

# Chapter 1

## Endophytic Fungi: Biodiversity, Ecological Significance, and Potential Industrial Applications



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**Abstract** Endophytic fungi are abundant and have been reported from all tissues such as roots, stems, leaves, flowers, and fruits. In recent years, research into the beneficial use of endophytic fungi has increased worldwide. In this chapter, we critically review the production of a wide range of secondary metabolites, bioactive compounds from fungal endophytes that are a potential alternative source of secondary plant metabolites and natural producers of high-demand drugs. One of the major areas in endophytic research that holds both economic and environmental potential is bioremediation. During their life span, microbes adapt fast to environmental pollutants and remediate their surrounding microenvironment. In the last two decades, bioremediation has arisen as a suitable alternative for remediating large polluted sites. Endophytic fungi producing ligninolytic enzymes have possible biotechnological applications in lignocellulosic biorefineries. This chapter highlights the recent progress that has been made in screening endophytic fungi for the

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production and commercialization of certain biologically active compounds of fungal endophytic origin.

## 1.1 Introduction

Microbes such as fungi, bacteria, cyanobacteria, and actinomycetes belonging to a class of plant symbionts residing within plant tissue are referred to as “endophytes” (De Bary 1866). From the germination of seeds to the development of fruits, endophytic microorganisms are associated with different parts of the plant, such as the spermosphere (in seeds), rhizosphere (roots), caulosphere (in stems), phylloplane (in leaves), anthosphere (in flowers), and laimosphere and carposphere (in fruits) (Clay and Holah 1999). To adapt to abiotic and biotic stress factors, endophytic microbes produce bioactive substances (Guo et al. 2008). The associations of endophytic microbes with plants, and in many cases their tolerance to biotic stress factors, have correlated with fungal natural products or biologically active metabolites, such as enzymes, phytohormones, nutrients, and minerals, and also enhance the resistance of the host against herbivores, insects, disease, drought, phytopathogens, and variations in temperature and salinity (Breen 1994; Brem and Leuchtman 2001; Schulz et al. 2002). Endophytic microbes enhance the resistance of plants to abiotic stress factors such as increasing drought tolerance, high temperature, low temperature, low pH, high salinity, and the presence of heavy metals in the soil (Jalgaonwala et al. 2017). On the other hand, plants provide a protective environment for the growth and multiplication of endophytic microbes, protection from aridness, and longevity via seed transmission to the next generation of host (Khan et al. 2015). One widespread phenomenon in nature is the symbiotic association between fungus and plant.

Initial information about fungal endophytes was found during the year 1904, from endophytes isolated from the seeds of darnel ryegrass (Bezerra et al. 2012; Freeman 1904). Endophytic fungi are a diverse and useful group of microorganisms reported to colonize plants in different parts of world, such as the Arctic (Fisher et al. 1995) and Antarctic (Rosa et al. 2009), and in geothermal lands (Redman et al. 2002), deserts (Bashyal et al. 2005), oceans (Wang et al. 2006b), rainforests (Strobel 2002), mangrove swamps (Lin et al. 2008b), and coastal forests (Suryanarayanan et al. 2005). Various secondary metabolites, for instance, alkaloids, cyclohexanes, flavonoids, hydrocarbons, quinines, and terpenes, have been reported to be synthesized by fungal endophytes and have various biological properties including antimicrobial, antioxidant, antidiabetic, anticancer, antihypercholesterolemic, and antiproliferative activities and cytotoxicity, and they are used in biofuel manufacturing (Fernandes et al. 2015; Naik and Krishnamurthy 2010; Ruma et al. 2013). Endophytic fungi produce various kinds of extracellular enzymes, i.e., hydrolases, lyases, oxidoreductases, and (Traving et al. 2015). In another study, endophytic microbes producing enzymes could help to initiate the symbiotic process (Hallmann et al. 1997). Fungal endophytes have been reported to produce hydrolytic enzymes

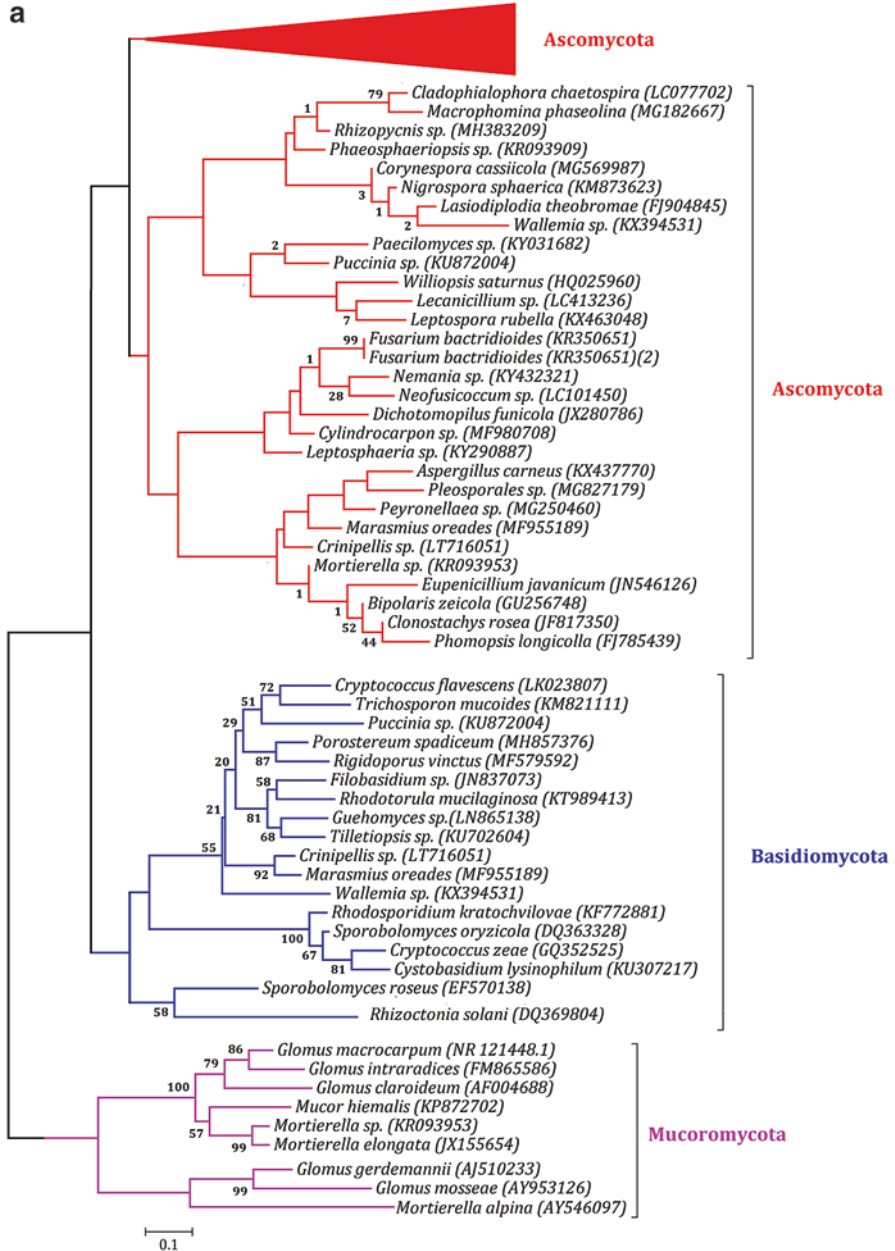
such as cellulase, lipoidase, pectinase, proteinase, and phenol oxidase so as to overcome the defense response against the host (Krishnamurthy and Naik 2017; Naik et al. 2009; Oses et al. 2006). Various organic compounds, for instance, cellulose, glucose, hemicelluloses, keratin, lignin, lipids, oligosaccharides, pectin, and proteins, have been reported to be degraded by the endophytic fungi (Kudanga and Mwenje 2005; Tomita 2003). Endophytic microbes have been reported in almost all plant studies (Suman et al. 2016; Verma et al. 2013, 2014a, 2015a). This chapter describes the biodiversity of endophytic fungi from diverse plants, producing wide groups of extracellular hydrolytic enzymes, bioactive compounds, and secondary metabolites useful for plant growth and soil health for sustainable agriculture, for environment bioremediation, and for different processes in industry.

## 1.2 Biodiversity and Distribution of Fungal Endophytes

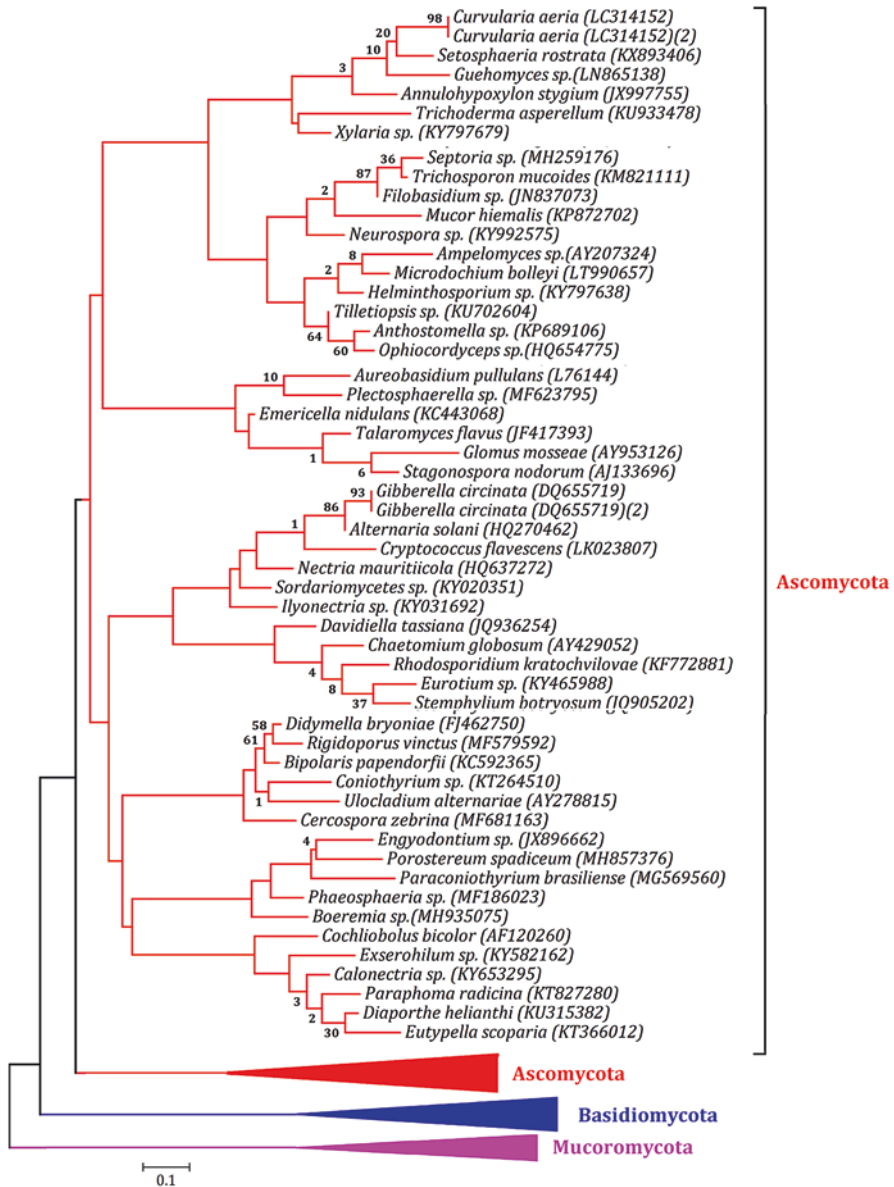
Recently, a greater progress has been made in fungal endophytic research. Fungal endophytes have been found to colonize land plants everywhere on earth. They have been isolated from boreal forests, tropical climates, diverse xeric environments, extreme arctic environments, ferns, gymnosperms, and angiosperms (Mohali et al. 2005; Selim et al. 2017; Šraj-Kržič et al. 2006; Suryanarayanan et al. 2000). Endophytic fungi play an important role in protecting their host from attack by phytopathogens and also facilitate the solubilization of the macronutrients phosphorus, potassium, and zinc; the fixation of atmospheric nitrogen; and the production of various hydrolytic enzymes, ammonia, siderophore, and hydrogen cyanide (HCN) (Maheshwari 2011; Rana et al. 2016a, b, 2017; Verma et al. 2015b, c, 2016a, b).

From a review of the diverse research on endophytic fungi diversity, it can be concluded that reported fungi belong to diverse phyla including Ascomycota, Basidiomycota, and Mucoromycota (Fig. 1.1a). Figure 1.1b presents the biodiversity and abundance of endophytic fungi reported from chick pea, common pea, maize, pigeon pea, rice, soybean, tomato, and wheat. Figure 1.1c presents the relative distribution and biodiversity of endophytic fungi reported from different host plants, showing the common and host-specific endophytic fungi. Figure 1.1d is a Venn diagram showing the endophytic fungal diversity of leguminous and nonleguminous crops. There are many reports of the microbiomes as niche-specific diversity caused by diverse environmental conditions, including low temperature (Yadav 2015; Yadav et al. 2015a, b, 2016, 2017c), high temperature (Kumar et al. 2014; Sahay et al. 2017), salinity (Yadav et al. 2015c, 2018a), drought (Verma et al. 2014a, 2016b), pH (Verma et al. 2013), and multiple extreme conditions (Saxena et al. 2016; Verma et al. 2017; Yadav et al. 2015c, 2018b). Suman et al. (2016) reported niche-specific endophytic microbes from 17 different host plants. Table 1.1 presents the biodiversity of endophytic fungi reported from these diverse host plants.

Impullitti and Malvick (2013) reported fungal endophytes such as *Alternaria* sp., *Cladosporium* sp., *Davidella* sp., *Diaporthe* sp., *Epicoccum* sp., *Fusarium* sp., *Phialophora* sp., *Phoma* sp., *Phomopsis* sp., *Plectosphaerella* sp., *Trichoderma* sp.,



**Fig. 1.1** (a) Phylogenetic tree shows the relationship among different groups of endophytic fungi isolated from different host plants. (b) Abundance of endophytic fungi belonging to diverse phyla isolated from different host plants. (c) Diversity and distribution of endophytic fungi of different crops. (d) Venn diagram showing niche-specific microbes reported from leguminous and nonleguminous crops. Wheat (*Triticum aestivum*): (Colla et al. 2015; Comby et al. 2017; Fisher and Petrini 1992; Keyser et al. 2016; Köhl et al. 2015; Larran et al. 2002, 2007, 2018; Ofek-Lalzar et al. 2016; Sieber et al. 1988; Spagnoletti et al. 2017; Wakelin et al. 2004); rice (*Oryza sativa*): (Naik et al. 2009;



**Fig. 1.1** (continued) Potshangbam et al. 2017; Tian et al. 2004; Wang et al. 2016; Yuan et al. 2010); tomato (*Solanum lycopersicum*): (Bogner et al. 2016; Chadha et al. 2015; Larran et al. 2001; Tian et al. 2014); maize (*Zea mays*): (Amin 2013; Köhl et al. 2015; Nassar et al. 2005; Pan et al. 2008; Potshangbam et al. 2017; Renuka and Ramanujam 2016; Saunders and Kohn 2008; Xing et al. 2018); chickpea (*Cicer arietinum*): (Narayan et al. 2017; Singh and Gaur 2017); soybean (*Glycine max*): (de Souza Leite et al. 2013; Fernandes et al. 2015; Hamayun et al. 2017; Impullitti and Malvick 2013; Khan et al. 2011b, 2012b; Rothen et al. 2017; Tenguria and Firodiya 2013; Yang et al. 2014, 2018; Zhao et al. 2018); common bean (*Phaseolus vulgaris*): (dos Santos et al. 2016; Gonzaga et al. 2015; Marcenaro and Valkonen 2016; Parsa et al. 2016; Pierre et al. 2016); pigeon pea (*Cajanus cajan*): (Gao et al. 2011, 2012; Zhao et al. 2012, 2013, 2014)

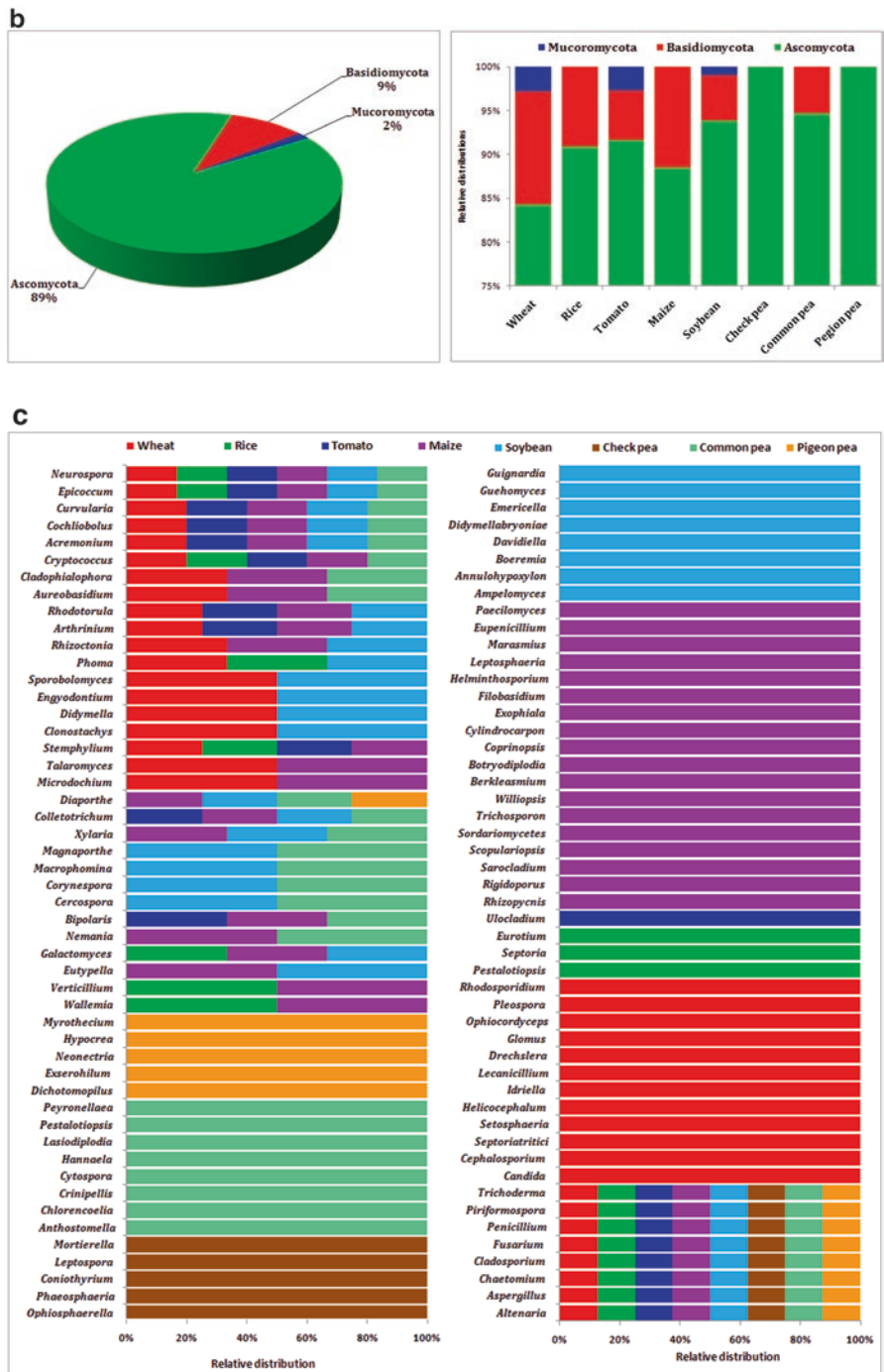
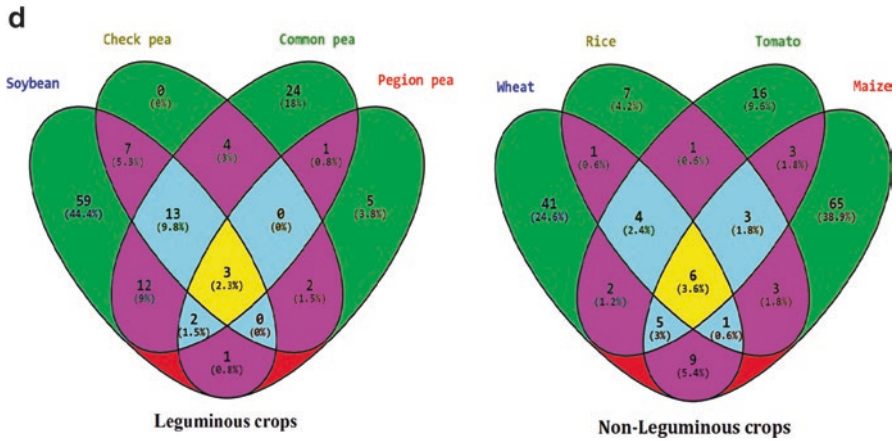


Fig. 1.1 (continued)



**Fig. 1.1** (continued)

and *Verticillium* sp. in soybean plants; these were found by using culture-dependent and culture-independent methods. Tenguria and Firodiya (2013) isolated endophytic fungi, including *Acremonium* sp., *Alternaria alternate*, *Aspergillus* sp., *Colletotrichum* sp., *Emericella nidulans*, *Fusarium* sp., *Penicillium* sp., and *Phoma* sp. from leaves of fresh *Glycine max* collected from the central region of Madhya Pradesh, India. Fernandes et al. (2015) reported the diversity of fungal endophytes in the leaves and roots of *G. max* (dos Santos Souza and dos Santos 2017). In that study, *Ampelomyces* sp., *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides*, *Diaporthe helianthi*, *Guignardia mangiferae*, and *Phoma* sp. were isolated from the leaves, and the dominance of *Fusarium oxysporum*, *Fusarium solani*, and *Fusarium* sp. was greater in the roots (Fernandes et al. 2015). Hamayun et al. (2017) reported *Porostereum spadiceum* AGH786 as a novel gibberellin (GA)-synthesizing fungal endophyte that promoted the growth of soybeans and was capable of producing six types of GAs (Onofre et al. 2013).

Larran et al. (2007) isolated *Alternaria alternata*, *Cladosporium herbarum*, *Epicoccum nigrum*, *Cryptococcus* sp., *Rhodotorula rubra*, *Penicillium* sp., and *Fusarium graminearum* with the highest colonization frequency from wheat (dos Santos Souza and dos Santos 2017). Amin (2013) isolated *Acremonium* sp., *Aspergillus* sp., *Botryodiplodia* sp., *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp. from the roots of *Zea mays* (Azevedo et al. 2000). Chadha et al. (2015) isolated endophytic fungi identified as *Aspergillus niger*, *Aspergillus* sp., *A. versicolor*, *Chaetomium globosum*, *Fusarium fusarioides*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *F. solani*, *Mucor hiemalis*, *Mucor* sp., and *Trichoderma pseudokoningii* from the roots of tomato, and further screened for different plant growth-promoting attributes. All the isolates showed that they were capable of solubilizing phosphorus, 7 showed siderophore production, 4 produced HCN, and 3 produced ammonia. The production of indole acetic acid (IAA) was found to be highest in *Fusarium fusarioides*. Renuka and Ramanujam (2016) determined *Acremonium zeae*, *Coprinosopsis cinerea*, *Fusarium fujikuroi*, *Gibberella moniliformis*, *Nemania* sp.,

**Table 1.1** Biodiversity of endophytic fungi isolated from diverse host plants worldwide

Host plant	Endophytic fungi	Reference
<i>Thuja plicata</i>	Xylaria	Adnan et al. (2018)
<i>Eremophila longifolia</i> , <i>Eremophila maculata</i>	<i>Alternaria</i> , <i>Preussia</i>	Zaferanloo et al. (2018)
<i>Oxalis corniculata</i>	<i>Aspergillus</i> , <i>Fusarium</i>	Bilal et al. (2018)
<i>Populus trichocarpa</i>	<i>Cladosporium</i> , <i>Penicillium</i> , <i>Trichoderma</i>	Huang et al. (2018)
<i>Catolopis procera</i>	<i>Alternaria</i> , <i>Aspergillus</i> , <i>Boeremia</i> , <i>Chaetosphaeromena</i> , <i>Cladosporium</i> , <i>Curvularia</i> , <i>Fusarium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Phoma</i> , <i>Phyllosticta</i> , <i>Pleosporales</i> , <i>Preussia</i> , <i>Pseudodiplodia</i> , <i>Pseudopithomyces</i> , <i>Purpureocillium</i> , <i>Rhizopus</i> , <i>Schizothecium</i> , <i>Talaromyces</i> , <i>Trichoderma</i> , <i>Truncatella</i>	Pieterse et al. (2018)
<i>Pinus wallichiana</i>	<i>Acremonium</i> , <i>Acremonium</i> , <i>Cercospora</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Curvularia</i> , <i>Diplodina</i> , <i>Glomerella</i> , <i>Microascus</i> , <i>Phaeoramularia</i> , <i>Rhodotorula</i> , <i>Xylaria</i>	Nascimento et al. (2015)
<i>Brassica napus</i>	<i>Alternaria</i> , <i>Anthostomella</i> , <i>Aspergillus</i> , <i>Cadophora</i> , <i>Cladosporium</i> , <i>Cochliobolus</i> , <i>Coniochaeta</i> , <i>Coniothyrium</i> , <i>Epicoccum</i> , <i>Fimetiariella</i> , <i>Fusarium</i> , <i>Geopyxis</i> , <i>Lecythophora</i> , <i>Leptosphaeria</i> , <i>Lophiostoma</i> , <i>Lophodermium</i> , <i>Microdiplodia</i> , <i>Neurospora</i> , <i>Nigrospora</i> , <i>Paraconiothyrium</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Preussia</i> , <i>Pseudoplectanitia</i> , <i>Rachicladosporium</i> , <i>Rosellinia</i> , <i>Sclerotagonospora</i> , <i>Sordaria</i> , <i>Sporormiella</i> , <i>Therrya</i> , <i>Tricharina</i> , <i>Trichoderma</i> , <i>Thielavia</i> , <i>Tritirachium</i> , <i>Truncatella</i> , <i>Xylaria</i>	Qadri et al. (2014)
<i>Taxus x media</i>	<i>Acremonium</i> , <i>Alternaria</i> , <i>Arhrinium</i> , <i>Aspergillus</i> , <i>Aureobasidium</i> , <i>Botrytis</i> , <i>Chaetomium</i> , <i>Clonostachys</i> , <i>Cryptococcus</i> , <i>Dioszegia</i> , <i>Dothidea</i> , <i>Dothiorella</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Hypoxylon</i> , <i>Leptosphaeria</i> , <i>Macrophomina</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Periconia</i> , <i>Phoma</i> , <i>Rhizoctonia</i> , <i>Rhizopus</i> , <i>Simplicillium</i> , <i>Sporidiobolus</i> , <i>Sporobolomyces</i>	Zhang et al. (2014)
<i>Stellera chamaejasme</i>	<i>Alternaria</i> , <i>Colletotrichum</i> , <i>Gibberella</i> , <i>Glomerella</i> , <i>Guignardia</i> , <i>Nigrospora</i> , <i>Phoma</i> , <i>Phomopsis</i>	Xiong et al. (2013)
<i>Panax ginseng</i>	<i>Acremonium</i> , <i>Alternaria</i> , <i>Aporospora</i> , <i>Ascochyta</i> , <i>Aspergillus</i> , <i>Bionectria</i> , <i>Botryotinia</i> , <i>Cadophora</i> , <i>Colletotrichum</i> , <i>Dothiorella</i> , <i>Emerickellopsis</i> , <i>Eucasphaeria</i> , <i>Eupenicillium</i> , <i>Fusarium</i> , <i>Geomyces</i> , <i>Ilyonectria</i> , <i>Leptosphaeria</i> , <i>Mucor</i> , <i>Nectria</i> , <i>Neonectria</i> , <i>Paecilomyces</i> , <i>Paraphoma</i> , <i>Penicillium</i> , <i>Schizophyllum</i> , <i>Scytalidium</i> , <i>Sordaria</i> , <i>Sporormiella</i>	Jim et al. (2013)
	<i>Aspergillus</i> , <i>Cladosporium</i> , <i>Engyodontium</i> , <i>Fusarium</i> , <i>Penicillium</i> , <i>Plectosphaerella</i> , <i>Verticillium</i>	Wu et al. (2013)



<i>Kigelia africana</i>	<i>Alternaria</i> , <i>Aspergillus</i> , <i>Botryodiplodia</i> , <i>Chaetomium</i> , <i>Colletotrichum</i> , <i>Curvularia</i> , <i>Drechslera</i> , <i>Fusarium</i> , <i>Mucor</i> , <i>Nigrospora</i> , <i>Nodulisporium</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Rhizopus</i> , <i>Trichoderma</i>	Maheswari and Rajagopal (2013)
<i>Jatropha curcas</i>	<i>Alternaria</i> , <i>Chaetomium</i> , <i>Colletotrichum</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Nigrospora</i>	Kumar and Kaushik (2013)
<i>Glycine max</i>	<i>Alternaria</i> , <i>Annelomyces</i> , <i>Amulohypoxylon</i> , <i>Arthrinium</i> , <i>Cercospora</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Cochliobolus</i> , <i>Colletotrichum</i> , <i>Curvularia</i> , <i>Davidiella</i> , <i>Diaporthe</i> , <i>Dichymella</i> , <i>Epicoccum</i> , <i>Eutypella</i> , <i>Fusarium</i> , <i>Gibberella</i> , <i>Guignardia</i> , <i>Leptospora</i> , <i>Magnaporthe</i> , <i>Myrothecium</i> , <i>Nectria</i> , <i>Neofusicoccum</i> , <i>Nigrospora</i> , <i>Ophiognomonia</i> , <i>Paraconiothyrium</i> , <i>Phaeosphaeriopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Rhodotorula</i> , <i>Sporobolomyces</i> , <i>Stemphylium</i> , <i>Xylaria</i>	de Souza Leite et al. (2013)
<i>Cannabis sativa</i>	<i>Aspergillus</i> , <i>Chaetomium</i> , <i>Eupenicillium</i> , <i>Penicillium</i>	Kusari P et al. (2013a)
<i>Vitis vinifera</i>	<i>Absidia</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Aureobasidium</i> , <i>Botrytis</i> , <i>Cladosporium</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Mortierella</i> , <i>Mucor</i> , <i>Penicillium</i> , <i>Pithomyces</i> , <i>Rhizopus</i> , <i>Trichoderma</i> , <i>Umbelopsis</i> , <i>Zygorhynchus</i>	Pancher et al. (2012)
<i>Trichilia elegans</i>	<i>Cordyceps</i> , <i>Diaporthe</i> , <i>Phomopsis</i>	Rhoden et al. (2012)
<i>Timospora sinensis</i>	<i>Acremonium</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Botryosphaeria</i> , <i>Botrytis</i> , <i>Cladosporium</i> , <i>Chaetomium</i> , <i>Colletotrichum</i> , <i>Curvularia</i> , <i>Drechslera</i> , <i>Emericella</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Humicola</i> , <i>Monilia</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Pseudofusicoccum</i> , <i>Trichoderma</i> , <i>Veronaea</i>	Mishra et al. (2012)
<i>Stryphnodendron adstringens</i>	<i>Alternaria</i> , <i>Arthrobotryx</i> , <i>Aspergillus</i> , <i>Botryosphaeria</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Coniochaeta</i> , <i>Cytospora</i> , <i>Diaporthe</i> , <i>Guignardia</i> , <i>Fimetaria</i> , <i>Massaria</i> , <i>Muscodor</i> , <i>Neofusicoccum</i> , <i>Nigrospora</i> , <i>Paraconiothyrium</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phomopsis</i> , <i>Preussia</i> , <i>Pseudofusicoccum</i> , <i>Sordaria</i> , <i>Sporormiella</i> , <i>Trichoderma</i> , <i>Xylaria</i>	Carvalho et al. (2012)
<i>Sapindus saponaria</i>	<i>Alternaria</i> , <i>Cochliobolus</i> , <i>Curvularia</i> , <i>Diaporthe</i> , <i>Phoma</i> , <i>Phomopsis</i>	Garcia et al. (2012)
<i>Reynoutria japonica</i>	<i>Alternaria</i> , <i>Arthrinium</i> , <i>Bionectria</i> , <i>Colletotrichum</i> , <i>Didymella</i> , <i>Glomerella</i> , <i>Nigrospora</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Phylloticta</i> , <i>Septoria</i> , <i>Xylaria</i>	Kurose et al. (2012)
<i>Piper hispidum</i>	<i>Alternaria</i> , <i>Bipolaris</i> , <i>Colletotrichum</i> , <i>Glomerella</i> , <i>Guignardia</i> , <i>Lasiodiplodia</i> , <i>Marasmius</i> , <i>Phlebia</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Schizophyllum</i>	Orlandelli et al. (2012)
<i>Picea abies</i>	<i>Acephala</i> , <i>Chalara</i> , <i>Cistella</i> , <i>Cladosporium</i> , <i>Entomocorticium</i> , <i>Fomitopsis</i> , <i>Lophodermium</i> , <i>Mollisia</i> , <i>Mycena</i> , <i>Neonectria</i> , <i>Ophiostoma</i> , <i>Phacidomyces</i> , <i>Phacidium</i> , <i>Phialocephala</i> , <i>Rhizoscyphus</i> , <i>Rhizosphaera</i> , <i>Sarea</i> , <i>Scleroconioma</i> , <i>Sirococcus</i> , <i>Valsa</i> , <i>Xylomelasma</i> , <i>Zalerion</i>	Koukol et al. (2012)

(continued)

Table 1.1 (continued)

Host plant	Endophytic fungi	Reference
<i>Opuntia ficus-indica</i>	<i>Acremonium</i> , <i>Aspergillus</i> , <i>Cladosporium</i> , <i>Fusarium</i> , <i>Monodictys</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Tetraploa</i> , <i>Xylaria</i>	Bezerra et al. (2012)
<i>Nyctanthes arbor-tristis</i>	<i>Acremonium</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Drechslera</i> , <i>Humicola</i> , <i>Fusarium</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Phomopsis</i> , <i>Rhizoctonia</i>	Gond et al. (2012)
<i>Ginkgo biloba</i>	<i>Alternaria</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Fusarium</i> , <i>Pestalotiopsis</i> , <i>Peyronellaea</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Phyllosticta</i>	Thongsandee et al. (2012)
<i>Echinacea purpurea</i>	<i>Ceratobasidium</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Fusarium</i> , <i>Glomerella</i> , <i>Mycoleptodiscus</i>	Rosa et al. (2012)
<i>Cinnamomum camphora</i>	<i>Alternaria</i> , <i>Arthrinium</i> , <i>Arthrobotrys</i> , <i>Aspergillus</i> , <i>Chaetomium</i> , <i>Chaetophoma</i> , <i>Cladosporium</i> , <i>Curvularia</i> , <i>Drechslera</i> , <i>Gliomastix</i> , <i>Humicola</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Periconia</i> , <i>Pestalotiopsis</i> , <i>Phacidium</i> , <i>Phomopsis</i> , <i>Phyllosticta</i> , <i>Stachybotrys</i> , <i>Trichoderma</i>	Kharwar et al. (2012)
<i>Acer tataricum</i> subsp. <i>ginnala</i>	<i>Alternaria</i> , <i>Cladosporium</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Neurospora</i> , <i>Penicillium</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Trichoderma</i>	Qi et al. (2012)
<i>Tylophora indica</i>	<i>Alternaria</i> , <i>Chaetomium</i> , <i>Colletotrichum</i> , <i>Nigrospora</i> , <i>Thielavia</i>	Tamura et al. (2011)
<i>Taxus globosa</i>	<i>Alternaria</i> , <i>Aspergillus</i> , <i>Annulohyphylon</i> , <i>Cercophora</i> , <i>Cochliobolus</i> , <i>Colletotrichum</i> , <i>Conoplea</i> , <i>Coprinellus</i> , <i>Daldinia</i> , <i>Hypocrea</i> , <i>Hypoxylon</i> , <i>Lecytophthora</i> , <i>Letendrea</i> , <i>Massarina</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Phialophorophoma</i> , <i>Phoma</i> , <i>Polyporus</i> , <i>Sporormia</i> , <i>Trametes</i> , <i>Trichophaea</i> , <i>Xylaria</i> , <i>Xylomelasma</i>	Rivera-Orduña et al. (2011)
<i>Solanum cernuum</i>	<i>Arthrobotrys</i> , <i>Bipolaris</i> , <i>Botryosphaeria</i> , <i>Candida</i> , <i>Cercospora</i> , <i>Colletotrichum</i> , <i>Coprinellus</i> , <i>Cryptococcus</i> , <i>Curvularia</i> , <i>Diatrypella</i> , <i>Edenia</i> , <i>Eutypella</i> , <i>Fusarium</i> , <i>Glomerella</i> , <i>Leptosphaeria</i> , <i>Mucor</i> , <i>Petriella</i> , <i>Phoma</i> , <i>Meyeromyza</i> , <i>Flavodon</i> , <i>Haplospilus</i> , <i>Hohenbuehelia</i> , <i>Kwonitella</i> , <i>Oudemansiiella</i> , <i>Phanerochaete</i> , <i>Phlebia</i> , <i>Phlebiopsis</i> , <i>Schizophyllum</i>	Vieira et al. (2011)
<i>Lippia sidoides</i>	<i>Alternaria</i> , <i>Colletotrichum</i> , <i>Corynespora</i> , <i>Curvularia</i> , <i>Drechslera</i> , <i>Fusarium</i> , <i>Guignardia</i> , <i>Microascus</i> , <i>Paecilomyces</i> , <i>Periconia</i> , <i>Phoma</i> , <i>Phomopsis</i>	de Siqueira et al. (2011)
<i>Ledum palustre</i>	<i>Arthrinium</i> , <i>Fusarium</i> , <i>Lecytophthora</i> , <i>Penicillium</i> , <i>Sordaria</i> , <i>Sphaerotothyrium</i>	Tejesvi et al. (2011)
<i>Dendrobium thyrsiflorum</i>	<i>Alternaria</i> , <i>Colletotrichum</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Glomerella</i> , <i>Leptosphaerulina</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Rhizopus</i> , <i>Xylaria</i>	Xing et al. (2011)
<i>Dendrobium devonianum</i>	<i>Acremonium</i> , <i>Arthrinium</i> , <i>Cladosporium</i> , <i>Fusarium</i> , <i>Glomerella</i> , <i>Leptosphaerulina</i> , <i>Phoma</i> , <i>Pestalotiopsis</i> , <i>Rhizopus</i> , <i>Trichoderma</i> , <i>Xylaria</i>	Xing et al. (2011)

<i>Aquilaria sinensis</i>	<i>Chaetomium</i> , <i>Cladosporium</i> , <i>Coniothyrium</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Hypocrea</i> , <i>Lasiodiplodia</i> , <i>Leptosphaerulina</i> , <i>Paraconiothyrium</i> , <i>Phaeoacremonium</i> , <i>Phoma</i> , <i>Pichia</i> , <i>Rhizomucor</i> , <i>Xylaria</i>	Cui et al. (2011)
<i>Theobroma cacao</i>	<i>Acremonium</i> , <i>Arthrinium</i> , <i>Aspergillus</i> , <i>Clonostachys</i> , <i>Colletotrichum</i> , <i>Coniothyrium</i> , <i>Curvularia</i> , <i>Cylindrocycladium</i> , <i>Fusarium</i> , <i>Gliocladium</i> , <i>Lasiodiplodia</i> , <i>Myrothectium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Septoria</i> , <i>Talaromyces</i> , <i>Tolypocladium</i> , <i>Trichoderma</i> , <i>Verticillium</i>	Hamada et al. (2010)
<i>Dendrobium loddigesii</i>	<i>Acremonium</i> , <i>Alternaria</i> , <i>Ampelomyces</i> , <i>Bionectria</i> , <i>Cercophora</i> , <i>Chaetomella</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Davidiella</i> , <i>Fusarium</i> , <i>Lasiodiplodia</i> , <i>Nigrospora</i> , <i>Paraconiothyrium</i> , <i>Pyrenochaeta</i> , <i>Sirodesmium</i> , <i>Verticillium</i> , <i>Xylaria</i>	Chen et al. (2010)
<i>Colobanthus quitensis</i>	<i>Aspergillus</i> , <i>Cadophora</i> , <i>Davidiella</i> , <i>Entrophospora</i> , <i>Fusarium</i> , <i>Geomyces</i> , <i>Gyoeffella</i> , <i>Microdochium</i> , <i>Mycocentrospora</i> , <i>Phaeosphaeria</i>	Rosa et al. (2010)
<i>Dracena cambodiana</i> , <i>Aquilaria sinensis</i>	<i>Botryosphaeria</i> , <i>Calcarisporium</i> , <i>Cephalosporium</i> , <i>Colletotrichum</i> , <i>Fusarium</i> , <i>Geotrichum</i> , <i>Gonytrichum</i> , <i>Guignardia</i> , <i>Mortierella</i> , <i>Rhinochladia</i> , <i>Mycelia</i> , <i>Pleospora</i>	Gong and Guo (2009)
<i>Artemisia</i>	<i>Alternaria</i> , <i>Colletotrichum</i> , <i>Phomopsis</i> , <i>Xylaria</i>	Huang et al. (2009)
Medicinal plants	<i>Alternaria</i> , <i>Colletotrichum</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Xylariales</i>	Huang et al. (2008)
<i>Aegle marmelos</i>	<i>Alternaria</i> , <i>Aspergillus</i> , <i>Chaetomium</i> , <i>Curvularia</i> , <i>Drechslera</i> , <i>Emmericella</i> , <i>Fusarium</i> , <i>Nigrospora</i> , <i>Rhizoctonia</i> , <i>Stenella</i>	Gond et al. (2007)

*Penicillium* sp., *Cladosporium oxysporum*, *Rigidoporus vinctus*, *Colletotrichum boninense*, *Sarocladium zeae*, *Epicoccum sorghinum*, *Curvularia lunata*, *Scopulariopsis gracilis*, and *Colletotrichum gloeosporioides* from the leaf, stem, and root fragments of different varieties of maize. Wang et al. (2016) isolated endophytic fungal and bacterial strains from sprouts, stems, and roots simultaneously in rice plants. *Aspergillus*, *Cryptococcus*, *Eurotium*, *Fusarium*, *Penicillium*, *Septoria*, and *Wallemia* were the most frequently detected genera in rice plants. The dominant fungal genera, including *Aspergillus*, *Penicillium*, and *Trichosporon*, coexisted in the stems and roots. Furthermore, *Cryptococcus*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, and *Verticillium* were detected in the sprouts, stems, and roots simultaneously. Xing et al. (2018) isolated *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Bipolaris zeicola*, *Chaetomium murorum*, *Cladosporium sphaerospermum*, *Fusarium proliferatum*, *F. verticillioides*, *Penicillium aurantiogriseum*, *P. oxalicum*, *P. polonicum*, *Sarocladium zeae*, and *Trichoderma gamsii* from maize seeds.

### 1.3 Biotechnological Applications of Endophytic Fungi

Over the past several decades, endophytic fungi separated from numerous plant sources have been recognized as valuable sources of natural products for agronomy, industry, and biomedical development, and also produce extracellular hydrolase enzymes, such as pectinases, cellulases, lipases, amylases, laccases, xylanase, and proteases, as one of the resistance mechanisms against pathogenic organisms and for gaining nutrition from the host. From medicinal plants, endophytic fungi synthesizing hydrolytic enzymes have been reported (Khan et al. 2017; Saxena et al. 2015a; Sunitha et al. 2013; Yadav et al. 2012). Extracellular enzymes target various macromolecules, e.g., lignin, proteins, carbohydrates, sugar-based polymers, to break them down into simpler ones. The production of extracellular enzymes has been measured qualitatively and quantitatively, from using agar plate-based to applying advanced spectrophotometric methods (Yadav et al. 2017a, b).

#### 1.3.1 Bioresources of Hydrolytic Enzymes

Endophytic microorganisms are well known, as they spend the whole of their life cycle inhabiting the inside of tissues in host plants without causing them any obvious harm (Bezerra et al. 2012; Kaul et al. 2013; Tan and Zou 2001; Yadav et al. 2016). The endophytic microbes guard their host plants against attack by other microbes, insects, and herbivore animals, furthermore providing other benefits, for instance, the production of numerous plant growth regulators, enzymes, and other chemical compounds (Azevedo et al. 2000; Bezerra et al. 2012). In addition, these endophytic microbes have also been reported to produce diverse metabolites, including alkaloids, flavonoids, isocoumarin derivatives, peptides, phenolic acids,

phenols, quinones, steroids, and terpenoids (Rana et al. 2016b; Yadav et al. 2015). In recent times, fungal endophytes have become responsive, as they are an appropriate reserve for the degradation of polycyclic aromatic hydrocarbons, which are well known as a toxic class of environmental contaminants (Bezerra et al. 2012; Dai et al. 2010). Additionally, endophytes are also known for the production of various extracellular enzymes, such as cellulases, esterases, lipases, pectinases, proteases, and xylanases, which play an important role in protecting themselves from the defense response of the host or in attaining nourishment from the soil (Bezerra et al. 2012; Suto et al. 2002). Therefore, endophytes are an enormous source of naturally active products that are of marked significance to the agricultural, industrial, and medical sectors (Hazalin et al. 2012). The major industries that utilize microbial enzymes include biomaterials, cellulose, cosmetics, detergents, energy, fine chemicals, food, leather, paper, pharmaceuticals, and textiles, (Bezerra et al. 2012; Suto et al. 2002; Yadav et al. 2015). Table 1.2 shows the diversity and abundance of diverse extracellular hydrolytic enzyme production by different groups of endophytic fungi reported from diverse host plants worldwide.

### 1.3.1.1 Cellulases

Cellulases are basically the enzymes that catalyze cellulolysis, which involves the degradation of the cellulose and certain related polysaccharides. Certain bacteria, fungi, and protozoans are known to synthesize the enzyme (Singh 2006). Different types of cellulases are known that differ from each other structurally and mechanistically, and these include endocellulases, exocellulases, also known as cellobiohydrolases, cellobiases or beta-glucosidases, oxidative cellulases, cellulose phosphorylases. Cellulases from microbes find diverse applications such as use with a supplement of hemicellulases, pectinases, ligninases, and associated enzymes (Adav and Sze 2014). In addition to lignocellulosic bioenergy, cellulase are important in the agricultural, animal feed, brewing, food, laundry, paper and pulp, textile, and wine industries (Adav and Sze 2014; Bhat and Bhat 1997; Mandels 1985; Ryu and Mandels 1980). The most commonly studied cellulolytic fungi include the species of *Aspergillus*, *Humicola*, *Penicillium*, and *Trichoderma* (Sukumaran et al. 2005).

Peng and Chen (2007), obtained 141 isolates of fungal endophytes from the stems of seven oleaginous plant species. These isolates belonged to genera including *Cephalosporium*, *Microsphaeropsis*, *Nigrospora*, *Phomopsis*, and *Sclerocystis*. The oil content of these isolates ranged from 21.3% to 35.0% of dry cell weight. Further, the strains also produced cellulase in addition to microbial oil when cultured on solid-state medium consisting of steam-exploded wheat straw, wheat bran, and water. The yield of cellulase ranged from 0.31 to 0.69 filter paper unit per gram of initial dry substrate. Bezerra et al. (2012) isolated 44 isolates of fungal endophytes from *Opuntia ficus-indica* and assessed their ability to synthesize hydrolytic enzymes such as cellulases, pectinases, proteases, and xylanases. The cellulase producers were identified as *Acremonium terricola*, *Aspergillus japonicas*,

**Table 1.2** Production of hydrolytic extracellular enzymes from fungal endophytes

Fungal endophyte	Enzyme	Plant host	References
<i>Colletotrichum</i> , <i>Fusarium</i> , <i>Phoma</i> , <i>Penicillium</i>	Asparaginase	<i>Cymbopogon citratus</i> , <i>Murraya koenigii</i>	Chow and Ting (2015)
<i>Aspergillus</i>	Amylase		Khan et al. (2017)
<i>Pochonia chlamydsoporia</i>	Protease		Escudero et al. (2016)
<i>Colletotrichum gloeosporioides</i>	Amylase, chitinase, protease	<i>Camellia sinensis</i>	Rabha et al. (2014)
<i>Acremonium</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Cylindrocephalum</i> , <i>Discosia</i> , <i>Drechslera</i> , <i>Fusarium</i> , <i>Fusicoccum</i> , <i>Mycelia sterilia</i> , <i>Myrothecium</i> , <i>Nigrospora sphaerica</i> , <i>Paecilomyces</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phyllosticta</i> , <i>Talaromyces emersonii</i> , <i>Xylaria</i>	Amylase, cellulase, laccase, lipase, pectinase, protease	<i>Alpinia calcarata</i> , <i>Bixa orellana</i> , <i>Calophyllum inophyllum</i> , <i>Catharanthus roseus</i>	Sunitha et al. (2013)
<i>Aspergillus</i> , <i>Bisporus</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Curvularia</i> , <i>Fusarium</i> , <i>Rhizoctonia</i>	Amylase, cellulose, lipase, protease	<i>Azadirachta indica</i> , <i>Citrus limon</i> , <i>Gossypium</i> , <i>Magnolia</i>	Patil et al. (2015)
<i>Cladosporium cladosporioides</i> , <i>Colletotrichum carssipes</i> , <i>C. falcatum</i> , <i>C. gloeosporioides</i> , <i>Curvularia brachyspira</i> , <i>Drechslera hawaiiensis</i> , <i>Lasiodiplodia theobromae</i> , <i>Nigrospora sphaerica</i> , <i>Phyllosticta</i> , <i>Xylariales</i>	Amylase, cellulase, laccase, lipase, protease	<i>Adhatoda vasica</i> , <i>Coleus aromaticus</i> , <i>Costus igneus</i> , <i>Lawsonia inermis</i>	Amirita et al. (2012)
<i>Amanita muscaria</i> , <i>Boletus luridus</i> , <i>Hydnum rufescens</i> , <i>Lactarius cerrimus</i> , <i>Piceirhiza bicolorata</i> , <i>Piloderma byssinum</i> , <i>P. fallax</i> , <i>Russulachloroides</i> , <i>Suillusluteus luteus</i>	Protease	Sporocarp	Nygren et al. (2007)
<i>Colletotrichum</i> sp., <i>Fusarium solani</i> , <i>Macrophomina phaseolina</i> , <i>Nigrospora sphaerica</i>	Amylase, cellulase, protease	<i>Catharanthus roseus</i>	Ayob and Simarani (2016)
<i>Acremonium curvulum</i> , <i>Aspergillus niger</i> , <i>Cochliobolus lunatus</i> , <i>Gibberella baccata</i> , <i>Myrmecridium schulzeri</i> , <i>Myrothecium verrucaria</i> , <i>Penicillium commune</i> , <i>Phoma putaminum</i> , <i>Pithomyces atro-olivaceus</i> , <i>Trichoderma piluliferum</i>	Cellulase, lipase, protease, xylanase	<i>Bauhinia forficata</i>	Bezerra et al. (2015)
<i>Alternaria alternate</i> , <i>Penicillium chrysogenum</i>	Amylase, cellulase	<i>Asclepias sinaica</i>	Fouda et al. (2015)

(continued)

**Table 1.2** (continued)

Fungal endophyte	Enzyme	Plant host	References
<i>Aspergillus terreus</i>	L-asparaginase	<i>Sueada monoica</i>	Kalyanasundaram et al. (2015)
<i>Hebelomaincarnatulum</i> , <i>Laccaria bicolor</i> , <i>Phialocephala fortinii</i> , <i>Umbelopsis isabellina</i>	Protease		Mayerhofer et al. (2015)
<i>Hormonema</i> sp., <i>Neofusicoccum luteum</i> , <i>Neofusicoccum australe</i> , <i>Ulocladium</i> sp.	Laccase	<i>Eucalyptus</i>	Fillat et al. (2016)
<i>Acremonium</i> sp., <i>Alternaria</i> sp., <i>Aspergillus</i> sp., <i>Fusarium</i> sp., <i>Pestalotiopsis</i> sp.	Amylase, cellulase, lipase	<i>Acanthus ilicifolius</i> , <i>Acrostichum aureum</i>	Maria et al. (2005)

*Cladosporium cladosporioides*, *Fusarium lateritium*, *Nigrospora sphaerica*, *Penicillium aurantiogriseum*, *P. glandicola*, *Pestalotiopsis guepini*, and *Xylaria* sp.

Cabezas et al. (2012) isolated 100 fungal endophytes from *Espeletia* sp. and estimated their cellulolytic potential. The research showed that only four isolates could synthesize cellulases, of which *Penicillium glabrum* displayed the highest cellulolytic activity, with the highest CMCase, exoglucanase, and  $\beta$ -glucosidase enzyme activities of 44.5 U/ml, 48.3 U/ml, and 0.45 U/ml respectively. Syed et al. (2013) identified the endophytic fungus *Penicillium* sp. CPF2 (NFCCI 2862). Different substrates were assessed for optimal synthesis of cellulase by CPF2. The best activities for FPase (1.2 IU/ml), endocellulase (19 IU/ml), xylanase (40 IU/ml), and  $\beta$ -glucosidase (2.8 IU/ml) with a protein content of 0.86 mg/ml were detected when cellulose (1.5 % w/v) was used in association with peptone (0.2 % w/v) in the growth medium. Optimal temperature and pH for the extracellular cellulase production were 28 °C and 5.5 °C respectively. Onofre et al. (2013) evaluated the production of cellulases by endophytic fungi, *Fusarium oxysporum* isolated from *Baccharis dracunculifolia*. The results showed that after 55 days of fermentation, the maximum peak of enzyme production with a yield of  $55.21 \pm 10.54$  IU/g of fermented substrate was at pH 5.96.

Patil et al. (2015) screened *Aspergillus* sp., *Bisporus* sp., *Chaetomium* sp., *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., and *Rhizoctonia* sp., isolated from seven medicinal plants and screened both qualitatively and quantitatively for the synthesis of hydrolytic extracellular enzymes, such as amylases, cellulases, lipases, and proteases. The study revealed that *Aspergillus* sp., *Bisporus* sp., *Cladosporium* sp., and *Colletotrichum* sp. showed cellulase production qualitatively, whereas quantitatively, *Rhizoctonia* sp. produced maximum cellulase of about 0.3 U/ml. However, other isolates, including *Bisporus* sp., *Chaetomium* sp., and *Fusarium* sp., exhibited moderate to low activity. Toghueo et al. (2017) reported the fungal endophytes from Cameroonian medicinal plants and screened for their extracellular cellulase activity. The two assays, enzyme and plate-clearing, were used for the screening of effective cellulolytic fungal endophytes. *Penicillium* sp., and *P. chermesimum* were the most effective producers.

### 1.3.1.2 Xylanase

Xylanases are glycosidases comprising endo-1,4-b-xylanase and  $\beta$ -xylosidase and catalyzing the endohydrolysis of 1,4-b- D-xylosidic linkages in xylan (Collins et al. 2005; Thomas et al. 2017). These enzymes basically cause the hydrolysis of the xylan present in the hemicelluloses of plants and convert them into monomeric sugars; this function is not performed alone, but rather with the assistance of certain other hydrolytic enzymes, for instance, acetyl xylan esterase,  $\alpha$ -L-arabinofuranosidase,  $\alpha$ -glucuronidase, and phenolic acid, including ferulic and p-coumaric acid esterase (Collins et al. 2005; Thomas et al. 2017). The chief substrate of xylanases is xylan, which is the key structural polysaccharide of plant cells and the second most abundant polysaccharide in nature, accounting for approximately one third of all renewable organic carbon on earth (Collins et al. 2005; Prade 1996). Xylanases possess numerous applications in the food, de-inking, biofuels, baking, animal feed, and paper and pulp industries (Kumar et al. 2017a; Polizeli et al. 2005; Singh et al. 2016; Suman et al. 2015; Thomas et al. 2017). In the baking industry, xylanases improve the strength of the gluten and ultimately the superiority of the bread as they are capable of absorbing water and collaborating with gluten (Butt et al. 2008; Gray and Bemiller 2003; Harris and Ramalingam 2010; Nuyens et al. 2001). Xylanases are also used with other enzymes to improve the yield of juices from fruit and vegetables; the firmness of fruit pulp; and the regaining of aromas, edible dyes, essential oils, hydrolysis substances, mineral salts, etc. (Polizeli et al. 2005). These enzymes have been reported from different microorganisms such as algae, arthropods, bacteria, fungi, gastropods, and protozoa (Collins et al. 2005).

Wipusaree et al. (2011) isolated 54 endophytic fungi from the Thai medicinal plant, *Croton oblongifolius* Roxb, and screened the isolates for xylanase production. In primary screening, xylanase activity was found in 30 isolates by growing them on solid xylan agar plates. After secondary screening for xylanase activity in xylan liquid culture, the isolate with the highest xylanase production, identified as *Alternaria alternata*, was selected for further evaluation. The study revealed this xylanase to be monomeric, possessing molecular weight of 54.8 kDa. It showed a broadly similar substrate affinity to other xylanases, with a  $K_m$  of 2.37 mg/ml, and was thermostable up to 40 °C. The enzyme was also shown to be inhibited to some extent by all tested divalent metal cations, but especially by  $Hg^{2+}$  and  $Cu^{2+}$ . Sorgatto et al. (2012) characterized xylanase synthesized by the endophytic fungus *Aspergillus terreus*, isolated from *Memora peregrine*. The research revealed an optimal temperature of 55 °C and a pH value of 4.5. The enzyme was thermotolerant at 45 °C and 50 °C, with a half-life of 55 and 36 min respectively. Tasia and Mellawati (2017) found *Acremonium* sp. and a member of the class *Coelomycetes* to be xylanase producers. The study by Marques et al. (2018) also reported *Acremonium* sp., *Botryosphaeria* sp., *Chaetomium* sp., *Cladosporium cladosporioides*, *Colletotrichum crassipes*, *Coniella petrakii*, *Coniothyrium minitans*, *Myrothecium gramineum*, *Paecilomyces* sp., *Phomopsis stipata*, *Saccharicola* sp., *Trichoderma viridae*, and *Ustilaginoidea* sp. to be xylanase producers.



### 1.3.1.3 Lipase

Lipases belong to serine hydrolases and do not require any cofactors. They are involved in diverse conversion reactions, such as transesterification, inter esterification, esterification, aminolysis, alcoholysis, and acidolysis (Gopinath et al. 2013; Panjari et al. 2017; Savitha et al. 2007; Yadav et al. 2017a). Triacylglycerol acyl hydrolases are lipases that are involved in the hydrolysis of fats and oils (Gopinath et al. 2013; Singh and Mukhopadhyay 2012). Lipases are of great importance to the food industry. Phospholipases are being used in treating egg yolk, which is useful for the processing of baby foods, custard, dressings, and mayonnaise; for dough preparation; and for sauces, such as Hollandaise, Béarnaise, and Café de Paris (Aravindan et al. 2007; Reimerdes et al. 2004). Lipase-modified butter fat has extensive applications in different food processes (Aravindan et al. 2007; Uhlig 1998). Chocolates with cocoa butter substitutes, bread, structured lipids such as human milk fat replacers, low calorie health oils, and nutraceuticals are some of lipase-mediated food products available (Aravindan et al. 2007). The addition of lipases to noodles results in appreciably softer textural characteristics (Undurraga et al. 2001). Furthermore, lipases are also used to increase the flavor content of bakery products (Ray 2012).

Lipases are produced by bacteria, yeasts, protozoans, molds, and even viruses are known to encode genes for lipases (Abrunhosa et al. 2013; Anbu et al. 2011; Ginalska et al. 2004). The production of lipases has been demonstrated in ascomycetes and coelomycetes (Gopinath et al. 2013). Lipolytic activity has been shown in *Rhizopus* sp., *Penicillium* sp., *Mucor* sp., *Lipomyces starkeyi*, *Humicola lanuginosa*, *Cunninghamella verticillata*, *Candida rugosa*, *Acremonium strictum*, and *Aspergillus* sp. (Tsujioka et al. 1973; Jacobsen et al. 1990; Petrović et al. 1990; Sztajer and Maliszewska 1989). Microbial lipases are of commercial importance because of the broader availability, greater stability, and low production costs compared with plant and animal lipases.

Torres et al. (2003) rendered a mycelium-bound lipase from *Rhizopus oryzae* that catalyzed the esterification of fatty acids in iso-octane. The enzyme was active over the entire pH range studied, from pH 3 to pH 8, but maximal activity was obtained at pH 4 and pH 7. The study by Costa-Silva et al. (2011) deals with improvement in the production and stabilization of lipases from the endophytic fungi *Cercospora kikuchii* isolated from *Tithonia diversifolia*. Amirita et al. (2012) reported *Colletotrichum falcatum*, *Curvularia brachyspora*, *Curvularia vermiformis*, *Drechslera hawaiiensis*, and *Phyllosticta* sp. to be producers of lipase enzymes from different medicinal plants. Panuthai et al. (2012) screened 65 endophytic fungal isolates for the production of lipases, of which only 10 were found to produce extracellular lipases, with *Fusarium oxysporum*, isolated from the leaves of *Croton oblongifolius* Roxb. (Plao yai), yielding the highest level. The enriched lipase showed optimal activity at 30 °C and pH 8, and was reasonably stable up to 40 °C and at a pH of 8.0–12. Venkatesagowda et al. (2012) isolated species of *Trichoderma*,

*Stachybotrys*, *Sclerotinia*, *Rhizopus*, *Phyllosticta*, *Phomopsis*, *Phoma*, *Pestalotiopsis*, *Penicillium*, *Mucor*, *Lasiodiplodia*, *Fusarium*, *Drechslera*, *Curvularia*, *Colletotrichum*, *Cladosporium*, *Chalaropsis*, *Aspergillus*, and *Alternaria*, showing strong lipolytic activity. Sunitha et al. (2013) isolated lipase-producing *Acremonium implicatum*, *Alternaria* sp., *Aspergillus niger*, *Chaetomium* sp., *Colletotrichum falcatum*, *C. gleosporoides*, *C. truncatum*, *Cylindrocephalum* sp., *Drechslera* sp., *Fusarium oxysporum*, *Isaria* sp., *Mycelia streilia* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Phoma* sp., *Phomopsis longicolla*, and *Xylaria* sp. from *Alpinia calcarata*, *Bixa orellana*, *Calophyllum inophyllum*, and *Catharanthus roseus*. Fareed et al. (2017) revealed *Aspergillus calidouustus*, *A. fumigatus*, *Microsporium gypseum*, *Penicillium marneffeii*, *P. viridicatum*, and *Trichophyton tonsurans* to be lipase producers.

#### 1.3.1.4 $\beta$ -glucosidase

*Periconia* sp. produce a thermotolerant  $\beta$ -glucosidase. This enzyme shows high activity toward cellobiose and carboxymethylcellulose.  $\beta$ -glucosidase hydrolyzes rice straw into simple sugars. Hydrolytic enzymes have the potential to convert lignocellulosic biomass to biofuels and chemicals (Harnpicharnchai et al. 2009). The major decomposers of lignocelluloses are fungi, which play an essential role in the cycling of carbon and other nutrients. Exo- and endoglucanases, exo- and endoxylanases,  $\beta$ -xylosidases, and  $\beta$ -glycosidase are the main hydrolytic enzymes involved in the degradation of lignocelluloses (Van Dyk and Pletschke 2012).

#### 1.3.1.5 Tannases

Tannases comprise two classes of enzymes, tannin acyl hydrolases and ellagitannin acyl hydrolases, also called ellagitannases. Tannin acyl hydrolases are used in the beverage, food, leather, and pharmaceutical industries (González et al. 2017). Vegetable and animal tissues are easily available sources of tannases; however, on an industrial scale, microbial sources are preferred. Tannases have been obtained from fungi, including *Aspergillus* sp., *Paecilomyces variotii*, and *Penicillium* sp. (Battestin and Macedo 2007; González et al. 2017). There are some reports of tannase production by endophytic fungi. Cavalcanti et al. (2017) isolated 16 endophytic fungal strains and screened them for the production of tannases. All the isolates produced tannases, with *Aspergillus fumigatus* and *A. niger* being the highest producers. The study revealed that the optimal temperature and pH of enzymes from the two strains were 30 °C and 4.0 respectively.

### 1.3.1.6 Pectinases

Pectinase is an enzyme that actually breaks down pectin, which is a polysaccharide found in plant cell walls. This enzyme has shown a robust rise on the market and has also held a leading position amongst commercially produced industrial enzymes (Garg et al. 2016). In the industrial sector, this enzyme plays an important role in decreasing viscosity and improving yield (Garg et al. 2016; Makky and Yusoff 2015). In the processing of citrus juice, the enzyme helps to eliminate the cloudiness of the juice and stabilize it (Braddock 1981; Garg et al. 2016).

In wine processing, pectinases are used to promote filtration, increase the juice yield, and strengthen the flavor and color (Chaudhri and Suneetha 2012; Garg et al. 2016). Additionally, in biorefineries, pectinases used to hydrolyze pectin are present in agro-industrial waste (Biz et al. 2014; Garg et al. 2016). The agro-waste is converted into simple sugars and bioethanol, or could also be used as fermentable sugars (Alshammari et al. 2011; Garg et al. 2016). The fermentation of tea can be speeded up by breaking down the pectin present in the cell walls of tea leaves (Garg et al. 2016). Further, pectinases are used in textile processing, the extraction of vegetable oil, the processing of animal feed, the biobleaching of kraft pulp, and the recycling of wastepaper (Garg et al. 2016). The most important sources of pectinases include bacteria, fungi, and plants, and recently microbial pectinases have been gaining a lot of attention.

Sunitha et al. (2013) reported *Acremonium implicatum*, *Aspergillus fumigatus*, *Colletotrichum gleosporoides*, *Coniothyrium* sp., *Cylindrocephalum* sp., *Drechslera* sp., *Fusarium chlamydosporum*, *F. oxysporum*, *Fusicoccum* sp., *Nigrospora sphaerica*, *Paecilomyces variotii*, *Pestalotiopsis disseminata*, *Phoma* sp., *Pyllosticta* sp., *Talaromyces emersonii*, and *Xylaria* sp. to be pectinase producers isolated from *Alpinia calcarata*, *Bixa orellana*, *Calophyllum inophyllum*, and *Catharanthus roseus*. Fouda et al. (2015) isolated pectinase producers, including *Alternaria alternata*, *Penicillium chrysogenum*, and the third fungal strain, described as sterile hyphae from the medicinal plant of *Asclepias sinaica*. Heidarizadeh et al. (2018) produced pectinases from *Piriformospora indica*. After 6 days, the maximum dry cell weight was 10.21 g/L, the growth yield was about 0.65 g/g, the specific growth rate 0.56 day<sup>-1</sup>, and pectinase activity was found to be 10.47 U/mL on pectin-containing medium (P<sup>+</sup>). In another case of pectin-free medium (P<sup>-</sup>), all parameters were kept lower than for P<sup>+</sup> medium. It was found in the study that the synthesis of pectinase on P<sup>+</sup> was 2.7 times greater than on the P<sup>-</sup> medium (Maheshwari 2011). About 5 and 50 °C are the ultimate pH and temperature required for polygalacturonase activity respectively (Kirti and Reddy 2013; Singh 2006). Indeed, this is the leading note of synthesis of pectinase by *Piriformospora indica*; the optimal pH of enzyme was additionally submitted and noted as a would-be contender for imminent use in the fruit juice industries (Bezerra et al. 2012; Mercado-Blanco et al. 2016). Uzma et al. (2016) reported *Aspergillus* sp., *Cladosporium* sp., *Colletotrichum*

sp., *Fusarium* sp., *Mucor* sp., *Mycelia sterilia*, *Penicillium* sp., *Phoma* sp., *Phomopsis* sp., and *Rhizopus* sp. and found that these fungal species exhibited pectinase production attributes (Kaul et al. 2013; Kirti and Reddy 2013).

### 1.3.1.7 Phytases

Phytases, or myoinositol hexakisphosphate phosphohydrolase, are phytate-degrading enzymes. Phytases catalyze the hydrolysis of phytic acid to inositol phosphates, myoinositol, and inorganic phosphate (Gontia-Mishra and Tiwari 2013; Kaur et al. 2017; Kumar et al. 2016, 2017b; Mitchell et al. 1997). Phytases have been gaining a lot of interest and have become a center of focus for scientists and entrepreneurs in the fields of nutrition, environmental protection, and biotechnology (Yadav 2018; Yadav et al. 2017b, d). In plants, these enzymes are usually expressed during seed germination, bring about the degradation of the phytate, provide the growing seedling with orthophosphate, and lower inositol polyphosphates, free myoinositol, and previously bound cations, including  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Zn}^{2+}$ , and hence provide nutrition for plant growth (Gontia-Mishra and Tiwari 2013; Reddy et al. 1989). In animals, phytases play a role in the maintenance of the cell's metabolic reservoirs of inositol hexaphosphate and other inositol polyphosphates. Phytases have many applications. The activity of some yeasts and fungi is generally regarded as safe for consumption by humans and animals, for example, *Saccharomyces cerevisiae* (Gontia-Mishra and Tiwari 2013; Nayini 1984) could be used as a probiotic in a range of food formulations to improve the utilization of phosphate. Phytases can also be utilized in bakery products, especially in the bread-making process (Gontia-Mishra and Tiwari 2013; Haros et al. 2001). The addition of phytase is known to reduce the phytate content in dough and shorten the fermentation time. Further, it improves the bread shape, volume, and softness of the crumb. More phytases are also added in the fractionation of cereal bran, the absorption of iron, and in animal nutrition. In fact, numerous microbial phytases are already on the market and expansively used as animal feed supplements, for instance, phytase from *Aspergillus ficuum* as Natuphos, *A. niger* as Allzyme, *A. awamori* as Finase and Avizyme, *A. oryzae* as AMAFERM, SP, SF, TP, and Phyzyme, and *Peniophora lycii* as Ronozyme, Roxazyme, and Bio-Feed phytase (Gontia-Mishra and Tiwari 2013). Additionally, phytases are utilized in feed for fish, poultry, and pigs, as bio-fertilizers, in paper manufacturing, and in the wet milling of maize (Gontia-Mishra and Tiwari 2013). Although phytases have been described in plants, animals, and in a range of bacteria, filamentous fungi, and yeasts, here we concentrate primarily on those from endophytic fungi (Venugopalan and Srivastava 2015).

Marlida et al. (2010) obtained 34 isolates of fungal endophytes and screened them for phytase synthesis. Renuka and Ramanujam (2016) reported that phytase production could be achieved only in *Fusarium verticillioides* and *Rhizoctonia* sp., which were also best induced by phytic acid and rice bran compared with other inducers in the submerged fermentation medium used. The phytases produced by *Fusarium verticillioides* and *Rhizoctonia* sp. showed optimal pH of 5.0 and 4.0 respectively. Phytase from *F. verticillioides* showed an optimal temperature of 50 °C

and stability up to 60 °C, optimal pH at 5.0 and pH stability at 2.5–6.0. Mehdipour-Moghaddam et al. (2010) isolated *Azospirillum* strains from rice and wheat and screened the strains for cellulase, pectinase, and phytase activity. The study revealed that the *Azospirillum* strain isolated from rice showed considerably greater phytase activity than that isolated from wheat. In fact, to our knowledge, this is the first study to report phytase activity and its zymogram for *Azospirillum* with different activity profiles exhibited by various isolates.

### 1.3.1.8 Ligninolytic Enzymes

White rot fungi are the most efficient ligninolytic organisms described to date. Owing to the extracellular nonspecific and nonstereoselective enzyme system in white rot fungi, the ability to degrade lignin is more efficient (Barr and Aust 1994). Recently, some microorganisms isolated from the hardwood forests of Zimbabwe (Tekere et al. 2001), Tunisia (Dhouib et al. 2005), Spain (Barrasa et al. 2009), Northern China (Sun et al. 2011a), and Norway (Kim et al. 2015) have been reported in the production of ligninolytic enzymes. For the study of lignin-degrading enzymes in endophytes, different substrates such as ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid), naphthol, and Poly R-478 have been isolated from living plants (Fillat et al. 2016; Oses et al. 2006; Sun et al. 2011a). From tree species *Drimys winteri* and *Prumnopitys andina*, endophytic fungi producing lignocellulolytic enzymes have been isolated. In *D. winteri*, *Bjerkandera* sp. and *Mycelia sterilia* (Dw-2) were identified, whereas in *P. andina*, an unidentified basidiomycete (Pa-1) and also *M. sterilia* (Pa-2) were recognized (Oses et al. 2006). Rodriguez et al. (2009) reported in the forest region that a basidiomycete and a deuteromycete use a combination of enzymes, 1,4-b-D-glucan cellobiohydrolases, endo-1,4-b-D-glucanases, and 1,4-b-D-glucosidase, which break glycoside linkages between B-D-xylopyranosyl and glucopyranosyl residues, thus promoting the biodegradation of wood.

The endophytic community of *Acer truncatum*, the main woody tree species of northern Chinese forests, was investigated, with 17 isolates belonging to the taxa *Alternaria alternata*, *A. arborescens*, *Ascochytopsis vignae*, *Coniothyrium olivaceum*, *Diaporthe* sp. 2, *Drechslera biseptata*, *Glomerella miyabeana*, *Gnomoniella* sp. 1, *Leptosphaeria* sp. 1, *Melanconis* sp. 1, *Melanconis* sp. 2, *Microsphaeropsis arundinis*, *Paraconiothyrium brasiliense*, *Phoma* sp. 4, *P. glomerata*, *Sirococcus clavignenti-juglandacearum*, and *Coelomycetes* sp. reported to oxidize the substrate naphthol (Sun et al. 2011a). The medicinal plants *Adhatoda vasica*, *Costus igneus*, *Coleus aromaticus*, and *Lawsonia inermis* were collected from Sathyamangalam, Tamil Nadu (India) for the isolation of endophytic fungi and screened for the synthesis of laccase enzyme (Kaul et al. 2013; Vasundhara et al. 2016; Venugopalan and Srivastava 2015). The fungal isolates were grown on GYP agar medium amended with 1-naphthol. Out of 12 different species, only two endophytes, *Xylaria* sp. and *Curvularia brachyspora*, were positive in naphthol (Amirita et al. 2012). From the medicinal plants *Alpinia calcarata* Roscoe, *Calophyllum inophyllum* L., *Bixa orellana* L., and *Catharanthus roseus*, 50 strains of fungal

endophyte were isolated. Very few endophytic strains, *Phomopsis longicolla* (Bo13), *Discosia* sp. (Ci5), *Fusicoccum* sp. (Ac26), and *Chaetomium* sp., were able to produce laccase, i.e., showed oxidation of naphthol (Sunitha et al. 2013).

A total of 127 strains of fungal endophytes were isolated from *Eucalyptus globulus* trees of Spain: Cantabria, Asturias 128 (AS), Seville, (SE), Extremadura (EX), and Toledo (TO). Out of 127 strains of endophytic fungi, 21 showed positive ABTS oxidation in an agar plate medium containing ABTS. *Hormonema* sp., *Pringsheimia smilacis*, *Ulocladium* sp., *Neofusicoccum luteum*, and *N. australe* in liquid medium confirmed laccase production. Copper sulfate and ethanol were examined as inducers for increasing the production of laccase. *Pringsheimia smilacis* belonging to the family Dothioraceae were reported for the first time for the production of laccase (Bezerra et al. 2012; Fillat et al. 2016). *Trametes* sp. I-62 was optimized for the production of laccase and was applied to solve problems associated with pulp bleaching. Maximal laccase activity was obtained on the addition of wheat straw and copper sulfate in combination as inducers (Martin-Sampedro et al. 2013). Ligninolytic fungi were collected in Huejutla and characterized as having laccase activity as part of their fundamental enzymatic pool to mineralize lignin. Out of the 100 fungal isolates, 60 had laccase activity, indicating that the isolated fungi have great biotechnological potential (Ramírez et al. 2012).

Two isolates of *Fusarium proliferatum* from different global locations and ecological sites were reported to display similar abilities to degrade natural lignin from wheat (14C-MWL) and synthetic polymers (Anderson et al. 2005). Shi et al. (2004) demonstrated that the fungal endophyte *Phomopsis* sp. almost decays straw by degrading lignin. In another study, laccase and peroxide synthesized by fungal endophytes contributed reliably to the decomposition of litter lignin (Dai et al. 2010; Krishnamurthy and Naik 2017). Various researchers have observed the laccase activity of fungal endophytes in liquid medium: *Phomopsis liquidambari* (Diaporthaceae), *Xylaria* sp. (Xylariaceae), *Fusarium* sp., *F. proliferatum* (Nectriaceae), *Chaetomium* sp., *C. globosum* (Chaetomiaceae), *Podospora anserina* (Lasiosphaeriaceae), *Colletotrichum gloeosporioides* (Glomerellaceae), *Trichoderma harzianum* (Hypocreaceae), *Botryosphaeria* sp., *Neofusicoccum australe*, *N. luteum*, *Botryosphaeria rhodina* (Lasiodiplodia theobromae), *Botryosphaeria obtuse*, *B. dothidea*, *B. ribis* (Botryosphaeriaceae), *Monotospora* sp. (Hysteriaceae), and *Hormonema* sp. (Dothioraceae) (Anderson et al. 2005; Durand et al. 2013; El-Zayat 2008; Fillat et al. 2016; Muthezhilan et al. 2014; Sara et al. 2016; Srivastava et al. 2013; Urairuj et al. 2003; Wang J et al. 2006a; Xie and Dai 2015).

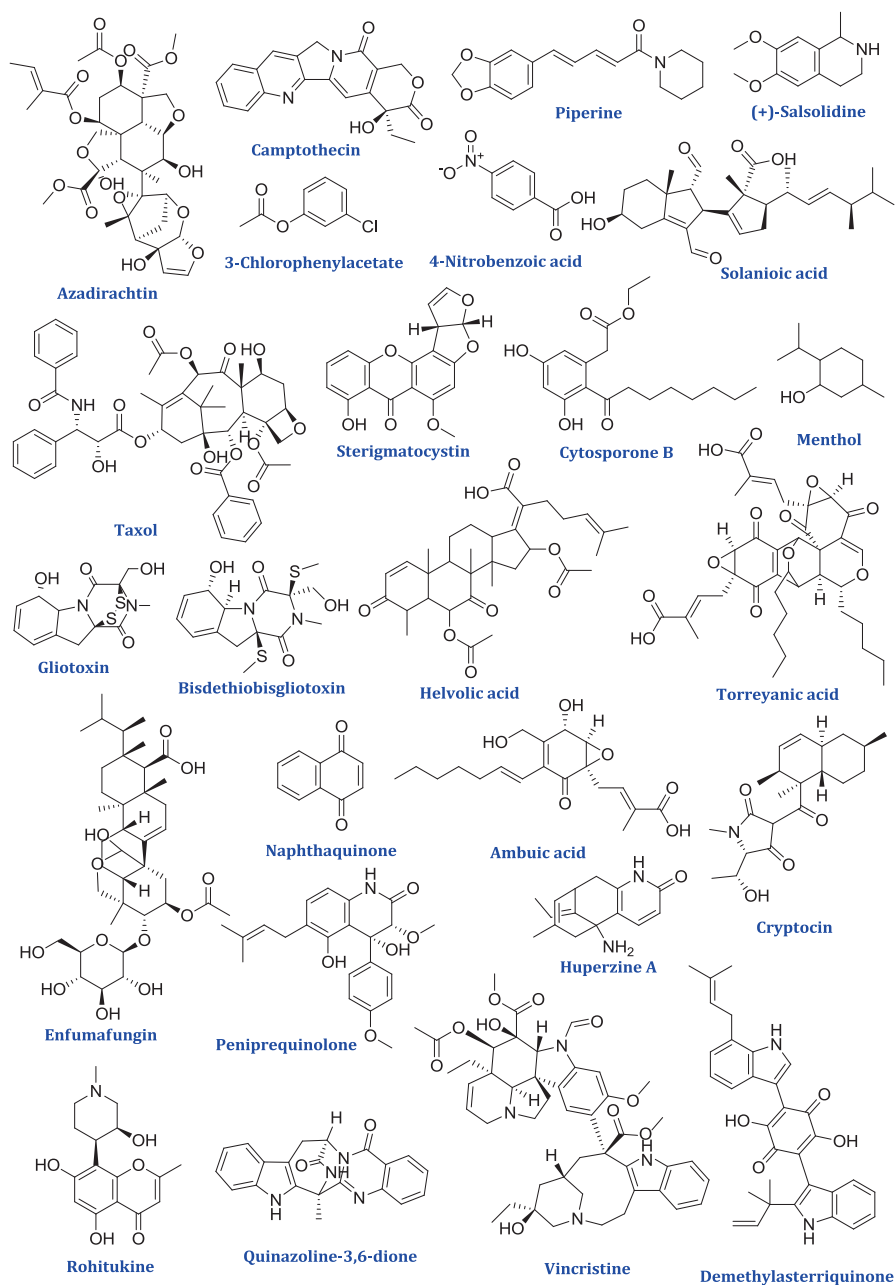
### 1.3.2 Bioresources for Secondary Metabolites

It is evident that numerous important compounds in the pharmaceutical and agronomy industries are synthesized by endophytes (Arora and Ramawat 2017). Numerous vital medicines have been acquired from plants, for instance, camptothecin, quinine, Taxol, vincristine, and vinblastine (Ramawat et al. 2009), whereas

more than 8500 bioactive metabolites with fungi as a source are well-known (Arora and Ramawat 2017; Demain and Sanchez 2009; Goyal et al. 2016). With the fungal endophyte *Taxomyces andreanae*, research into fungal endophytes was initiated, synthesizing certain bioactive molecules such as Taxol (Nicoletti and Fiorentino 2015). There are numerous tasks that have been encountered to synthesize these commercialized bioactive molecules (Arora and Ramawat 2017; Kusari and Spiteller 2011). In *Oryza sativa*, *Fusarium oxysporum* was reported to cause foolish seedling disease, as the fungus was also reported to produce gibberellin (Arora and Ramawat 2017). Further, for the synthesis of secondary metabolites, the bio-transformation process has been efficaciously realized using endophytes (Pimentel et al. 2011; Wang and Dai 2011). The process of chemical variation of any substance is referred to as bio-transformation in a biological system (Arora and Ramawat 2017; Wang and Dai 2011). The changes or transformation in the basic molecule result in a further effective active compound, e.g., semisynthetic compounds established from Taxol and podophyllotoxin have a supplementary effect to the basic molecule (Arora and Ramawat 2017; Ramawat et al. 2009). Figure 1.2 presents the chemical structures of secondary metabolites produced by different groups of endophytic fungi. Table 1.3 shows the diversity and abundance of diverse bioactive compound or secondary metabolite production by different groups of endophytic fungi reported from diverse host plants worldwide.

### 1.3.2.1 Azadirachtin

Azadirachtin is a recognized insecticide found in three species of the neem tree, *Azadirachta indica* A. Juss., *A. excelsa* Jacobs, and *A. siamensis* Valetton (Verma et al. 2014b). Azadirachtin is a highly oxygenated tetranortriterpenoid (Verma et al. 2014b). It contains 16 stereogenic centers, 7 of which are fully replaced (Ley et al. 1993). It takes about 16 years for its first structural interpretation and improvements (Butterworth et al. 1972) and 25 years for its chemical synthesis (Veitch et al. 2007a) to take place. Azadirachtin has been synthesized chemically from a common intermediate “epoxide-2”; this molecule alone has the potential as an intermediate to synthesize compounds from all three groups of limonoids: azadirachtin, azadirachtol, and meliacarpin from the neem tree (Kusari et al. 2012; Veitch et al. 2007b, c). Inside the cellular metabolism, azadirachtin is designed via the “iso-prenoid pathway” (IPP) (Kraus et al. 1985). Azadirachtin acts as an antifeedant, insect growth regulator, and sterilant in insects (Jennifer Mordue et al. 1998). Azadirachtin functions at a cellular level by disrupting protein synthesis, more precisely at the molecular level by altering the transcription and translation of protein expressed during rapid protein synthesis (Nisbet 2000). Azadirachtin has several structurally related isomers. Azadirachtin A and its several congeners have significant biological activity, specifically insecticidal and nematocidal (Klenk et al. 1986). To enable the synthesis of potential bioactive compounds, some novel biotechnological approaches have been used, such as callus culture (Krishnamurthy and Naik 2017; Prakash et al. 2002; Rafiq and Dahot 2010), cell culture (Jarvis et al. 1997), and hairy root culture of neem plants (Pimentel et al. 2011; Satdive et al. 2007).



**Fig. 1.2** Structures of compounds presenting several novel bioactive secondary metabolites isolated from fungal endophytes



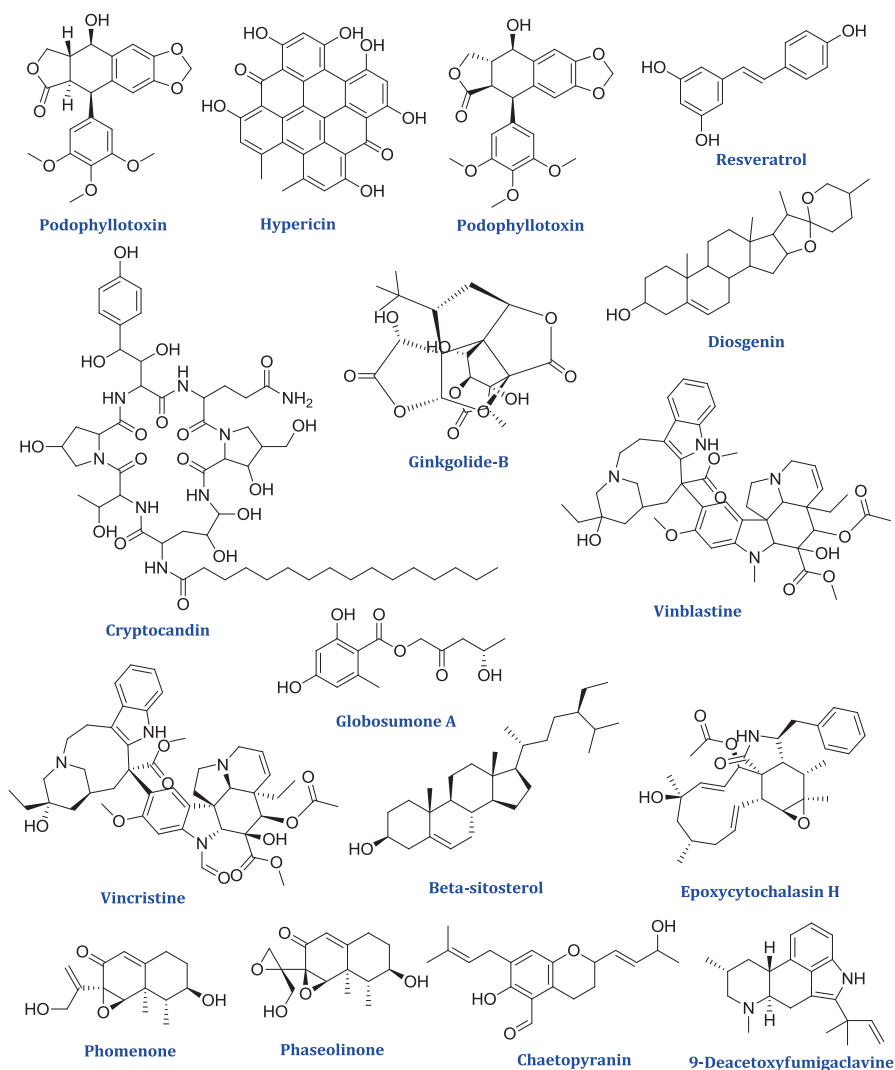
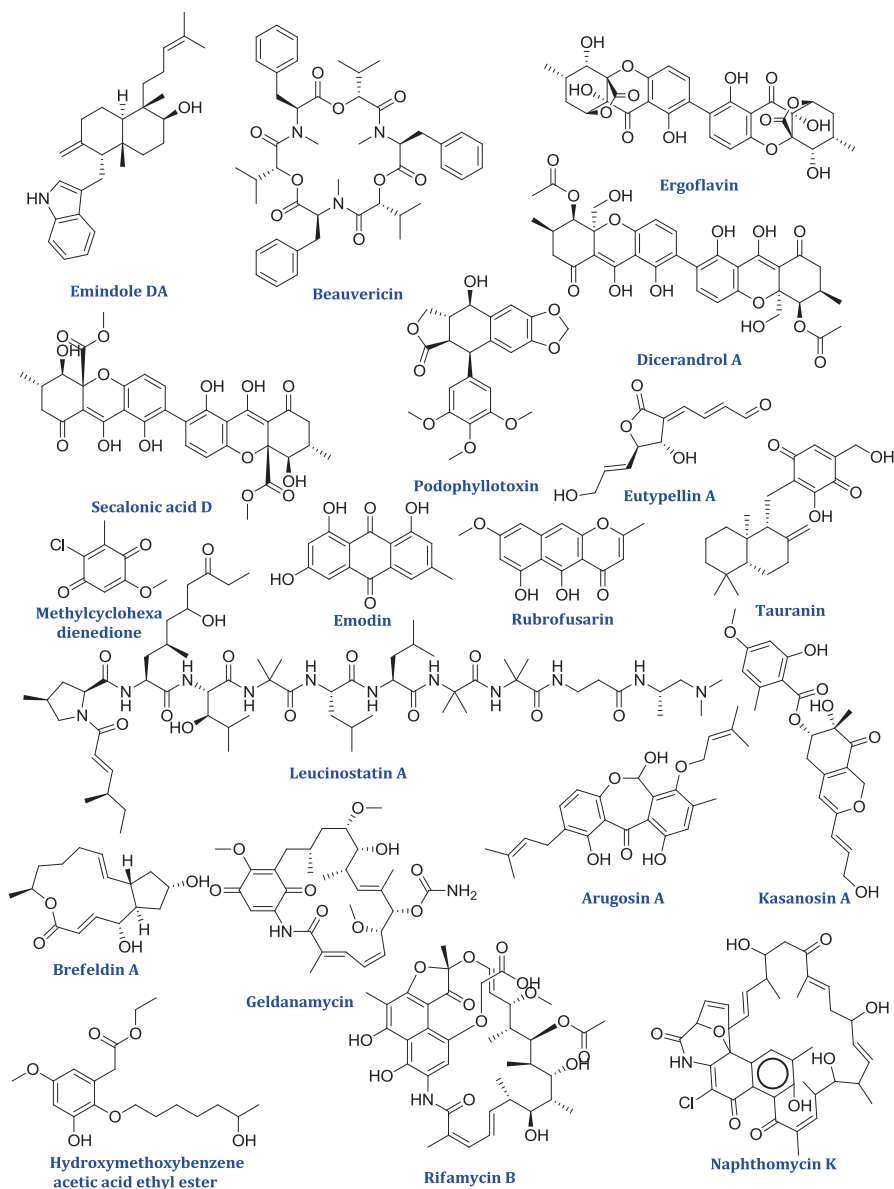


Fig. 1.2 (continued)

### 1.3.2.2 Camptothecin

Camptothecin (CPT) is a quinoline alkaloid mainly isolated from *Camptotheca acuminata*, a deciduous tree native to China and Tibet (Kumara et al. 2014). The bark of the tree was extensively used in traditional Chinese medicine (Wall et al. 1966). Later, camptothecin was discovered in several other species belonging to the families Icacinaceae, Rubiaceae, Apocynaceae, and Loganiaceae, with the maximum



**Fig. 1.2** (continued)

concentration described in *Nothapodytes nimmoniana* (0.3% by dry weight from its bark) (Govindachari and Viswanathan 1972; Kumara et al. 2014). The biosynthetic pathway of CPT in plants is simply moderately categorized (Yamazaki et al. 2003, 2004). Further, Sun et al. (2011b) cloned and categorized three putative genes involved in CPT biosynthesis; namely, geraniol-10-hydroxylase, secologanin

**Table 1.3** Production of bioactive compounds by endophytic fungi

Bioactive compounds	Host endophytic fungi	References
<i>Antibacterial</i>		
Altenuisol	<i>Alternaria</i> sp. Samif01	Tian et al. (2017)
Bacteriocins	<i>Bacillus subtilis</i>	Sansinenea and Ortiz (2011)
Chaetoglobosin A	<i>Chaetomium globosum</i>	Dissanayake et al. (2016)
Naphthaquinone	<i>Chloridium</i> sp.	Kharwar et al. (2009)
Polyketide citrinin	<i>Penicillium janthinellum</i>	Marinho et al. (2005)
Polyketide citrinin	<i>Penicillium janthinellum</i>	Marinho et al. (2005)
Phenols, flavonoids	<i>Pestalotiopsis neglecta</i>	Sharma et al. (2016)
Ambuic acid derivative	<i>Pestalotiopsis</i> sp.	Ding et al. (2008)
Phomodione (43)	<i>Phoma pinodella</i>	Hoffman et al. (2008)
Dicerandrol C (24)	<i>Phomopsis longicolla</i>	Erbert et al. (2012)
Solanioic acid	<i>Rhizoctonia solani</i>	Ratnaweera et al. (2015b)
Infectopyrones A and B	<i>Stemphylium</i> sp.	Zhou et al. (2014)
Ethanollic extract	<i>Trichoderma stromaticum</i>	Ratnaweera et al. (2015b)
Ophiobolin P	<i>Ulocladium</i> sp.	Wang et al. (2013)
Helvolic acid	<i>Xylaria</i> sp.	Ratnaweera et al. (2014)
<i>Anticancer</i>		
Vinblastine	<i>Alternaria</i>	Guo et al. (1998)
Capsaicin	<i>Alternaria alternata</i>	Clark and Lee (2016).
Resveratrol	<i>Aspergillus niger</i>	Liu et al. (2016)
Baccatin III	<i>Diaporthe phaseolorum</i> ,	Li et al. (2015)
Secoemestrin D	<i>Emericella</i> sp.	Xu et al. (2013)
Camptothecin	<i>Entrophospora infrequens</i>	Puri et al. (2005)
Asparaginase	<i>Eurotium</i> sp.	Jalgaonwala and Mahajan (2014)
Vincristine	<i>Fusarium oxysporum</i>	Kumar et al. (2013)
Vincristine	<i>Fusarium oxysporum</i>	Zhang et al. (2000)
Camptothecin	<i>Fusarium solani</i>	Shweta et al. (2010)
Camptothecin	<i>Fusarium solani</i>	Shweta et al. (2010)
10-hydroxycamptothecin	<i>Fusarium solani</i>	Shweta et al. (2010)
9-methoxycamptothecin	<i>Fusarium solani</i>	Shweta et al. (2010)
Torreyanic acid	<i>Pestalotiopsis microspora</i>	Lee et al. (1996)
Torreyanic acid	<i>Pestalotiopsis microspore</i>	Lee et al. (1996)
Podophyllotoxin	<i>Phialocephala fortinii</i>	Eyberger et al. (2006)
Podophyllotoxin	<i>Phialocephala fortinii</i>	Eyberger et al. (2006)
Taxol (paclitaxel)	<i>Taxomyces andreanae</i>	Kusari et al. (2014)
<i>Antifungal</i>		
Leucinostatin A	<i>Acremonium</i> sp.	Strobel et al. (1997)
Asperamide A, B	<i>Aspergillus niger</i>	Zhang et al. (2007)
Bacillomycin	<i>Bacillus amyloliquefaciens</i>	Aranda et al. (2005)
Bacilysocin	<i>Bacillus subtilis</i>	Tamehiro et al. (2002)
Gliotoxin	<i>Chaetomium globosum</i>	Li et al. (2011)
Cryptocandin	<i>Cryptosporiopsis quercina</i>	Strobel et al. (1999)

(continued)

**Table 1.3** (continued)

Bioactive compounds	Host endophytic fungi	References
Cryptocin	<i>Cryptosporiopsis quercina</i>	Li and Strobel (2001)
Cryptocandin	<i>Cryptosporiopsis quercina</i>	Strobel et al. (1999)
Cytosporone B	<i>Dothiorella</i> sp.	Xu et al. (2005)
Enfumafungin	<i>Hormonema</i> sp.	Aly et al. (2011)
Microsphaerophthalide A	<i>Microsphaeropsis arundinis</i>	Sommart et al. (2012)
Sclerin	<i>Microsphaeropsis arundinis</i>	Sommart et al. (2012)
Ambuic acid	<i>Monochaetia</i> sp.	Li et al. (2001)
Myxodiol A	<i>Myxotrichum</i> sp.	Yuan et al. (2013)
Solanapyrone C	<i>Nigrospora</i> sp. YB-141	Wu et al. (2009)
Pinazaphilones A and B	<i>Penicillium</i> sp.	Liu et al. (2015)
Quinazoline alkaloid	<i>Penicillium vinaceum</i>	Zheng et al. (2012)
Jesterone	<i>Pestalotiopsis jester</i>	Li and Strobel (2001)
Pestaloside	<i>Pestalotiopsis microspora</i>	Lee et al. (1995)
Ambuic acid	<i>Pestalotiopsis</i> sp.	Li et al. (2001)
b-sitosterol	<i>Phoma</i> sp.	Wang et al. (2012)
Cytochalasin N	<i>Phomopsis</i> sp.	Fu et al. (2011)
Phomenone	<i>Xylaria</i> sp.	Silva et al. (2010)
<i>Antimicrobial</i>		
Altenusin	<i>Alternaria</i> sp.	Kjer et al. (2009)
Altersolanol A	<i>Ampelomyces</i> sp.	Aly et al. (2008)
Deoxydopodophyllotoxin	<i>Aspergillus fumigatus</i>	Kusari et al. (2009)
Cephalosol	<i>Cephalosporium acremonium</i>	Zhang et al. (2008)
Javanicin	<i>Chloridium</i> sp.	Kharwar et al. (2009)
Methanol	<i>Colletotrichum</i> sp.	Arivudainambi et al. (2011)
Cytonic acid	<i>Cytospora</i> sp.	Guo et al. (2000)
Cytonic acid B	<i>Cytospora</i> sp.	Li et al. (2007b)
Eupenicinols	<i>Eupenicillium</i> sp.	Li et al. (2014)
Equisetin	<i>Fusarium</i> sp.	Ratnaweera et al. (2015a)
Gliotoxin	<i>Hypocrea virens</i>	Ratnaweera et al. (2016)
Bisdethiobis gliotoxin	<i>Hypocrea virens</i>	Ratnaweera et al. (2016)
Botralin	<i>Microsphaeropsis olivacea</i>	Li et al. (2007b)
Graphis lactone A	<i>Microsphaeropsis olivacea</i>	Li et al. (2007b)
Ulocladol	<i>Microsphaeropsis olivacea</i>	Li et al. (2007b)
Isocaryophyllene	<i>Muscodor sutura</i>	Kudalkar et al. (2012)
Octadecylmorpholine	<i>Muscodor tigerii</i>	Saxena et al. (2015b)
Phomol	<i>Phomopsis</i> sp.	Weber et al. (2004)
<i>Antioxidant</i>		
Campothecin	<i>Entrophospora</i>	Puri et al. (2005)
3- <i>epi</i> -dihydroaltenuene	<i>Alternaria</i> sp.	Tian et al. (2017)
Pestacin and isopestacin	<i>Pestalotiopsis microspora</i>	Harper et al. (2003)
Isopestacin	<i>Pestalotiopsis microspora</i>	Strobel et al. (2002)
Pestacin	<i>Pestalotiopsis microspora</i>	Strobel et al. (2002)

(continued)

**Table 1.3** (continued)

Bioactive compounds	Host endophytic fungi	References
<i>Antitumor</i>		
Naptha-y-pyrone	<i>Aspergillus niger</i>	Zhang and Qi-Yong (2007)
Cytochalasins	<i>Rhinochadiella</i> sp.	Wagenaar et al. (2000)
Cytochalasins	<i>Xylaria</i> sp.	Wagenaar et al. (2000)
<i>Antiviral</i>		
Podophyllotoxin	<i>Alternaria</i> sp.	Eyberger et al. (2006)
Cytotoxic acid A	<i>Cytosphaera</i> sp.	Guo et al. (2000)
Pestalol A–E	<i>Pestalotiopsis</i> sp.	Sun et al. (2014)
<i>Cytotoxic</i>		
Cordyheptapeptides C–E	<i>Acremonium persicinium</i>	Chen et al. (2012)
Desmethyldiaportinol	<i>Ampelomyces</i> sp.	Aly et al. (2008)
8-O-methylversicolorin	<i>Aspergillus versicolor</i>	Dou et al. (2014)
Xanthoquinodin	<i>Chaetomium elatum</i>	Chen et al. (2013)
Coniothiepinol A	<i>Coniochaeta</i> sp.	Wang et al. (2010a)
Conioxepinol B	<i>Coniochaeta</i> sp.	Wang et al. (2010b)
Myxotrichin A	<i>Myxotrichum</i> sp.	Yuan et al. (2013)
Myxotrichin D	<i>Myxotrichum</i> sp.	Yuan et al. (2013)
Terricollene A	<i>Neurospora terricola</i>	Zhang et al. (2009a)
Ginsenosin	<i>Penicillium melinii</i>	Zheng et al. (2013)
Penicillanols A <sub>1</sub> and B <sub>1</sub>	<i>Penicillium</i> sp.	Lin et al. (2008a)
Torreyanic acid	<i>Pestalotiopsis microspora</i>	Lee et al. (1996)
Phaeosphaerin A	<i>Phaeosphaeria</i> sp.	Li et al. (2012a)
Preussochrome C	<i>Preussia africana</i>	Zhang et al. (2012)
Atrichodermone A, B, C	<i>Trichoderma atroviride</i>	Zhou et al. (2017)
<i>Immunosuppressive</i>		
Subglutinol A and B	<i>Fusarium subglutinans</i>	Lee et al. (1995)
Periconicins	<i>Fusarium subglutinans</i>	Kim et al. (2004)
<i>Insecticidal</i>		
Azadirachtin A	<i>Eupenicillium parvum</i>	Kusari et al. (2012)
Azadirachtin	<i>Eupenicillium parvum</i>	Kusari et al. (2012)
Nodulisporic acid A	<i>Nodulisporium</i> sp.	Ondeyka et al. (1997)
1,3-oxazinane	<i>Geotrichum</i> sp. AL4	Li et al. (2007a)
4-hydroxybenzoic acid	<i>Fusarium oxysporum</i>	Bogner et al. (2017)

synthase, and strictosidine synthase from *C. acuminata*. In recent times, an effort was made to unravel the CPT biosynthetic gene from a CPT-producing endophytic fungus, *Fusarium solani*, isolated from *C. acuminata* (Kusari et al. 2011; Kaul et al. 2013; Kumara et al. 2014). However, the endophyte was revealed to synthesize CPT. Kusari et al. (2011) suggested that the endophyte might be using the host STR to synthesize CPT. However, as Sudhakar et al. (2013) debated, this proposition is unbelievable, because the endophyte was able to produce CPT in axenic cultures for numerous generations in the absence of the host tissue, where evidently the fungus

cannot access the host *STR*. Anticancer drugs isolated from endophytic fungi include camptothecin, which is a potent anti-neoplastic agent isolated from *C. acuminata* Decaisne (Nyssaceae) from China (Premjanu and Jayanthi 2012; Wall et al. 1966).

### 1.3.2.3 Taxol

Paclitaxel, a greatly functionalized diterpenoid, occurs in *Taxus* plants (Suffness 1995). Derivatives of paclitaxel signify a leading group of anticancer agents that were earlier reported to be synthesized by endophytes (Kumara et al. 2014). In plants, the synthesis of Taxol occurs by the involvement of three genes, namely, *ts* (involved in the formation of the taxane skeleton), *dbat* (involved in baccatin-III formation), and *bapt* (involved in phenylpropanoyl side chain formation at C-13) (Xiong et al. 2013). Zhang P et al. (2009b) reported the gene 10-deacetyl baccatin-III-10-O-acetyl transferase to be accountable for the biosynthesis of Taxol in the endophyte *Cladosporium cladosporioides* MD213 isolated from *Taxus media* (yew species). In recent times, Xiong et al. (2013) revealed that in three Taxol-synthesizing endophytes isolated from *Anglojap* Yew, or *T. media*, the fungus resulted in positive successes for the three key genes, *ts*, *dbat*, and *bapt*. The fungus *Taxomyces andreanae*, an endophyte isolated from *T. brevifolia*, was found to produce Taxol (Stierle et al. 1993), subsequently drawing the attention of microbiologists to endophytes. Each plant is a repository of one or more fungal endophytes, and one endophytic species may possess several to a few hundred strains (Huang et al. 2007; Strobel and Daisy 2003). In recent years, the biosynthetic potential of endophytic fungi has gained more significance. It is thus imperative to study the complex relationship of endophytes with existing endophytes, host plants, insect pests, and other definitive herbivores, which standardizes the ability of endophytes to synthesize compounds similar to their hosts (Kusari et al. 2013b). *Aegle marmelos*, an extensively used medicinal plant, shelters Taxol-producing fungi (Gangadevi and Muthumary 2008). Taxol is an important and expensive anticancer drug generally used in clinics. The endophytic fungus *Bartalinia robillardoides* (strain AMB-9) produces 187.6 l g/l of Taxol. This confirms that the fungus can be genetically upgraded to increase the synthesis of Taxol. Taxanes such as Taxol are plentifully synthesized by members of the coniferous family Taxaceae (Wang and Dai 2011). It was found that a number of fungal endophytes isolated from yew trees (*Taxus* spp., Taxaceae) produce Taxol under in vitro conditions (Zhou et al. 2010).

### 1.3.2.4 Gibberellic Acid and Indole Acetic Acid

The biosynthetic pathway of gibberellic acid (GA) is compared with other secondary metabolites (Kumara et al. 2014). In plants, the conversion of GGDP to active GA requires the presence of three terpene synthases, two 450s, and a soluble 20 DDS.

Compared with the fungus, the synthesis is made by only one bifunctional terpene cyclase (copalyl synthase/kaurene synthase) and by P450s. These results suggest that the biosynthetic pathways in plants and fungi might have evolved independently (Bömke and Tudzynski 2009; Kumara et al. 2014). GA production has also been reported from the endophytic fungus *F. proliferatum*, isolated from orchid roots. Research has specified that this fungus obtained the genes for GA biosynthesis from higher plants by horizontal gene transfer. Endophytic microorganisms have been found to produce phytohormones such as GA, abscisic acid, auxins, cytokinins, and ethylene (Kaul et al. 2013).

Hamayun et al. (2009b) isolated *Cladosporium sphaerospermum* from the roots of *G. max* (L.), which indicated the presence of bioactive GA3, GA4, and GA7. The endophytes isolated from medicinal plants have been found to encourage plant growth and development. Waqas et al. (2012) studied the endophytic fungi *Phoma glomerata* and *Penicillium* sp. in the promotion of shoot growth, related vegetative growth, and other characteristics of GA-deficient dwarf mutant Waito-C and Dongjin-byeo rice. Therefore, if cultured endophytes were to produce the same rare and important bioactive compounds as their host plants, this would diminish the harvesting of slow-growing rare plants, and also help to restore the world's biodiversity (Waqas et al. 2012). Jerry (1994) revealed that during seed germination, the symbiotically associated endophytic fungi degrade cuticle cellulose and make carbon available to seedlings, which improves seed germination, vigor, and establishment. Endophytes have the ability to produce plant growth regulators and thereby promote seed germination in crop plants (Bhagobaty and Joshi 2009). Plant growth promotion is the major contribution of fungal symbiosis (Hassan et al. 2013). However, fungal endophytes enhance plant growth by the production of ammonia and plant hormones, particularly IAA (Bal et al. 2013). IAA acts as a plant growth promoter that enhances both cell elongation and cell division, and is essential for plant tissue differentiation (Taghavi et al. 2009). The ability of soil microorganisms to become involved in the production of IAA in culture plates and in soil has been recorded (Spaepen and Vanderleyden 2011). The endophytic microorganisms isolated from various plants have shown a high IAA production level compared with those isolated from root-free soil (Spaepen et al. 2007). Owing to the impact of IAA on the plant tissues, the ability of fungal endophytes to produce IAA has provoked a great response (Hamayun et al. 2010). Only a few fungi linked with plants have been stated to synthesize gibberellin (Kawaide 2006; Vandebussche et al. 2007), for instance, *Cladosporium sphaerospermum* and *Penicillium citrinum* (Hamayun et al. 2009b; Khan et al. 2008). Hamayun et al. (2010) examined gibberellin production and the growth-promoting potential of a fungal strain belonging to *Cladosporium* sp. isolated from the roots of the cucumber. Khan et al. (2008) isolated *P. citrinum*, which showed growth promotion activity in dune plants owing to the presence of bioactive gibberellins in the filtrate of the fungi (Khan et al. 2008). Hasan (2002) revealed the growth promotion activity of endophytic *Phoma herbarum* and *Chrysosporium pseudomerdarium* in the soybean and proved that some endophytes are host-specific. Ahmad et al. (2010) studied the plant growth-promoting activity and stress resistance capability of endophytic *Penicillium* sp. and

*Aspergillus* sp., which were shown to produce physiologically active gibberellins. Many fungal endophytes, such as *Neurospora crassa* (Rademacher 1994), *Sesamum indicum* (Choi et al. 2005), *Penicillium citrinum* (Khan et al. 2008), *Scolecobasidium tshawytschae* (Hamayun et al. 2009b), *Arthrinium phaeospermum* (Khan et al. 2009a), *Chrysosporium pseudomerdarium* (Hamayun et al. 2009a), *Cladosporium sphaerospermum* (Hamayun et al. 2009c), *Cladosporium* sp. (Hamayun et al. 2009c), *Gliomastix murorum* (Khan et al. 2009b), *Fusarium fujikuroi*, *Sphaceloma manihoticola* (Shweta et al. 2010), *Phaeosphaeria* sp. (Kawaide 2006), *Phaeosphaeria* sp., *Penicillium* sp. (Hamayun et al. 2010), *Aspergillus fumigatus* (Khan et al. 2011a), *Exophiala* sp. (Khan et al. 2011b), and *P. funiculosum* (Khan et al. 2011b), have been reported to be gibberellin producers. Hasan (2002) demonstrated gibberellin production with molds such as *Aspergillus flavus*, *A. niger*, *Penicillium corylophilum*, *P. cyclophilum*, *P. funiculosum*, and *Rhizopus stolonifera*.

### 1.3.2.5 Siderophores

Endophytes help plants to take up solubilized phosphate (Wakelin et al. 2004), enhancing hyphal growth and mycorrhizal colonization (Will and Sylvia 1990), and by producing siderophores (iron-chelating molecules that increase the availability of phosphate to plants) (Costa and Loper 1994). Endophytic bacteria were found to be responsible for the allelopathic effects on maize observed with these plants, causing reduced plant emergence and plant height (Sturz et al. 1997). Dutta et al. (2008) reported improvement of plant growth and disease suppression in pea plants co-inoculated with fluorescent pseudomonads and *Rhizobium*. Hung et al. (2007) studied the effect of endophytes on soybean growth and development, and proved them to have a positive influence on root weight. Plant growth-promoting endophytic bacteria influence seed germination, root and hypocotyl growth and increase seedling vigor. Root endophytes in the cortical parenchymatous tissue of vetiver were used for the enhancement of essential oil metabolism (Del Giudice et al. 2008). Harish et al. (2009) studied the effect of the bio-formulations of consortial combinations of the rhizobacteria *Pseudomonas fluorescens* (Pf1) and endophytic *Bacillus* sp. (EPB22), which enhanced the yield of bananas. One of the bacterial endophytes, *B. subtilis* HC8, isolated from hogweed, *Heracleum sosnowskyi*, found the potential to stimulate plant growth and the biological control of foot and root rot diseases in tomato (Malfanova et al. 2011).

In field experiments, inoculation with endophytic bacteria resulted in sugarcane plants that were more superior in terms of plant height and shoot counts. Conventional manipulation of soil microorganisms has been practiced for decades. For example, sewage and manure applications for the enhancement of soil fertility dramatically affect autochthonous communities of soil biota (Biswas et al. 2018). The practice of monoculture is in itself instrumental in altering soil microbial populations at the field level. Thus, it may be possible to influence plant endophytic populations by seed bacterization, by soil inoculation, and by identifying the genetic (bacterial) component responsible for their beneficial effects. Endophytic microbes have merit over rhizospheric bacteria, as they deliver fixed nitrogen straight to the host plant



tissue and are able to fix nitrogen more competently than free-living bacteria because of the lower oxygen pressure in the interior of plants than in soil. Jha et al. (2013) explored the potential of endophytic association with plants in agricultural sustainability in particular and yield enhancement in general. The potential of bio-fertilizers was formulated using endophytic bacteria for the enhanced production of bananas in a sustained way (Ngamau et al. 2014).

### 1.3.3 Pharmaceutically Important Bioactive Compounds

Throughout the year, natural products from microorganisms, plants, or animals play a key role in the search for novel drugs. These naturally derived products are non-toxic and inexpensive, and have been exploited for human use. The biggest store of bioactive compounds is fungal endophytes. Alexander Fleming, in 1928, discovered the first bioactive compound from *Penicillium notatum*, i.e., penicillin. During the 1990s one of the most useful anticancer drugs was paclitaxel. An endophyte of *T. brevifolia*, *Taxomyces andreanae* was reported to produce the drug paclitaxel. Later research suggested lateral gene transfer from host to fungus (Stierle et al. 1993). The fungal endophyte *Fusarium* was reported to produce subglutinol A and diterpene pyrones, providing immunosuppressive activity. The endophyte was isolated from the stem of *Tripterygium wilfordii* (Strobel and Pliam 1997). Isobenzofuranone as isopestacin, obtained from the fungal endophyte *Pestalotiopsis microspora*, possesses antifungal and antioxidant activity (Strobel et al. 2002). Antimicrobial activity of fungal endophytes was screened against the pathogenic organisms *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans*. Fungal endophytes were isolated from the leaves and branches of five different species of *Garcinia* plants. The fungal endophytes *Phomopsis* sp. and *Botryosphaeria* sp. showed antibacterial activity against *Staphylococcus aureus*. *Botryosphaeria* sp. also showed antifungal activity against *M. gypseum*. The results specify that the endophytic fungus of *Garcinia* plants are a potential source of antimicrobial compounds (Phongpaichit et al. 2006).

Endophytic fungi can be isolated from the bark of *Juglans mandshurica*. On the basis of the internal transcribed spacer sequence and morphological identification, the fungal endophyte belongs to Deuteromycotina, Hyphomycetes, Moniliales, and *Trichoderma longibrachiatum*. The fermentation of fungus FSN006 provides a possible mechanism for producing anticancer drugs with lower toxicity and greater efficiency (Li et al. 2009). The crude extract of the endophytic fungus *Pichia guilliermondii* was separated using bioassay-guided fractionation. Helvolic acid exhibited strong, broad-spectrum, antimicrobial activity (Zhao et al. 2010). Developments in screening technologies have received much attention; thus, fungal endophytes are an outstanding source of biologically active compounds with applications in medicine and agriculture (Aly et al. 2011). A large number of bioactive compounds produced by fungal endophytes are alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, xanthenes, chinones, isocoumarins, benzopyranones, tetralones, cytochalasines, perylene derivatives, furandiones, depsipeptides,

and enniatins (Elfita et al. 2011) Tenguria et al. (2011) reported that endophytic fungi of *Tinospora crispa* (L.) was a probable candidate for the synthesis of bioactive compounds. *Plasmodium* species cause the most acute diseases in human beings, and even death. Hypericin isolated from fungal endophytes of medicinal plants possess antimicrobial activity against *Staphylococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteric*, and *Escherichia coli* (Kusari and Spiteller 2012).

Khan et al. (2012a) reported five fungal endophytes isolated from *Capsicum annum*, *Cucumis sativus*, and *G. max* roots. Using phylogenetic analysis, the isolate was found to belong to *Paraconiothyrium* sp. and produce the phytotoxic compound ascotoxin characterized using gas chromatography-mass spectrometry and the nuclear magnetic resonance technique. On seed germination of *Echinochloa crus-galli* and *Lactuca sativa*, 100% inhibitory effects were shown by ascotoxin. The buds and leaf of Malabar Embelia, found in peninsular India, were subjected to the isolation of fungal endophytes. Four different fungal endophytes were identified, *Cladosporium cladosporioides*, *Penicillium* sp., *Aspergillus niger*, and *Alternaria* sp., and were characterized for phytochemical analysis and antibacterial activity against *Pseudomonas aeurogenosa*, *Bacillus subtilis*, and *Shigella flexneri*. The four different fungal endophytes exhibited the presence of phytochemicals at different concentrations: cardiac glycoside, flavonoids, phenols, tannins, terpenoids, cardenolides, and saponins. Endophytic microbes are a great source of bioactive compounds to satisfy the demands of the pharmaceutical and medical industries (Chandruppa et al. 2013). *Pinellia ternata* is used as a traditional medicine for antiemetic and sedative effects, and as an antitussive and analgesic. Su et al. (2014) isolated 193 endophytic microbes from Chinese medicinal plants, *Camptotheca cuminata* Decne, *Gastrodia elata* Blume, and *Pinellia ternata*. On the basis of morphological and rDNA sequences, the fungal isolates belong to Ascomycota, Basidiomycota, and Mucoromycotina. Endophytes produce various types of compounds, for instance, essential oils, azadirachtins, terpenes, flavonoids, lignins, cytochalasins, steroids, and alkaloids (Nicoletti and Fiorentino 2015).

### 1.3.4 Lignocellulosic Biorefineries: Biofuel Production

One of the main renewable materials on earth is wood. The cell walls of wood are composed of cellulose microfibrils covered with hemicelluloses and lignin hemicellulose matrices (Higuchi 2012). In 1813, Swiss botanist, A. P. de Candolle, mentioned lignin for the first time. About 20–30% of the dry weight of wood is made up of lignin (Abdel-Raheem and Shearer 2002). It is covalently linked to hemicellulose and confers mechanical strength to the cell wall (Chabannes et al. 2001). Owing to the chemical complexity and recalcitrant properties of lignin, very few microbes are able to degrade it (Guillén et al. 2005). In biorefinery processes, such as the production of ethanol and cellulose-based papers, the degradation of lignin is a central issue (Cañas and Camarero 2010).

An array of extracellular oxidative enzymes are produced by white-rot fungi (basidiomycetes), as they are the main wood rotters that synergistically and proficiently degrade lignin. Ligninolytic enzymes include lignin peroxidases (LiPs), manganese peroxidases (MnPs), versatile peroxidases, and laccases (Wong 2009). On the basis of macroscopic features, wood-rotting basidiomycetes are categorized into white-rot and brown-rot fungi (Schwarze et al. 2000). In the mid-1980s, LiP and MnP were discovered in *P. chrysosporium* and termed true ligninases because of their high redox potential (Evans et al. 1994). *Pleurotus eryngii* were reported to produce versatile peroxidase that showed catalytic properties similar to LiP and MnP (Ruiz-Dueñas et al. 1999). Other extracellular enzymes involved in wood lignin degradation are oxidases generating H<sub>2</sub>O<sub>2</sub>, aryl-alcohol oxidase (AAO), glyoxal oxidase, aryl-alcohol dehydrogenases (AAD), and quinone reductases (QR) (Guillén et al. 1997; Gutierrez et al. 1994).

Laccases have been known for many years to play a variety of roles, including production of pigments, fruit body morphogenesis, lignification of cell walls, and detoxification in plants, fungi, and insects (Mayer and Staples 2002). The preliminary steps in the biodegradation of lignin must be extracellular. LiP is also called a ligninase. First discovered in *Phanerochaete chrysosporium*, this enzyme is a heme peroxidase with a remarkably high redox potential and low optimal pH (Tien 1987). Laccase enzymes are copper-containing oxidases that mostly oxidize only those lignin model compounds with a free phenolic group, forming phenoxy radicals (Bourbonnais and Paice 1990). The most common laccase-producing endophytic fungi are *Chaetomium* sp., *C. globosum*, *Podospira anserina*, *Botryosphaeria* sp., and *Neofusicoccum austral* (Fillat et al. 2016; Sara et al. 2016).

Laccase enzymes produced by endophytic fungi have extensive substrate specificity and generally act on small organic substrates, such as polyphenols, methoxy-substituted phenols, and aromatic amines. Fungal laccases are used in paper manufacture for delignification, bioremediation of phenolic compounds, and biobleaching (Kunamneni et al. 2008). Exoglucanases, endoglucanases,  $\beta$ -glycosidase, exoxylanases and endoxylanases, and  $\beta$ -xylosidases are the main hydrolytic enzymes involved in lignocellulose degradation (Van Dyk and Pletschke 2012). For complete degradation of lignocellulose materials, laccases, MnP and LiP (oxidative enzymes), and additional hemicelluloses (e.g., acetyl esterase, b-glucuronidase, endo-1, 4- $\beta$ -mannanase, and  $\alpha$ -galactosidase) and oxidoreductases (aryl-alcohol oxidase, glucose-1-oxidase, glyoxal oxidase, and pyranose-2-oxidase) are necessary (Correa et al. 2014).

### 1.3.5 Endophytic Fungi in Bioremediation

Bioremediation is a process used to treat contaminated media, including water, soil, and subsurface material, by varying the conditions of the environment to stimulate the growth of microorganisms (fungi or bacteria) and degrade the target pollutants into simpler compounds. Biological treatment of the contaminated site is the least

expensive method (Barranco et al. 2012). To optimize the conditions for the microorganisms, additional nutrients, vitamins, minerals, and pH buffers are added. The prime goal of bioremediation is to create an optimal environment for the microbes to degrade pollutants. Although it is a cost-effective option, it is a very slow process, sometimes taking weeks to months for results to appear. Technologies can be generally classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site, whereas ex situ involves the removal of the contaminated material to be treated elsewhere.

To restrain the growth of endophytes, the plant synthesizes a range of toxic metabolites and endophytes over a period of co-evolution, progressively establishing a genetic system as a tolerant mechanism by generating exoenzymes and mycotoxins (Mucciarelli et al. 2007; Pinto et al. 2000). Fungal endophytes synthesizing the enzymes degrade the macromolecules into simpler compounds, including amylases, lipases, pectinase, cellulase, proteinase, phenol oxidase, and lignin catabolic enzymes (Oses et al. 2006; Tan and Zou 2001; Zikmundova et al. 2002). In general, fungal endophytes have been stated to have the ability to use various organic compounds, such as glucose, oligosaccharides, cellulose, hemicelluloses, lignin, keratin, pectin, lipids, and proteins, allowing the degradation of structural components into simpler forms (Kudanga and Mwenje 2005; Tomita 2003; Urairuj et al. 2003).

One of the methodologies in which green plants are used for the process of bioremediation is referred to as phytoremediation. It has been documented to be a promising technology for the in situ remediation of contaminated soils. Numerous studies have demonstrated that endophytes produce various enzymes for the degradation of organic contaminants and reduce both the phytotoxicity and evapotranspiration of volatile contaminants (Li et al. 2012b). Soleimani et al. (2010) reported the infection of *Festuca pratensis* and *Festuca arundinacea*, two grass species, by two endophytic fungi, *Neotyphodium coenophialum* and *Neotyphodium uncinatum*, increasing the ability of the plants to accumulate more Cd in roots and shoots and decreasing stress in the plants in addition to increasing the production of biomass. Rabie (2005) reported the phytoremediation efficiency of wheat, mung beans, and eggplant grown in soil contaminated with hydrocarbons. He concluded that the plants provided a larger sink for the contaminants, because they were better able to survive and grow, leading to the significance of treatment with arbuscular mycorrhizal fungi.

Since the industrial revolution, there has been a widespread rise in the discharge of waste into the environment, which is mostly collected in soil and water, comprises heavy metals, and generates distressing conditions for human life and aquatic biota. Heavy metals are metals with relatively high densities, atomic weights, or atomic numbers. Some heavy metals are either vital nutrients, such as iron, cobalt, and zinc, or comparatively harmless, such as ruthenium, silver, and indium, but in higher amounts or definite forms they can be toxic. Cadmium, mercury, and lead are reported to be highly poisonous heavy metals. Salem et al. (2000) reported that

arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc, etc., are not only cytotoxic, but also carcinogenic and mutagenic (Ahluwalia and Goyal 2007). In heavy metal-polluted habitats, microorganisms are known to change different detoxifying mechanisms, such as biosorption, bioaccumulation, biotransformation, and biomineralization (Gadd 2000; Lim et al. 2003; Malik 2004).

One of the biological processes in which chemical changes on compounds take place is referred to as biotransformation. The endophytic fungus *Phomopsis* sp. (VA-35), obtained from *Viguiera arenaria*, was reported to biotransform the tetrahydrofuran lignan, (-)-grandisin, into a new compound, 3,4-dimethyl-2-(4'-hydroxy-3',5'-dimethoxyphenyl)-5-methoxy-tetrahydrofuran (Verza et al. 2009). In another study, endophytic fungi *Fusarium sambucinum*, *Plectosporium tabacinum*, *Gliocladium cibotii*, and *Chaetosphaeria* sp., isolated from the roots and shoots of *Aphelandra tetragona*, were capable of transforming the benzoxazinones 2-benzoxazolinone (BOA) and 2-hydroxy-1,4-benzoxazin-3-one (HBOA). Aminophenol was formed as a key intermediate during the metabolic pathway for HBOA and BOA degradation (Zikmundova et al. 2002). On the basis of 18S rRNA gene sequencing, *Lasiodiplodia theobromae* isolated from the leaves of *Boswellia ovalifoliolata*, an endemic medicinal plant from the Tirumala Hills, was reported to show resistance to all four heavy metals, Co, Cd, Cu, and Zn, up to 600 ppm (Sani et al. 2017).

### 1.3.6 Endophytic Fungi in Agriculture

A lot of research into fungal endophytes is underway, which signifies that they are the most important source of biocontrol agents. They have a considerable effect on the physiological actions of their host plants. Further, various environmental factors, including rainfall and humidity, may have an influence on the occurrence of some fungal endophytic species (Khiralla et al. 2017; Petrini 1991; Selvanathan et al. 2011). According to Schaechter (2012), endophytic fungi have frequently been categorized into two major groups, including clavicipitaceous endophytes, which are known to infect some grasses, and nonclavicipitaceous endophytes. The Clavicipitaceae family of fungi include free-living and symbiotic species in association with insects and fungi or grasses, rushes, and sedges (Bacon and White 2000; Khiralla et al. 2017). Many of its members produce alkaloids, which are toxic to animals and humans, whereas nonclavicipitaceous endophytic fungi, mainly in association with leaves of tropical trees, have been discovered to play an important role in defending the host from abiotic stress, fungal pathogens, and an increase in the biomass (Fröhlich and Hyde 1999; Gamboa and Bayman 2001; Khiralla et al. 2017; Yadav and Yadav 2018; Yadav 2019)

Endophytic fungi play vital roles in host plants, protecting them from stress conditions, making nutrients, such as phosphorus, potassium, and many more,

available, producing auxins, cytokinins, gibberellins, siderophores, ammonia, HCN, and diverse hydrolytic enzymes, and ultimately promoting the growth of host plants. A number of studies suggest that inoculating crops with endophytic fungi might improve growth by diverse plant growth-promoting traits and might also mitigate the effect of stress conditions. Khan et al. (2011b) demonstrated the role of a newly isolated endophytic fungus, *Penicillium funiculosum*, with diverse plant growth-promoting attributes in *G. max* growing under salinity stress. The study revealed that the fungus ameliorated the effect of salinity stress. Kedar et al. (2014) studied the growth promotion potential of *Phoma* sp. isolated from *Tinospora cordifolia* and *Calotropis procera* for maize. The fungal endophytes were found to enhance growth in inoculated maize plants compared with non-inoculated plants. In the study by Rinu et al. (2014), *Trichoderma gamsii* isolated from the lateral roots of lentil with multifarious plant growth-promoting attributes showed its potential in plant growth promotion conducted under greenhouse conditions using two cereals and two legumes, suggesting its potential to be developed as a bioformulation for application under a mountain ecosystem. Yuan et al. (2017) studied the effect of *Penicillium simplicissimum*, *Leptosphaeria* sp., *Talaromyces flavus*, and *Acremonium* sp. isolated from cotton roots with wilt disease caused by the defoliating *Verticillium dahliae* (Vd080). The study demonstrated that all treatments considerably reduced disease incidence and the disease index. The results clearly signified that these endophytes not only delayed, but also led to a reduction in, wilt symptoms in cotton.

In the study by Asaf et al. (2018), *Aspergillus flavus* CHS1, an endophytic fungus, isolated from the roots of *Chenopodium album* with multiple growth-promoting activities, was assayed for its ability to promote the growth of mutant Waito-C rice. The results revealed an increase in chlorophyll content, root–shoot length, and biomass production. Furthermore, the strain was used to evaluate its potential to improve the growth of soybean under salinity stress. Dastogeer et al. (2018) evaluated whether the colonization of two fungal endophytes isolated from wild *Nicotiana* species from areas of drought-prone northern Australia, and a plant virus, yellowtail flower mild mottle virus, could improve water stress tolerance in *N. benthamiana* plants. Inoculation with the fungal strains and the virus considerably increased the tolerance of the plants to water stress. Inoculation with the fungal strains alone resulted in an increase in the relative water content, soluble sugar, soluble protein, proline content, plant biomass, and enzymatic activity, and a decrease in the production of reactive oxygen species and electrical conductivity. Furthermore, there was noteworthy upregulation of numerous genes that had previously been identified as drought-induced. The influence of the virus was similar to that of the fungi in terms of increasing the plant osmolytes, antioxidant enzyme activity, and gene expression. Fungal endophytic communities associated with plants play a vital role in balancing the ecosystem and in enhancing the growth of hosts. They have been shown to be potent biocontrol agents; furthermore, they produce a large number of fungal metabolites that could protect the host from disease, insects, and mammalian herbivores. They have been known to increase the tolerance of their host to abiotic stress.

Thus, fungal endophytes are gaining greater attention and are of greater interest to chemists, ecologists, and microbiologists as a treasure of biological resources, because of their diverse vital roles in the ecosystem.

## 1.4 Future Prospects and Conclusion

For the previous two and half decades, the scientific community has been aware of the effective role of fungal endophytes in agriculture, ecology, biotechnology, and industry. Fungal endophytes are also an alternative to existing industrial processes of transformation of lignocellulosic biomass, possessing great potential for application in the lignocellulosic industry. The ability of hydrolytic enzymes to synthesize can be employed in enzyme fermentation industries. New techniques with advanced sensitivity are required for enzyme quantification, such as fluorescence spectrophotometry, near-infrared-, and Fourier-transform infrared-based methods. The consequences of enzymes generating endophytes with distinctive consideration of remediating environmental pollutants, such as metals, polyaromatic hydrocarbons, and polychlorinated hydrocarbons, have been understated at the very minimum. Production of secondary metabolites of interest to the pharmaceutical industry is a very attractive field of research using biotechnological methods. The integration of genetic manipulation technology to progress the research to recognize the regulatory gene/s of numerous biosynthesis pathways of metabolite construction can lead to an increase in growth production of the compounds to be used for human welfare. The participation of fungal endophytes in the cycling of nutrients has significant consequences for living organisms and human health. For future research, there are still many areas that need to be explored, including new technologies and new crops with endophytes. Modern techniques of molecular biology, involving metagenomes, proteomes, and transcriptomes, will help to define the characteristics of endophytes and to find novel products for industrial development. The future of research into endophytes is bright, as demand for pharmaceutical products and agricultural produce is increasing day by day with an ever-increasing population.

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