be positive or negative (Aguilar-Méndez et al. 2011). One of the important consequences for nanomaterials' applications in seed germination is their phytotoxicity. The type and concentration of nanomaterial determine the level of phytotoxicity. The applicability of fluorescein isothiocyanate (FITC)-labeled silica nanoparticles and photostable cadmium-selenide (CdSe) quantum dots were used as bio-labels for promoting and assessment of seed germination. They found that FTIC-labeled silica nanoparticles induced seed germination in rice, whereas quantum dots arrested the seed germination. Root length of radish, rape canola, ryegrass, lettuce, corn, and cucumber species were found to inhibit the use of 200 mg/L nano-Zn and ZnO. The phytotoxic behavior of the nanomaterials needs to be meticulously understood before utilizing under field conditions. It has always been a debate regarding the applicability and phytotoxicity of silver nanomaterials in agriculture (Nair et al. 2011).

The citrate-coated colloidal Ag-NPs were not genotoxic (genetic), cytotoxic (cell), and phototoxic (toxicity through photodegradation) to humans; however, citrate-coated silver nanoparticles in powder form were found to be toxic. It was also found that the phototoxicity of the powdered Ag-NPs was interestingly repressed when they were coated with biocompatible polyvinyl pyrrole. Exploring such biocompatible coatings to reduce or inhibit the toxicity of nanomaterials would increase the chances of applying nanomaterials in plant germination and growth. However, it is also needed to explore the undesirable effect of such coatings on the desired seed or plant properties and the effectiveness of nanomaterials (Lu et al. 2010).

20.7.2 Nanofungicides

The common plant pathogens are fungi compared to viruses and bacteria (Zameer et al., 2016, 2010a, b). These plant pathogens such as species of *Aspergillus, Fusarium,* and *Phytophthora* can be used as a nanomaterial for the synthesis of nanoparticles. The silver nanoparticle can be used as an effective antifungal agent in the treatment of different plant pathogens (Manjunatha et al. 2013; Singh et al. 2016). In-vitro assay was performed on a Petri dish. They used 18 plant pathogenic fungi for treating the silver nanoparticles on malt extract agar, potato dextrose agar, and cornmeal agar plates. The fungal inhibition was calculated to evaluate the antifungal activity of silver nanoparticles against the pathogens. The results revealed that silver nanoparticles possess antifungal properties against these plant pathogens at different levels (Yadav et al. 2015).

In vitro as well as field conditions trials were conducted. They demonstrated the effects of silver nanoparticles using *Colletotrichum* species and pepper anthracnose disease. Silver nanoparticle solution at different concentrations, viz., 10, 30, 50, and 100 ppm, were used for the in vitro assay. The maximum inhibition rate was at 100 ppm silver nanoparticles solution with a percentage of 93.50%, while the lowest inhibition was found to be 11.33% at 10 ppm. In the case of field trial analysis, an experiment was conducted before and after the pepper was infected. Two positive controls, i.e., commercial fungicide NSS-F and chemical fungicide Feneri, were used. The leaves of the plants were treated with silver nanoparticles 3–4 weeks before and after the outbreak of the disease. Results were analyzed 1 and 4 weeks after the final treatment respectively (Lamsal et al. 2011). Each experiment was performed in triplicates. Moreover, after the disease outbreak treatments, disease incidence was higher compared to before with NSS-F 72.1% and Fenari 63.4%. The lowest disease incidence was noticed on plants treated with 50 ppm silver nanoparticles before the disease outbreak with 9.7%, whereas the highest disease incidence was observed on plants treated with NSS-F after the disease outbreak with 72.1%. The results show that, before the outbreak of the disease, silver nanoparticle treatment was applied which suppressed the pathogen attack (Lamsal et al. 2011). Silica-based silver nanoparticles were prepared, and antifungal activity was performed against *Rhizoctonia solani*, *Botrytis cinerea*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides,* and *Pythium ultimum*. The antifungal effect was performed on powdery mildew in the field. The results suggest that since silver and silica are non-toxic and safe for human health, the cost is much lower than the commercial fungicide (Bhattacharyya et al. 2016). This nano-formulation is highly useful for managing different fungal plant diseases (Prasad et al. 2012).

20.7.3 Nano-pesticides

Plant diseases have reduced agriculture production. Various methods have been employed in combating the different diseases of plants such as natural or artificial methods. Excessive use of pesticides can cause environmental hazards. Therefore, scientists have been investigating the replacement of chemical-based pesticides. Due to its durability, and high efficacy, nano-pesticides represent the next-generation pesticides. Nano-pesticides can be prepared in two ways: organic ingredients which are polymers and inorganic, i.e., metal oxides (Bhattacharyya et al. 2016). Cetyl trimethyl ammonium bromide and copper nitrate were used to synthesize stable copper nanoparticles at room temperature. A significant antifungal activity was determined against three *Fusarium* spp. crop pathogens namely *F. oxysporum*, *F. culmorum,* and *F. equiseti* (Devi et al. 2019). Useful properties of nanomaterials, i.e., stiffness, solubility, thermal stability, permeability, crystallinity, and biodegradability are needed for formulating nano-pesticides. They possess a larger surface area to volume ratio and a greater absorption rate and higher affinity to the target. The use of these nano-pesticides can reduce the runoff of organic solvents and the movement of overdosed pesticides by increasing the dispersion of these formulations (Devi et al. 2019; Yadav et al. 2015).

The rapid development in nano-pesticide research over the last few years has stimulated several international organizations to consider potential issues relating to the use of nanotechnology for crop protection. Microorganisms have caused marvelous ecological changes. This is the result of the ingress of new diseases into the environment leading to the disease and death of plants. Crops and forestry should, therefore, be protected against the invasions of insects, pests, and pathogens (Bergeson 2010). A sustainable mechanism for disease control is therefore essential, and the improvement of nano-pesticides can facilitate the control plant diseases. Nano-pesticides enhance the diffusion and wettability of agricultural formulations. The different delivery techniques of nano-pesticides, like nano-emulsions, nano-encapsulates, nano-containers, and nano-cages are used for plant protection. Recently, nanotechnology has increased the effectiveness of Ag-NPs because of its antimicrobial activity and non-toxicity to humans. A mechanism for disease control is therefore required (Archarya et al. 2014). Ag-NPs caused damage to fungal hyphae of *Raffaelea* sp. causing oak wilt, by reducing microbial absorption, and increased inhibition of fungal growth and conidial germination. Similarly, it was found that Ag-NPs inhibited the hyphal growth of *R. solani*, *S. sclerotiorum,* and *S. minor* in a dose-dependent manner in vitro (Kar et al. 2014). The antifungal activity of different forms of silver ions and NPs against *B. sorokiniana* and *M. grisea* was observed. They found that both silver ions and Ag-NPs could manipulate the colony formation of spores and disease progress of phytopathogenic fungi (Bergeson 2010; Aziz et al. 2016). These results advocate that Ag-NPs may have a huge impend of nano-pesticides on the control of phytopathogens.

20.7.4 Nano-fertilizers

Fertilizers are those vital nutrients in disguise for the growth of the plants. Excessive consumption and continuous use of chemical fertilizers decrease the fertility of the soil and crop production. Therefore, nano-fertilizers can replace in regaining and protecting the fertility of the soil. The use of nano-fertilizers leads to an increase in nutrient efficiencies and reduces the toxicity of the soil. Zinc nanoparticle was employed as a nano-fertilizer in pearl millet *Pennisetum americanum L.* for enhancing crop production. Zinc oxide solution reacted with the fungus *R. bataticola* for 62 h resulted in the extracellular synthesis of high mono-dispersed zinc nanoparticles with an average size of 18.5 nm as confirmed by transmission electron microscopy (Prasad et al. 2017). To determine the effect of the synthesized zinc nanoparticle as a nano-fertilizer, seeds of pearl millet were sown at 3 cm depth in the field. The field experiment was conducted with three treatments such as control, i.e., without any treatment, nanosize, and normal size zinc oxide. After 2 weeks of germination, the foliage was sprayed with the normal size zinc oxide and nano-zinc. Results were observed after 4 weeks of spray in which significant improvement was observed in shoot length, root area, and root length (Prasad et al. 2017; Liu and Lal 2015).

Besides, the chlorophyll content, dry biomass of plant, total soluble leaf protein, dehydrogenase, and enzyme activities of acid phosphatase were also estimated in 6-week-old plants. The hydrothermal method was employed for the synthesis of zinc oxide nanoparticles and was characterized by powder X-ray diffraction, and field emission electron microscopy provided the size range with a diameter of 20–30 cm and is spherical. Zinc oxide nanoparticle was used during the root growth and seed germination of *Cicer arietinum* which resulted in an increased level of Indole acetic acid in the roots, therefore bringing out an increase in the rate of plant's growth (Singhal et al. 2017).

20.7.5 Hydroponics

Hydroponics is a technique of agriculture that is not very well known for a common man. But it is also a fact that several fruits and vegetables in superstores are products of hydroponics. It is a branch of agriculture including the technique of growing plants without soil. It is a widely used technique. It is being used for the production of lettuce, tomatoes, cucumber, melons, broccoli, sweet pepper, chilies, and eggplant. Research is also going on the hydroponic procedure of biofuel and fodder crops. Scientists have used nanotechnology to harvest nanoparticles in crops as nutrients. It is the future of man when agricultural land is going to be limited (Sekhon 2014). Nutrient management in agricultural production is increasingly important and is more effective in hydroponic than in soil-based production. Recent work on nano-phosphor-based on electroluminescence lighting devices has shown that its use can reduce energy consumption significantly. Such nanotechnology-based light could reduce energy costs and encourage photosynthesis in indoor, hydroponic agriculture (Witanachchi et al. 2012).

20.7.6 Nano-herbicides

Weeds are unwanted plants growing in soil and taking the essential nutrients which are added for the growth and development of crops to get better yield. These weeds not only survive but spread in the soil through tubers and deep roots. Conventional methods are available to remove weeds as removal by hands, but these are laborious and time-consuming jobs. Weeds in the soil can be destroyed and their germination can be prevented while the conditions become suitable for their growth. For this purpose, nano-herbicides can be used as being very small they will blend with soil easily and eradicate weeds in an eco-friendly way without leaving any toxic residues and prevent the growth of weeds. Herbicides can be applied in the form of an active ingredient combined with any smart delivery system purposed by nanotechnology according to requirement. The use of nano-herbicides can improve crop production by reducing the competition for nutrients between the weeds and plants without harming the agricultural land, environment, and the workers who have direct exposure to weeds while working in the field (Mukherjee et al. 2015).

20.7.7 Sustainable Water Use

Scientists have been working on the optimized use of water in agriculture and its conservation for the future. Various approaches have been introduced, i.e., sprinkler irrigation, gun, and drip irrigation. More precise systems are needed to be developed for the delivery of water in the field. Water holding ability of the soil should be increased to prevent the leaching of water (Cross et al. 2009). Proper distribution of water near the roots is a key factor of maximum water absorption by plants. Nano-hydrogels, a product introduced by nanotechnology, can be used for efficient use of water. These gels can absorb more water than normal soil and release it on demand. These can be used to store rain and irrigation water. It is especially useful in dry areas. This is highly needed as drought is considered the largest environmental risk for crop production. Nanosensors, distributed in the field, can measure the amount of already present water and determine the time of its requirement (Vundavalli et al. 2015). Nanoparticles like zeolite, metal oxides, alumina, silver, zinc, carbon nanotubes and fibers, enzymes, and titanium are also good for water treatments.

20.7.8 Field Sensing Systems to Monitor Crop Condition and Environmental Stresses

Nanomaterials also play an important role in promoting sustainable agriculture and provide better agro-products worldwide. In developing countries, nanotechnology has received significant importance for enhancing agricultural productivity, along with other emerging technologies such as biotechnology including genetic engineering, plant breeding, disease control, fertilizer technology, precision agriculture, and other associated fields. Nanotechnology can be used for combating the plant diseases either by the controlled delivery of functional molecules or as an indicative tool for disease detection (Scott and Chen 2012). Signaling networks of wireless nanosensors placed across cultivated fields afford essential data leading to the best agronomic proficient processes resulting in minimizing resource utilization and maximizing the product output. Such kind of signals can provide information about optimal timing for planting and harvesting crops and the level of water; time of application of agrochemicals like fertilizers, pesticides, and herbicides; and other treatments that need to be administered for a specific plant physiological, pathological, and environmental conditions. The crop nutrient status, insects, pathogens, weeds, moisture level, soil fertility, soil temperature, etc., can be recognized and measured with the help of nanosensors and other field-sensing devices which in turn help in real-time monitoring of the crop growth and provide indispensable data for precision farming practices leading to diminishing agricultural inputs and maximizing resource output and yield (Singh et al. 2015).

20.8 Challenges

While nanotechnology provides a solution to many of the issues faced by the agricultural industry to date, still further research is needed to assuage the questions that the public and policymakers have a concern about the effect of these materials on humans and the ecosystem. Some of the key concerns that plague the application of nanotechnology in the agricultural industry are as follows:

1. Non-target interactions: There is the possibility that these nanoparticles may also interact with non-target surfaces or cells. For instance, if a nanoparticle is being used as an antimicrobial agent, these particles may also act on non-target organisms or even on other compounds creating non-desired outcomes (Chaudhary and Misra 2017).
2. Effect on human and environment: Although much research is being directed toward the development of new nanoparticles in various industries, there is insufficient research on determining the effect of these nanoparticles on humans and the environment. Nanotechnology is the way forward as it has enormous potential that remains to be harnessed for the agricultural industry (Singh et al. 2015).
3. Cost effect: This technology is a state of the art and therefore not a cheap alternative to be adopted by all agricultural nations. Investment by the government and the industry players is limited, and this can limit the adoption of this technology. Research in this area also requires funding which is also limited.
4. Public awareness: Making the general public and policymakers aware of the application and providing evidence on the safety and positive outcomes of utilizing this platform is lacking.
5. Regulations and ethical concerns: These are a new platform for the agroindustry, and therefore, regulations need to be in place to ensure all safety procedures have been adhered to and the products using nanomaterials are labeled accordingly.
6. Each one of the abovementioned constraints can deter or slow the process of acceptance of this technology in the agroindustry.

20.9 Future Perspectives

Sustainable agriculture must be taken as an eco-friendly method, where abiotic and biotic-living beings live in accord with coordinated stability of food chains and their related energy balances. New technologies, modernization, increased use of nano-chemicals, specialization, and government policies are adapted to maximize production in agriculture. Nanotechnology helps in the food supply chain (from the field to table: crop production, use of agrochemicals such as nano-fertilizers, nano-pesticides, nano-herbicides, etc., precision farming techniques, intelligent feed, enhancement of food texture and quality, and bioavailability/nutrient values, packaging and labeling, etc.) around the world agricultural sector (Satapathy et al. 2019). The bio-sensors-related nanotechnology has an effective role in insect pest control and food products of agriculture. Consumers always can get actual information on the state of certain food products via intelligent food packaging corporate with nanosensors.

- The properties of nanomaterials such as size, dose, exposure time, surface chemistry, structures, immune response, accumulation, and retention time should be accessed carefully. New analytical methods are needed to develop to detect, validate, and access the effects of each nanomaterial/and nanofood.
- Shelf-life analysis of nanomaterials/nanofoods should be done. Improvement of wide-ranging databank as well as international collaboration for policy, idea, and regulation are needed for manipulation of this knowledge. Additionally, the authorities should provide clear guidelines and roadmaps for reducing risks of the use of nanotechnological products.
- New communication channels and debates should be opened with the participation of different sides such as consumers, researchers, authorities, and industrial sectors to discuss impacts of this technology in human life, economy, and science.

20.10 Conclusion

Mycogenic synthesis of nanoparticles has attracted great interest in recent years, although most of the mechanisms related to their synthesis have not been elucidated yet; it is supposed that fungi will take measures when the toxic ions are present in their growth environment for protection. Since the cell surface of fungal biomass is of a negative charge, and the secretion of cells is sticky, the ions will get adhered to the cells due to the electrostatic interaction. The functional reducing agents, metabolites, and enzymes released by fungi to convert the toxic ions into non-toxic matters may have a specific role in nanoparticle synthesis. Besides, it cannot rule out the possibility that the nanoparticles were formed due to the precipitation. The better control of particle size, shape, and mono-dispersity of nanoparticle synthesis by fungi is still being sought. In terms of the results of related studies, it can be understood that variations of fungal strains, growth medium, and synthesis conditions are responsible for the size and mono-dispersity of nanoparticles.

The mycological methods to produce nanoparticles are still in the developing stage. Extracellular methods are appropriate for entrapment and immobilization of nanomaterials on the desired support. Intracellular methods may be suitable for bio-inorganic composite films. The strategy of the utilization of enzymes secreted by the fungi for subsequent formation of nanoparticles in-vitro opens up the new exciting possibility of biosynthesis of nanoparticles of predefined chemical composition and developing a rational, eco-friendly fungal enzyme-based large scale bioprocess for nanoparticle synthesis. With the recent progress in improving nanomaterial(s) (QDs, nano-wires, nano-emulsions, nano-sensors, nano-fibrous mats, nano-biopesticides) synthesis efficiency, exploring their applications in agro-industry and assessment of environmental risks associated with these particles, it is hopeful that the implementation of myco-nanotechnology strategies on a large scale and their commercial applications in agriculture and allied sectors will take place in the coming years.

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