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Sumita Jha *Editor*

Endophytes and Secondary Metabolites

Springer

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Sumita Jha
Editor

Endophytes and Secondary Metabolites

With 101 Figures and 53 Tables

Springer

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Foreword

The human-plant-microbe tripartite relationship goes back ages. This has resulted in human curiosity to search for microbes in nearly every habitat, be it soil, freshwater, or marine surroundings. Over the years, human curiosity has delved in rather normal habitats a bit more extensively because of their close relationships ammensal, commensalism and mutualists of both plants and animals.

What was known to the researchers from the time of deBary about microbes associated with plants has gradually turned into a goldmine of phenotypes with their hidden partners and their associated metabolites. The world of both epiphytes and endophytes as silent partners of living plants is an interesting saga of ecosystem dynamics and resultant exploratory pathways leading to recovery of some very precious drugs, such as taxol. However, simple questions of endophytic adaptations of bacteria and fungi in various plant tissues still baffle researchers. Also, unlike pathogenic microbiota, how the silent hidden microbial partners live in various plant tissues without disturbing the normal plant metabolic machinery is an open research question!

Considering the unique niche occupied by endophytes and possible application of such resident microbiota, a great deal has been published on this subject during the last couple of decades. However, use of new tools and strategies and even larger surveys of unique ecosystems and their resident floristics always throws up new information. Thus, continuity in published literature on endophytes is constantly updated. The present volume being brought out by Springer is a testimony to this need. It is edited by a very distinguished biotechnologist of international repute, Professor Sumita Jha, a NASI Senior Scientist, who has attempted to put together authors who are not only well versed in the endophytic research arena but also those who are masters of chemical scrutiny of microbiota and product development. While each chapter is unique in its own way, I find the study of marine endophytes of special significance on account of earlier uniqueness of bioactives from this habitat and their industrial applicability. In addition, the study of volatiles of endophytes is a new direction that is of considerable relevance not only in terms of bioactives but also from the angle of environmental pollution and climate change. Additionally, a couple of chapters lay emphasis on the role of endophytes under stressed environmental conditions, something of great virtue in sustainable plant production systems. In line with this approach is the discussion of biodegradation of pollutants by

endophytic microbes that has direct bearing on the current scenario of maintenance of balanced ecosystems.

From the floristics point of view, the study describing endophytes from Ginkgo stands out since this plant is of not only great evolutionary significance but is also well known for ginseng – a medicinal plant of much value. It would therefore be of interest to assess the role of Ginkgo's transient endophytes in the secretion of ginseng in plants or alternately under in vitro conditions.

I find that Professor Jha has done a commendable job in bringing together a very valuable volume on endophytes that will interest not only researchers and teaching fraternity but also industrial chemists.

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Preface

The biosynthesis of secondary metabolites with characteristic structural diversity occurs abundantly in microorganisms, fungi, plants, and sessile animals. In the evolution of secondary metabolism, evidence suggests that plants may have acquired some genes of key enzymes of biosynthesis of relatively ancient origin by horizontal gene transfer. Ectomycorrhizal and endophytic fungi might have transferred their pathway genes into the genome of their host plants times ago. Because of their intimate intra- or intercellular association with plants for competence, survival, and reproduction, endophytes have become a class of interesting and curious microorganisms. This book aims to present a comprehensive account of the fast-expanding field of endophytes and their applications for the welfare of human beings. Novel and beneficial effects of endophytes are being developed besides several already existing ones from the agriculture to industrial levels. This book includes original research reviews and case studies on diversity and ecology of major groups of endophytes, plant–endophyte interactions, identification of endophytes by conventional and molecular tools, biologically active compounds from endophytes, and applications in agriculture and industry. This book will be useful for all those concerned with microorganisms – from students and researchers in the field of botany, pathology, biotechnology, and agriculture to entrepreneurs involved in developing industrial applications.

Finally, I would like to acknowledge all our contributors who have made immense efforts to ensure the scientific quality of this book. We hope that the book will be useful for researchers in academia and industry.

I am grateful to respected Professor B.N. Johri, for agreeing to write the foreword for this book, and to Professor K.G. Ramawat for constant encouragement and support.

We thank all our colleagues at Springer, particularly Sylvia Blago and Clifford Nwaeburu for excellent support.

June 2019 Sumita Jha

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About the Editor

Sumita Jha (nee Mukherjee) received her M.Sc. (1975) and Ph.D. (1981) from the University of Calcutta, Kolkata, in India. She joined the same University as UGC (New Delhi) Research Scientist in 1985 and as a faculty member in Botany in 1990. She was appointed Associate Professor in 1993 and became Professor in 2001. She served as Program Coordinator of UGC Centre of Advanced Study in Botany (2004–2015), as Head Department of Botany (2007–2009), and as Head Department of Genetics (2009–2011; 2013–2014), Calcutta University. She has been involved in teaching courses on plant biology, plant genetics, and biotechnology.

Prof. Jha's group has developed transgenic cell and organ cultures in a number of rare, endangered indigenous medicinal plants for the production of high-value pharmaceuticals. Her research is supported by funding from the Department of Science and Technology and the Department of Biotechnology, Government of India.

Prof. Jha, under an Indo-French Project funded by IFCPAR/CEFIPRA (2002–2005), developed a strong collaboration with Dr. David Tepfer, INRA, Versailles, France, leading to the development and exchange of novel methods and information on secondary metabolism in transformed plant cell and organ cultures, and proposing that natural transformation operates as an adaptive resource in evolution, particularly in plant–microorganism interactions. She also visited AFRC Institute of Food Research (Plant Biotechnology Group), Norwich, UK (1991), and Plant Science Division, Nottingham University, Nottingham, UK (1999), as Visiting Scientist under Royal Society–Indian National Science Academy scientist exchange program.

Prof. Jha has published more than 130 research papers in internationally recognized journals and 20 book chapters. To her credit, Professor Jha has mentored 20 students toward their Ph.D., and 5 are working on their Ph.D. thesis. She has served on various advisory committees and has garnered prestigious awards for her contributions to academic excellence. Notable among these are the INSA Science Academy Medal for Young Scientist (1983), the Prof. Hiralal Chakravarty Award by ISCA (1989), the UGC Career Award for young teachers (1994–1997), Fellow of the National Academy of Sciences, India (2008), and Fellow of the West Bengal Academy of Science and Technology (2015).

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Part I

Biology of Major Groups of Endophytes

Biologically Active Compounds from Bacterial Endophytes

1

Pablo R. Hardoim

Contents

Abstract

The phytomicrobiome plays a key role in incrementing the fitness of the host. The interactions between plants and their microbes yield a vast and diverse assortment of secondary metabolites. The myriad of genes within bacterial cells thriving inside plant tissues (i.e., endophytes) contributes to the production and conversion of small molecules into bioactive compounds, and the genome mining can be a powerful tool to extract this knowledge from large amounts of data sets. In this chapter, annotated biosynthetic gene clusters ($n = 4614$ unique within 60,632 genes) from genomes of endophytes assigned to *Actinobacteria* ($n = 26$), *Bacteroidetes* ($n = 6$), *Firmicutes* ($n = 15$), and *Proteobacteria* ($n = 99$) were analyzed and predicted to be involved in the biosynthesis of 4766 types of secondary metabolites classified within 22 families. The vast majority of secondary metabolites was predicted as putative ($n = 3684$), followed by those involved

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in the biosynthesis of nonribosomal peptide synthetase ($n = 293$), polyketide synthases ($n = 268$), and terpene ($n = 120$) compounds. This reveals that the community of endophytes conceals a great source of potential proteins with novel enzymatic activities and novel families of secondary metabolites.

Keywords
Bacterial endophytes · Bioactive compounds · Nonribosomal peptide synthetase (NRPS) · Polyketide synthases (PKSs) · Improved plant fitness

1 Introduction

Plant forms associations with microbes and both partners interact concomitantly with the environment as a holobiome rather than a single living organism. In nature, these multitrophic interactions among plants and their microbial players, represented largely by archaea, bacteria, and fungi, are the rule. These symbiotic microbes augment an extra layer of complex complementary functions that often increase host plasticity and fitness, especially in challenge conditions [1, 2]. Until today no individual plant investigated was reported without a microbial community associated with it. The presence of bacteria in long-term in vitro propagated plants from meristematic cells, which are believed to be free of microbes [3], emphasis that is extremely difficult, if not at all impossible, to segregate the host cells from their microbes. The importance of these mutualistic bacteria in micropropagated plants becomes more evident during seedling acclimatization where the inoculation of previously isolated strains significantly enhances root and shoot development [4]. These benefits for plant growth, development, and even reproduction have been positively correlated with investigated bacterial properties of inoculated strains, suggesting that these mutualistic bacteria interact intimately with the host and might be even thrive inside host cells as stable endosymbionts [5, 6]. The importance of these microbes for the host growth and fitness has been long overlooked, and only recently we started to scratch the surface of what is a promising field for production of food in sustainable agriculture [7].

Plant growth-promoting bacteria were also shown to modify the host biosynthesis of primary and secondary metabolites [8]. For instance, the endophyte alphaproteobacterium *Azospirillum* sp. B510 might enhance plant resistance against pathogenic fungi in the host rice (*Oryza sativa*) by inducing the biosynthesis of phenolic compounds such as flavonoids, hydroxycinnamic acid derivatives, and alkylresorcinols [9]. The endophyte actinomycete *Pseudonocardia* sp. YIM 63111, isolated from surface-sterilized tissue of the medicinal plant *Artemisia annua*, stimulates the biosynthesis of the antimalarial compound artemisinin in its host plant *A. annua* by inducing the expression of genes cytochrome P450 monooxygenase and cytochrome P450 oxidoreductase [10]. The inoculation of the endophyte betaproteobacterium *Paraburkholderia phytofirmans* PsJN induces the plant grapevine tolerance to low temperatures by modulating its host carbohydrate metabolism [11]. Furthermore, endophytic bacteria are likely to be adapted to the presence

and are capable to metabolize complex organic molecules [12, 13]. These features are especially interesting for biodegradation activities, and the application of symbionts to improve phytoremediation strategy is in high demands for contaminated and marginal soils [14]. Therefore, the production and secretion of novel enzymes and metabolites involved in pharmaceutical therapy, biopolymers and biofuel production, wastewater treatment, farming, and human well-being are of high interest for industrial and farming applications.

In this context, endophytic fungi offer an enormous potential for discovering novel products with pharmaceutical and agrochemical applications [15, 16]. Nevertheless, bioactive secondary metabolites produced by endophytic bacteria have been mostly overlooked. A classic example is the production of maytansinoid compounds, which once was thought to be a plant metabolite since these were originally isolated from members of the higher plant families *Celastraceae*, *Rhamnaceae*, and *Euphorbiaceae*. The extraordinarily potent antitumor maytansinoid ansamitocin is indeed originated from the biosynthetic gene clusters of the actinomycete *Actinosynnema pretiosum* ssp. *auranticum* ATCC 31565 [17]. Here, a genome mining approach was applied to identify the diversity and distribution of biosynthetic gene clusters assigned to secondary metabolite families among bacterial endophytes. Unfortunately with this approach, the link between gene clusters and identification of characterized bioactive compounds is not always possible. Nevertheless, I summarize the main findings on the gene clusters involved in the biosynthesis of known secondary metabolites, discuss their biological and ecological functions when allowed, and speculate on plausible functions of few putative-based bioactive compounds detected among bacterial endophytes.

2 Methods of Analysis

Genomes from bacterial communities capable to live inside host plants without causing any apparent harm have been compiled. Only genomes of bacteria published in peer-reviewed journals and deposited in the Pubmed repository (as of May 01, 2018) were included. The endophyte genome data set was generated by using the string "endophyt* AND genome." This was further refined by strains available in the Integrated Microbial Genomes & Microbiomes and Atlas of Biosynthetic Gene Clusters (IMG/ABC) data mart [18]. To avoid bacterial species redundancy, a pairwise genome-wide average nucleotide identity (gANI) was performed. Genome sequences with more than 96.5% for gANI and an alignment fraction more than 0.6 were computed as an intraspecies cluster [19]. When more than one bacterial strain, including a single strain with more than one genome sequenced, was assigned to a single intraspecies cluster, a representative genome was selected based on the following criteria: (i) sequence status "finished" and (ii) the highest number of putative genes encoding proteins. By removing intraspecies genome sequences, the collected data on secondary metabolite become more reliable. Genome sequences from singular species of bacterial endophytes were used for detection of biosynthetic gene clusters (BGCs) potentially involved in the biosynthesis of

bioactive compounds with implementation of cluster finder algorithm in the IMG/ABC data mart [18]. A feature-by-sample contingency table with the values of secondary metabolites assigned to its respective family in each genome was generated (i.e., SM-by-sample) as well as a second contingency table with genomic features with all data statistics annotated by JGI from each genome sample (i.e., genomic-by-sample). Unconstrained (simple) and constrained (canonical) ordinations were performed with principal coordinate analysis of the dissimilarity matrix. All multivariate statistical analyses were conducted using the vegan package [20] in R Program [21]. Distance-based redundancy analysis was used to further assess how genomic features of endophytes affected the composition of SMs. Permutational multivariate analysis of variance was used to evaluate the effects of taxonomy on secondary metabolite composition using adonis function from vegan package with 999 permutations.

3 Identification of Endophytes with Potential to Synthesize Bioactive Products

The massive DNA sequencing of microbial genomes by thousands of next-generation sequencing projects has provided unprecedented opportunities to explore the diversity and distribution of natural products originated from biosynthetic gene clusters. These bioactive compounds play many important physiological roles, including communication between and among species, enhance competition for nutritional elements, and improve fitness, especially to survive in adverse conditions. Additionally, these compounds have a chemical structure complexity unmatched by synthetic chemistry, and their attractive functional properties, such as antimicrobial, anticancer, antidiabetic, antioxidant, somatic fat reducing, and immune suppressive, are very appealing for pharmaceutical and farming applications. There are many studies reporting novel and known biological activities of secondary metabolites synthesized by endophytic fungi and how these bioactive compounds might improve fitness of plants and symbionts alike [15, 16, 22, 23]. Although bacterial strains are also involved in the biosynthesis of secondary metabolites, only few studies, mostly describing products originated from strains of actinomycetes, are reported [24–27]. In this context, the genomic sequences of 146 bacterial endophytes, including strains from *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla, were investigated with a genomic mining approach to predict the identification of BGCs encoding proteins putatively involved in the biosynthesis of secondary metabolites [18, 28, 29]. Within phylum *Actinobacteria* ($n = 26$), the genera *Arthrobacter* ($n = 4$), *Curtobacterium* ($n = 1$), *Frankia* ($n = 9$), *Frigoribacterium* ($n = 1$), *Jiangella* ($n = 1$), *Kibdelosporangium* ($n = 1$), *Microbacterium* ($n = 2$), *Micromonospora* ($n = 1$), *Nocardia* ($n = 2$), *Rhodococcus* ($n = 2$), and *Streptomyces* ($n = 2$) are explored. Members of phylum *Bacteroidetes* ($n = 6$) comprise strains from *Chitinophaga* ($n = 1$), *Chryseobacterium* ($n = 3$), *Elizabethkingia* ($n = 1$), and *Flavobacterium* ($n = 1$) genera, while the phylum *Firmicutes* ($n = 15$) is represented by *Bacillus* ($n = 7$), *Brevibacillus* ($n = 2$),

Enterococcus ($n = 1$), *Lactococcus* ($n = 1$), *Lysinibacillus* ($n = 1$), *Paenibacillus* ($n = 2$), and *Staphylococcus* ($n = 1$). Members of the genera *Azorhizobium* ($n = 1$), *Azospirillum* ($n = 1$), *Bradyrhizobium* ($n = 2$), *Gluconacetobacter* ($n = 1$), *Martelella* ($n = 1$), *Mesorhizobium* ($n = 1$), *Methylobacterium* ($n = 4$), *Phyllobacterium* ($n = 1$), *Rhizobium* ($n = 15$), *Sinorhizobium* ($n = 5$), and *Sphingobium* ($n = 1$) are investigated within class *Alphaproteobacteria* ($n = 33$). The phylum *Betaproteobacteria* ($n = 18$) is represented by strains of the genera *Acidovorax* ($n = 2$), *Azoarcus* ($n = 2$), *Burkholderia* ($n = 3$), *Herbaspirillum* ($n = 5$), *Paraburkholderia* ($n = 2$), *Polaromonas* ($n = 1$), and *Variovorax* ($n = 3$). The most diverse of all investigated group is represented by strains of the genera *Acinetobacter* ($n = 1$), *Enterobacter* ($n = 5$), *Erwinia* ($n = 1$), *Klebsiella* ($n = 3$), *Kosakonia* ($n = 4$), *Pantoea* ($n = 4$), *Pseudomonas* ($n = 26$), *Raoultella* ($n = 1$), *Rheinheimera* ($n = 1$), *Serratia* ($n = 1$), and *Stenotrophomonas* ($n = 1$) of class *Gammaproteobacteria* ($n = 48$). In this genome mining, only strains from genera *Bacillus, Frankia, Pseudomonas,* and *Rhizobium* have more than five genome sequences investigated. Strains from these genera are remarkable and versatile with broad spectrum of functional traits [30–32] and have adapt a facultative lifestyle by colonizing diverse habitats and hosts, by forming symbiosis with plants and animals, and by living freely as soil dwellers.

4 Biosynthetic Gene Clusters Content and Their Distribution Across Bacterial Class

Biosynthetic gene clusters putatively involved in the biosynthesis of secondary metabolites were identified within genome sequences of strains assigned to phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Table 1). The percentage of genes putatively involved in the biosynthesis of secondary metabolites in relation to the total number of genes in each genome is significantly larger ($p < 0.001$) in strains of *Actinobacteria*(up to 26%) when compared to any other investigated phyla (13–1%). This result emphasizes the importance of actinobacterial strains as the largest producers of secondary metabolites when compared to any other investigated class of bacterial endophytes and might reflect their facultative lifestyle strategies for thriving as free-living soil inhabitants as well as plant symbionts [30].

Strains *Frankia inefficax* EuI1c^T, *Frankia* sp. CN3, and *Nocardia casuarinae* BMG51109 reveal between 24% and 26% of their coding sequences dedicated to the biosynthesis of secondary metabolites, while strain *Microbacterium foliorum* 122 has the lowest proportion (2.2%) across *Actinobacteria* endophytes. *Frankia* species are notorious nitrogen fixer symbionts of actinorhizal plants. However, it is interesting that these strains of *Frankia* have the genome represented by atypical *Frankia* species [30], which are unable to reinfect actinorhizal plants (strain CN3) or when it is able to infect plants; this is restricted to plants of family *Elaeagnaceae* and lacks the capability to establish effective root nodules or even to fix atmospheric nitrogen inside the host plants (strain EuI1c^T) [33, 34]. The strains from this atypical

Table 1 Distribution of identified secondary metabolite families across bacterial classes of endophytes

BiG-SCAPE class	Secondary metabolite families	Actino	Chitino	Flavo	Bacilli	Alpha	Beta	Gamma
		Bacteria		Phaga	Bacteria	Proteobacteria		
NRPS	nrps	98	1	0	46	25	26	97
PKS I	t1pks	132	1	2	3	11	15	8
PKS other	t2pks	24	0	0	5	0	1	2
PKS other	t3pks	20	1	1	2	9	0	1
PKS other	t4pks	25	0	2	1	0	1	1
PKS other	Transatpks	6	0	0	14	0	3	3
RiPPs	Bacteriocin	16	1	6	16	11	6	23
RiPPs	Lantipeptide	35	0	6	10	2	0	2
RiPPs	Thiopeptide	4	0	0	0	0	0	0
Terpene	Terpene	60	1	6	12	11	17	13
Saccharides	Amglyccycl	1	0	0	0	0	0	0
Saccharides	Oligosaccharide	3	0	0	0	0	0	0
Others	Blactam	1	0	0	0	0	0	0
Others	Butyrolactone	9	0	0	1	0	0	6
Others	Ectoine	9	0	0	0	2	1	2
Others	Hserlactone	1	0	0	0	15	4	15
Others	Melanin	3	0	0	0	0	0	0
Others	Phenazine	1	0	0	0	0	0	0
Others	Phosphonate	1	0	0	1	2	2	1
Others	Siderophore	18	0	5	7	6	4	19
Others	Other	51	0	3	7	8	7	21
Others	Putative	1020	10	53	230	1021	519	831
Total		**1538**	**15**	**84**	**355**	**1123**	**606**	**1045**

Secondary metabolites were identified using cluster finder algorithm from IMG/ABC (https://img.jgi.doe.gov/cgi-bin/abc/main.cgi) and classified according to BiG-SCAPE (https://git.wageningenur.nl/medema-group/BiG-SCAPE/wikis/home) and AntiSMASH (https://antismash.secondarymetabolites.org/) tools

Frankia cluster also have the largest genome size across *Frankia* species. The inability to establish a fruitfully mutualistic interaction with the host plant might be correlated with their adaptation to cosmopolitan lifestyle. These strains are capable of thriving inside the host plants as well as outside in the soil environment, where competition for resources is generally tough, such as in the rhizosphere. The biosynthesis of intra- and interspecies communication molecules or even of deterrent compounds under highly competitive conditions might be an essential edge for the fitness of these microorganisms, therefore optimizing niche adaptation.

Strains of *Bacillus amyloliquefaciens* 629 from *Bacilli* and *Burkholderia gladioli*3A12 (also known as UCD-UG_CHAPALOTE) from *Betaproteobacteria* classes also reveal a large proportion of their genomes (13% and 11.5%, respectively) dedicated to genes encoding sequences involved in the biosynthesis of

bioactive products. *Burkholderia gladioli* strain 3A12was isolated from an ancient maize (*Zea mays* subsp. *mays*) landrace as part of a project investigating the antifungal properties of bacterial endophytes [35].

Within actinomycetes, strains from genera *Frankia*, *Kibdelosporangium*, and *Nocardia* have significantly ($p < 0.05$) larger percentage (20.4%, 22.3%, and 23.8%, respectively) of dedicated genes putatively involved in the biosynthesis of secondary metabolites than any other strains of actinomycetes, with exception of *Micromonospora lupini* Lupac_08 with 13%. No significant ($p < 0.05$) difference in the amount of genes involved in the biosynthesis of secondary metabolites is observed across strains of each genus assigned to *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Overall, these results reinforce the current knowledge available from the literature, in which strains from actinomycetes are important source of bioactive compounds. They also reveal that some strains in this group are capable to dedicate a large proportion of their DNA coding signature to the biosynthesis of secondary metabolites. The energetic costs to maintain up to one-fourth of their total coding genes for the biosynthesis of natural products must be enormous and certainly reflects both the capacity to colonize diverse habitats and their lifestyle strategy. From an ecological perspective, the boundaries between groups of endophytes and epiphytes are not always clear, because bacteria thriving inside plant host tissues might also colonize the external layers of the plant and even the surrounding soil substrates [1].

Biosynthetic gene clusters of endophytes ($n = 4614$) identified in this study are assigned to 22 families of secondary metabolites. Predicted bioactive products from antibiotic families aminoglycoside/aminocyclitol, β-lactam, and a heterocyclic organic compound of phenazine class are detected only once among actinobacterial genomes of *F. alni* ACN14a, *Streptomyces* sp. LUP30, and *Nocardia* sp. BMG111209, respectively. A canonical correspondence analysis performed on the distribution of all 4763 bioactive compounds assigned to remaining 19 families of secondary metabolites across 146 endophyte genomes reveals major separations on the distribution of secondary metabolites by bacterial classes (Fig. 1). On the ordination triplots, the distribution of bioactive compounds synthesized by *Actinobacteria* differs largely from those of *Gammaproteobacteria*, whereas secondary metabolites synthesized by communities of *Alpha* and *Betaproteobacteria* contrast with those of *Bacilli* and *Flavobacteria* classes. Despite these differences, only the distribution of bioactive compounds synthesized by actinobacterial strains differs significantly ($p < 0.001$) from all other classes of endophytes.

Actinobacterial strains have their peculiarities. For instance, BGCs involved in the production of sugar (oligosaccharide, $n = 3$), pigment melanin ($n = 3$), and antibiotic thiopeptide ($n = 4$) metabolites are exclusively detected among genomes of *Actinobacteria*. Oligosaccharide compounds have multiple roles in signaling transduction systems in plants, regulating host defense response and also developmental processes, including the formation of specialized symbiotic organelles. A major component of fungal cell wall, the oligosaccharide chitin, is recognized as a general elicitor by plants, animals, and insects and activates innate immune system response in these organisms [36]. Interestingly, specific modifications on the extracellular glucosamine oligosaccharide molecules by addition of fatty acids, sulfates,

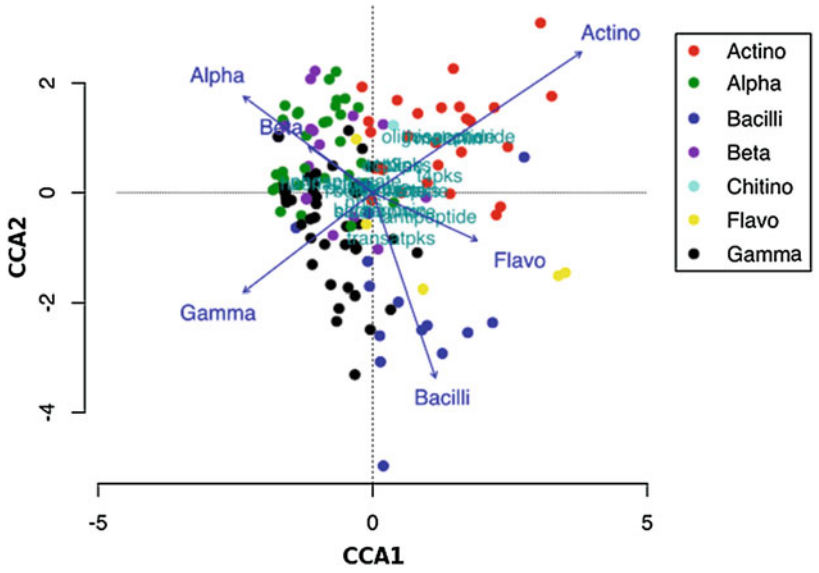

Fig. 1 Canonical correspondence analysis triplots of Hellinger-transformed secondary metabolites distribution matrix constrained by bacterial classes. The ordination plot was generated with results of secondary metabolite distribution across 146 bacterial endophyte samples. Classes of secondary metabolites were projected as weighted averages, whereas only significantly different ($p < 0.05$) classes of bacteria were projected a posteriori as explanatory factor using function envfit(). Actino, *Actinobacteria*; Alpha *Alphaproteobacteria*; Beta, *Betaproteobacteria*; Chitino, *Chitinophaga*; Flavo, *Flavobacteria*; Gamma, *Gammaproteobacteria*

acetyl groups, or even some sugars, generate "NOD factor" that elicit root hair deformation and nodulation in legume roots in the symbiotic interaction with rhizobial bacteria [37]. There are several mechanistic similarities in the perception and transduction of signals by plants when interacting with beneficial and detrimental microbes. Furthermore, signaling of arbuscular mycorrhizal (AM) fungi, rhizobial, and actinorhizal nodules occurs via a common symbiotic signaling pathway, where root plasma membrane receptor kinases recognize rhizobial and actinorhizal "NOD factor" and signal factors of AM fungi. These receptors activate signal transduction pathway, which leads to nuclear calcium spiking reads and further activation of transcription factors putatively involved in the infection and organogenesis pathways [38]. Therefore, it is plausible that within the course of evolution, initial similar responses were further adapted to distinguish between these relationships. In addition to the function of cell wall surface and signaling molecules, oligosaccharides, such as trehalose, amino acids, and sugar alcohol, are also important osmotic regulators in many Gram-negative bacteria. At this moment, the functional characterization of oligosaccharides synthesized by *Frankia saprophytica* CN3, *Kibdelosporangium phytohabitans* KLBMP1111, and *Streptomyces* sp. LUP30 was not accomplished. However, it is very likely that these metabolites are not involved in organogenesis development [34].

5 Secondary Metabolites Synthesized by *Actinobacteria*

Actinobacteria represents one of the most dominant phyla among *Bacteria* and comprises of heterogeneous Gram-positive and even few Gram-negative species (e.g., *Thermoleophilum* sp., *Gardnerella vaginalis*, and *Saccharomonospora viridis* $P101^T$) with a high G + C content in their DNA. Their cells exhibit a wide variety of morphologies, ranging from coccoid (e.g., *Micrococcus*) or rod-coccoid (e.g., *Arthrobacter*) to fragmenting hyphal forms (e.g., *Nocardia* spp.) or permanent and highly differentiated branched mycelium (e.g., *Streptomyces* spp.). Their physiological and metabolic capabilities are also very diverse. They can be either aerobes or anaerobes, motile or non-motile, spore or non-spore-forming bacteria, and reproduce by mycelia (vegetative mode) or by formation of spores or conidia (asexual mode). They are ecologically important in the mineralization of organic matter in the soil, where the majority of *Actinobacteria* is able to synthesize various classes of extracellular enzymes including nucleases, lipases, glucanases, xylanases, amylases, proteinases, peptidases, peroxidases, chitinases, cellulases, ligninases, pectinase, hemicellulase, and keratinase, which facilitate the process organic decompositions. Members of *Actinobacteria* are well adapted to thrive in diverse ecological niches such as terrestrial and aquatic ecosystems (both fresh and marine waters) and are reported to adopt diverse lifestyles such as pathogens (e.g., *Actinomyces* spp., *Corynebacterium* spp., *Gordonia* spp., *Mycobacterium* spp., *Nocardia* spp., *Propionibacterium* spp., and *Tropheryma* spp.), plant growth promoters (*Arthrobacter* spp., *Azotobacter* spp., *Microbacterium* spp., *Microbacteriumlactium* spp., *Micromonospora* spp., *Nocardiopsis* spp., *Rhodococcus* spp., and *Streptomyces* spp.) nitrogen-fixing symbionts (*Agromyces* spp., *Arthrobacter* spp., *Corynebacterium* spp., *Frankia* spp., *Micromonospora* spp., *Mycobacterium* spp., *Propionibacteria* spp., *and Streptomyces* spp.), mycorrhizal symbionts (*Streptomyces* spp.), and health-promoting gastrointestinal tract inhabitants (*Bifidobacterium* spp.) [39, 40]. Inside the host plants, *Actinobacteria* strains are largely isolated from root tissues [27], emphasizing the role of soil as an important source for actinobacterial endophytes.

Actinobacteria also play a major role in plant and human health as biocontrol agents. They are the primary source of most naturally biosynthesized antibiotic compounds and are notably known for their capability to synthesize secondary metabolites. For instance, in agriculture, at least 3000 potent bioactive compounds were reported with pesticide, herbicide, plant growth regulatory, insecticide, larvicide, acaricide, algicide, and nematicide activities [39]. Furthermore, the importance of this group of bacteria for the discovery of new drugs was recently revised in a comprehensive genome mining study, where the capacity of actinobacterial strains to encode hundreds of thousands of bioactive compounds was reported [41]. Therefore, *Actinobacteria* remains an extensive pool for bioprospecting relevant natural biomolecules for industrial, pharmaceutical, and farming interest. Among endophytes, actinobacterial strains have the capacity to synthesize a vast amount of bioactive compounds (Table 1). They are the largest and the most diverse producers of bioactive compounds. BGCs involved in the biosynthesis

of bioactive compounds of NRPS, type I PKS, other types of PKS (type II pks, type III pks, type IV pks, and *trans*-AT pks), ribosomally synthesized and post-translationally modified peptides (RiPPs, such as lasso peptide, thiopeptide, and bacteriocin), terpenes, saccharides (amglyccycl,oligosaccharide), and other (including blactam, butyrolactone, ectoine, hserlactone, melanin, phenazine, phosphonate, siderophore, and secondary metabolite-related proteins that do not fit into any other assigned category, denominated as "others" or as "putatives") families are detected (Fig. 2).

Almost 2/3 of all bioactive products identified among actinobacterial strains are assigned to putative family, which is significantly larger ($p < 0.05$) than the abundance detected among *Bacilli*, *Flavobacteria*, and *Gammaproteobacteria* strains (Table 1). This is a class of putative biosynthetic gene clusters with unknown type identified in the cluster finder algorithm [18]. As these bioactive compounds are only predicted in silico, further characterization by experimental assays should resolve their chemical structures and biological functions. This could increment the discovery of new drugs and even new types of secondary metabolites family. Strains of *Nocardia*, *Frankia*(except *Frankia casuarinae* CcI3), and *Micromonospora*, which are often isolated from actinorhizal plants and capable to induce nodule structure on the roots of selected hosts, are the largest producers of putative secondary metabolites with more than 60 bioactive compounds frequently detected among their strains (Fig. 2). These putative bioactive compounds might be directly involved in competent plant-microbial mutualistic interactions. Previously result reported here showed that *Frankia* strains (CN3 and EuI1c^T) equipped with the largest percentage of genes encoding secondary metabolite proteins in their genomes are inefficient mutualistic symbionts [30]. These results reveal the importance of a combination or even a single uncharacterized gene cluster rather than the amount of genes encoding secondary metabolite proteins for a strain to be a successfully mutualistic symbiont. This assumption is further supported by the observation that none of other investigated actinobacterial endophyte strains have the ability to induce root nodule formation on legume plants and by the fact that the genome of *F. casuarinae* CcI3 has only 29 putative BGCs detected, which is less than a half of that detected among strains able to induce root nodulation in a wide range of actinorhizal plants. It is interesting that this strain possesses one of the smallest genomes across *Frankia* species and is capable to nodulate only few *Casuarina* and *Allocasuarina* species in the *Casuarinaceae* host family, which grow in restricted geographic regions of Australia and the Pacific islands [42]. Events of gene loss, gene duplication, and chromosomal rearrangements are very likely to participate on the adaptation of this strain to this host plant speciation. This drastic reduction in the numbers of putative BGCs was not observed on any other family of secondary metabolites, emphasizing the assumption that the loss of putative BGCs directly affects the capabilities of *F. casuarinae* CcI3 to establish effective mutualistic interactions with a broad variety of host plants. This hypothesis is worth exploring in the future studies. Experimental characterization of these bioactive compounds should demonstrate whether or not they are essential for plant host beneficial interactions.

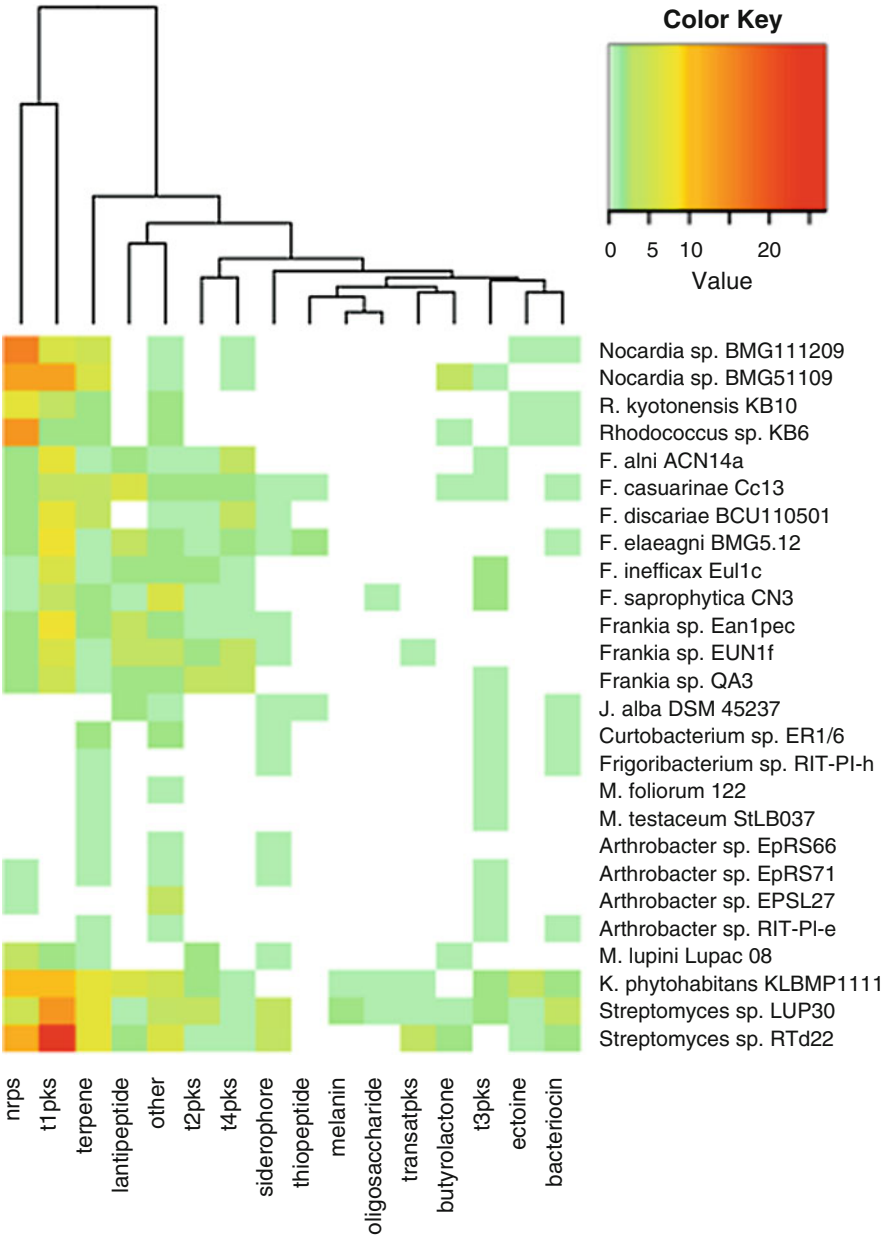

Fig. 2 Heat map shows abundance values of 16 secondary metabolite families detected across actinobacterial strains. Putative-based family of secondary metabolites was removed for visual clarity. Species names of bacteria were abbreviated as R. for *Rhodococcus*, F. for *Frankia*, J. for *Jiangella*, M. for *Microbacterium testaceum* and *Micromonospora lupini*, and K. for *Kibdelosporangium*

Biosynthetic gene clusters of NRPS are the third most abundant family among endophytic actinobacterial strains (Table 1). Genomes of *Arthrobacter, Curtobacterium*, *Frankia*, *Frigoribacterium*, and *Microbacterium* strains are equipped only with maximum of two NRPS clusters, whereas genomes of *Nocardia, Rhodococcus* sp. KB6, *K. phytohabitans* KLBMP1111^{T}, and *Streptomyces* sp. RTd22 strains have at least ten NRPS clusters. The importance of NRPS and NRPS-PKS hybrid clusters for the biosynthesis of bioactive compounds among actinobacteria is further demonstrated in a comprehensive genome mining study with sequences of830 actinobacterial strains [41]. The largest amount of NRPS clusters was detected among members of *Streptomycetales* and *Pseudonocardiales*, whereas members of *Propionibacteriales*, *Micrococcales*, and *Frankiales* have only few NRPS clusters. Earlier assessment of *K. phytohabitans* KLBMP1111^{T} genome, a member of *Pseudonocardiales* with plant growth- promoting properties isolated from the oil seed plant *Jatropha curcas* L., has allowed the identification of gene clusters responsible for nonribosomal peptide synthesis of at least 12 natural products as well as genes involved in phytohormone synthesis and modulation [43].

It is interesting that despite the dominance of NRPS-based products among the 830 actinobacterial strains, it was predicted by data extrapolation that with the increment in genome sequencing of novel actinobacterial strains, type I PKS would be the most abundant family of secondary metabolites [41]. This bias toward NRPS-based products was consistent with the nonrandom nature, with over 40% of the genomes derived from a few medically relevant genera, data set investigated in their study.

Here, bioactive compounds assigned to type I PKS are the second most abundant class with 132 entries detected (Table 1). The largest producers are actinobacterial endophytes *Nocardia sp. BMG51109*, *K. phytohabitans* KLBMP1111^{T}, *Streptomyces sp. LUP30*, and *Streptomyces* sp. RTd22 ($n \geq 10$), whereas all investigated strains of *Arthrobacter*, *Curtobacterium*, *Frigoribacterium*, *Jiangella*, and *Microbacterium* reveal null capability to synthesize type I PKS-based bioactive products (Fig. 2). Unfortunately at this point, the molecular characterization of these type I PKS-based secondary metabolites was not performed, and their biological and ecological functions inside host plants remain unknown. However, these polyketide compounds are structurally diverse natural products with antibiotic, chemotherapeutic, and phytotoxic activities [44].

Biosynthetic clusters of terpene products are the fourth most commonly detected family among actinobacterial strains and are significantly higher ($p < 0.05$) than any other investigated bacterial class (Table 1). These natural compounds might be largely detected among strains of *Nocardia*, *K. phytohabitans* KLBMP1111^{T}, and *Streptomyces*. Terpenes are probably the largest class of small-molecule natural products on earth with more than 55,000 members identified so far, the epitome of molecular biodiversity [45]. Terpenes are formed within the mevalonate or deoxyxylulose phosphate pathways with units of dimethylallyl pyrophosphate and isopentenyl pyrophosphate. Throughout the tree of life, all organisms are capable to synthesize terpene molecules, although they are best known as plant metabolites. The biological and ecological roles of bacterial terpenes remain largely unknown,

even for the volatile sesquiterpene geosmin, one of the most well-known terpenes emitted by *Streptomyces*. This is certainly extended to actinobacterial endophytes, where no biological and ecological functions have been reported so far for the synthesized terpene molecules. One exception to this observation is met in the powerful sesquiterpene albaflavenone synthesized by *Streptomyces albidoflavus* strain DSM 5415, which was isolated from corn (*Zea mays* L.) seeds [46]. Albaflavenone has a characteristic odor of an earthy camphor-like aroma and exhibits antibacterial activity; however, its ecological function on host seeds remains to be elucidated.

In addition to these three classes (type I PKS, NRPS, and terpene), endophytic actinobacterial BCs involved in the biosynthesis of lasso peptide, type II, type III, type IV PKS, and other clusters are detected in significantly larger ($p < 0.05$) amounts among strains of *Actinobacteria* than any other investigated bacterial class of endophytes. Lasso peptides (also known as lantipeptides) are cyclic peptides ribosomally synthesized followed by extensive posttranslational modifications. Their chemical structures have characteristic lanthionine and/or methyllanthionine thioether cross-links that are often unsaturated with amino acids dehydroalanine and dehydrobutyrine [47]. Not all lantipeptides have antimicrobial activity. Only those that are confirmed experimentally are called lantibiotics. Genome mining approach of lanthionine synthetase, a single posttranslational promiscuous enzyme capable to transform ribosomally synthesized linear peptides into a multitude of polycyclic peptides with highly diverse ring topologies, reveals that lantipeptide biosynthesis might occur in single-celled strains of planktonic marine cyanobacteria*Prochlorococcus* around the world oceans [48]. This result demonstrates that biosynthesis of lantipeptide compounds is not restricted to Gram-positive or soil bacteria as long believed and that other bacteria might have acquired the capability to synthesize these cyclic peptides. Among endophytic actinobateria, lasso peptide clusters are largely detected in strains of *Frankia* and *K. phytohabitans* KLBMP1111^{T}, but their biological functions are yet to be revealed.

Compounds of tetracycline are aromatic antibiotic polyketides produced via type II polyketide synthases. These tetracyclines exert their antibiotic effect primarily by binding to the bacterial ribosome and consequently halting protein synthesis in susceptible bacteria [49]. Endophytic strains of *Frankia* are the potential largest producers of tetracycline compounds (Fig. 2). The gene *ctcP* encoding a tetracycline 7-halogenase (EC: 1.14.19.49), an enzyme responsible for chlorination of tetracycline compound in the last step of chlortetracycline biosynthesis, was detected among these strains. The gene *oxyR* encoding a 5a,11a-dehydrotetracycline reductase (EC: 1.3.98.4), an enzyme involved in the biosynthesis of oxytetracycline – another natural product of tetracyclines, was not detected among endophyte strains. The genes *actI1*, *actI2*, and *actI3* encoding a minimal PKS ketosynthase (EC: 2.3.1.235) that is involved in backbone biosynthesis of type II polyketide and tetracyclines were detected mainly among strains of *Frankia* spp., *K. phytohabitans* KLBMP1111, *M. lupini* Lupac 08, *Nocardia* sp. BMG51109, and *Streptomyces* sp. LUP30. Although the biosynthesis of tetracycline compounds is an important feature to reduce resource competition in diverse niches, it seems that is not a

mandatory strategy to interact with plant. It is interesting that the gene *tetX* encoding a key enzyme involved in the resistance to tetracycline (EC, 1.14.13.231, tetracycline 11a-monooxygenase) was detected exclusively in the *Flavobacteriales* order of *Bacteroidetes* within genera *Chryseobacterium* and *Elizabethkingia*. This enzyme catalyzes efficiently the degradation of a broad range of tetracycline natural products both in vitro and in vivo, decreasing locally its concentration [50].

Biosynthetic gene clusters encoding signal molecules of butyrolactone, high-affinity iron-chelating compounds of siderophore, and NRPS classes are detected in significantly larger ($p < 0.05$) numbers among *Actinobacteria* than among strains of *Alphaproteobacteria* (Table 1). The quorum sensing butyrolactone system is almost exclusively limited to *Actinobacteria* and plays a major role in the biosynthesis of secondary metabolites [51]. Despite this fact, only a limited number of actionobacterial strains ($n = 6$) seem to employ this strategy of communication (Fig. 2). Metabolites with osmolyte properties (i.e., capable to ameliorate extreme osmotic stress) of ectoine family are significantly more abundant among *Actinobacteria* than among *Gammaproteobacteria* strains (Table 1). Strain *K. phytohabitans* KLBMP1111 is equipped with four copies of this gene cluster, while other five strains have a single copy of ectoine family gene cluster (Fig. 2). The ecological function of these compounds inside the host plant is largely unknown; however, the concentration of metabolites inside certain plants, including sugarcane and mangrove plants, or during specific developmental stages, such as extensive drought season, might be extremely exacerbate, therefore favoring the growth of strains with osmotic protective mechanism.

6 Secondary Metabolites Synthesized by *Bacilli*

Members of *Bacilli* class generally form a Gram-positive type of cell wall with relative low G + C content in their DNA when compared to *Actinobacteria*. Their physiological and metabolic capabilities are very diverse ranging from aerobes to anaerobes, motile to non-motile, and may or may not form endospores. The majority of *Bacilli* species are harmless, free-living saprophytes ubiquitous in soil. This ever-competitive environment for nutrient sources might have shaped cells to morphological and physiological differentiations providing beneficial adaptation for their survival, including the biosynthesis of bioactive compounds. Members of this group that are beneficial to crops have been commercialized as plant bioinoculants mainly for protection against herbivores and for promotion of plant growth by diverse mechanisms. The class *Bacilli* is the fourth bacterial group most abundant among endophytes [1], with members of *Bacillus*, *Paenibacillus*, and *Staphylococcus* being the most abundant 16S rRNA gene sequences (more than 100 for each genus) detected. The entomopathogenic *Bacillus thuringiensis* is the best-known and best-studied biocontrol agent, mostly for its capacity to synthesize parasporal crystal proteins, which have selective insecticidal properties toward different species of invertebrate phyla. Strains of *B. thuringiensis* also synthesize a vast assortment of bioactive products, including photoprotective compounds of class melanin,

siderophores, and antibiotics from nonribosomal peptide synthetase/polyketide synthase (NRPS-PKS) hybrid and from ribosomally synthesized posttranslationally modified peptide pathways, such as bacteriocins and lipopeptides [52].

Biosynthetic gene clusters of unknown secondary metabolite families (putative BGCs) are the most common class identified among *Bacilli* endophytes, with strains of *Bacillus* spp., *Brevibacillus* sp., and *Paenibacillus* sp. being the largest potential producers (Table 1). These three genera are known as aerobic endospore-forming bacteria (AEFB) and play a large role in the field of agriculture. Hence, the potential of these uncharacterized compounds urges for a fully elucidation to improve sustainable food production.

Bacilli endophytes have also a great capability to synthesize NRPS-based compounds (Fig. 3). NRPS gene clusters are the second most abundant family potentially synthesized by strains of AEFB, although the biological and ecological functions of these compounds remain largely unknown. The biocontrol agent *Bacillus amyloliquefaciens* 629 produces and secretes a range of multifunctional secondary metabolites including cyclic lipopeptide antibiotics, such as surfactin, fengycin, and iturin A. These cyclic lipopeptides are synthesized by modular enzymes, such as NRPSs, PKSs, and their hybrid structures (NRPSs-PKSs), and were found to be a major factor suppressing plant disease caused by pathogenic fungi and bacteria [53, 54]. It is interesting that gene cluster assigned to *trans*-AT PKS family is significantly ($p < 0.05$) more common among strains of *Bacilli* than from any other investigated bacterial class (Table 1). *Bacillus amyloliquefaciens* 629 and *Paenibacillus polymyxa* Mc5Re-14 have four BGCs of polyketides assigned to *trans*-AT PKS in each genome and are capable to suppress fungi and bacteria phytopathogens. However, the biological roles of *trans*-AT PKS-derived polyketides are not limited to antimicrobial activity. Indeed these bioactive compounds are highly diverse with broad-spectrum antibiotic, antibacterial, antifungal, antiviral, cytotoxic, antitumor, dermatotoxic, and neuroprotective activities. Some compounds of this family have also plant virulence, biocontrol, and deterrent properties [55]. These observations and the fact that *B. amyloliquefaciens* strain 629 has the largest percentage of its genome content dedicated to biosynthesis of bioactive compounds emphasize the importance of this strain for the discovery of novel bioactive compounds, which might improve plant growth and even ameliorate the host biotic and abiotic stresses.

In addition to their impressive capacity to synthesize nonribosomal secondary metabolites, *Bacilli* strains are also capable of synthesizing bioactive compounds with antimicrobial activity via ribosome. Bacteriocins are posttranscriptionally modified peptides synthesized by the ribosome with a bacteriocidal mode of action that are only toxic to bacteria closely related to the producing strain [56]. Biosynthetic gene clusters of bacteriocin family are detected among *Bacilli* endophytes, especially in the genome of *B. thuringiensis* Bc601, *Bacillus toyonensis* BAC3151, and *P. polymyxa* Mc5Re-14, each with four, three, and two BCs assigned to this family, respectively (Fig. 3). Members of this family of peptides are likely to be widely spread among the *Bacillus*/*Paenibacillus* taxon [57] and might confer competitive advantage in niche colonization among closely related species.

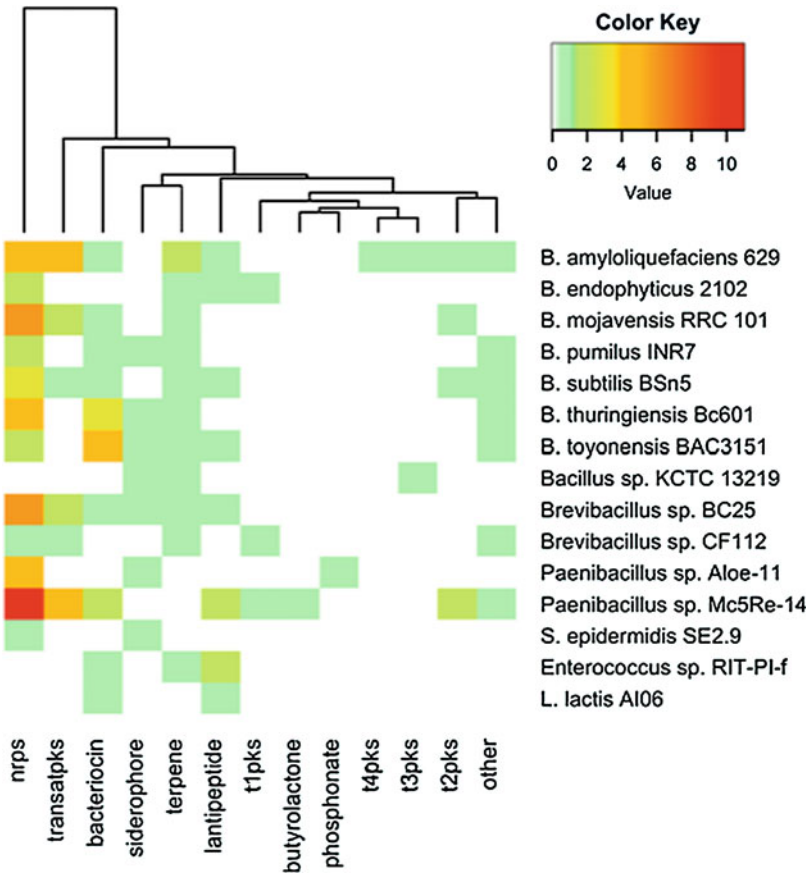

Fig. 3 Heat map shows abundance values of 13 secondary metabolite families detected across *Bacilli* strains. Putative-based family of secondary metabolites was removed for visual clarity. Species names of bacteria were abbreviated as B. for *Bacillus*, S. for *Staphylococcus*, and L. for *Lactococcus*

7 Secondary Metabolites Synthesized by *Alphaproteobacteria*

The phylum *Proteobacteria* is by far the largest phylogenetically, metabolically, and ecologically diverse group of bacteria. They are Gram-negative bacteria of considerable importance for overall ecosystem functioning and function as plant and animal pathogens or mutualistic symbionts. Members of *Alphaproteobacteria* are mostly oligotrophs (i.e., capable of growing at low nutrient levels). They commonly establish close associations with plants by colonizing virtually all tissues of the host plants and are able to modify host physiology, biochemistry, and even morphology, which might improve host fitness, growth, and yield, especially in adverse

conditions [1]. *Alphaproteobacteria* is the third most commonly detected class of bacterial endophytes. The importance of members of this class is well documented in the legume – *Rhizobium* mutualistic symbiosis. Selected rhizobial species capable to induce root nodule formation on legumes may successfully provide nitrogen to the host by fixation of atmospheric nitrogen. This mutualistic interaction is tightly regulated by both patterns and requires the exchange of specific chemical signals to facilitate plant infection and the establishment of effective cooperation [58]. For instance, each rhizobial species is adapted to recognize the repertoire of flavonoids, a family of secondary metabolites derived from phenylpropanoid pathway in the plant host, for initiation of compatible interaction. The community of *Alphaproteobacteria* also synthesizes large amount of secondary metabolites, of which more than 90% is assigned to putative BGCs (Table 1). This amount is significantly larger ($p < 0.05$) in *Alphaproteobacteria* than among *Gammaproteobacteria* strains. All investigated strains of alphaproteobacterial endophytes have more than ten putative BGCs, with exception of *Rhizobium smilacinae* CCTCC AB 2013016 and *Rhizobium populi* CCTCC AB 2013068, which have no putative BGCs identified. In general rhizobial-related strains of genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*, as well as nitrogen-fixing strains of non-rhizobial genera *Azospirillum*, *Methylobacterium*, and *Phyllobacterium*, are the largest potential producers of this type of secondary metabolites. The biological and ecological functions of these bioactive compounds remain to be investigated; however, their roles in mutualistic interactions with the host plant are plausible as discussed above.

The bioactive communication molecules of family homoserine lactone are responsible for cell-to-cell signaling that coordinate community activities. These compounds are detected in significantly higher ($p < 0.05$) quantities among strains of *Alphaproteobacteria* than among *Actinobacteria* and *Bacilli* communities. This is consistent with the fact that these "autoinducers," name given for the self-producing signal molecules involved in the luminescence control of marine Gram-negative *Vibrio fischeri*, are largely restricted to members of *Proteobacteria* [59]. Nevertheless, not all alphaproteobacterial strains are able to synthesize these compounds. Indeed, only a few strains of *Rhizobium* and *Sinorhizobium* as well as *Gluconacetobacter diazotrophicus* PAL 5, *Methylobacterium* sp. AMS5, and *Phyllobacterium* sp. YR531have in their genome a single copy of the biosynthetic gene cluster for the production of homoserine lactone-based compounds (Fig. 4). Many bacteria that lack the gene for biosynthesis of autoinducer molecules might have genes encoding signal receptors (*luxR*) and proteins involved in their response (*lux*box) and therefore are still able to coordinate their behavior to function as a group. In this study, all strains involved in the biosynthesis of autoinducer homoserine lactone compounds are diazotrophic bacteria (i.e., bacteria able to fix atmospheric nitrogen). Biological nitrogen fixation is an energy-intensive process, and the enzyme nitrogenase, which catalyzes this reaction, is oxygen sensitive. Therefore, considerable physiological constraints are imposed on these diazotrophs, which must communicate to function as a united community to protect nitrogenase from oxygen damage. When this community communication is impaired, such was done in knockout mutants of *Rhizobium etli* CNPAF512 for an autoinducer synthase (*cinI*) and a transcriptional

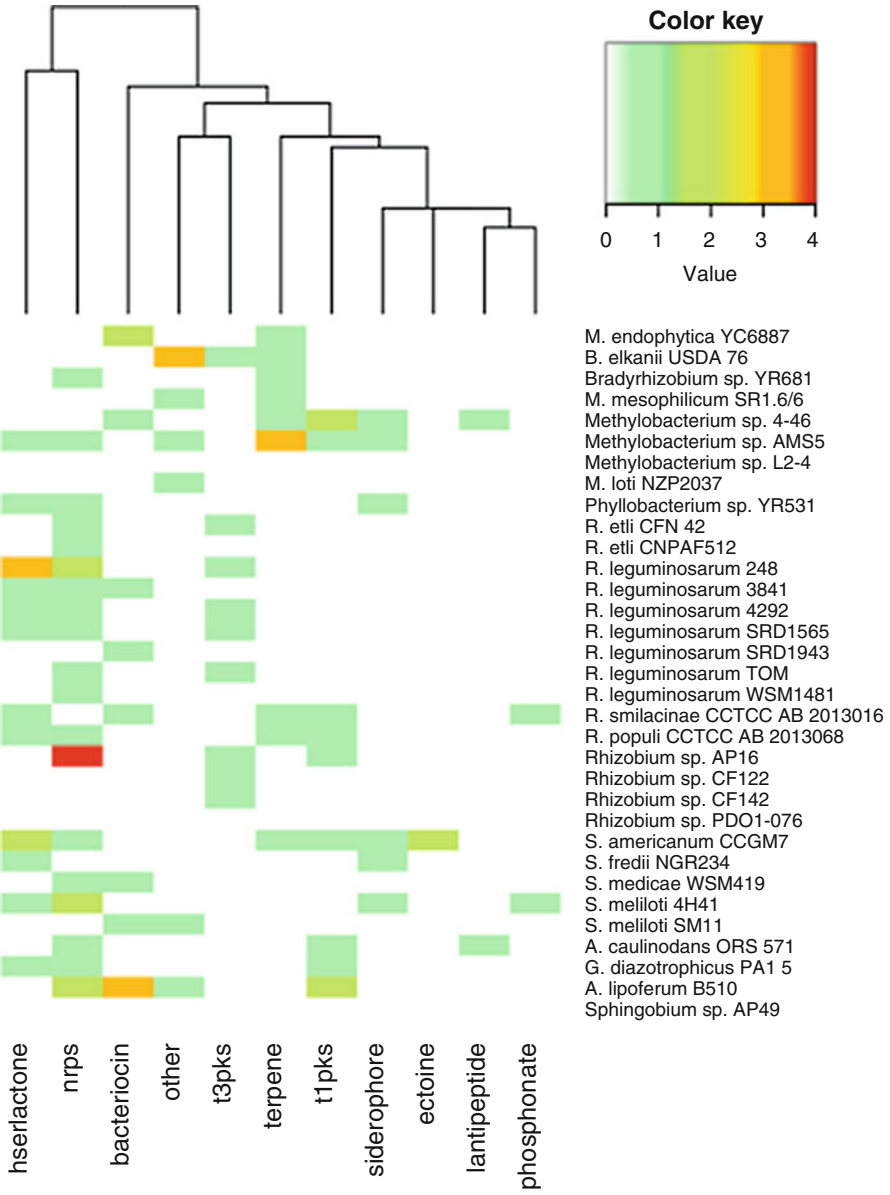

Fig. 4 Heat map shows abundance values of 11 secondary metabolite families detected across alphaproteobacterial strains. Putative-based family of secondary metabolites was removed for visual clarity. Species names of bacteria were abbreviated as M. for *Martelella endophytica*, *Methylobacterium mesophilicum*, and *Mesorhizobium loti*, B. for *Bradyrhizobium*, R. for *Rhizobium*, A. for *Azorhizobium caulinodans* and *Azospirillum lipoferum*, S. for *Sinorhizobium*, and G. for *Gluconacetobacter*

regulator (*cinR*), the activity of nitrogen fixation is reduced by 60–70% in bean nodules [60], emphasizing the role of autoinducer homoserine lactone compounds in the process of nitrogen fixation. Further microscopic analysis reveals that *cinI* mutant bacteroids were always individually packed in the symbiosome membrane, whereas multiple bacteroids were detected in wild-type symbiosomes, suggesting that mutant bacteroids could not fully differentiate. It is interesting that *R. etli*CNPAF512 produces at least seven different autoinducer molecules [60], of which only one type was detected in this study (Fig. 4).

8 Secondary Metabolites Synthesized by *Betaproteobacteria*

Betaproteobacteria are commonly detected as the most dominant members of freshwater ecosystems. They are also encountered in relative high percentage (10%) inside host plants [1]. Few species of *Betaproteobacteria* are able to establish an intimate association with plant host. For instance, species of *Burkholderia* and *Cupriavidus* are able to induce nodule formation and to effectively fix atmospheric nitrogen in selected legume hosts [61]. They are also among the largest producers of bioactive compounds (Table 1), although more than 85% of all BGCs detected in this group are identified as putative without biological or ecological function predicted. All investigated betaproteobacterial strains have at least 20 putative BGCs, with exception of *Variovorax paradoxus* strain KB5, which has no putative BGCs detected. In addition to this family of bioactive compounds, BGCs of NRPS and terpene families are detected in relatively high numbers, mainly among *Burkholderia cenocepacia* strain 869 T2 and *Burkholderia gladioli* strain 3A12 (Fig. 5). These strains have great potential for production of diverse bioactive compounds. *Burkholderia* species are known to suppress soil-borne fungal phytopathogens by their ability to synthesize diverse antifungal compounds. Strain 869 T2 has the ability to reduce significantly the incidence of *Fusarium* wilt in banana plants growing in the field as well as to increment significantly banana growth when compared to mock-inoculated plants [62]. Strain3A12 is able to suppress the phytopathogenic fungus *Sclerotinia homoeocarpa* by the production of an unidentified antifungal compound [63]. Ironically, *B. cenocepacia* strain 869 T2 belongs to a group of phenotypically heterogeneous species of *Burkholderia*, referred as the *Burkholderia cepacia* complex, with opportunistic plant and human pathogens. Indeed, some strains of *B. cenocepacia* and *B. gladioli* pose potential threat to both plant and human health. Strains of *B. cenocepacia* other than 869 T2 are considered the causal agent of banana fingertip rot as well as cause severe infections in cystic fibrosis and immunocompromised patients, while strains of *B. gladioli* other than 3A12 might induce rice panicle blight disease, and they are well-known pathogen of plants and animals. Therefore, the great potential for farming applications of these strains should be considerably diminished to avoid potential health concerns for plants and humans alike.

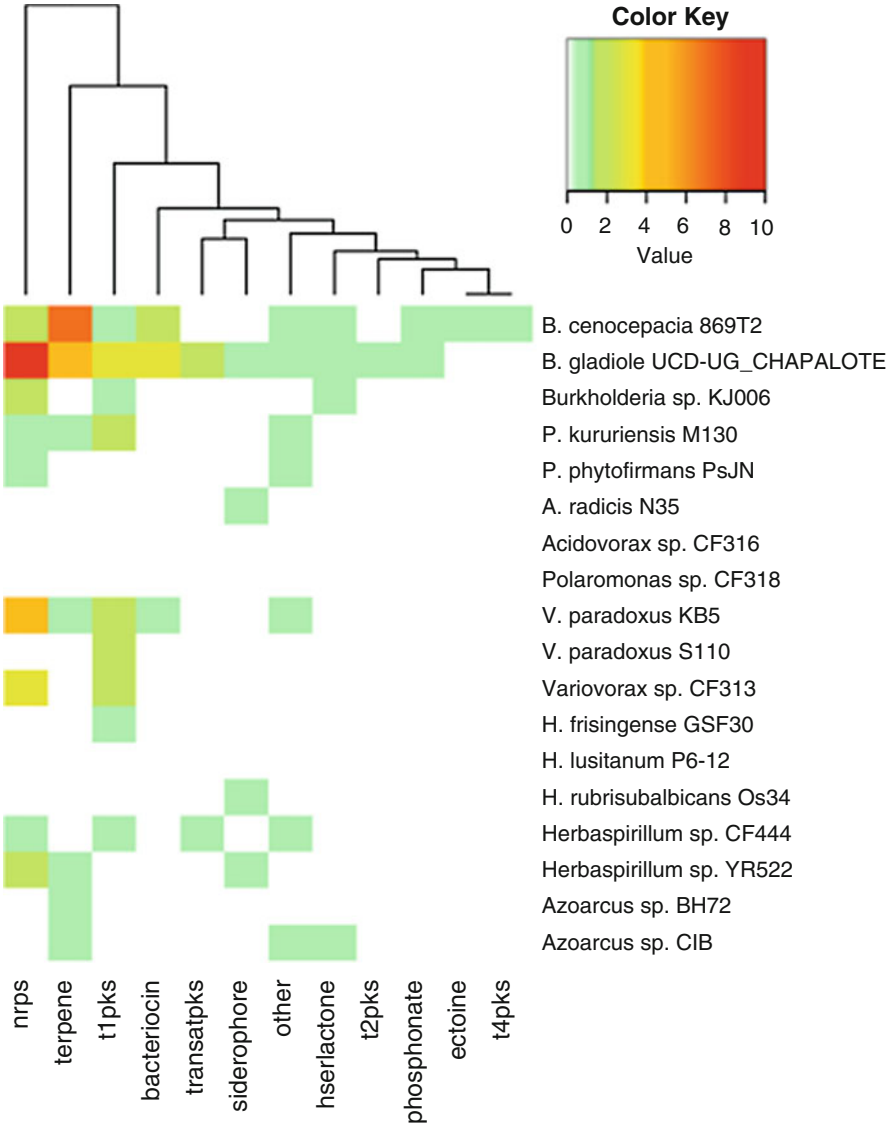

Fig. 5 Heat map shows abundance values of 12 secondary metabolite families detected across betaproteobacterial strains. Putative-based family of secondary metabolites was removed for visual clarity. Species names of bacteria were abbreviated as B. for *Burkholderia*, P. for *Paraburkholderia*, A. for *Acidovorax*, V. for *Variovorax*, and H. for *Herbaspirillum*

9 Secondary Metabolites Synthesized by *Gammaproteobacteria*

The *Gammaproteobacteria* constitute the largest subgroup of *Proteobacteria* with extraordinary variety of physiological, morphological, and metabolical properties. They are well-known for their ubiquity in natural habitats, colonizing a broad range of environments, including soil, water, organic matter, and plant and animal systems. They have the capacity to utilize a remarkable variety of organic compounds as energy sources, show resistance to a spectrum of structurally diverse antimicrobial compounds, and are able to synthesize a remarkable array of bioactive compounds. Members of *Gammaproteobacteria* exhibit varied lifestyles and can even establish close associations with host plants. Indeed, most of the prokaryotic endophytes (26%) comprise species of *Gammaproteobacteria*, whereas many species of this class are also described as plant pathogens [1]. Members of this class, such as plant growth-promoting species of *Pseudomonas* and *Stenotrophomonas*, are used as potential health indicators of banana plants cultivated on *Fusarium* wilt-infested soils in Central America [64]. Strains of these genera are competent plant colonizers and show various plant growth-promoting properties, which are especially valuable when hosts are challenged by stress conditions.

Pseudomonas is a diverse group of *Gammaproteobacteria* with astonishing metabolic capacity for biosynthesis and catabolism of secondary metabolites including nonribosomally produced peptide, polyketides and fatty acid derived, hybrid NRPSs-PKSs, and alkaloid-derived compounds [44]. In this study more than a half of the investigated *Gammaproteobacteria* strains are species of *Pseudomonas*. This is consistent with the overwhelming number of genomes sequenced in this genus. It is interesting that even strains from the same species have a large genetic variation, which justify the need to sequence multiple isolates to access their functional properties [65]. The genomes of endophytic *Pseudomonas* species are equipped with a wide range of putative BGCs, which are not functionally characterized. Indeed, almost 80% of biosynthetic gene clusters from *Gammaproteobacteria* are assigned to this putative family (Table 1). All but five gammaproteobacterial strains have putative BGCs, with numbers of gene clusters per genome ranging from seven to 38. *Pseudomonas* strains GM21, GM79, GM17, GM80, GM78, and *Raoultella terrigena* R1Glyare most likely the largest producers of putative bioactive compounds with more than 30 putative BGCs per genome, whereas *Erwinia* sp. ErVv1, *Kosakonia sacchari* SP1, *Klebsiella* strains LTGPAF-6F and RIT-PI-d, *Pseudomonas ananatis* strains AMG521, B1-9, and GB1, *Pseudomonas fluorescens* strains L228 and L321, *Pseudomonas stutzeri* A1501, *Pseudomonas* sp. EpS/L25, and *Rheinheimera* sp. EpRS3 have less than ten putative BGCs per genome. This result

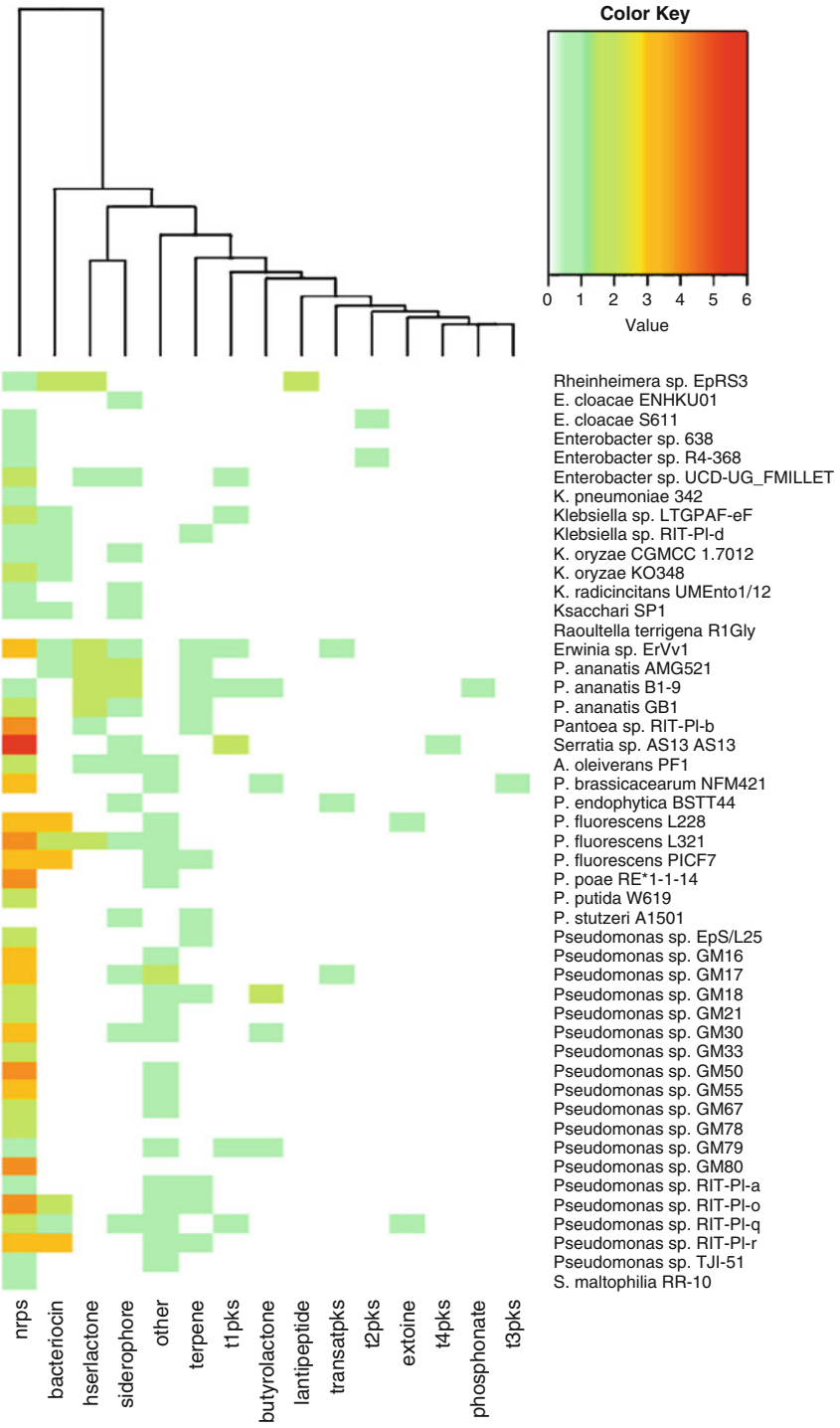

Fig. 6 (continued)

suggests that there are no correlation between strain taxonomy or even genome size and the quantity of putative-based bioactive compounds.

Biosynthetic gene clusters of NRPS family are the second most commonly detected among gammaproteobacterial strains (Table 1). *Serratia* sp. AS13 is the potentially largest producer of NRPS-based bioactive compounds, followed by *P. fluorescens* strains GM80 and L321, *Pseudomonas poae* RE*1-1-14, *Pseudomonas* sp. strains GM50 and RIT-PI-o, and *Pantoea* sp. RIT-PI-b, each with four identified BGCs (Fig. 6). A biosynthetic gene cluster region encoding an NRPS-PKS-fatty acid (FA)-based natural product with a potent antimicrobial activity toward Gram-positive and Gram-negative bacterial pathogens was identified in a closely related species of *Serratia* sp. AS13, named *Serratia plymuthica* RVH1 [66]. This BGC is almost identical (99% identities, 99% similarities) between both strains and even across other plant-associated *Serratia* strains, suggesting a plausible role of horizontal gene transfer on the dissemination of zeamine-related antibiotics among plant beneficial bacteria. In addition of antibiotic compounds, multi-functional proteins of NRPS family are also involved in the biosynthesis of Indole-3-acetic acid compounds. These indole-based phytohormones called auxins participate virtually in every aspect of plant morphology and physiology; therefore, they play a major role on host development and growth. Auxins might also function as signaling molecules on plant defense strategies as well as to coordinate physiological response on prokaryotes. Auxins are produced by many microorganisms including beneficial and phytopathogen strains. It was observed that host response to auxin is dependent on its concentration. High concentrations may function as virulence factor and are often produced by pathogen species to facilitate host colonization, whereas low levels are produced by strains beneficial to plants. These interactions are further complicated by the ability of certain strains to catabolize auxins and thus to interfere with these relationships. Some strains might even overwhelm host defense system to facilitate pathogen colonization. There are at least five possible biosynthetic routes for auxin production; however, Indole-3-acetamide (IAM), Indole-3-pyruvic acid (IPyA), and tryptophan side-chain oxidase (TSO) pathways are the most commonly detected. The beneficial plant growth-promoting strains use primarily the latter two pathways [44]. The key gene *ipdC*, encoding Indole-3-pyruvate decarboxylase (EC, 4.1.1.74), in the IPyA pathway was detected in 34 genomes of endophytes, of which 18 are of gammaproteobacterial strains. All investigated *Kosakonia* strains have a single copy of this gene, whereas none is detected among *Pseudomonas* genomes, a common producer of auxins [44]. Further investigation reveals that none of the endophytes harbor the gene encoding the enzyme tryptophan 2′-dioxygenase (EC, 1.13.99.3) involved in TSO pathway.

Fig. 6 Heat map shows abundance values of 15 secondary metabolite families detected across gammaproteobacterial strains. Putative-based family of secondary metabolites was removed for visual clarity. Species names of bacteria were abbreviated as E. for *Enterobacter*, K. for *Klebsiella pneumoniae* and *Kosakonia*, P. for *Pantoea ananatis* and *Pseudomonas*, A. for *Acinetobacter*, and S. for *Stenotrophomonas*

Genetic clusters of bacteriocin family are also detected within *Gammaproteobacteria*, mainly among strains of *Pseudomonas fluorescens*, *Pseudomonas* spp., *Kosakonia*, *Klebsiella*, as well as *Erwinia* sp. ErVv1 and *Rheinheimera* sp. EpRS3 (Fig. 6). Strains of *P. fluorescens*, including Pf-5 the first genome sequenced for this species, are notorious biocontrol agents against (i) fungal phytopathogens via production of a wide spectrum of secondary metabolites including 2,4-diacetylphloroglucinol, hydrogen cyanide, pyrrolnitrin, pyoluteorin, pyoverdine, and pyochelin compounds, as well as against (ii) closely related bacterial species via production of bacteriocins [44, 67]. The ecological role of bacteriocin-producing bacteria is evident when outcompeting closely related bacterial neighbors; however, the involvement of bacteriocin compounds in the biocontrol of distantly related phytopathogens has not been sufficiently investigated.

Additionally to the synthesis of secondary metabolites, many endophytes participate in the catabolism of secondary metabolites, and therefore these endophytes might disrupt the biological functions of these bioactive compounds. For instance, the catabolism of terpenoid compounds is restricted to very few microorganisms, mostly of the genus *Pseudomonas*, which have genes ($n = 8$) involved in the catabolism of acyclic terpenoids and genes ($n = 4$) encoding enzymes involved in *B*-oxidation. Acyclic monoterpenes, such as citronellol and geraniol, are aroma compounds frequently produced in plants. These citral compounds belong to the family of acyclic methyl-branched molecules derived from isoprene and are commonly used in food and perfume industries. These terpenoid compounds are known to have important biological activities in living organisms such as repellents against mosquitoes (citronellol), as alarmones for some ants (citral), and as antitumor properties on mammalian cells (geraniol). In this study, only investigated genomes of *Pseudomonadales* strains (18 *Pseudomonas* and 1 *Acinetobacter*) carried genes (at least 11 out of 12) involved in the catabolism of citronellol. This result corroborates the observation that the ability to utilize citronellol and related acyclic monoterpenes as a sole source of carbon and energy is restricted to the *Pseudomonadales* order [68].

Ethylene is a simple volatile plant hormone compound synthesized from methionine in many tissues and functions as a short-, from cell to cell, as well as long-distance, from one plant to neighbor plants, signaling molecule in response to abiotic and biotic stresses and fruit ripening. Ethylene-deficient transgenic plants grow apparently normally under optimal conditions but lack the ethylene-induced disease resistance response upon invasion of pathogen microorganisms and also lack the ability to cope with abiotic stresses, including salt and drought tolerances. Ethylene is also involved in plant-microbe interactions. The application of exogenous ethylene, or its direct precursor 1-aminocyclopropane-1-carboxylate (ACC), in legume plants inhibits the elongation of infection threads and, consequently, the formation of nodule organelles. Ethylene-insensitive mutants of *Medicago truncatula* are hypercolonized by endophytes when compared to normal plant genotype. Similar results are also observed on normal *M. truncatula* genotype when treated with exogenous ethylene inhibitor, 1-methylcyclopropene, whereas the addition of ACC drastically reduces the endophytic colonization in non-transgenic *Medicago sativa* plants [69]. In this context, bacterial endophytes with functionally active ACC deaminase, enzyme that cleaves ACC into ammonia and α-ketobutyrate, might ameliorate plant stress by efficiently blocking the

extended period of ethylene production. In this study all strains of *Pseudomonas* and *Rheinheimera* sp. EpRS3 have the gene *acdS* encoding the enzyme ACC deaminase (EC, 3.5.99.7) that are likely to confer direct plant growth promotion ability [70].

10 Concluding Remarks

This chapter has dealt with the report of secondary metabolite families encountered among bacterial endophytes and their potential beneficial roles to plants. The biological activities of secondary metabolites far exceed their functions as inhibitory or killing molecules of microorganisms. They also can have toxic and deterrent effects against multicellular organisms like animals and plants. Some secondary metabolites are agents of symbiosis between microbes and plants, nematodes, insects, and higher animals, while others will play hormone-like roles for microbial community behavior and plant developmental differentiation, reproduction, or even activation of host immune response. Others have a role as metal transport agents. Additionally, there is an enormous amount of uncharacterized bioactive compounds present among bacterial endophytes, suggesting that many other mechanisms than biosynthesis of antimicrobial compounds are important for endophytes to thrive inside the host plants. Indeed, the great majority of identified BGCs involved in the biosynthesis of secondary metabolites is assigned to putative family with unknown biological and ecological functions. This pattern is observed for all the investigated bacterial classes of endophytes. Consequently, these communities of endophytes are a truly potential untapped source of novel bioactive compounds that might be further explored for biotechnological, pharmaceutical, and farming applications. With the advance of high-throughput whole genome sequencing efforts, new opportunities for genome mining of biosynthetic gene clusters were opened, and the number of these genes has undoubtedly surpassed by far the number of known secondary metabolites detected among fungal and bacterial cells. Technological developments, including "omics" approaches, will definitely improve our understanding on how these secondary metabolites are used as chemical communication molecules between microbial endophytes and their host plants for harmonious signaling in these multitrophic interactions. It is very likely that some of these putative bioactive compounds also participate on host colonization and in the establishment of successful mutualistic relationship, which is very interesting and worth some consideration. A significant reduction in the number of these putative compounds might reduce drastically the bacterial ability to establish a productive mutualistic interaction with host plants. On the other hand, a significant increase in the number of overall BGCs in the bacterial genome is not correlated with an augmentation on mutualistic interaction , but rather with bacterial facultative lifestyle strategy. Bacterial strains with large percentage of their genome encoding enzymes involved in the biosynthesis of secondary metabolites have a competitive advantage to colonize various environments, including soil and plants. Indeed, many bacterial endophytes are also detected thriving on soil substrates or in animal guts. Because a vast majority of endophytes are commensal,

their ecological roles in the context of these multitrophic interactions with other "superior" organisms remain largely unknown.

Strains of *Actinobacteria* are the largest and more diverse producers of secondary metabolites among all investigated bacterial classes. Many important pharmaceutical and farming bioactive compounds are originated from *Streptomyces* strains, which are prolific producers of multiple secondary metabolites [27]. The urge to outcompete their biological competitors in the soils, because they are not easily able to evade in their saprophytic lifestyle, might have lead *Streptomyces* strains to synthesize multiple synergistically acting antibiotics as well as contingently acting siderophores [71]. Nevertheless, one might have notice that only two strains of *Streptomyces* (strains LUP30 and RTd22) are analyzed in this data set. This is not natural, especially when considering that strains of *Streptomyces* are the most frequently isolated genus of *Actinobacteria* inside plants [25, 27]. This dominance is also observed when analyzing sequences of 16SrRNA gene based on cultivation dependent and independent methods [1]. At this moment, there are only few genomes of endophytic *Streptomyces* species sequenced and even fewer deposited in the IMG/ABC data mart. The addition of *Streptomyces* genome sequences into IMG/ABC data mart will certainly enhance both the number and diversity of BGCs among endophytes.

The beneficial properties of many endophytes species have attracted much recent attention, not least because analysis of microbial genome sequences has suggested that these properties may be far more complex than was previously thought. Evidences emerging here and from the recent literature suggest that endophytes employ diverse number of hitherto putative bioactive compounds that appeared to be essential for them to establish the successful mutualistic relationship with the host plants. Indeed, as the study of endophyte secondary metabolism continues, our understanding of their ecological roles will consistently improve further.

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Endophytic Pseudomonads and Their Metabolites

2

Apekcha Bajpai and Bhavdish N. Johri

Contents

Abstract

Plant microbiome is crucial in maintaining both plant health and ecosystem functioning. Rapid advance in next-generation sequencing technology has brought about a paradigm shift in our understanding of plant microbiome. This has especially shed light on selective colonization of microbes in root

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compartments, i.e., rhizosphere, rhizoplane, and endosphere. A growing body of evidence reveals the predominance of the phylum Proteobacteria in endomicrobiome of several crop plants. Additionally, *Pseudomonas* is found to be a widely distributed genus within Proteobacteria which exists in both above and below ground plant parts. Pseudomonads are extensively exploited for their metabolic potential and adaptability toward endophytic lifestyle in contrast with their rhizospheric counterpart and fungal endophytes. This together develops a better understanding of the genus *Pseudomonas* as key determinants in plant health including their role as biocontrol agents. In this chapter, we discuss pseudomonads with endomicrobiome perspectives, their atypical characteristics with respect to rhizospheric microbes, and influence of metabolites in context with their role in plant growth and biocontrol. A comprehensive understanding about selection of endophytic lifestyle will perhaps provide better opportunities to improve plant performance and pathogen resistance.

Keywords
Pseudomonas · Endosphere · Biocontrol · Plant growth promotion · Rhizosphere

Abbreviations

2, 4-DAPG	2, 4-diacetylphloroglucinol
ACC	1-aminocyclopropane-1-carboxylate
AntiSMASH	Antibiotic and secondary metabolite cluster analysis
CNN	Competition for niches and nutrients
DGGE	Denaturing gradient gel electrophoresis
HCN	Hydrogen cyanide
IAA	Indole acetic acid
ISR	Induce systemic resistance
NRPS	Non-ribosomal peptide synthases
OTUs	Operational taxonomic units
PGPR	Plant growth promotory rhizobacteria
TAD	Take-all disease
TRIS	Tracking root interaction system

1 Introduction

Pseudomonas genus is ubiquitous in natural soils exhibiting diverse metabolic activities and remarkable adaptation to various niches [1]. Along with other microbial communities, they reside in close proximity to plants and are often termed as "second genome" of plants [2]. These communities together comprise plant microbiome which is further classified as rhizobiome and endomicrobiome. Within endomicrobiome, microbes exist as neutral, commensal, and/or beneficial [3], and these have been termed as endophytes. With the advent of next-generation sequencing technology, detailed characterization of both culturable and unculturable microbial communities associated with agronomically important plants [4–7] has now been

studied with greater depth. However, research on these hidden microbes dates back to the nineteenth century when De Bary [8] coined the term endophytes. Since then several definitions have been proposed. In brief endophytes could be best described as "any microbe which occur within plant tissue for at least part of their life cycle without causing disease under any known circumstances" [9].

The endophytes are known to colonize nearly 300,000 plant species on earth. However, the most predominant and studied endophytes belong to three major phyla, viz., Proteobacteria, Actinobacteria, and Firmicutes [10]. *Pseudomonas* is categorized as a representative of the phylum Proteobacteria which is found commonly as an endophyte transmitted either horizontally from plant to plant or vertically through host seeds [11]. In contrast, with the report of Koehorst et al. [12], the genus *Pseudomonas* possesses a closed pan genome indicating that the genomic content is perhaps not shaped by horizontal gene transfer. There are 236 known species of *Pseudomonas* (http://www.bacterio.net/pseudomonas.html) occupying various niches out of which only 432 strains distributed over 33 species have now complete and draft genomes submitted to public databases [12]. However, for endophytic strains complete genome sequence is far more limited, represented by a small fraction of 23 strains from 5 species reflecting a lack of attention paid on these microbes as they are often assumed to be similar to rhizospheric pseudomonads. The use of denaturing gradient gel electrophoresis (DGGE) [4] and high-throughput sequencing platforms [6, 7] has uncovered a greater diversity of endophytes; their analysis using bioinformatic tools provides microbiologists now with several novel genomic insights.

The most commonly found nonpathogenic endophytic colonizers from the genus *Pseudomonas* include *P. fluorescens*, *P. putida*, *P. fragi*, *P. gingeri*, *P. stutzeri*, *P. borealis*, *P. citronella*, *P. corrugata*, *P. brassicacearum*, *P. pseudoalcaligenes*, *P. pavonaceae*, *P. trivialis*, *P. tolaasii*, *P. viridiflava*, *P. aureofaciens*, and *P. poae* and pathogenic *P. syringae* and *P. aeruginosa*. Besides being good root colonizers, *Pseudomonas* genus is an excellent metabolite secretor which exhibits multiple plant growth promotory effects and also confers induce systemic response [1]. While living in plant parts such as intracellular spaces, within cells, or in vascular system, they either produce secondary metabolites or are associated with microbe-assisted secondary metabolite production from plant (Fig. 1). The latter is a widespread phenomenon which could be well exemplified by the fact that an endophyte from phylum *Actinobacteria*, *Pseudonocardia* sp. strain YIM63111, is able to enhance the production of artemisinin, an antimalarial compound, in its host plant *Artemisia annua* [13]; a Nobel prizewinning drug sets the stage where the importance of endophytes cannot be undermined.

Endophytic pseudomonads confer some direct and indirect benefits to host which are mediated through metabolites. Direct benefits include nutrient acquisition like iron, nitrogen, and phosphorous via siderophores, diazotrophy, phosphate solubilization, phytohormone production (auxin, cytokinin, gibberellins), reducing stress condition (1-aminocyclopropane-1-carboxylate deaminase), and phytopathogen suppression (antibiotics, HCN, siderophores). Similarly, indirect benefits include helping plant with induced resistance to confer protection against foliar pathogens by

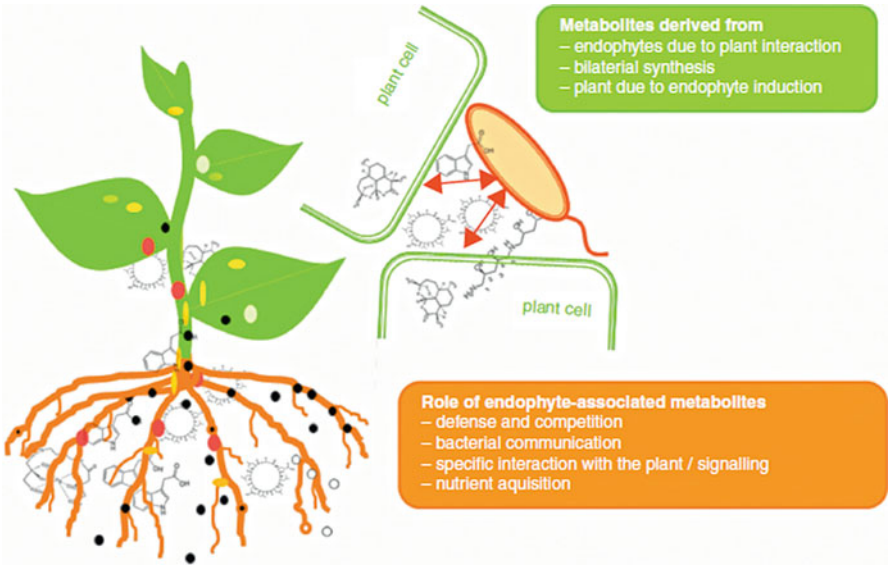

Fig. 1 Schematic overview showing the different types of plant-endophyte interactions leading to the synthesis of metabolites, which are in many cases not produced by the macro- or microsymbiont alone or in different quantities. Furthermore, the different known functions of endophyte-associated metabolites are presented [28] with permission https://creativecommons.org/licenses/by/4.0/

boosting the immune system of the plants [14]. Information on microbes living in these specialized niche is far less than the soil bacteria. But they are of special interest as they harbor some novel genes and contrasting genomic features with bacteria thriving in rhizosphere [1, 15].

Metabolites secreted by pseudomonads exhibit numerous applications including antimalarial, antiviral, antitumor, antioxidants, and immunosuppressant activities; they also possess immense potential in pharmaceutical, agrochemical, and biotechnology industry as a source of novel antibiotics [16]. This chapter deals specifically with pseudomonads discussing their selectivity to colonize endosphere, adaptability to endophytic lifestyle, and how in turn they influence metabolite production along with their role in plant growth promotion and biocontrol.

2 The Endomicrobiome

Using next-generation sequencing technology, microbial communities within plants could be studied and together termed as “endomicrobiome.” Previous studies suggest that it is less diverse than the rhizomicrobiome and is controlled by several factors like plant genotype [17], geographical location [18], etc., but the mechanism of acquisition of microbes in this specialized niche is still a matter of speculation. A plausible hypothesis laid forward suggests that acquisition of

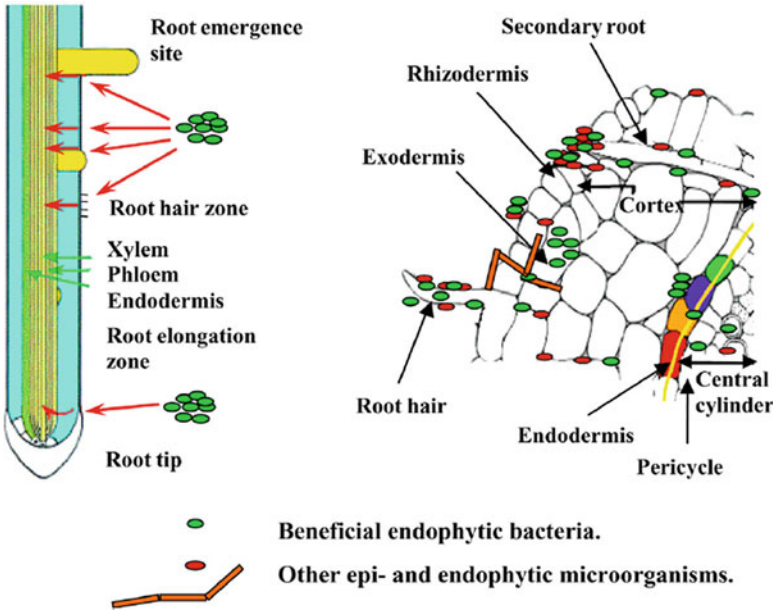

Fig. 2 Sites of plant colonization by endophytic bacteria. (Reprinted from Soil Biology and Biochemistry, 42, [22], Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved, and prospects for utilization, 669–678, 2009 with permission from Elsevier)

microbiomes is completed in two steps: first there is a general recruitment of microbes in the rhizosphere, followed by entry inside root tissue that involves species-specific general factors [5]. Microbial colonization in endosphere is a rapid process that starts within a day, and the relative level of microbes approaches steady state within 2 weeks; this has been revealed through time-staged profiling experiments on rice plants [17, 19]. Bacteria gain entry through lateral roots or root hair, more frequently through root wounds, cracks, or stomata on leaves or lenticels [20]. Various sites colonized by endophytes are schematically represented in Fig. 2.

It has been well documented that endophytic bacteria are a subset of rhizospheric microbial community, dominated by phylum Proteobacteria which becomes apparent from the discussion that follows underneath. A study by Edwards et al. [17] comprehensively described the selective role of each niche, i.e., rhizosphere, rhizoplane, and endosphere, in harboring microbes. Proteobacteria usually increase gradually from bulk soil to endosphere, whereas *Acidobacteria* and *Gemmatimonadetes* followed a reverse trend, i.e., least in endosphere compared to rhizosphere or bulk soil. Similar study on endophytic bacterial sequences of *Populus* reported abundance of *Pseudomonas*-like operational taxonomic unit (OTU), a gamma Proteobacteria to be about 34% [21]. Such trends are observed not only in roots but also when communities are analyzed using different plant parts and sequencing approaches.

Through 16S-rRNA pyrosequencing, [6] revealed the predominance of *Proteobacteria* (90%), *Actinobacteria* (1.5%), *Planctomycetes* (1.4%), *Verrucomicrobia* (1.1%), and *Acidobacteria* (0.5%) on tomato leaf. Illumina sequencing uncovered bacterial endophytic diversity at tuber and rosette formation stage and detected 146 and 109 OTUs, respectively. The phylum Proteobacteria dominated (98%) among other microbial communities and mainly comprised of *Enterobacteriales*, *Pseudomonadales*, *Rhizobiales*, *Sphingomonadales*, *Xanthomonadales*, *Burkholderia*, *Actinomycetales*, and *Flavobacteriales* [7]. Thus, it can be hypothesized that this trend is found generally in endosphere of most land plants.

3 Contrast with Rhizospheric Pseudomonads

Endophytic bacteria are mostly derived from rhizosphere and are generally regarded as subset of rhizospheric microbes. But the niche they select to live largely influences their behavior [22]. Endophytic life depends upon the availability of nutrients provided by the plant, and the viability of bacteria is strongly influenced by plant metabolism [4]. This is unlike rhizospheric bacteria which are recruited and dependent upon root exudates for nutrition [17].

Selective nutrient inclination toward specialized niche is strongly supported by the report of Malfanova et al. [23] which shows that those *Pseudomonas* strains that possess the capacity to utilize L-arabinose have greater propensity to become endophytes; such strains were able to oxidize L-arabinose significantly more than rhizospheric group. Therefore, the authors concluded that L-arabinose might be a trait contributing to the endophytic lifestyle of the *Pseudomonas* isolated from cucumber plants. Previously, similar substrate utilization-dependent switching of nutritional behavior was reported by Prakamhang et al. [24]. Such studies must also be extended to other endophytes and crop plants to generate information on different nutrients apart from L-arabinose which might help in unraveling mechanism involved in switching from free-living to endophytic lifestyle.

Another comprehensive study carried out by Edwards et al. [17] on rice microbiome sheds light on the assembly of microbes in roots which clearly depicts a compartmentalization between these specialized niches. Significantly, a greater proportion of Proteobacteria (alpha, beta, and delta), *Spirochaetes* (*Chloroflexi*, *Bacteroidetes*), and low alpha diversity were found in endosphere as compared to rhizosphere, and also there was a reduction in relative abundance of *Acidobacteria*, *Planctomycetes*, and *Gemmatimonadetes* in the endosphere. This study draws attention toward similarity of endosphere and rhizoplane being the most identical compartments and enriched with phyla, namely, *Fibrobacters* and *Spirochaetes*, which are well-known cellulose degraders. Microbes in endosphere mostly contain genes encoding for plant polymer-degrading enzymes such as cellulases, xylanases, cellobiohydrolases, and pectinases [25].

The key difference between rhizosphere and endosphere reported is that the former has least effect on excluding microbes (only 17 OTUs, mainly *Proteobacteria* and *Acidobacteria* phyla) than the latter (1961 OTUs, mainly

Acidobacteria, *Planctomycetes*, *Chloflexi*, and *Verrucomicrobia* phyla). In other words, the first level of exclusion is determined by root exudates which facilitate recruitment of microbes in rhizosphere [17], or it can be mediated by microbial interaction of root-colonizing bacteria, for instance, relative quantification of bacterial fluorescence intensity showed a strong negative correlation between both bacterial concentrations when *E. coli*, a common root colonizer of *Arabidopsis thaliana*, excluded itself when co-inoculated with *B. subtilis* [26]. Similarly, second level of exclusion occurs at rhizoplane by selectively recruiting microbes for colonization into endosphere. Rhizoplane acts as a critical gate that permits only a subset of microbes recruited in rhizosphere to bind to rhizoplane, and a fraction of these are allowed to penetrate and proliferate in endosphere. Thus, entry of microbes largely depends upon (i) molecular signals from plant, (ii) composition of root exudates, and (iii) cell wall components of root and membrane proteins [17].

To explore their metabolic and ecological diversity, an in-depth comparative reanalysis of *Pseudomonas* genome has been carried out in several studies. In this regard endophytic *P. putida* TJ151 provided information regarding adaptation to endophytic fitness which might be linked with alginate involved in bacterial colonization within plant tissues. Additionally, the genome was found to contain several regions novel to this strain encoding enzymes [27]. Interestingly, it has been found that endophytes (*P. syringae*/*P. fluorescens*) host a higher number of secondary metabolite clusters compared to free-living *P. putida* strains [28]; a key difference has been pointed out after analyzing their complete genomes using antibiotic and secondary metabolite cluster analysis (AntiSmash) (Fig. 3). Furthermore, a comparative finding of 58 endophytic *Pseudomonas* species based on their complete genome sequence is presented as a phylogenetic tree in Fig. 4. The presence and absence of protein domains were deterministic in assessing strain diversity, and this strategy was found to be successful in reconstructing evolutionary history [12].

Comparative genomic and functional analysis by Wu and coworkers [1] has identified some unique traits that are crucial to endophytic lifestyle of *P. putida* strain W619, in contrast to rhizospheric strain KT2440. According to these authors, the major differences lie in DNA rearrangement, lesser IS elements, and change in stress level due to reactive oxygen species. To overcome such stress strain, W619 possesses genes encoding enzymes not shared by rhizospheric strains: for example, *sodc* gene (superoxide dismutase), *acnA* (aconitase, oxidation metabolic enzyme), *katB*, and *ahpD* (catalase). Furthermore, the presence of *ndvB*, gene involved in production of β-(1,2)-glucan, catalyzes initiation, elongation, and cyclization required in attachment of *A. tumefaciens* to plant cells. This could be an important factor to help establish the bacterium in root interior.

4 Contrast with Fungal Endophytes

Despite occupying similar niche, colonization of endophytic bacteria and fungi varies with the pattern of distribution wherein plant parts like rhizosphere close to soil are heavily colonized by bacteria than phyllosphere. More tissue specificity is

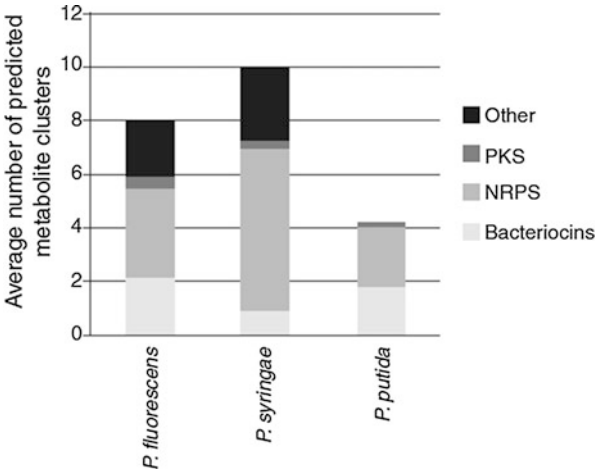

Fig. 3 Average numbers of metabolite gene clusters predicted by AntiSmash 2.0. The numbers are the mean of six *P. fluorescens* (plant-associated) strains, five *P. syringae* (plant pathogens), and nine *P. putida* strains (no association with plants) and contain all fully sequenced and published genomes in the given category [28], with permission https://creativecommons.org/licenses/by/4.0/

encountered with endophytic fungi in contrast to bacterial counterparts, e.g., leaves are found heavily colonized with fungi attributable to the fact that they are derived from aerial fungal spores, whereas decreased bacterial diversity in leaf could be due to lack of nutrients and exposure of leaf to direct UV radiation [29]. Bacterial abundance and diversity in terms of OTU's is generally higher in roots than stems and also in regions with low fungal infection [30, 31]. Compared with fungi, bacteria are more diverse and tolerate a wider pH range although it is not a determining factor for community structure [32]. 16S taxonomic marker along with DGGE profiling [4] has been found to be more deterministic in uncovering diversity at much greater depth (species level) in contrast with ITS region targeting at higher taxonomic level (genera or families) [33]. The mode of colonization of bacteria uses hyphae for penetration inside root as a vehicle to gain entry to the sites which might be difficult to reach by bacterial cells alone [34]. Moreover, fungi often outplay bacteria in terms of improving overall plant biomass as compared to bacterial endophytes as is evident from a comparative study by Hassan [35]. Dependence on environmental factors for both the endophytes differs considerably, for example, in *Ilex paraguariensis*, humidity plays a crucial role in determining the fungal diversity, recorded highest during fall season and low in winter, whereas no such dependence was observed with bacterial diversity [29].

5 Metabolic Potential

A very relevant question that has been raised is, while living in noncompetitive environment, why do endophytes need to secrete metabolites? Residing inside plant tissues, these microbes face less competitive environment as compared to soil bacteria, and this enables bacteria to produce some other special metabolites required to support and build interaction with the host [36]. Thus, endophytic pseudomonads

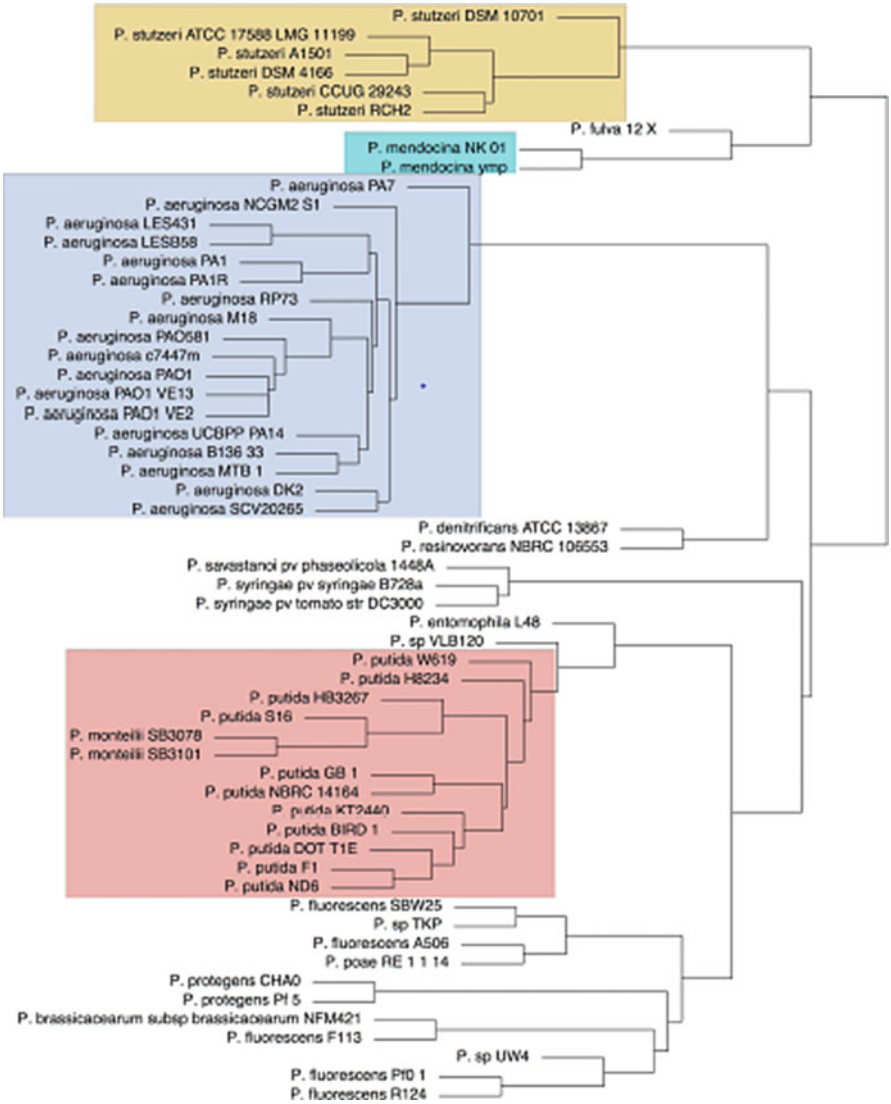

Fig. 4 Domain-based distance tree of 58 *Pseudomonas* strains. The tree was build considering the pattern of presence/absence of protein domains using an average clustering approach. Only completely sequenced genomes are considered. The phylogenetic clusters corresponding to the most abundant species (*P. stutzeri*, *P. mendocina*, *P. aeruginosa*, and *P. putida*) are color-shadowed. (Reprinted by permission from Nature Springer: (Scientific Reports) [12], copyright (December, 2016) https://creativecommons.org/licenses/by/4.0/)

are equipped with PGPR (plant growth promotory rhizobacteria) activity and bio-control attributes (Table 1). Their genome is much more reduced [47] which is evident upon a comparison of obligate endophytes with genomes of rhizospheric

Table 1 Natural products that are known to be produced by endophytic pseudomonads

S. No	Species	Compounds	Antagonism	References
1.	*P. syringae*	Pseudomycins A-D	*Candida albicans*	[37]
2.	*P. syringae*	Antimycotics: syringomycin, syringotoxin, and syringostatins	*Candida tropicalis* and *Candida rugosa*	[38]
3.	*P. viridiflava* EB 273	Ecomycins: novel lipopeptides unusual amino acid like homoserine and beta-hydroxyaspartic acid	*Cryptococcus neoformans* and *Candida albicans*	[39]
4	*P. putida* BP25	Volatile compounds: 1-undecene; disulfide dimethyl; pyrazine, methyl-pyrazine, 2,5-dimethyl-; isoamyl alcohol; pyrazine, methyl and dimethyl trisulfide	*Phytophthora capsici*, *Pythium myriotylum*, *Gibberella moniliformis*, *Rhizoctonia solani*, *Athelia rolfsii*, *Colletotrichum gloeosporioides*, and *Radopholus similis*	[40]
5.	*Pseudomonas* P2	Volatile compounds: dimethyl disulfide and dimethyl trisulfide, siderophore production	*Rhizoctonia solani*	[41]
6.	*P. aeruginosa* strain UICC B-40	(2E,5E)-phenyltetradeca-2,5-dienoate antibacterial compound	*Staphylococcus aureus* and *B. cereus*	[42]
7.	*Pseudomonas* sp. p21	Two pyoverdine biosynthetic gene clusters	*Aspergillus niger*	[43]
8.	*P. aeruginosa*	Phenazine 1-carboxylic acid	Rhizome protective effect	[44]
9.	*P. chlororaphis* Zong1	Siderophore, phosphate solubilization, organic acid, IAA, and antifungal activity	*Fusarium oxysporum*, *Magnaporthe grisea*, *Botrytis cinerea* Pers., *Valsa mali* Miyabe et Yamada, *Alternaria alternata*	[45]
10.	*Pseudomonas* isolates	Gluconic acid which solubilize phosphate		[46]
11.	*P. syringae*	Coronatine, a plant hormone-acting agent that mimics the structure of the actual plant hormone (+)-7-iso-jasmonoyl-L-isoleucine		[28]

bacteria or microbes that have switched their lifestyle to endophytic form [22]. The available complete genome sequences of endophytic pseudomonads suggest a relatively higher number of secondary metabolite clusters [28]. To date,

Table 2 Endophytic *Pseudomonas* with complete genome sequences

S. No	Endophyte	Host plant	Accession number	Important features	Genome size	References
1.	*Pseudomonas* sp. strain C9	*Brassica oleraceae* L	MPAK00000000	GacS/GacA two-component system, siderophore-iron reductase, Fe-S cluster protein, siderophore biosynthesis protein SbnG, NADPH-dependent ferric siderophore reductase, and iron-siderophore ABC transporter permease	6,350,161 bp	[48]
2.	*P. punonensis* strain D1–6	*Erodium hirtum*	LWHA00000000	Possess herbicide resistant gene and plant growth promotory	4,534,589 bp	[49]
3.	*P. fluorescens* strains (L111, L228, and L321)	*Miscanthus giganteus*	CP015637, CP015638, CP015639, and CP015640	Type 3 secretion system, ACC deaminase, and IAA producing capability	6.72 Mb, 6.28 Mb, 6.75 Mb	[50]
4.	*P. poae* RE*1-1-14	*Beta vulgaris*	CP004045	Produce hydrolytic exoenzymes and cyclic lipopeptides suppress late root rot in the sugar beet and ACC deaminase	5.5 Mb	[51]
5.	*15 strains of Pseudomonas* sp.	*Populus deltoides*	AKJV00000000	Plant growth promotory bacteria	~6.5 Mb	[52]
			AKJR00000000			
			AKJB00000000			
			AKJO00000000			
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			AKJD00000000			
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			AKJS00000000			
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			AKJT00000000			

(*continued*)

Table 2 (continued)

S. No	Endophyte	Host plant	Accession number	Important features	Genome size	References
6.	*P. putida W619*	*Populus trichocarpa deltoides* cv. "Hoogvorst"	NC_010501	IAA synthesis and ACC deaminase activity	5,774,330 bp	[53]

Pseudomonas sp. strain C9, *P. punonensis* strain D1-6, *P. fluorescens* strains (L111, L228, and L321), *P. poae* RE*1-1-14, *Pseudomonas* sp., and *P. putida* W619 have been completely sequenced (Table 2). In general, they all show metabolic capabilities, and genes in particular involved in adaptation to endophytic lifestyle. Features that underpin endophytic lifestyle include flagella, nod genes, plant polymer-degrading enzymes, detoxification mechanisms, type IV pili, LPS, and absence of type 3 secretion system [22]. To find novel genomic traits, such studies should be extended to other species too. In spite of 432 pseudomonad strains that have been completely sequenced, considerably smaller numbers of endophytes are sequenced to date, and even fewer studies emphasize on the comparative genomics between these two forms. In this regard Ali et al. [15] studied nine endophytes from a single clade of Proteobacteria and compared their unique genes that perhaps are involved in endophytic behavior such as transporter proteins, secretion/delivery systems, plant polymer degradation, transcriptional regulation, detoxification, redox potential maintenance, 2-isopropylmalatesynthase, and diaminopimelate decarboxylase functionality.

The key factor often responsible for metabolite production is the environment wherein the bacterium resides; otherwise rhizospheric and endophytic forms secrete nearly similar metabolites (Fig. 5). Endosphere is a highly favorable niche for metabolically potential microbes; *Methanobacterium* is unexpectedly found in rice endosphere, an oxygen-rich environment compared to rhizosphere. However, whether this bacterium is involved in methane production or not is still unclear [17]. Hence, a better metagenomics and metatranscriptomic sequencing to understand the metabolic potential of endophytes is required. Similarly, Song et al. [54] for the first time reported that genus *Pseudomonas* is also capable of producing an enzyme agarase; this bacterium is important in two ways: first it is derived from a nonmarine source (plant endosphere), and, secondly, this strain was selectively found in endosphere but not in rhizosphere. It would be of interest to study further why some strains exclude themselves from rhizosphere and preferentially inhabit root interior.

Not all endophytic *Pseudomonas* species isolated from plant parts are potential suppressor of pathogens which is well exemplified by the fact that out of seven species of *P. fluorescens* and *P. putida*, only three (strain CS1, CR2, CR3) were able to suppress tomato foot and root rot [23]. The authors suggested that the possible mechanisms of biocontrol consisted of induce systemic resistance (ISR)

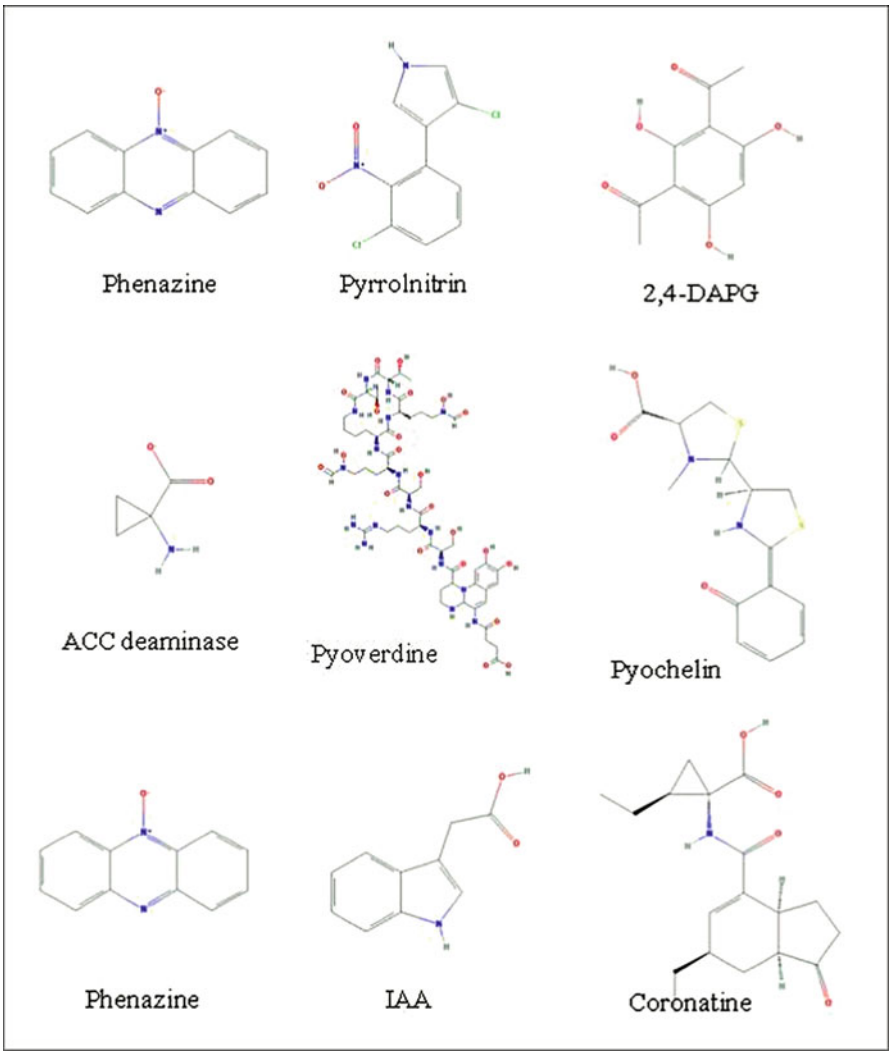

Fig. 5 Major metabolites of endophytic pseudomonads (https://www.ncbi.nlm.nih.gov/pubmed)

and competition for niche and nutrition (CNN). This finding leaves a question to speculate the mechanism of CNN-mediated biocontrol by selected endophytic pseudomonads only? An answer to such question lies in those studies which highlight the community shift during pathogen attack and possible mechanisms behind biocontrol. The conclusion from such findings supports the fact that altered plant metabolites induce only selective endophytic pseudomonads to respond to pathogen attack [4]. However, not all endophytes respond to phytopathogens; therefore, exploiting endophytes for metabolites could be attained by shedding

light into their genomic DNA which would reveal whether they are capable of producing novel metabolites or not.

6 Metabolites Involved in Plant Growth Promotion

Endophytic pseudomonads secrete secondary metabolites which are required for nutrient uptake, controlling abiotic stress and regulating signaling mechanisms. Traits possessed by endophytic pseudomonads to promote plant growth include associative nitrogen fixation [55], phosphate solubilization [46], siderophore production [43], phytohormone release [50], and ACC deaminase production [56]. There are several evidences which suggest that endophytes are rich source of metabolites that underlie the intricate regulatory mechanisms between microbe and plant interaction.

6.1 Nitrogen Fixation

Nitrogen fixation or diazotrophy is a well-studied phenomenon in the genus *Pseudomonas*; such strains are effective in fixing nitrogen for plants or found as rhizobium helper bacteria [55]. Nitrogenase expression and activity in *P. stutzeri* A1501 have been studied in greater detail comprising of 49 kb nitrogen fixation cluster containing 59 genes with a distinct G+C ratio and might be horizontally transmitted. *nif* and *pnfA* genes are involved in nitrogen fixation, while *nifHDK* is involved in encoding nitrogenase enzyme. *nifHDK* and *pnfA* are both involved in nitrogen fixation and regulated by a common sigma factor [57]. A recent investigation by Pham et al. [55] on a nitrogen deficient mutant *P. stutzeri* A15 unable to fix nitrogen and reduced ability to promote plant growth compared to wild type throws light on diazotrophic behaviour of Pseudomonas. Apart from showing diazotrophy, *Pseudomonas* also served as a rhizobium helper bacterium in enhancing number of nodules, nitrogen content, and plant biomass [58].

6.2 Indole Acetic Acid (IAA)

Metabolites like phytohormones have been reported in various studies involved in cell division, cell expansion, differentiation, shoot branching, cell death [14], and significant adventitious root development of plants [59]. IAA from pathogenic *P. syringae* is produced from tryptophan via the intermediate indoleacetamide; however nonpathogenic beneficial bacteria synthesize IAA predominantly by an alternate tryptophan-dependent pathway through indolepyruvic acid [59]. An endophytic strain W619 was a better producer of phytohormone IAA than rhizospheric strain encoding two putative tryptophan-dependent IAA synthetic pathways with three genes encoding for auxin efflux carriers [1]. Completely sequenced endophytic strains L111, L228, and L321 which produce IAA

were further investigated to be involved in plant stem growth and root development [50]. There is a synergistic role of IAA, siderophore, and HCN in plant growth promotion and suppression of charcoal rot disease of chickpea [60]. Chen et al. [61] using an endophytic strain *P. fluorescens* Sasm5 reported a positive correlation between the increment of exogenous IAA and plant biomass, chlorophyll levels, and also expression of metal transporter families (ZRT/IRT-like proteins, natural resistance-associated macrophage protein, and heavy metal ATPase); these were largely improved suggesting a probable role in phytoremediation.

6.3 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

ACC deaminase, a multimeric pyridoxal phosphate-dependent enzyme of tryptophan synthase family, has crucial role in relieving plant from abiotic stresses by catalyzing cleavage of ACC into cyclopropane ring and deaminase to form α-ketobutyrate and ammonia. Majority of such studies have dealt with soil bacteria, but now endophytes are also reported to reduce the levels of stress ethylene and ACC by two- to fourfold which in turn promotes shoot and root growth [59]. Stress conditions encountered by *Pseudomonas* are salt, low and high temperature, drought, water logging, mechanical wounding, and the presence of heavy metals and other organic and inorganic toxic compounds [62]. It has also been reported that ACC deaminase while involved in downregulation of ethylene-related genes can also upregulate genes involved in IAA synthesis for plant growth promotion [56]. The gene *acdS* encodes an inducible enzyme ACC deaminase whose production is in tight regulation of LRP protein-encoding genes, *acdB* or *LysR* genes, and various environmental conditions such as oxygen levels, substrate concentration, and product accumulation. The enzyme works best at optimum pH between 8.0 and 8.5 and temperature 30 °C; it is also found to be induced by amino acid L-alanine, DL-alanine, and D-serine [63]; however, the enzyme is highly specific for D- and L-form of amino acids [64]. Mechanism of action of enzyme involves opening of cyclopropane ring of ACC by a series of nucleophilic addition and elimination [65]. Breakdown of ACC follows either of the two steps: first, direct opening of ring at Lys51 and second by nucleophilic attack on β-carbon atom of ACC [64]. *acdR* act as a regulatory gene together with promoter region like Lrpbox, AcdB box, FNR box, CRP box controls the expression of *acdS*. Additionally, *acdS* gene present in the core genome is also regulated by a product of *nif* and *rpoN* genes [66]. It has been proposed that ACC and protein AcdB bind to the octamer of LRP protein which in turn binds to the promoter region and then activates *acdS* transcription [67]. α-Ketobutyrate formed as a by-product synthesizes leucine which is involved in negatively controlled regulation of ACC deaminase gene. The *AcdS* gene is generally found in *Actinobacteria*, *Deinococcus-Thermus*, and Proteobacteria and are also spread in fungi and archaeal species and helps plant in phytoremediation and detoxification of heavy metals [68].

6.4 Siderophores

Siderophore-mediated plant growth is essential when plant lives under stressed environment with limited iron. Here endophytic pseudomonads acquire the trait to combat stressed condition and release siderophores in the environment. These molecules bind to ferric state of iron and transport it to root surface and then make it available to plant to be utilized in ferrous state. From our previous work on a rhizospheric *Pseudomonas*, strain GRP3A was shown to reduce chlorotic symptoms and enhancement of chlorophyll level in mung bean plants compared to uninoculated plants [69]. An endophyte *Pseudomonas* sp. p21 analyzed for siderophore through antiSMASH and RAST server indicates putative secondary metabolite clusters for pyoverdine and two incomplete putative biosynthetic clusters for bacteriocin and others. It is presumed that together with other traits and iron-chelating siderophores, they exhibit antagonism against *Aspergillus niger* [43]. Siderophores are chelating fluorescent molecules with a characteristic presence of quinoline as a chromophore and having high affinity for iron. Two major classes of siderophores produced by genus *Pseudomonas* are pyoverdine and pyochelin. Pyoverdine is composed of three structural parts: an invariant dihydroxyquinoline chromophore; a dicarboxylic acid, amide, or α-ketoglutaric acid attached to the NH_2 group of the chromophore, and a variable peptide side chain comprising of 6–12 amino acids which is species specific [70]. Two iron-binding sites are on the peptide chain and one on the catecholate of the chromophore [71]. Pyoverdine is synthesized by non-ribosomal peptide synthases (NRPS) like PvdL, PvdI, PvdJ, and PvdD that together form nonfluorescent molecule ferribactin. Enzymes such as ornithine hydroxylase PvdA, the amino transferase PvdH, and hydroxyornithine transformylase PvdF product are involved in the formation of substrate for NRPS. Precursor PVDI peptide synthesis occurs in cytoplasm and is exported to the periplasm by PvdE where siderophore maturation and formation of chromophore moiety occurs [72]. Siderophores are generally involved in disease suppression, metal uptake, scavenging oxygen free radicals, and biomineral formation [73]. The production of siderophores occurs at low level and are therefore expensive; high-throughput methods like flash chromatography are developed to scale up pyoverdine at industrial level [74].

6.5 Phosphate Solubilization

Phosphate is stored in plants as phytic acid (inositol hexakisphosphate) important in seed germination. However, insoluble phosphate in complex form present in environment is not readily available to plants. For this the role of phosphate solubilizing microbes comes into play; they secrete various organic acids that solubilize complex forms of phosphate to H_2PO^{4-} and HPO_4^{2-} ions. Commonly secreted acids analyzed through high-performance liquid chromatography are gluconic acid, 2-α-ketogluconic acid, lactic acid, succinic acid, formic acid, malic acid, and citric acid [75]. The main purpose of organic acid is lowering of pH which finds a major

role in phosphate solubilization. Park et al. [76] proposed two mechanisms of phosphate solubilization in *P. fluorescens* RAF15; first by proton extrusion by ammonium assimilation and second by organic acid secretion. However, the former mechanism is of lesser importance in phosphate solubilization indicating involvement of some other mechanism like the presence of phenol hydroxylase gene probably involved in solubilization of complex phosphorous [77]. Studies have shown that phosphate solubilizing endophytic *Pseudomonas* isolates increase both phosphate content and plant growth in *Pisum sativum*. Gluconic acid analyzed through HPLC possesses higher phosphate solubilization capacity of ~400–1300 mgL^{-1}; this is controlled genetically by *pqq* operon and glucose dehydrogenase gene (*gcd*) present in *Pseudomonas* isolates [46]. In contrast with other bacteria, gluconic acid production is highly specific to carbon source used and environmental factors in *Pseudomonas* [76]. Moreover, a kind of organic acid produced also depends upon adequate supply of soluble phosphorous; insufficient supply produces gluconic acid as predominant compound, whereas sufficient supply induces formation of formic acid, butyrate, and propanedioic acid [77].

7 Metabolites Involved in Biocontrol

Endophytic pseudomonads are known to be an excellent biocontrol agent that include those that reduce *Verticillium* wilt of olive [78], *F. solani* root rot [58], late root rot in sugar beet [51], and corn rootworms [79]. Biocontrol in pseudomonads is mediated both directly by antibiosis, siderophores, biosurfactants, hydrogen cyanide (HCN), etc. and indirectly through ISR [14]. Beyond these metabolites, recently, it has been observed that some non-proteinogenic amino acid like furanomycin [80] and protein IPD072Aa [79] also exhibit biocontrol and insecticidal properties, respectively.

7.1 Phloroglucinols

It is a broad-spectrum phenolic molecule formed by the decarboxylative condensation of monomers such as acetyl-CoA, malonyl CoA, and methylmalonyl CoA catalyzed by polyketide synthases [81]. Naturally, phloroglucinol and its derivatives constitute around 700 known compounds among which 2, 4-diacetylphloroglucinol (2, 4-DAPG) is most prominent and its role in suppression is well established [81, 82]. Production of 2, 4-DAPG is genetically under tight regulation of an operon containing six structural genes *phlABCDEI* and three regulatory genes *phlF*, *phlG*, and *phlH*. 2, 4-DAPG is formed from the precursor monoacetyl phloroglucinol through transacetylation reaction. Monoacetyl phloroglucinol on the other hand is formed from three molecules of acetyl CoA with one molecule of malonyl CoA through condensation reaction [82].

One of the best examples of biocontrol mediated by *P. fluorescens* harboring *phlD* gene is the disease-suppressive soil against take-all disease (TAD) caused by

Gaeumannomyces graminis var. *tritici*. The strain present in soil as monoculture above a threshold value of 10^5 CFU/g of root successfully suppresses the pathogen [2]. However, in contrast with this report, recently PhyloChip-based metagenomics study has revealed representatives of 33,000 bacterial and archaeal species involved in disease suppression especially members of the group gamma Proteobacteria synthesizing NRPS rather than a single species as previously reported [83]. Patel and coworkers [84] have recently scaled up the production of 2, 4-DAPG in a diazotrophic endophytic *Pseudomonas* sp. WS5. There was a sevenfold increase in the antibiotic production that checked the hemibiotrophic infection of *Magnaporthe oryzae* B157 as also *Rhizoctonia solani* and significantly elevated the levels of NPR1 and PR10a gene expression. Recent finding highlights the utilization of high-throughput techniques for the detection of metabolites such as in situ detection as described for *P. fluorescens* CHA0 lipopeptides [85]. Mendes et al. [83] described a PhyloChip-based metagenomics method to detect a secondary metabolite synthesized by a NRPS of *Pseudomonas* sp. strain SH-C52 which was identified to be involved in suppressing sugar beet disease caused by *R. solani*.

7.2 Phenazines

Besides *Pseudomonas*, phenazines are also synthesized by *Burkholderia*, *Brevibacterium*, *Streptomyces*, and archaeal phylum of *Euryarchaeota*. There are around 180 naturally occurring nitrogen containing heterocyclic compounds containing amide nitrogen of glutamine of phenazine derivatives [86]. The major derivatives of phenazine are pyocyanin, phenazine 1-carboxylic acid, phenazine 1-carboxamide, and 1-hydroxyphenazine. Phenazine 1-carboxamide acts as a key intermediate in the production of other derivatives [87]. Phenazine synthesis is controlled by the operon *phzABCDEFG* encoding enzymes required for condensation of two molecules of chorismic acid with phenazine nucleus. Biocontrol against phytopathogens through phenazine 1-carboxylic acid is mediated by generation of oxygen free radical or hydrogen peroxide by superoxide dismutase in the cell by interfering with normal electron transport system [88]. Phenazines are highly effective and therefore marketed at commercial level to control phytopathogens, e.g., shenqinmycin, a commercial product, is employed to control rice sheath blight and bacterial blight [89]. An endophytic strain *P. aeruginosa* secreting phenazine 1-carboxylic acid suppresses phytopathogens and is also considered plant growth promotory perhaps due to a combined effect of various other traits involved in improvement of overall plant health [44].

7.3 Pyoluteorin

Pyoluteorin is a polyketide antibiotic comprising of resorcinol ring linked to a bichlorinated pyrrole moiety [90]. L-Proline acts as a precursor to dichloropyrrole moiety of pyoluteorin following a series of condensation and oxidation reaction all

catalyzed by a multienzyme complex [91]. Pyoluteorin biosynthesis requires an operon *pltABCDEFG* that contains ten genes. L-Proline is activated by L-prolyl-AMP ligase PltF and is subsequently attached to the peptidyl carrier protein PltL. PltE and PltL perform oxidation and chlorination of the intermediate to form 4, 5-dichloropyrrolyl-S-PltL. This chlorinated product is catalyzed by type I polyketide synthase PltBC and yields resorcinol which in turn further catalyzes synthesis of pyoluteorin [92]. The antibiotic production is often considered to be coordinated; an intermediate phloroglucinol in 2, 4-DAPG production together with the help of halogenase encoded by the pyoluteorin gene forms mono- and dichlorinated phloroglucinols which induces and controls the novel mechanism of pyoluteorin production [93]. Pyoluteorin shows inhibitory action against broad range of phytopathogens especially oomycetous fungi, *Pythium ultimum*, suppressing the symptoms of *Pythium* damping off [90].

7.4 Pyrrolnitrin

Pyrrolnitrin is a broad-spectrum halogenated antifungal metabolite secreted by several representatives of Proteobacteria. It was first described for *Burkholderia* and later for *Pseudomonas*, *Enterobacter*, *Myxococcus* and *Serretia*. Pyrrolnitrin biosynthesis is genetically controlled by a small operon of 5.5b *prnABCD*, and tryptophan acts as a precursor to its synthesis [94]. Additionally, it is also differentially regulated by carbon source used; reduced levels are obtained with glucose [95]. Endophytic *Pseudomonas* strain ESR94 also harbors *prn* gene confirmed by easily locating them through gene-specific primers. Further to confirm that the suppression of disease is mediated by the sole action of antibiotic could be carried out by experiments like site-directed mutagenesis followed by thin-layer chromatography [96]. Pyrrolnitrin does not show nemocidal property [97]; however, it is widely utilized against management of phytopathogens like *R. solani*, commercially available as fludioxonil and phenyl pyrroles [98]. Its inhibitory action involves inhibiting an enzyme, glycerol kinase resulting in cell membrane lysis, and accumulation of glycerol [99].

7.5 Biosurfactant

Pseudomonads are well-known to secrete biosurfactants like viscosin, putisolvin, amphisin, syringofactin, rhamnolipids, arthrofactin, etc. [100]. Besides *Pseudomonas*, rhamnolipids are also produced by *Burkholderia* [101], *Thermos* and *Meiothermus* sp. [102], and *Streptomyces* [103]. However, rhamnolipids have been extensively studied from *P. aeruginosa* and are mainly classified as monorhamnolipids (Rha C_{10}) and di-rhamnolipids (Rha-Rha C_{10}-C_{10}). So far, 25 different rhamnolipid congeners are reported depending upon the chain length. Rhamnolipids help in swarming motility, and the one obtained from endophytic strain exhibits unusual temperature-dependent production [19]. However, this property is

associated with endophytesr or exhibited by other species occupying various niches is still unclear! In rhizospheric bacteria rhamnolipid synthesis is controlled by *rhlAB* operon. RhlA catalyzes the formation of fatty acid dimer moiety and free 3-(3-hydroxyalkanoyloxy) to alkanoic acid (HAA), whereas RhlB catalyzes the transfer of dTDP-L-rhamnose to HAA [104]. Several gene regulatory factors control rhamnolipid production at transcriptional level (PQS, RsaL, Vfr, PtxR, AlgR, RpoN, RpoS, BqsS-BqsR, RhoB, VqsR, VqsM) and posttranscriptionally (QScR, GacS-GacR, DksA, GidA) [100]. Du et al. [105] showed that recombinant production in *E. coli* expressing *rhlAB* and *rhlC* genes resulted in increased production of di-rhamnolipid suggesting a way to circumvent the problems associated while using pathogenic strains. An endophytic strain PaBP35 upon genetic and phenotypic characterization was found to possess *rhlA*, *rhlC*, *rhlI*, and *rhlR* genes essential for rhamnolipid production which were also involved in zoosporic activity against *P. palmivora* and *P. megakarya* [19].

7.6 HCN

Pseudomonads involved in cyanogenesis inhibit the enzyme cytochrome c oxidase, an enzyme of electron transport system that renders phytopathogens inactive. However, *Pseudomonas* strains rescue themselves from the harmful effect of HCN because of the presence of RhdA, a thiosulphate/cyanide sulfurtransferase (rhodanese) that is involved in cyanide to thiocyanate conversion [106]. HCN also shows nematocidal activity [97]. Endophytic forms are also reported to produce HCN but in low concentration [19]. Its production is often limited to certain genus; out of 63 endophytes isolated from oak tree, only two genera *Pseudomonas* and *Stenotrophomonas* were found to produce HCN [107]. This result is in contrast with the belief that none of the endophytes produce HCN which is a characteristic feature of antagonistic forms. Genetically, HCN production in endophytic pseudomonads is regulated by *hcnABC* synthase gene cluster following a mechanism similar to that reported for rhizospheric strains. Using amino acid glycine as a precursor, glycine synthase carries out a decarboxylation to yield the secondary metabolite HCN and CO_2 [97].

7.7 Induce Systemic Resistance

Pseudomonas possesses capacity to induce a defense state in plants to combat pathogens known as induced systemic resistance [108]. In addition to direct suppression of phytopathogens, it has been found that endophytic pseudomonads possess capacity to prevent foliar pathogens by activating the defense pathways at distance parts of the plants through ISR which is mediated by jasmonic acid/ethylene signaling pathway [109]. In one instance, endophyte such as *P. fluorescens* PICF7 has been found to induce ISR against *Verticillium* wilt of olive mediated by induction of genes such as lipoxygenase 2, catalase,

1-aminocyclopropane-1-carboxylateoxidase, and phenylalanine ammonia-lyase involved in the defense pathways of plant using suppression subtractive hybridization cDNA library of upregulated genes through real-time PCR [78]. Pseudobactin-type siderophore also elicit ISR against *M. oryzae*; an increased expression of defense response at sites of pathogen entry through secretion of phenolic compounds, hydrogen peroxide formation, and increased expression of structural defenses was noticed [110].

8 Conclusion and Perspectives

Like rhizospheric *Pseudomonas*, endophytic forms possess capacity to improve plant performance and often serve as replacement to chemical fertilizers. Recent studies with a focus on analyzing microbial diversity have revealed endosphere as a niche consistently enriched with Proteobacteria than previously imagined. Such trend of predominance of certain phyla in endosphere is generally followed by majority of crop plants. Both increased power and lowered down cost of next-generation sequencing technology have immensely helped in adding information about novel genomic islands, IS elements, genes, and enzymes particularly unique to endophytic forms. Using complete genome sequencing data to unravel novel genes and to interpret its information to provide functional basis can be further linked to endophytism. However, more such comparative studies are required to establish such links. Accurately analyzed complete genome sequences from curated databases could serve as a platform to check key processes, genomic adaptation acquired during endophytic lifestyle, novel genes and their regulation, or constitutive expression resulting in exploitation for biotechnological application.

Endophytes share niches with other microbial communities, and it would be of interest to study whether the metabolites are induced under the influence of other microbes, pathogen invasion or its sole presence enhances plant metabolites! Based on the evidence that endophytic forms could produce novel metabolite sets a challenge for future research not just on detection and characterization but rather aims at concentration and circumstances under which they are produced. To date several findings emphasize on the contrasting features of endophytic microbes, but making sense of how some selective strains show endophytic behavior and why only a few of them show antagonism against phytopathogens is a real question. An appropriate strategy would be possible with complete genome sequencing with a focus on unraveling the key processes and genomic information acquired during endophytic lifestyle. Secondly, studying root-microbe interaction through a recently designed microfluidic device tracking root interaction system (TRIS) [26] which has been successfully employed to study root microbe interaction in the rhizosphere could be used to study root endophytes and their mechanism of acquisition in endosphere. To find prevalence of social interaction between endophytic community structured well in space and time could be built upon previous studies on free living soil pseudomonads [111]. Nevertheless, these available approaches must be employed to study endosphere, a complex and fascinating ecological niche.

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Diversity, Ecology, and Significance of Fungal Endophytes

3

Kandikere R. Sridhar

Contents

Abstract

Fungal mutualistic association with plant species has become one of the important emerging contemporary issues in biology. Non-mycorrhizal endophytic fungal studies have multifold interest owing to their basic and applied value. Various tissues (leaf, stem, bark, seed, root, rhizome, and tuber) of a wide array of phototrophs (forest trees, plantations, shrubs, medicinal plants, vegetables, macrophytes, seaweeds, seagrass, ferns, and orchids) occurring in different ecosystems (terrestrial, riparian, freshwaters, mangroves, marine, marshes, and coastal sand dunes) have attracted the attention of researchers. The main focus of such interest is to understand their coevolution, life history, lifestyle, diversity, ecology, stress tolerance, natural products, biological control, bioprospects, and bioremediation. Climate change and anthropogenic interference on biodiversity have dramatic impact on the mutualistic association between plant species and

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endophytic fungi. The purpose of this review is to provide a brief overview on endophytic fungal studies carried out in different plant species, ecological perspectives, methods, and applications in different fields.

Keywords
Mutualism · Natural products · Biological control · Bioprospects · Bioremediation · Techniques

Abbreviations

AM	Arbuscular mycorrhizal
BLAST	Basic local alignment search
CAZymes	Carbohydrate active enzymes
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DSE	Dark septate endophytes
EM	Ectomycorrhizal
HIV	Human immunodeficiency virus
ITS	Internal transcribed spacer
LC-MS	Liquid chromatography-mass spectrometry
LSU	Large subunit
MAP	Mitogen-activated protein
OTU	Operational taxonomic unit
PCWDE	Plant cell wall degrading enzyme
RBP	Retinal-binding protein
RFLP	Restriction fragment length polymorphism
RIA	Radioimmunoassay
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
SSU	Small subunit
TEF	Transcription enhancer factor
UPLC-ESI-MS/MS	Ultra-performance liquid chromatography-electrospray ionization mass spectrometry
VOC	Volatile organic compound

1 Introduction

Fungi being widespread have continuum of lifestyles in order to survive and compete with other life systems in nature. They exhibit different lifestyles like biotrophy (dependence leading to parasitic), hemibiotrophy (initially biotrophic transform into necrotrophic), nectrotrophy (pathogens cause cell death and continue to exploit dead tissue), endotrophy (mutualistic without causing harm to the host), and saprotrophy (live on dead and decaying tissues) [1]. Their lifestyle is mostly dependent on the specific morphological features and the metabolites produced by them. The record of mutualistic or endophytic association (endotrophy) of fungi in roots of fossil tree

Amyelon radicians has been traced to the Paleozoic era [2–4]. Studies on fungal endophytes have a long history over a century by designation as "endophyte" by [5]; however, intensified efforts were seen only for a few decades.

The definition of endophytes has undergone a series of changes along with research advancement with several disparities [6]. Endophytic fungi have been broadly defined as those colonizing the live tissues of plant species at some time in their life without causing disease and pathological symptoms [7–9]. Almost all plant species are known for their mutualistic association with fungal endophytes [10, 11]. Although the exact molecular mechanism which triggers mutualistic association of plants and fungi is not clear, several hypotheses on signaling mechanisms have been proposed. For instance, flavonoids, isoflavonoids, and phenolic compounds serve as signaling molecules in rhizosphere [12], while stress-activated mitogen-activated protein (MAP) kinase pathway is responsible for stability of mutualistic association [13]. Research on the endophytic fungi although revealed that they are the major fungal community associated with plant species, focus on endophytic fungi is not as much as mycorrhizal association [14, 15]. For example, highly diverse interactions of the obligate biotrophs within one order *Sebacinales* (*Agaricomycetes*, *Basidiomycota*) have been overlooked especially transitions between saprophitism and endophytism [15, 16].

Fungal guilds (=functional groups) are attractive to the ecologists owing to assemblage or association with fungi which are specialized to live or exploit a specific category of environmental resource [17, 18]. Based on the phylogeny and life history strategies, fungal endophytes have been divided into two major groups such as clavicipitaceous and non-clavicipitaceous with narrow as well as broad host ranges, respectively [19] (Fig. 1). The clavicipitaceous group represents a small number of species, which are fastidious and confined to cool- and warm-season grass [20]. The non-clavicipitaceous endophytes are highly diverse and further classified into three distinct functional groups. The first group colonizes above-ground as well as below-ground parts of plants (ascomycetes and basidiomycetes), while the second and third groups are confined only to above-ground and below-ground parts, respectively. The diversity of the first group of non-clavicipitaceous endophytes is limited and shows highly localized colonization, while the second (ascomycetes and basidiomycetes) and third groups (mycorrhizal and dark septate) have traits for extensive colonization and wide host range (vascular and nonvascular) and possess high transmission potential. For instance, the second group of non-clavicipitaceous endophytes colonize extensively as much as over 20 species in a single tropical leaf [21], while the third group (mainly dark septate endophytes, DSE) is known to colonize up to 600 plant species in wide geographic zones [22].

The advent of anticancer drug Taxol from the endophytic fungus *Taxomyces andreanae* with the host species (*Taxus brevifolia*) [23] was the main stimulus to study endophytic fungi from various ecological niches. There are several contributions on endophytes as overviews, evolution, lifestyle, diversity, ecology, techniques, natural products, bioprospects, biological control, and bioremediation. The purpose of this review is to provide a glimpse of different facets (diversity and ecology) on non-clavicipitaceous and non-mycorrhizal endophytic fungi associated with plant species in different ecosystems with emphasis on bioprospect avenues.

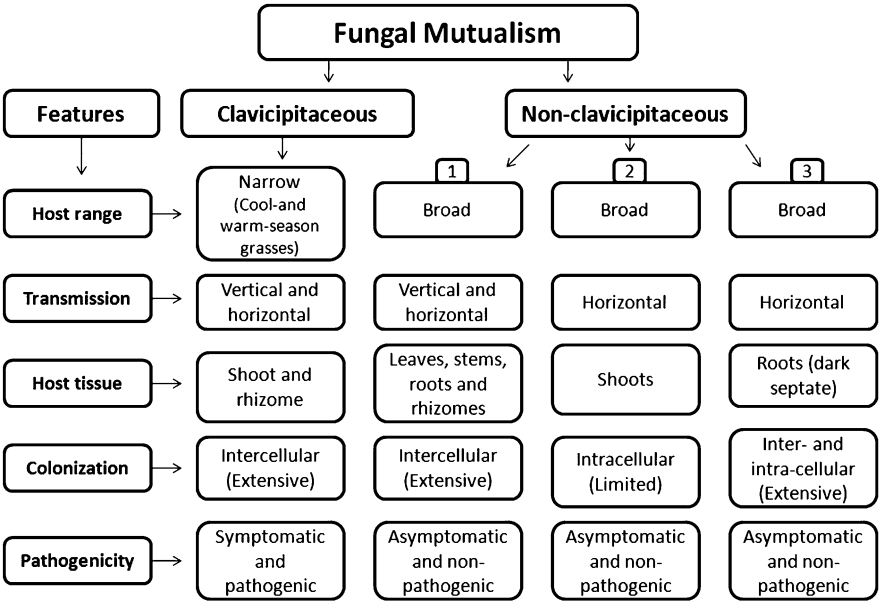

Fig. 1 Classification with specific features of fungal mutualism with host species in different ecosystems. (Modified from Rodriguez et al. [19])

2 Diversity and Distribution

In the evolutionary context, the study of endophytic fungal community is necessary in different plant species worldwide for diversity and ecosystem functioning [24]. Studies on the diversity and distribution of endophytic fungi have been carried out in wide geographical regions (e.g., terrestrial, semiaquatic, freshwater, and marine habitats). Similarly, a variety of phototrophs have been screened for endophytic fungi (e.g., forest trees, medicinal plants, mangroves, seaweeds, seagrass, vegetables, ferns, and orchids) (Fig. 2). The interest on endophytic fungi has mainly channeled toward applications rather than taxonomic novelty. However, many fungal endophytes have been recorded as new to science [25]. Despite dominance and diversity of fungal endophytes in different biomes of wide geographic regions, understanding their functions and applications is still in the experimental phase. The following sections provide available information on the richness and diversity of endophytic fungi in terrestrial and aquatic ecosystems.

2.1 Terrestrial Ecosystem

Association of fungi with terrestrial plant species has received major attention compared to aquatic habitats [26]. Angiosperms and gymnosperms of wide

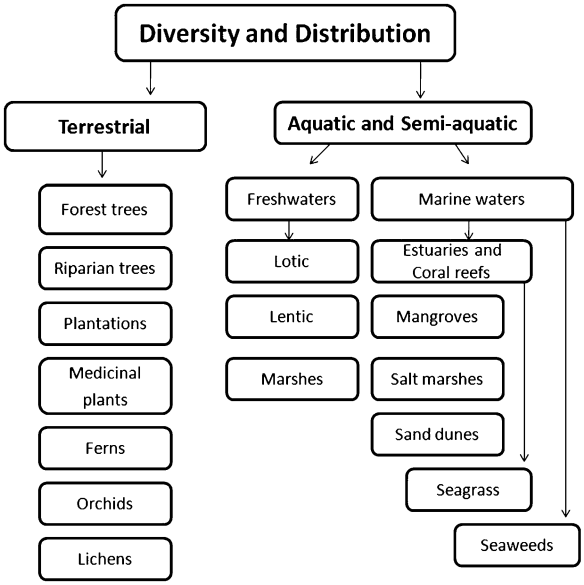

Fig. 2 Diversification and distribution of endophytic fungi in different ecosystems

geographic areas have been assessed for the occurrence of endophytic fungi in forest trees in tropical, subtropical, and temperate regions [27]. Endophytic fungal association has been assessed in trees of rain forests, forests in arid/semiarid regions, alpine, dry deciduous, dry thorn, moist deciduous, pine plantations, palms, riparian, shola, and dipterocarps. The tissues assessed for endophytic fungi include foliage, bark, xylem, and root. Although roots of tree species are known to harbor arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi, the DSE fungal association has been widespread and needs further study.

2.1.1 Trees and Shrubs

This section discusses endophytic fungal association with trees/shrubs other than medicinal and mangrove tree species. Tropical angiosperms are the major niches for expansion of diversity of endophytic fungi. Based on the extent of colonization of mutualistic fungi, Arnold and Lutzoni [28] considered tropical trees, and their leaves are the special hotspots of endophytic fungal diversity. Irrespective of habitat or tree species in dry tropics, the endophytic fungal diversity was not exceptionally high, but there is a trend that dominance of a set of core group fungi (e.g., *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, and *Xylaria*) [29]. Endophytic fungi associated with palms have also been evaluated [30, 31]. Twenty-four tree hosts belonging to 17 families of 2 dry topical forests of southern India showed 81 endophytes in 3600 segments [32]. A total of 56 species were distributed in more than 1 host species. Two groups of fungi were found: (i) the first group was ubiquitous in many host species; (ii) the second group is composed of less frequent forms. In Chinese oil pine (*Pinus tabulaeformis*) of northern China revealed 2065 isolates of endophytic fungi from 4320 tissue segments (needles, bark, and xylem) [33]). Isolation

frequency was significantly increased with increase in age of the tree. In the bark and needles, *Alternaria alternata*, *Phoma* sp., *Phomopsis archeri*, and *Leptostroma* were dominant. Guo et al. [34] reported the influence of season and tissue age on the endophytic fungal diversity in *Pinus*. Sun et al. [27] performed quantitative assessment of endophytic fungi among tree species and tissues of *Betula platyphylla*, *Quercus liaotungensis*, and *Ulmus macrocarpa* from woodland habitats of China. The overall colonization ranged between 48.5% and 65.6%; however, the rate of colonization was significantly higher in twigs compared to leaves. Based on the morphology and internal transcribed spacer (ITS) sequence analysis, a total of 61 species were identified. The impact of host was stronger than the impact of tissues. Studies on endophytic fungi in the tropical wet forests of Costa Rica at different altitudinal range (400–2900 m msl) revealed that endophytic fungi varied along the gradient with highest in the lowest stratum compared to high elevation supporting the idea that the environment rather than tree species controls the endophytic fungal colonization [35].

In *Juniperus procera* of Saudi Arabia, 26 endophytic fungi were recovered [36], and the overall colonization rate was 36%. Molecular assessment of rRNA revealed six distinct operational taxonomic units (OTUs). The dominant fungi include *Aspergillus fumigatus*, *Penicillium oxalicum*, *Preussia* sp., *Peyronellaea eucalyptica*, *Peyronellaea sancta*, and *Alternaria tenuissima*. Phellophytic (=bark-inhabiting) endophytic fungi were investigated in 15 dicotyledonous tree species in dry thorn, dry deciduous, and stunted montane evergreen forests of the Western Ghats [37]. Montane forest consists of higher species diversity compared to other forests. *Fusarium*, *Paecilomyces*, *Phoma*, *Phomopsis*, and two sterile forms (EGS1 and EGS3) were found in high frequency. The authors predicted that the environmental factors rather than tree species determine endophytic fungal assemblages in the forests surveyed. Leaf, bark, and stem of *Tectona grandis* in different geographical locations and seasons were assessed for endophytic fungi in tropics by [38]. A total of 5089 isolates were assigned to 45 distinct morphotypes with 43 ascomycetes and 2 basidiomycetes. The leaves possess the highest diversity in all locations and seasons and concluded that all three factors (tissue, spatial, and temporal) are responsible for structuring the endophytic fungi. The woody angiosperm *Populus trichocarpa* endowed with three distantly related genera belongs to *Cladosporium*, *Penicillium*, and *Trichoderma* [39]. Conidia of endophytes germinated on leaf discs in the laboratory with high humidity, developed hyphae on surface, and entered via stomatal openings. Such events were visualized in living leaf discs with the help of light microscopy and scanning electron microscopy (SEM).

The DSE constitute a specific group of fungi colonizing the roots of different tree species. These fungi exhibit melanization within the root tissues, and some are also known to produce hyaline structures [40]. They are usually asexual filamentous ascomycetes belonging to different orders (*Helotiales*, *Pleosporales*, and *Xylariales*) [41]. Their distribution is worldwide and frequent in harsh and nutrient-deficient environments like arid/semiarid regions. Even though they are important components of soil ecosystem, their evolution, lifestyle, ecology, and interaction with host species are not well understood [42]. Knapp et al. and Bonfim et al. [43, 44] have

reviewed the research carried out on DSE. The DSE are endowed with plant cell wall-degrading enzymes (PCWDEs) and carbohydrate-active enzymes (CAZymes) [43]. Many strains of DSE are known to stimulate growth and vitality of tree species [45, 46]. The DSE have also been considered crucial in conserving rare, endemic, and endangered plant species [47]. Bonfim et al. [44] predicted that these DSE have several ecosystem functions especially nutrition, deterring root pathogens, and developing tolerance to environmental fluctuations.

Zubek et al. [48] reported co-occurrence of DSE with AM fungi in ten plant species of Pamir-Alay Mountains of Central Asia. Except for the *Spiraea baldschuanica*, the root colonization frequency of DSE in the rest of the species was low. Comparative genomics of two DSE (*Cladophora* and *Periconia macrospinosa*) revealed their origin from different evolutionary lines despite root colonization of same plant species (*Salix rosmarinifolia*). Many endophytes from the tree species became the source of new therapeutic compounds [49]. Bagyalakshmi et al. [50] assessed the DSE from the shola forest of the Western Ghats and found DSE association in six tree species. Diversity of DSE in the roots of seven native tree species of the Atlantic rain forest (southeast Brazil) in three seasons along the altitudinal gradient has been evaluated by Bonfim et al. [44]. A total of 151 isolates have been assigned to 35 OTU, representing 27 species by sequencing ITS regions of rDNA. The most frequent genera were *Alternaria*, *Ascochyta*, *Cladosporium*, *Coniothyrium*, *Nigrospora*, *Microdiplodia*, and *Phoma*.

2.1.2 Medicinal Plants

In the interest of natural product drug discovery, exploring medicinal plants for endophytic fungi is more pertinent. Statistics during the last three decades reveal about 50% of new drugs were formulated from natural product derivatives, among them up to 75% constitute anti-infective agents [51]. To initiate such ventures, ethnic knowledge on medicinal plants which are used in therapeutics will be of immense value. In spite of advancement in combinatorial chemistry, traditional medicinal compounds and medicinal plants will serve as starting point to inquire the potential of associated endophytic fungi. Natural products from the medicinal plants are highly promising owing to varied biological activities. Many endophytic fungi in medicinal plants are involved in plant growth promotion, tolerance to adverse conditions, nutrient supply, and preventing the herbivore attack. There are many instances that some of the value-added metabolites derived from medicinal plants will also be produced by the inhabiting endophytic fungi [52, 53]. Some secondary metabolites will be co-produced by the host and endophytic fungi (e.g., the anticancer drugs, camptothecin, and podophyllotoxin [54–56]; bioinsecticide, azadirachtin [57]). There are instances that some of the hormones and anticancer drugs will be synthesized independently by the host as well as the endophyte (e.g., gibberellin and Taxol) [23, 58].

Leaf, petiole, stem, and root of 6 medicinal herbs of central India yielded 46 endophytic fungi based on morphological and molecular techniques, while 3 medicinal shrubs consist of 34 endophytic fungi [59]. From the leaf, bark, and stem segments of 5 medicinal plants of the Western Ghats yielded 18 species of

endophytic fungi with highest colonization in leaf segments indicating the tissue specificity [60]. Plant-colonized fungi produce a wide variety of medicinally valuable metabolites and further lead to the discovery of new compounds of therapeutic interest. There are several endophytic fungi that harbor medicinal plants which are capable to produce host-independent production of natural products [53] (Table 1). Evidences suggest that such capability of endophytic fungi or the host plant species is the outcome of biochemical convergence or horizontal transfer of gene [53, 85].

Owing to continued loss of biodiversity of medicinal plants exploited for the purpose of natural products by anthropogenic activity, low output of products of interest, long periods for maturation of medicinal plants, and difficulty to cultivate are the major constraints to develop desired medicinal product [53, 86, 87]. Although many endophytic fungi could produce novel biochemical metabolites of applied value, the existent of yield of a specific metabolite is also important. Usually production efficiency of axenic cultures will be hampered in subcultures, and several reasons and solutions have been suggested to overcome those constraints. Coexistence of host and fungus get appropriate signals to produce a specific metabolite, which will be lost in axenic cultures [88]. Co-cultivation of two endophytic fungi or endophytic fungus and bacteria (mixed fermentation) showed enhancement of metabolites of applied value [53]. There is ample scope to identify the appropriate signaling molecules; co-culture of endophytic fungi, co-cultivation with host tissue/cells, and addition of host tissue extract into the medium are some of the possible alternatives to achieve success.

There are a series of articles and reviews dealing with therapeutic ethnic use of diverse plant species against many diseases like diabetes, cancer, human immunodeficiency virus (HIV), Alzheimer's, malaria, and so on [89–93] . For example, Ranade et al. [93] discussed about the impact of the association of endophytic fungi in specific plant species used ethnically to cure diabetes. Many medicinal plant species are traditionally used to cure diseases that have potential endophytic fungi, which are of immense value to generate novel metabolites. Instead of using the plant species, possibilities to employ the relevant endophytic fungi in the production of required metabolite will be an important task to conserve the endangered/endemic medicinal plants.

2.1.3 Ferns

Unlike angiosperms, pteridophytes have high proportion of epiphytes, and up to 13,000 species of epiphytic ferns have been reported in tropical and subtropical ecosystems [94]. Lehnert et al. [95] reviewed symbiotic fungal association with pteridophytes (AM-, EM-, and DSE-fungi). However, reports on the endophytic fungi with pteridophytes are scanty [10, 96–100]. Raviraja et al. [97] evaluated roots of four riparian ferns (*Angiopteris evecta*, *Cristela dentata*, *Diplazium esculentum*, and *Macrothelypteris torresiana*) in streams of the west coast and Western Ghats of India. Up to ten aquatic hyphomycetes colonized these pteridophytes, and three species were common to all ferns (*Triscelophorus acuminatus*, *T. monosporus*, and unknown sp.). Kumaresan et al. [99] evaluated endophytic fungi of five pteridophytes from the east coast of India. Forty species of endophytic fungi (331 isolates)

Table 1 Endophytic fungi in medicinal plants capable to produce different bioactive metabolites. (Modified from Venieraki et al. [53])

Medicinal plant	Metabolite and application	Endophytic fungi and tissue	References (or reference therein)
Cajanus cajan	Cajaninstilbene acid (anti-inflammatory and analgesic)	Three strains in leaves	[61]
Camptotheca acuminata	Camptothecine (anti-cancer)	Many isolates	[62]
Capsicum annuum	Capsaicin (cardio protective influence, anti-lithogenic effect, anti-inflammatory and analgesia and thermogenic)	*Alternaria alternata* in fruits	[63]
Catharanthus roseus	Vinca alkaloids (anticancer)	*Fusarium oxysporum, Talaromyces radicus* and *Eutypella* spp. in leaves	[64]
Cephalotaxus hainanensis	Homoharringtonine (myeloid leukemia)	*Alternaria tenuissima* in bark	[65]
Cinchona spp.	Quinine alkaloids (anti-malaria)	*Phomopsis, Diaporthe*, *Schizophyllum*, *Penicillium*, *Fomitopsis* and *Arthrinium* in stem	[66]
Coleus forskohlii	Forskolin (to treat glaucoma)	*Rhizoctonia bataticola* in stem and roots	[67]
Digitalis lanata	Digoxin (to treat atrial fibrillation, atrial flutter and heart failure)	Five strains in leaves and stem	[68]
Forsythia suspensa	Phillyrin (antioxidant, anti-inflammatory, anti-hyperlipidemia and antipyretic)	*Colletotrichum gloeosporioides* in fruits	[69]
Fritillaria cirrhosa	Peimisine and imperialine (antitussive and expectorant)	*Fusarium redolens* in bulb	[70]
Ginkgo biloba	Bilobalide (neuroprotective effects)	*Pestalotiopsis uvicola* in leaves	[71]
Ginkgo biloba	Ginkgolide B (platelet activating factor)	*Fusarium oxysporum* in leaves	[72]
Huperzia serrata	Huperzine A (anti-Alzheimer disease)	*Penicillium griseofulvum*, *Penicillium* sp., *Aspergillus flavus*, *Mycoleptodiscus terrestris*, *Trichoderma* sp., *Colletotrichum gloeosporioides* and *Shiraia* sp. in leaves	[73]
Macleaya cordata	Sanguinarine (antibacterial, antihelmintic, antitumor and anti-inflammatory)	55 isolates in leaves	[74]

(*continued*)

Table 1 (continued)

Medicinal plant	Metabolite and application	Endophytic fungi and tissue	References (or reference therein)
Miquelia dentata	Camptothecine (anti-cancer)	*A. alternata*, *Phomopsis* sp. and *Fomitopsis* sp. in fruit	[75]
Nerium indicum	Vincamine (cerebrovascular, precaution of chronic ischemic stroke, and reduction of vascular dementia)	One strain in stem and roots	[76]
Passiflora incarnata	Chrysin (including antibacterial, anti-inflammatory, anti-diabetic, anxiolytic, hepatoprotective, anti-aging, anticonvulsant and anticancer effects)	*Altenaria alternata*, *Colletotrichum capsici*, and *C. taiwanense* in leaves	[77]
Piper longum	Piperine (antibacterial, antifungal, hepato-protective, antipyretic, anti-inflammatory, anti-convulsant, insecticidal and antioxidant)	*Periconia* in leaves	[78]
Piper nigrum	Piperine (antibacterial, antifungal, hepato-protective, antipyretic, anti-inflammatory, anti-convulsant, insecticidal and antioxidant)	*Colletotrichum gloeosporoides* in stem	[78]
Rheum palmatum	Rhein (alleviate pain and fever and inhibits inflammation)	Two strains in stem and one strain in roots	[79]
Rhodiola spp.	Salidrosides and p-tyrosol (antioxidant and anti-aging properties, anti-microwave radiation, antihypoxia and adaptogenic activities)	*Phialocephala fortinii*	[80]
Salvia sp.	Salvianolic acid (for cardiovascular and cerebrovascular diseases)	Fungi in leaves and roots	[81]
Silybum marianum	Silybin A and silybin B (chemoprevention and hepatoprotective)	Two strains *of Aspergillus iizukae* in leaves and stem	[82]
Solanum nigrum	Solamargine (anticancer activity against colon, prostate, breast, hepatic and lung cancer cell lines)	*Aspergillus flavus* in stem	[83]
Vinca minor	Vincamine (cerebrovascular, precaution of chronic ischemic stroke, and reduction of vascular dementia)	One strain in leaves, stem and roots	[84]

were recorded with common occurrence of *Colletotrichum* sp., and the *Pteris* showed the highest number of fungi, while *Selaginella* ranked first in a number of isolates. Sati and Belwal [101] reported 11 species of aquatic hyphomycetes as endophytic in roots of several riparian pteridophytes occurring in two locations of Kumaun, Himalaya. Roots of *Botrychium* are colonized by a maximum of seven endophytic hyphomycetes. Leaflets, stolon, runners, and roots of an aquatic medicinal fern *Marsilea minuta* in southern India yielded 14 hyphomycetes, 3 *Coelomycetes*, and 1 non-sporulating endophytic fungus [102].

2.1.4 Orchids

Although orchids are obligatory associated with fungi to fulfill germination of seed and nutritional requirements, information on endophytic fungi are scanty [103, 104]. However, up to 200 genera of orchids have been evaluated to understand their association with mycorrhizal fungi [105].

Five species of Mediterranean orchids (83 samples of roots and tubers) were evaluated for endophytes and isolated *Fusarium*, *Papulaspora*, and *Rhizoctonia* [106]. Assessment of roots of five orchids from the rain forest of southern Ecuador based on ITS sequence (249 isolations) revealed frequent occurrence of several ascomycetes (*Helotiales*, *Hypocreales*, and *Xylariales*) [107]. Ten orchid species belong to the genera *Dendrobium* in China which yielded high diversity of endophytic fungi (37 genera, up to 80 species) based on morphological and molecular methods [108]. The dominant endophytes include *Acremonium*, *Alternaria*, *Ampelomyces*, *Bionectria*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Verticillium*, and *Xylaria* with varied degree of host specificity. Endophytic *Phomopsis* sp. was isolated from the orchid *Odontoglossum* sp. from the Northern Ecuador [109]. This endophyte has the capacity to control a wide range of plant pathogenic fungi by producing volatile organic compounds (VOCs). The VOCs of this fungus consist of sabinene, which has a monoterpene with flavor of pepper. Ninety segments from two dominant orchids (*Bulbophyllum neilgherrense* and *Vanda testacea*) of the Western Ghats were assessed for endophytic fungi [110]. Aerial roots, mature bulb/stem, and mature leaves yielded 118–130 endophytic fungal isolates with 17–20 anamorphic species. In both orchids, leaf segments yielded more endophytic fungi than bulb/stem or roots. Endophytic fungal diversities of three endangered Brazilian orchids (*Hadrolaelia jongheana*, *Hoffmannseggella caulescens*, and *H. cinnabarina*) have been assessed by [111]. These orchids were endowed with basidiomycetes which belong to *Sebacinales* (81.6%) followed by *Cantharellales* (12.1%), while ascomycetes belong to *Helotiales* (29.3%), *Capnodiales* (18.1%), and *Sordariales* (10.3%). The *Xylaria* sp. was endophytic in an orchid *Anoectochilus setaceus* found in Sri Lanka [112].

Roots of two orchids (*Cymbidium faberi* and *C. goeringii*) from Central China delivered 120 isolates consisting of 15 morphotypes and 21 representative strains (8 genera) based on ITS sequencing [113]. *Tulasnella* and *Umbelopsis* were the dominant endophytes in *C. faberi* and *C. goeringii*, respectively. Leaves and roots of

9 orchids from Thailand consist of 12 endophytic fungi [114]. However, many isolates could not be identified by mere morphological characteristics, and *Xylaria* sp. was dominant in these orchids. Leaf and root tissues of 11 orchids from the Arunachal Pradesh of northeastern India showed higher endophytic fungi in leaves than roots. Again the *Xylaria* was dominant in leaf as well as root tissues [104]. From the threatened epiphytic orchid (*Dendrobium aqueum*) of southern India (stem, leaf, and pseudobulb), the endophytic fungus *Colletotrichum* was isolated [115]. Colonization was higher in basal stem segments than other tissues. Leaf and root samples of an endangered orchid *Pomatocalpa decipiens* from forests of Orissa, eastern India, consist of 928 phosphate-solubilizing endophytic fungi in leaf samples [116]. Many fungi could not be identifiable based on morphology, while identifiable fungi consist of six genera (*Aspergillus*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Paecilomyces*, and *Penicillium*). Root tissues consist of 20 endophytic fungi belonged to 4 genera which were also phosphate-solubilizing potential (*Aspergillus*, *Fusarium*, *Paecilomyces*, and *Penicillium*).

2.2 Aquatic Ecosystem

Relatively studies on the diversity and distribution of endophytic fungi in aquatic plant species are scanty [26]. However, many typical aquatic fungi are also associated as endophytes with terrestrial plant species. The following sections provide information on the endophytic fungi in association with plant species in freshwater (macrophytes, ferns, and riparian plant species) and marine habitats (mangroves and coastal sand dunes).

2.2.1 Freshwater Habitats

Endophytic fungi in freshwater habitats are less explored compared to terrestrial and marine habitats. Several plant species grow under the influence of freshwaters such as streams, rivers, lakes, and marshes. Besides plant species adapted to aquatic habitats (e.g., macrophytes and marshes), many riparian tree species project their roots into the running waters that serve as potential sites for fungal colonization, which prevents total removal of fungi from the upper reaches of the streams or rivers [117, 118]. Submerged macrophytes (e.g., *Apium*, *Potamogeton*, and *Ranunculus*) serve as hosts in the absence of submerged leaf litter in streams [119, 120]. Endophytes from macrophytes of freshwater lake and reservoirs in Northern Arizona revealed low isolation frequency but differed in species richness, diversity, and community structure with similarity with proximate terrestrial communities [26]. Aquatic medicinal fern *Marsilea minuta* (leaflets, stolon, runners, and roots) of southern India yielded 14 hyphomycetes with 3 *Coelomycetes* and 1 sterile endophytic fungus [102].

Freshwater hyphomycetes are known as endophytes in roots exposed to streams and aerial plant parts [121–123]. Fisher and Petrini [124] first demonstrated the endophytic phase of two typical aquatic hyphomycetes (*Campylospora parvula* and *Tricladium splendens*). Subsequently, Fisher et al. [125] compared endophytic

aquatic hyphomycete population in submerged and terrestrial roots and demonstrated higher colonization in submerged (30%) than terrestrial (12%) roots. *Tetracladium marchalianum* was a root endophyte in *Fragaria* sp. [126], while *Tetracladium setigerum* was associated with roots of *Fragaria* sp. as well as *Gentiana* sp. [127]. Some species of *Gyoerffyella* were found to colonize the healthy roots of *Picea abies* [128]. Root bark and xylem of ten riparian tree species in two altitudinal ranges (475–500 and 765–800 m msl) of the Western Ghats were assessed for endophytic fungi [129]. These tree species yielded 20 species of aquatic hyphomycetes with dominance of *Anguillospora crassa*, *A. longissima*, and *Cylindrocarpon* sp. Xylem showed higher number of endophytic fungi than bark. The diversity of aquatic hyphomycetes was higher in mid-altitude than high-altitude streams which supported the notion of higher occurrence of saprophytes on plant detritus in mid-altitude streams (e.g., Sampaje stream) [130]. From the three aquatic (*Equisetum arvense*, *Myriophyllum verticillatum*, and *Ottelia acuminata*) and two riparian (*Cardamine multijuga* and *Impatiens chinensis*) plant species from the southwest China, 31 endophytic fungi were recovered with dominance *Cladosporium*, *Fusarium*, and *Geotrichum* [131].

While studying endophytic fungi, new species of aquatic hyphomycetes (*Filosporella fistucella*, *F. versimorpha*, *Fontanospora fusiramosa*, and *Tetracladium nainitalense*) were described in many riparian tree roots [132–135]. In addition, some anamorph-teleomorph connections of aquatic hyphomycetes were established [136, 137]. A teleomorphic state was induced by the anamorphic endophyte *Heliscus lugdunensis* subcultures exposed to fluorescent light [118]. Approximately, 60 species of freshwater hyphomycetes are known as endophytes in submerged roots of angiosperms, gymnosperms, and pteridophytes [98, 101, 102, 118, 119, 125, 129, 132–135, 138].

2.2.2 Marine Habitats

Endophytic fungi have been studied from a wide range of marine and marine-influenced habitats like mangroves/estuaries, coral reefs, sand dunes, and salt marshes. A brief outline on endophytic fungi in mangroves and coastal sand dunes is given in the following sections with emphasis on seaweeds and seagrass.

Mangroves

Plant species occurring in river mouths (estuaries and mangroves) of tropical and subtropical regions provide potential habitats for colonization of endophytic fungi. The common foliar endophytes include *Acremonium*, *Phomopsis*, *Phyllosticta*, and *Sporormiella minima* in mangrove plant species of the east coast of India [139]. Based on conventional and molecular approaches, the endophytic fungus *Diaporthe phaseolorum* has been isolated from the endangered mangrove trees of *Kandelia candel* in South China Sea coast [140]. Four mangrove plant species in southern China consist of 36 species of culturable endophytic fungi with differential colonization in hosts as well as higher colonization in twigs than leaves (30–58 vs. 6–25%) [141]. Mangrove trees in Thailand consists of 619 endophytic fungi with a highest colonization in *Bruguiera cylindrica* [142]. Seven endophytic fungi in the

roots of three mangrove tree species were isolated from the coast of Andaman and Nicobar Islands with dominance of *Aspergillus* sp. [143]. From the mangrove shrub *Acanthus ilicifolius* (leaf, stem, and root) and mangrove fern *Acrostichum aureum* (pinna, petiole, rhizome, and root), 25 endophytic fungi were isolated [144]. Interestingly, a typical marine fungus *Cumulospora marina* was endophytic in the roots of *A. ilicifolius*. Using three methods of assessment (direct-plating, damp-chamber, and bubble-chamber incubation) of root segments of four mangrove plant species in a southwest India showed a highest number in *Rhizophora mucronata* by direct- as well as damp-chamber incubation methods [145]. Interestingly, bubble-chamber incubation yielded two freshwater fungi (*Mycocentrospora acerina* and *Triscelophorus acuminatus*). Further study on the whole root segments of *R. mucronata* in three depths (low-tide, mid-tide, and high-tide) on direct-plating yielded higher fungi in mid-tide samples than samples from other depths. Root bark and decorticated root of *R. mucronata* from mid-tide level on direct-plating showed higher fungal colonization in bark than decorticated root. The result of these studies revealed endophytic fungi composed of a consortium of soil, marine, and freshwater fungi.

Leaf, stem, root, and pod of a mangrove legume *Sesbania bispinosa* of southwest India yielded 25 endophytic fungi with highest in root segments [146]. *Aspergillus niger* was the most dominant endophyte. Shreelalitha and Sridhar [147] studied endophytic fungi of wild legume *Sesbania bispinosa* adapted to mangroves and coastal sand dunes. Another mangrove legume, *Canavalia cathartica*, consists of 36 endophytic fungi with highest of 15 species in stem followed by 14 species in the root [148]. As seen in *S. bispinosa*, *A. niger* was dominant. Leaf, stem (top, middle, and basal), and rhizome of mangrove sedge *Cyperus malaccensis* of the southwest coast of India consist of 30 endophytes with highest species in middle stem [149]. The endophytic fungal association showed zonation in inflorescence, stem, and rhizome of *C. malaccensis* [150]. Many endophytic fungi were also pathogens, and this sedge serves as a potential collateral host for pathogenic fungi causing disease in paddy as well as vegetables grown around the estuarine habitats. In mangrove plant species, some endophytes have been considered as saprotrophs or opportunistic pathogens (*Chaetomium globosum* and *Paecilomyces variotii*) [6, 145, 151]. Endophytes in mangrove plant species are composed of plant pathogenic fungi (*Alternaria alternata*, *Curvularia clavata*, and *Drechslera halodes*), toxigenic fungi (*Aspergillus flavus*, *A. ochraceus*, and *Trichoderma harzianum*), and entomopathogenic fungi (*Paecilomyces* sp.) [144–146, 148].

Single species dominance of endophytic fungi has been reported in *Avicennia marina* (*Phoma* sp.), *Bruguiera cylindrica* (*Colletotrichum gloeosporioides*), *Rhizophora apiculata* (*Sporormiella minima*), *Rhizophora mucronata* (*Sporormiella minima*), and *Suaeda maritima* (*Camarosporium palliatum*) [139, 152, 153]. However, multispecies dominance was also evident in *Avicennia officinalis*, *Lumnitzera racemosa*, *Rhizophora mucronata*, and *Sonneratia caseolaris* [145, 153].

Coastal Sand Dunes

Root segments of three coastal sand dune plant species of the southwest coast of India on direct-plating and damp incubation showed the presence of 31 endophytic fungi [154]. Plating yielded consistently more endophytic fungi than damp incubation. Interestingly, in addition to terrestrial fungi coastal sand dune, plant species also consists of marine fungi (13%). Three age classes and five tissue classes of two wild legumes (*Canavalia cathartica* and *C. maritima*) on the coastal sand dunes of the southwest coast of India revealed 46 species of endophytic fungi (33 mitosporic fungi, 6 ascomycetes, 2 zygomycetes, and 5 sterile fungi) [155]. *Chaetomium globosum* was the most dominant fungus, which has significant role in plant protection. Only one marine fungus *Halosarpheia* sp. was endophytic in *Canavalia maritima*. Surface-sterilized 450 segments of another wild legume *Sesbania bispinosa* of coastal sand dunes and mangroves of the southwest coast of India consist of 39 endophytic fungi with dominance of 6 species [147]. The endophytic fungal composition was consortium of saprophytic, pathogenic, and toxigenic fungi which exist as endophytes as seen in mangrove habitats [145]. Although seeds of *S. bispinosa* yielded more endophytes, their richness and diversity were low; however, the richness and diversity were high in roots in spite of low colonization confirming the host and habitat specificity.

Seaweeds

Seaweeds of wide geographic regions host endophytic fungi, and they constitute the second largest niche for assemblage of marine fungi(Baltic Sea, Canada, China, India, Malaysia, North Sea, and the United Kingdom) [156–160]. The topic on endophytic fungi of seaweed served as a major subject matter of many reviews [157, 160–165]. About 100 endophytic fungi are known from the seaweeds distributed worldwide, and up to 75% of them have been reported from the Baltic Sea, Canada, China, India, North Sea, and the United Kingdom [160]. Nearly 100 seaweeds have been assessed for the endophytic fungi, and Suryanarayanan [164] compiled information on the diversity of fungal endophytes in red, brown, and green seaweeds mainly from the coastal region of India.

The endophyte *Mycophycias ascophylli* is associated throughout the life cycle of some seaweeds (*Ascophyllum nodosum* and *Pelvetia canaliculata*) [166–168]. Up to 25 species of green, red, and brown algae of the east coast of India harbored about 72 endophytic fungi with dominance in brown algae [162]. Many ubiquitous endophytes found in terrestrial plant species (e.g., *Colletotrichum*, *Pestalotiopsis*, *Phoma*, and *Phyllosticta*) were not represented in macroalgae which reveals the selection of different guilds. From the coastal regions of Mandapam and Pondicherry of the east coast of India, 10 seaweeds were assessed for endophytic fungi and found 156 isolates with a highest frequency of isolation from *Codium* (80%), while it was least in *Ulva fasciata* (10%) [169]. Flewelling et al. [159] studied seaweeds of the Atlantic coast of Canada and found 79 endophytic fungi from red, brown, and green algae (7, 4, and 3 species, respectively). The overall isolation frequency

was 26%, and two red algae showed frequency between 72 and 87%. Twenty endophytes were identified up to genus or species level, and the rest were coded based on morphological difference.

Fungi those that are capable to tolerate the conditions of the sea will colonize the seaweeds leading to evolution of generalist endophytes; thus, frequent isolates are seen in different seaweeds. There are some instances that many endophytic fungi were symbiotic in a specific seaweed or recurrence of single fungus as endophyte in many seaweeds [162, 170, 171].

Seagrass

Seagrass serves as potential niche for colonization of endophytic fungi, and studies have been carried out in Bermuda, Hong Kong, India, Puerto Rico, the Philippines, and Thailand. Leaf blade, petiole, and rhizome of *Halophila ovalis* of the east coast of India showed low colonization density of 14 endophytic fungi [172]. Venkatalachalam et al. [173] studied endophytes in leaf tissues and rhizome of ten seagrass species collected from the east coast of India. This culture-based study also showed low colonization frequency, which is lower than terrestrial plant species. The frequency of colonization was higher in rhizome than leaves, and major endophytic fungi include *Aspergillus*, *Paecilomyces*, and *Penicillium*. Another study by Raja et al. [174] employed direct method to detect endophytic fungi using acridine orange and aniline blue in three seagrass species derived from the reefs of Palk Bay, India. Culture-dependent fungal DNA and denaturing gradient gel electrophoresis (DGGE) have confirmed the occurrence of more than one taxon in the tissue of seagrass. Forty-two culture-dependent endophytic fungi have been isolated from nine seagrass species collected from the Palk Bay based on ITS1-ITS4 sequences [175]. They were represented by 15 genera (2 were identified only up to the order), and variations in the distribution of endophytic population were seen among the seagrass species without significant similarity.

About 26 endophytic fungi are known from 3 seagrass collected from Hong Kong and the Philippines (*Thalassia testudinum*, *Zostera japonica*, and *Z. marina*) [176]. Diversity of culturable endophytic fungi in seagrass *Enhalus acoroides* from Thailand consists of 47 isolates belonging to 17 phylogenetic genera based on morphology as well as molecular methods [177]. Three endophytes (*Fusarium*, *Penicillium*, and *Nigrospora*) were dominant. Interestingly, *Nigrospora* sp. showed antifungal property against the dermatophyte *Microsporum gypseum* (4–8 μg/ml). A thorough review on the phylogenetic community structure of endophytic fungi in four seagrass species (*Cymodocea serrulata*, *Enhalus acoroides*, *Halophila ovalis*, and *Thalassia hemprichii*) of southern Thailand has been published by [178]. A total of 81 culturable endophytes were isolated, and their phylogeny was studied by ribosomal rDNA sequences. Majority of endophytes belonged to the three classes of Pezizomycota (*Sordariomycetes*, 55.6%; *Dothideomycetes*, 38.3%; *Eurotiomycetes*, 4.9%), while one isolate belonged to *Saccharomycetes* (1.2%). These isolates were assigned under the clades of fungi occurring in terrestrial habitats, and each seagrass species hosted different fungal communities.

3 Ecological Perspectives

Endophytic fungi being ecologically diverse and versatile guild possess broad functional traits in host phytogeography, adaptation, and defense. Studies in association with endophytic *Pestalotiopsis* in the foliage of four different forest types in the Western Ghats revealed that it exists as a generalist owing to environmental conditions especially the host's taxonomic and habitat restrictions [179]. The genes responsible for such transition are ribosome biogenesis and MAP kinase signaling. In addition, upregulation of genes encoding enzymes for biosynthesis of amino acids (phenylalanine, tryptophan, and tyrosine) for production of secondary metabolites is found in host defense [180]. Such mechanism helps in understanding adaptation of *Phomopsis liquidambari* with host and the environment that in turn facilitates sustainable agriculture by carbon and nitrogen cycles. Molecular and cytological evidences suggest the adaptation of *P. liquidambari* with hosts (*Oryza* and *Arabidopsis thaliana*) for growth promotion under low N conditions.

Regarding the lifestyle, transitions of saprophytism toward endophytism and vice versa have been identified by researchers in the root endophytic basidiomycete *Piriformospora indica* [181]. Such phenotypic plasticity during the interaction between *P. indica* with *A. thaliana* was possible by the transcriptional regulation, which leads to changes in tissue morphology as well as lifestyle owing to the impact of signals by host as well as environment [182]. Promputtha et al. [183] provided evidences for functional switchover of endophytes into saprobes due to production of the same degrading enzymes by nine foliar endophytes and their saprobe counterparts isolated from *Magnolia liliifera*.

It is realized that colonization of endophytes leads to the slowdown of the rate of decomposition of twig and woody litter. A few studies have shown the impact of endophytic fungi on litter decomposition [184, 185]. Leroy et al. [184] demonstrated the impact of foliar endophytes on slow decomposition and in turn influence the rate of carbon and nutrient cycles in ecosystems. However, the endophytes occur in woody material and appear as early decomposers on senescence [186, 187]. Competition (negative interaction) between endophytic and secondary decomposer fungi has been reported by Dowson et al. and Fukasawa et al. [188, 189]. It is known that exclusion of secondary saprotrophic basidiomycetes (e.g., *Mycena polygramma* and *Phanerochaete filamentosa*) by the ascomycete fungal endophytes (e.g., *Phomopsis* spp. and *Xylaria* spp.) results in reduced decay rates [185]. On semi-defined malt extract agar medium, the above endophytes inhibit saprotrophic basidiomycetes [189]. Exploitation of organic resources during the late stage by endophytes is dependent on environmental factors and antagonistic mycelia [190].

The DSE belonging to the orders *Helotiales*, *Pleosporales*, and *Xylariales* develop hyphal structures in healthy roots with varying degrees of melanization [40, 191]. They have been considered as latent pathogens and showed adaptation to nutrient-poor arid or semiarid habitats [41]. The presence of CAZymes in DSE indicates their capability to degrade plant cell wall [192]. The genome analysis of two DSE (*Cadophora* sp. and *Periconia macrospinosa*) originated from the semiarid

environment along with 32 ascomycetes that showed different evolutionary lineages with functional differences although existing in the same ecological guild [43].

Investigations on the endophytic fungal association have exposed some tripartite relationships (e.g., tree-fungi-insects, sedge-fungi-mollusk; shrub-fungi-bees). Fungi are known to interact with gall-forming insects in multiple ways. Fungus-plant interaction with emphasis on insect control has been reviewed by Raman and Suryanarayanan [193]. A tripartite relationship has been observed among fungal endophytes, bees, and a shrub (*Baccharis dracunculifolia*) [194]. Physical damage caused by the bees (during resin collection) paves the way for endophytic fungal colonization and leads to phytochemical differences between the sexes of host plant. A huge biomass of giant marine gastropod mollusk (*Telescopium telescopium*) has intimate association with the sedge *Cyperus malaccensis* in a southwest estuarine and mangrove habitats of southwest India [150]. Being detritus feeder, this gastropod depends on these sward expanses in estuarine and mangrove habitats. Up to 30 species of endophytic fungi were recorded in the above ground (bracts and stem) and below ground (rhizome) tissues of this sedge [149]. There seems to be a tripartite association among sedge-endophytes-mollusk, which provides ample scope to focus research on this interesting trophic interaction.

4 Techniques of Evaluation

Endophytic fungal evaluation is also method dependent, and any flaws in the methodology result in accessing pseudo-endophytic or weedy fungi. The Box 1 represents various techniques employed or suggested to evaluate the endophytic fungi in plant tissues. Sun and Guo [27] reviewed the traditional and molecular methods employed for assessment of endophytic fungi. Successful results, acceptable conclusions, and outlook on the endophytic fungi depend on the application of foolproof and authentic methodology. Techniques of isolation and identification involve mainly direct and indirect methods. The direct methods involve surface sterilization followed by plating, histological (simple stains and fluorescent dyes), and microscopic studies. Various simple stains are useful in localizing the endophytic fungi in the live plant tissues [195]. Indirect methods involve biochemical (e.g., structural components like chitin and ergosterol), immunological (e.g., fungal-specific antigen-antibody reactions; radioimmunoassay, RIA), and molecular approaches (e.g., DGGE; restriction fragment length polymorphism, RFLP; DNA cloning; ITS sequence analysis, pyrosequencing, DNA barcoding, RNA applications, and liquid chromatography-mass spectrometry (LC-MS)).

Box 1 Techniques Proposed or Employed to Evaluate Endophytic Fungi

Direct methods

Surface sterilization methods [196]

Bulk surface sterilization method [197]

(*continued*)

Box 1 Techniques Proposed or Employed to Evaluate Endophytic Fungi (continued)

Histological methods [196]
Fluorescence microscopy [174, 180]

Indirect methods

Ergosterol [198, 199]
RIA [200]
DGGE [201, 202]
T-RLFP [203, 204]
DNA cloning [205–207]
Whole-community DNA [207]
ITS sequence analysis [27]
Pyrosequencing [27]
DNA barcoding [27]
RNA applications [180, 208]
UPLC-ESI-MS/MS [209]

Fröhlich et al. [31] suggested the need of pilot study using traditional method before attempting to study endophytic fungi in any plant species. Stringent sterilization protocol needs to be followed to overcome surface dwelling fungi or contaminating fungi. Sterilization schedule depends on the host, tissue, and tissue size. For example, thin leaves need shorter period of sterilization than thick stem or bark. A strong linear relationship was seen between the size of leaf fragments and number fungi isolated [210]. This suggests to consider the leaf size into account in isolation of endophytic fungi. Its likely different type of leaf or other tissues or its anatomy (e.g., angiosperm, gymnosperm, pteridophyte, and orchid) has great influence on isolation of endophytes. Success of surface sterilization method employed could be tested by imprinting the sterilized segment on antibiotic-amended sterile media [211]. If imprints show the growth of fungi, it means surface fungi have not been eliminated by the protocol employed. Similarly, the growth of endophytic fungi from the sterile segment requires 5–7 days to emerge as mycelia; if growth occurs within 2 days, they are likely contaminants owing to improper sterilization process (or it may also reflect the quality of chemicals used for sterilization).

Sieber [196] has extensively reviewed various techniques of tissue sterilization (chemical, physical, and combination of methods) for the isolation of endophytic fungi in a wide range of plant tissues. Insights are also given on the sample size of herbaceous and woody host plant species in endophyte isolation. Interestingly, the root segments of woody hosts yielded more endophytic fungi than herbaceous hosts. Usually a very large number of tissue segments need to be processed to isolate endophytic fungi; Greenfield et al. [197] devised a bulk sterilization method to scale up fungal endophyte isolation. This method is advantageous over conventional methods as 24 plant tissue samples could be surface sterilized separately and simultaneously. Isolation of endophytic fungi is dependent on the

type of medium used for isolation [212]. It is obvious that conventional methods lead to overcome slow-growing, non-culturable, and fastidious endophytic fungi which exist in the sterilized tissues. Many endophytes isolated will not sporulate on the culture media in spite of employing authentic sterilization methods, and in such instances to make those cultures to sporulate constitutes an interesting task. A method of promoting sporulation of sterile endophytic fungi has been proposed by [213]. Mycelia sterilia inoculated onto the agar media consisting of sterile petiole fragment of palm *Livistona chinensis* exposed to UV light and darkness (12-hr alternate duration) resulted in inducing fruit bodies on the petioles. Those fungi did not sporulate on semi-defined media where sporulated on petiole cultures is the advantage of this method. Thus, there is ample scope to modify or apply innovative methodology to obtain endophytic fungi from live tissues [6].

Several molecular approaches are useful in the isolation and identification of endophytes (e.g., DGGE, T-RFLP, DNA cloning, whole-community DNA, ITS analysis, pyrosequencing, and DNA barcoding). Recently, studies involving RNA applications are also employed in endophytic research [208]. To overcome the discrepancies of conventional methods of evaluation of phylogeny of endophytes, a recent study has employed the evaluation of six different gene regions (small subunit, SSU; large subunit, LSU; ITS; transcription enhancer factor, TEF; retinal-binding protein 2, RBP2; β-tubulin) [214]. Although these approaches provide a scope to identify the sterile isolates, several questions are posed mainly to consider sterile endophytes as new species. How to use the dried cultures of sterile endophytes as holotypes for validation? Should we use diversity of OTU as an appropriate taxonomic rank? It is difficult to totally switchover from conventional methods into molecular methods owing to misidentified fungal sequences in GenBank [215]. In addition to misidentification, chimeric sequences, static taxonomic assignments, and fungi with unknown taxonomic identity are also misleading [216]. Such databases lead to erroneous mismatch of fungi for unequivocal decisions in identification and propagate cumulative errors. Thus, the BLAST result needs additional evaluation like ITS sequences by careful steps by looking at the original author's sequences and its revisions. However, the ITS sequence analysis provides classification of endophytic fungi at least up to genus level in most instances [217].

Due to the complexity and so-called dead-end feature of molecular approaches in elucidating the diversity of endophytic fungi [214], recently the FUNGuild has been proposed [18]. However, it needs further expansion and refinement to make it fully useful in mycological research, which needs cooperative venture of conventional and molecular mycologists. Maciá-Vicente et al. [209] followed chemosystematic approach by untargeted ultra-performance liquid chromatography-electrospray ionization mass spectrometry (UPLC-ESI-MS/MS) to assess the natural products produced by 822 strains of root endophytic fungi from brassicaceous genus *Microthlaspi* in Europe and Turkey. This approach helped in searching new sources as well as novel natural products from the endophytic fungi.

5 Bioprospect Avenues

Endophytic fungi have already known as major source of novel metabolites and in turn useful in applications in different areas. Significance of natural products from biological source has been realized on the invention of anticancer drug Taxol from the *Taxomyces andreanae*, an endophytic fungus associated with trees of Pacific yew (*Taxus brevifolia*) [23]. Later, it has been understood that production of Taxol by *T. andreanae* was an evolutionary consequence of genetic recombination due to mutualism with the host species. Such novelties of endophytic fungi associated with plant species paved the way to protect the endangered and endemic plant species for want of extraction of drugs. Thus, fungi exist in unique, stressed, and unusual biological niches which have immense value in the discovery of novel metabolites. Interestingly, the cumulative number of patents granted by the US patent authority on endophytic fungal metabolites is steadily increasing from mid-1990s onward [218, 219]. Although the endophytic fungi derived from angiosperms and gymnosperms were assessed for value-added metabolites, other photosynthetic systems like algae, bryophytes, mosses, and pteridophytes have been fairly ignored.

5.1 Bioactive Metabolites

Endophytic fungi are prospective and reliable niche for novel bioactive compounds useful in medicine, agriculture, and industries (Box 2). Production of secondary metabolites by the endophytic fungi is mainly mediated by three metabolic pathways (e.g., mevalonic acid, polyketide, and shikimic acid pathways). A variety of antimicrobial products have been produced by the endophytic fungi, which are of immense value in combating the human health [220]. It is known that over 40% of drugs those prescribed are based on the natural products and more than 50% of anticancer, antimigraine, and antihypertensive compounds are originated from the natural products or their derivatives [221]. Even though many plant species possess cancer therapeutic potential, extraction and processing are labor intensive, while endophytic fungi on in vitro cultivation and fermentation techniques help deriving the desired metabolites.

Box 2 Some Examples of Bioactive Metabolites of Endophytic Fungi

Antibacterial compounds [159, 222, 223]
Antifungal compounds [222, 224, 225]
Anticancer compounds [56, 222, 226]
Cytotoxic compounds [227–229]
Anti-insect metabolites [230–232]
Pharmacological metabolites [233–235]
Secondary metabolites [25, 236, 237]
Volatile organic compounds [238–240]
Enzymes [241–243]

Endophytic strains of *Chaetomium* isolated from different plant species possess unique bioactive metabolites (anthraquinones, chaetoglobosins, chromones, desidones, steroids, terpenoids, and xanthones) with potential therapeutic activity (antitumor, anti-malaria parasite, and enzyme inhibition) [233]. Evaluation of fungal endophytes isolated from the medicinal plants has attracted the attention of several researchers in recent years. Nisa et al. [25] have reviewed the literature on phytochemicals and other natural products of endophytic fungi derived from the medicinal plants. Suryanarayanan et al. [234] have reviewed the natural products produced by the endophytic fungi having pharmaceutical significance (e.g., apicidin, aphidicolanatriol, aphidicolaneodiol, aphidicolanepentol, aphidicolene, aphidicolin, chaetoglobosin, cytochalasin, enniatin, phomopsolide, prosalanapyrone, and solanopyrone). Helaly et al. [236] evaluated the endophytic fungi of the order *Xylariales* to assess the diversity of biologically active metabolites in relation to biodiversity. These authors found a high metabolic diversity in three endophytic fungi (*Daldinia eschscholtzii*, *Hypoxylon rickii*, and *Pestalotiopsis fici*) and advocated to study the volatile secondary metabolites. Endophytes are known for production of a variety of additional secondary metabolites (e.g., antibacterial, antifungal, anti-plasmodial, anticancer) [244–246].

Literature on a wide range of endophytic fungi have been reviewed by Deshmukh et al. [247] for antibacterial compounds. In view of controlling multidrug-resistant bacteria, endophytic fungal antibiotics are potential alternatives. Flewelling et al. [159] evaluated 79 endophytic fungi isolated from 14 seaweeds of the Atlantic coast of Canada and found 43 and 32 strains which showed antibacterial (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and antifungal (*Candida albicans*) potential, respectively. Relatively, there is scarcity of antifungal than antibacterial antibiotics. A wide range of structurally diverse secondary metabolites of endophytic fungi harboring seaweeds have been documented based on recent literature search by [248]. Up to 182 metabolites of seaweed endophytes possess biological potential. Deshmukh et al. [238] reviewed the reports on antifungal metabolites of endophytic fungi isolated from medicinal plants for the last 5 years. Several compounds possessing diverse configurations have been documented, which will be of immense value in drug development. The VOCs are also commonly produced by the endophytic fungi, which has ample significance in agriculture and postharvest technology. It is interesting note that co-cultivation of endophytic fungi with bacteria results in novel compounds which could not be obtained on isolated cultures.

Endophytic fungi offer several potent anticancer drugs or their analogues or precursors (e.g., Taxol, podophyllotoxin, camptothecin, and vinca alkaloids). Kharwar et al. [226] reviewed anticancer agents isolated from endophytic fungi during 1990–2010. Up to 100 anticancer compounds possessing cytotoxicity have been reported from the endophytic fungi. Uzma et al. [227] reviewed clinically employed anticancer drugs, isolation, mode of action, characterization, and endophytic fungal improvement strategies. This review encompasses endophytic fungal products from different habitats (terrestrial, mangrove, and marine) and their cytotoxic potential on various cancer cell lines. An array of anticancer compounds (Taxol, podophyllotoxin, and camptothecin) produced endophytic fungi; their host

plant species and yield are also discussed. Four endophytic fungi isolated from leaf tissues of two Australian native plants (*Eremophila longifolia* and *E. maculata*) yielded potential anticancer agents. The endophyte *Chaetomella raphigera* isolated from the medicinal plant *Terminalia arjuna* produced Taxol (~80 μg/l) [249].

Debbab et al. [244] reviewed bioactive fungal metabolites derived from the endophytic fungi of marine origin (197 metabolites with 138 new natural products). A wide variety of plant species and macroalgae of maritime habitats were targeted for endophytic fungi as well as their metabolites using different screening strategies [157]. Based on literature, Singh et al. [160] compiled information on important metabolites produced by the endophytic fungi isolated from seaweeds. Schulz et al. [157] screened endophytic fungi of marine algae obtained from wide geographic regions for secondary metabolites. Interestingly, up to 42% of metabolites possess previously unknown structures. *Coniothyrium*, *Geniculosporium*, *Microsphaeropsis*, *Nodulisporium*, and *Phomopsis* were the promising isolates in the synthesis of novel metabolites. The dominance of metabolites of macroalgal endophytes was brown algae> red algae> green algae (i.e., 39 > 28 > 23%, respectively) [165]. Zuccaro and Mitchell [250] suggested that the poor performance of Chlorophyceae was due to their short life span. Endophytic fungi were better producers of secondary metabolites than other fungi isolated from marine algae. Hulikere et al. [251] reported angiosuppressive and antioxidant activity of an endophytic fungus *Cladosporium cladosporioides* isolated from *Sargassum wightii* from the coast of Kanyakumari, southern India.

Mangrove endophytic fungi are the potential source of novel metabolites [222]. Endophytic fungi isolated from *Acanthus ilicifolius* to *Acrostichum aureum* showed potential antibacterial, antifungal, and enzyme production [252]. The extracellular enzyme production under solid-substrate fermentation was highest during 5–10 days of incubation. Cellulase was produced by *Pestalotiopsis* during 6 days at pH 7 while xylanase during 10 days at pH 10. Thus, there is scope to harness cellulase-free xylanase by tuning the pH and duration of fermentation. The bioactive compounds include cytotoxic, anti-infective, radical-scavenging, enzyme-inhibiting, antifouling, and anti-parasitic activities. There seems to be several silent biosynthetic pathways in endophytic fungi, which function only under favorable natural conditions. Such so-called cryptic (or orphan) pathways may not be expressed under in vitro conditions owing to the lack of signal molecules. Thus, it is necessary to simulate natural or near natural conditions to stimulate endophytic fungi to produce rare metabolites. There is ample scope for innovative recipe as well as techniques to handle endophytic fungi for optimum harness of metabolic capabilities.

5.2 Bioprospect Potential

Various bioprospect avenues have been opened up due to the advancement of investigation on endophytic fungi such as agriculture, crop production, biological control, bioremediation, biopesticides, and biodegradation of recalcitrant compounds (Box 3). The current understanding of remediation enforced by plants

(phytoremediation) may happen due to the association of endophytic fungi, which provide stress resistance, production of enzymes, growth hormones, nutrient uptake, degradation of pollutants, and reduction of phytotoxicity of pollutants.

Box 3 Some Examples of Bioprospect Potential of Endophytic Fungi

Sustainable agriculture [220, 253, 254]
Biological control [151, 255–257]
Industrial enzymes [241–243]
Biofuels [109, 238, 239]
Bioremediation [235, 258, 259]

Endophytic fungi are well known for improving the plant fitness by inducing stress tolerance (biotic and abiotic) and supply of nutrients. Endophytes are also known for inducing tolerance against salinity and drought by production of phytohormones [260–262]. Such stress tolerance will be possible by regulation of phytohormones, enzymes, and oxygen-scavenging potential [19, 263]. Plant species face different types of biotic stress like insect attack, nematode infection, pathogenic fungal, and bacterial menace. It is known that the endophytic fungi decrease the severity of pathogen attack by upregulating defense genes of the host plant [264, 265].Although like mycorrhizal fungal role in nutrient uptake is not well known in endophytic fungi, there are many instances that endophytes are capable to enhance the supply important nutrients like phosphorus and nitrogen from soil. For example, *Piriformospora indica* in roots is known to enhance phosphate uptake, the DSE *Heteroconium chaetospira* capable to transfer nitrogen from organic matter [266, 267]. The fungal endophytes belong to the order *Sebacinales* have a broad host range, and they have promising avenues for sustainable plant production and in turn favor agricultural advancement [15].

Endophytic fungi are known for enzymes of industrial and pharmaceutical interest [235, 241, 242, 268]. They are the potential source of industrial enzymes (e.g., acidic protease, alkaline protease, asparaginase, chitinase, chitin deacetylase, chitosanase, laccase, tannase, and β-glucosidase) [241]. Nearly 38–84% of endophytes were positive for the production of a variety of enzymes (e.g., amylase, cellulase, chitinase, laccase, lipase, pectate lyase, pectinase, protease, and tyrosinase). Salt-tolerant chitinase and chitosanases were isolated from the endophytic fungus *Talaromyces stipitatus* from the roots of *Avicennia marina* of the east coast of India [269]. The chitinase activity was not altered drastically by varying salt concentrations.

Enzymes have immense applications in human health (e.g., chitin-modifying enzymes and L-asparaginase), food processing (e.g., alkaline protease, α-amylases, and tannases), energy (e.g., cellulases and lignocellulases), and bioremediation (e.g., chitinase, laccases, and polyurethanase) [241]. Interestingly, a recent study on the electron-beam irradiated (0.2 kGy) foliar endophyte (*Phomopsis* sp.) isolated from *Simarouba glauca* has shown enhanced detoxification potential of recalcitrant

anthraquinone dye by increased laccase production (1.6-fold compared to control) [258]. The dye on degradation by fungus was nontoxic to plants as well as microbes. The partially purified laccase has the ability to decolorize 200 mg/l dye within 20 min, which may be significantly higher than the physicochemical and photocatalytic degradations. Further, the irradiated endophytic fungus showed high tolerance to several metals (Zn^{2+}, Cu^{2+}, and Cr^{2+}) up to 10 mM concentration. Ling et al. [270] studied the heavy metal biosorption (Cu^{2+} and Zn^{2+}) capacity of endophytic fungi isolated from mangrove plant species.

Endophytic fungi are also known for the production of biofuels [110, 243, 271]. A wide array of VOCs have been reported from many endophytic fungi [238]. The endophyte *Muscodor albus* (collected from the jungles of the Caribbean coast) produced VOCs (mixture of gases consisting of alcohols, acids, esters, ketones, and lipids) with practical application to control a wide range of fungal and bacterial pathogens, which also helps preventing deterioration of fruits and vegetables [239]. Recently, Wang and Harper [240] reported endophytic fungal strain belonging to the genus *Hypoxylon* (BS15) as a potential producer of VOCs. The VOCs were produced in in vitro growth media on solid-phase extraction which consists of ground woody tissue of host plant species (*Taxodium distichum*). It is interesting to note that the wood tissue after extraction of VOCs on re-exposure serves as modulator to generate biofuel on the supply of growth media and such results predicted due to cellulose-degrading products. The dichloromethane as well as water extracts served as modulators with difference in products. Transformation of solid wastes by the endophytic fungal enzymes toward the improvement of manure qualities and production of biofuels warrants further exploration. Endophytic fungi isolated from medicinal plants are also good candidates for nanoparticle synthesis, which are of immense value in the control of human pathogens [272].

It is understood that entomopathogens are common colonizers of host plant species and endophytes. The first report on plant protection against the beetle (*Physocnemum brevilineum*) by the endophytic fungus (*Phomopsis oblonga*, which was known to spread Dutch elm disease) was during 1981 by Webber. Subsequently, Claydon et al. [273] confirmed that the endophytic fungi (*Xylariaceae*) synthesize secondary metabolites in host plant (*Fagus*) against the beetle larvae. Several entomopathogenic fungi have the endophytic lifestyle with many plant species and provide protection from the insect attack (e.g., *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys*, and *Isaria*) [151, 274]. The mechanism of insect control by the endophytic fungi was correlated with toxin production (insect deterring, loss of weight, reduction in growth, and increased rate of mortality) [230, 255]. Thus, studies have been intensified to trace the presence of endophytic entomopathogenic fungi in host plants for future applications. Several abiotic and biotic factors influence the function of entomopathogenic fungi as pesticides especially the toxin production (e.g., plant genotype, endophyte genotype, and soil fertility). Although *Beauveria bassiana* and *Metarhizium anisopliae* are commonly used to control the agricultural insect pests, other endophytic fungi are also known for controlling capacity (e.g., *Acremonium*, *Cladosporium*, *Clonostachys*, and *Paecilomyces*) [151]. Three among 150 foliar fungal endophytic fungi in needles

of red spruce (*Picea rubens*) were toxic to eastern spruce budworm (*Choristoneura fumiferana*) based on dietary bioassays [231]. Further detailed study on culture extract using spectroscopic and chromatographic methods revealed the presence of several new metabolites of fungal origin. Anti-insect metabolites of conifer tree endophytic fungi of the eastern North America revealed a rich source of new metabolites, which help improving the tolerance nursery seedlings to insect pests [232]. Suryanarayanan et al. [256] emphasized the importance of non-entomopathogenic endophytic fungi from foliage of forest trees in biological control (anti-pathogen/anti-pest metabolites and weedicide ability). To understand the intricacies of pest control by the entomopathogenic endophytic fungi, thorough knowledge on tripartite relationship and interaction among plant-insect-fungus is utmost important [193].

6 Concluding Remarks

Mutualism is one of the most essential endeavors essential for the diversity and productivity of the ecosystem. Nearly 300,000 plant species harbor one or more endophytic fungi [25]. Conventional estimates depict that at least one million species of endophytic fungi exist globally in various geographic and climatic zones [8, 275]. Jones [276] predicted that the number of endophytes in marine ecosystems is up to 6000 species, thus demanding further inquiry of marine communities. The *Sebacinales* (*Agaricomycetes*, *Basidiomycota*) are ubiquitous and highly diverse on interaction with plant systems which has been overlooked as endophytic association [15, 16]. Within this fungal order, transitions from saprotrophy to endophytism and nutrition via mycorrhizal association have been achieved. There seems to be an answer by the endophytic fungi for the current debate on the estimate (2.2–3.8 million; currently accepted species, 120,000) and whereabouts of cryptic species [277, 278]. Probably the missing fungi are hidden in phototrophs as mutualists, and certainly endophytic fungal research (morphology and molecular studies) will disclose the secret of cryptic fungal population.

The evolution of mutualism, association (tripartite), interactions (multi-trophic), and mode of transmission will answer many questions about the origin of new metabolites to support the mutualism to overcome the influence of pathogens. Multiple symbionts in one host plant species and single symbiont in different host plant species seem to be the law of nature, which needs further clarification. Selosse and Strullu-Derrien [279] predicted the origin and terrestrialization of flora (phototrophs) due to symbiotic association of fungi with algae based on fossil evidences. The Embryophyta (land plants) today in association with Glomeromycota (AM fungi) dates back to early Devonian period [280]. Association of algae with fungi (e.g., lichens) was evolved in Siluro-Devonian period, and those are the first players toward terrestrialization [281]. In view of association of several endophytic fungi in lichens [282], they are part and parcel of plant species with unimaginable functional roles. Thus, pattern of distribution of endophytes in plant species across different habitats or landscapes provide rich dividend of endophytic fungi with traits

of applied value. In the context of current global climate change, we need to understand the impact of such perturbations on the mutualistic linkages between endophytic fungi and their hosts.

Research on endophytes has already surpassed the preliminary stages (isolation, diversity assessment, ecology, and metabolites) as other issues like interactions (endophyte-plant, endophyte-host-insect, and endophyte-other fungi) and lifestyle endophytism pave the way for further progress [283]. The combinatorial chemistry has drawn much attention of researchers away from natural products owing to in vitro synthesis; the endophytic fungi continue to attract further due to highly valuable metabolites leading to novel drug discovery. Although diversity based on morphological features showed limited fungal taxa, metagenomics may have precise answer for this problem. In order to assess the diversity of fungi in environmental samples, a tool referred as FUNGuild has been introduced by Nguyen et al. [18]. This ecological guild operates as fungal OTUs independent of sequencing in evaluating different habitats like soils (saprotrophic and EM fungi), grasslands (saprotrophic and AM fungi), and decomposing wood (plant pathogenic fungi). Opposing to the unification of sequence-based taxonomy, the FUNGuild progresses toward unification of trophic guild-based taxonomy with continuous assessment.

Based on the current literature, it is possible to predict that (i) some endophytic fungi produce the compounds of our interest in in vitro cultures without host dependence, (ii) some endophytes need the host tissue extracts or substrates to produce desired compounds, and (iii) some fungi produce the compounds only in association with the host tissues in culture. There are several aspects that need specific explanation about the endophytic fungal association with host species. The critical aspects require further insight which include (i) the role of host in regulation of metabolic product of the endophyte, (ii) the genetic mechanism facilitating the mutualism between host and endophyte, (iii) the stimulation of silent genes which are responsible to produce the secondary metabolites, (iv) the role of metabolic product of endophyte on the host species, (v) the precise conditions (physiological and ecological) required by the endophyte to produce a specific metabolite, (vi) scale up the required endophyte metabolite to the industrial level, (vii) to test the benefit of endophytic fungi on inoculation to the plant species similar to probiotics, and (vii) to establish a repository to preserve endophytic fungi along with its host tissue for future use.

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Bioactive Metabolites from Turkish Marine Invertebrates and Associated Fungi

4

Belma Konuklugil and Hajar Heydari

Contents

Abstract

Nature provides a broad arsenal of structurally diverse and pharmacologically active compounds that serve as highly effective drugs with advanced chemical structures for the development of novel synthetic drugs to combat a multitude of diseases. Marine natural products are considered as promising sources of new secondary metabolites with pharmaceutical potential. Turkey has over 8300 km coastline with different geographical zones or habitats accounting for a great amount of diversity among its species. The largely unexplored Turkish seas with a wide range of biological diversity provide a lot of scope in future. In this section,

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we have tried to assemble studies of Turkish marine invertebrates and associated fungal species attempted so far.

Keywords
Bioactivity · Marine derived fungi · Marine invertebrates · Marine pharmacy · Turkish seas

1 Introduction

Like terrestrial species, the marine species also produce a great variety of secondary metabolites. Most of these secondary metabolites which are also found in terrestrial species include alkaloids, terpenoids, steroids, and some of these have extraordinary structures. The functional role of some of these secondary metabolites is known in the invertebrates. These metabolites have a vast range of different biological activities such as antioxidant, cytotoxic [1], anti-inflammatory [2], antiviral [3], antibacterial, antifungal [4], antifouling [5], and a range of other activities.

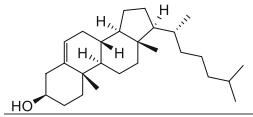
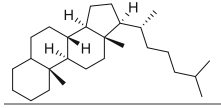
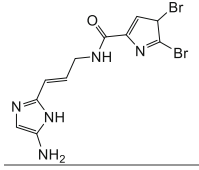
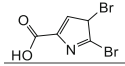
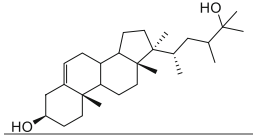
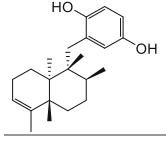
Among marine invertebrates, sponges are important sources for the discovery of new bioactive natural products. From sponges, 224 and 291 new compounds were reported in 2016 and 2015, respectively, that may prove important for drug discovery and development [6].

For the marketing of any drug derived from a natural source, 13–15 years would be required with an investment of 2–3 billion dollars on an average. Among all isolated marine natural products, 8 of these compounds have been approved as drugs and 12 of these compounds are in testing Phase III, II, or I of clinical pipeline. Plitidepsin (2016-EMEA), Terbectedin (2015-FDA), Berntuximab vedotin (2011-FDA), Eribulin mesylate (2010-FDA), Cytarabine (1969-FDA) have been approved as anticancerous drugs; Vidarabine (1976-FDA) has been approved as an antiviral drug, whereas Ziconotide (2004-FDA) and Omega-3-acid ethyl ester (2004-FDA) have been approved as analgesic and cardiovascular drugs [7].

Most of the marine natural products have shown anticancerous and cytotoxic properties. This is because of the fact that these natural products serve as chemical defenses in host marine species against adverse ecological conditions [8]. The different ecological conditions of marine ecosystem make biosynthetic pathways of marine organisms and microorganisms different and this is the reason why their genes potentially encode novel secondary metabolites. As a report of the United States (US) National Cancer Institute, marine invertebrates are a preferred source of cytotoxic compounds [9] (Table 1).

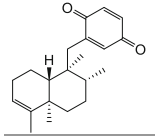
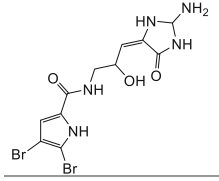
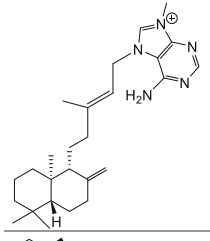
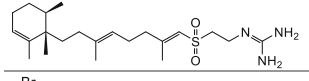
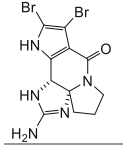
Spongothymidine and spongouridine were the first natural products derived from sponges, isolated from *Cryptotethya crypta* in the 1950s. There are more than 15,000 sponge species worldwide and more than 47% of marine natural products have been reported to be derived from sponges. Steroidal compounds, terpenoids, macrolides, anthraquinones, polyketides, fatty acids, nucleotides, cyclic peptides, and alkaloids are the main secondary metabolites which have been isolated from marine sponges [24].

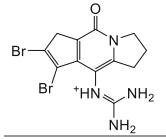
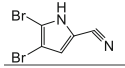
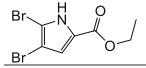
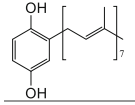
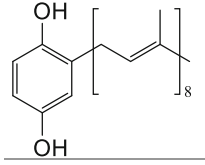
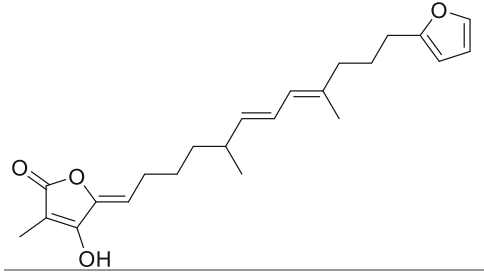
Table 1 Secondary metabolites isolated from Turkish marine invertebrates

Structure	Compound	Sponges	References
	Cholesterol **S1**	*Dictyonella incisa*	[10]
	Cholestane **S2**	*Dictyonella incisa*	[10]
	Oroidin **S3**	*Agelas oroides*	[11]
	4,5-Dibromo pyrrol-2-carboxylic acid **S4**	*Agelas oroides*	[11]
	25-Hydroxy-24-methyl cholesterol **S5**	*Agelas oroides*	[11]
	Avarol **S6**	*Dysidea avara*	[12]

(*continued*)

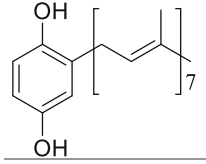
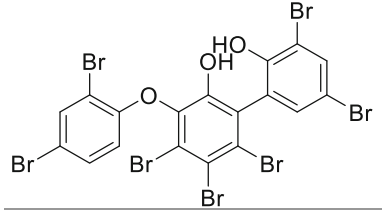
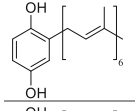
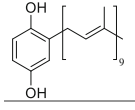
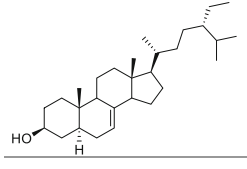
Table 1 (continued)

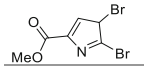
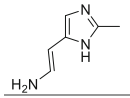
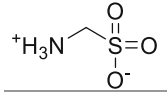
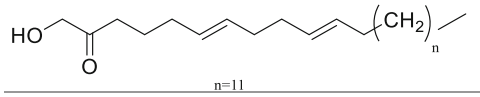
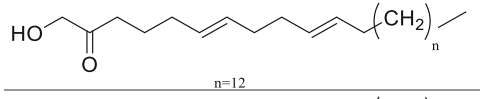
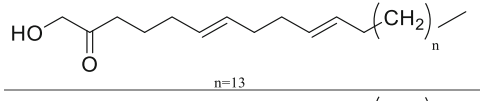
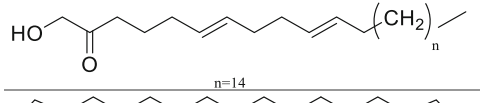
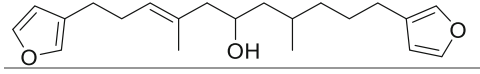
Structure	Compound	Sponges	References
	Avarone **S7**	*Dysidea avara*	[12]
	Dispacamide C* **S8**	*Agelas oroides (Hatay)*	[13]
	Agelasine D* **S9**	*Agelas oroides (Hatay)*	[13]
	(+)-Agelasidine C* **S10**	*Agelas oroides (Ayvalık)*	[13]
	Cylindradine A* **S11**	*Axinella cannabina* (Hatay)	[13]

	Ugibohlin*S12	*Axinella damicornis*	[13]
	4,5-Dibromo-1H-pyrrol-2-carbonitrile **S13**	*Agelas oroides*	[14]
	4,5-Dibromo-1H-pyrrol- 2-carboxylic acid ethyl ester **S14**	*Agelas oroides*	[14]
	Heptapregnyl hydroquinone **S15**	*Sarcotragus spinulosus*	[15]
	Octapregnyl hydroquinone **S16**	*Sarcotragus spinulosus*	[15]
	Fasciculatin **S17**	*Ircinia variabilis*	[14]

(*continued*)

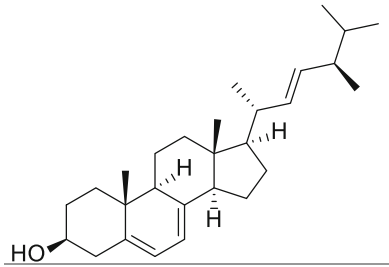
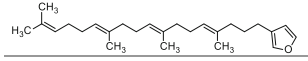
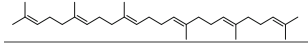
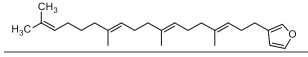
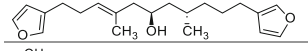
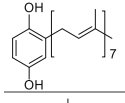
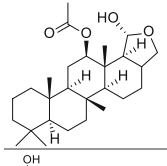
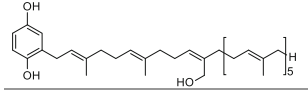
Table 1 (continued)

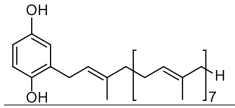
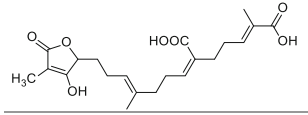
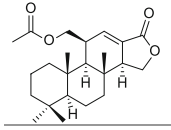
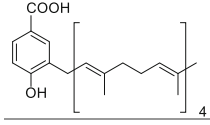
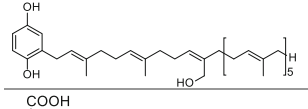
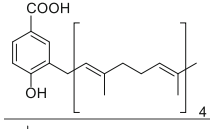
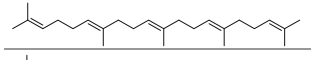
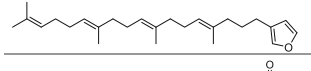
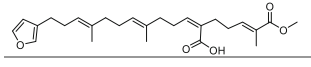
Structure	Compound	Sponges	References
	Heptaprenylhydroquinone **S18**	*Ircinia fasciculata*	[14]
	Ambigol A derivatives **S19**	*Sarcotragus muscarum*	[14]
	Hexaprenylhydroquinone **S20**	*Sarcotragus muscarum*	[14]
	Nonaprenylhydroquinone **S21**	*Sarcotragus muscarum*	[14]
	24-Ethyl-cholest-5a-7-en-3-β-ol **S22**	*Agelas oroides*	[16]

	4,5-Dibromopyrrole-2-carboxylic acid methyl ester **S23**	*Agelas oroides*	[16]
	(*E*)-Oroidin, 3-amino-1-(2-aminoimidazoyl)-prop-1-ene **S24**	*Agelas oroides*	[16]
	Taurine **S25**	*Agelas oroides*	[16]
	(5Z,9Z)-5,9-Tricosadienoic acid **S26**	*Agelas oroides*	[16]
	(5Z,9Z)-5,9-Tetracosadienoic acid **S27**	*Agelas oroides*	[16]
	(5Z,9Z)-5,9-Pentacosadienoic acid **S28**	*Agelas oroides*	[16]
	(5Z,9Z)-5,9-Hexacosadienoic acid **S29**	*Agelas oroides*	[16]
	Furospongin **S30**	*Spongia officinalis*	[17]

(*continued*)

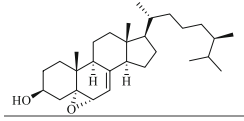
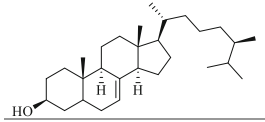
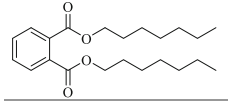
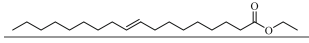
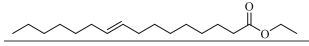
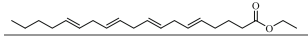
Table 1 (continued)

Structure	Compound	Sponges	References
	Ergosterol **S31**	*Spongia officinalis*	[18]
	Furospinulosin-II **S32**	*Spongia officinalis*	[18]
	Squalene **S33**	*Spongia officinalis*	[19]
	Furospinulosin-1**S34**	*Spongia officinalis*	[19]
	Furospongin-1 **S35**	*Spongia officinalis*	[19]
	Heptaprenyated *p*-quinol **S36**	*Spongia officinalis*	[19]
	12 *epi*-Deoxoscalarin **S37**	*Spongia officinalis*	[19]
	1,4,44-Trihydroxy-2-octaprenylbenzene **S38**	*Spongia officinalis*	[19]

	4-Hydroxy-3-tetra-prenylphenyl acetic acid **S39**	*Ircinia spimulosa*	[19]
	Dimethyl-furospongin-4 **S40**	*Ircinia spimulosa*	[19]
	11β-Acetoxyspongi-12-en-16-one **S41**	*Ircinia spimulosa*	[19]
	4-Hydroxy-3-octaprenylbenzoic acid **S42**	*Ircinia spimulosa*	[19]
	1,4,44-Trihydroxy-2-octaprenylbenzene **S43**	*Spongia* sp.	[20]
	4-Hydroxy-3-octaprenylbenzoic acid **S44**	*Ircinia* sp	[20]
	Squalene **S45**	*Spongia* sp.	[20]
	Furanospinulosin-1 **S46**	*Spongia* sp.	[20]
	Furospongin-4 **S47**	*Spongia* sp.	[20]

(*continued*)

Table 1 (continued)

Structure	Compound	Sponges	References
	5α- 6α- epoxyergosta 7-en- 3β- ol **S48**	*Microcosmus vulgaris*	[21]
	β-Sitosterol **S49**	*Paramuricea clavata*	[22]
	Diheptyl phthalate **S50**	*Paramuricea clavata*	[22]
	9-octadecenoic acid ethyl ester **S51**	*Anemonia viridis*	[23]
	9-hexadecenoic acid ethyl ester **S52**	*Anemonia viridis*	[23]
	5, 8, 11, 14, 17-docosapentaenoic acid ethyl ester **S53**	*Anemonia viridis*	[23]

The importance of terrestrial microorganisms as sources of therapeutic chemicals/drugs has been well known for several years. Penicillins, cyclosporin A, and adriamycine are examples of such compounds. Moreover, extreme marine ecological conditions such as changes in pressure, temperature, oxygen concentration, light, and sea salt force marine microorganisms to produce structurally different secondary metabolites. About 2% of 30,000 natural products isolated from marine environment has been obtained from microorganisms [25].

Sponges are most important sources of marine microorganisms (fungi and bacteria) when compared to algae or other marine species. These fungi have important role in the biosynthesis of bioactive secondary metabolites which are produced by sponges for defense [9].

Turkey has over 8300 km coastline in total with clusters of marine habitats like inter-tidal rocky, muddy and sandy shores, coral reefs. The ecological and pharmaceutical potential of Turkish marine habitat has remained largely unexplored. Turkey is surrounded by four seas, namely, the Black Sea, the Marmara, the Aegean, and the Mediterranean Sea with different ecosystems and noticeable marine and coastal diversity with several rare species. About 4.1% of terrestrial zone of Turkey belongs to coastal ecosystems and among all coastlines the Mediterranean region maximally reflect the rich diversity of fauna.

The purpose to write a chapter is to introduce Turkish marine organisms and microorganisms which are studied for their natural compounds. This information might help the researchers to develop potential drugs from Turkish coastlines [26].

Although most of studies on marine invertebrates are about sponges, there are limited studies about Turkish corals, tunicates, and sea anemones.

2 Turkish Marine Invertebrates

In this section, we tried to review the sponge species which were studied for their phytochemistry and bioactivity. 141 species including 82 species from the Aegean Sea, 63 species from the Sea of Marmara, 51 species from the Levantine Sea, and 18 species from the Black Sea were reported. Some of Turkish sponges which were studied for their secondary metabolites have been shown in Fig. 1.

2.1 *Agelas oroides*

Agelas oroides belongs family Agelasidae of the genus *Agelas*. Compounds **S3**, **S4,** and **S5** were obtained from *A. oroides* from Kemer [11], Turkey. From Gökçeada-Aegean Sea, compounds **S22** and **S23** were isolated and compounds **S24–S29** were detected from *Agelas oroides* [16]. Compounds **S8–S10** were isolated from *A. oroides* which were collected from different regions of Hatay and Ayvalik [13]. Compounds **S3**, **S4**, **S13,** and **S14** were isolated from *A. oroides* which were collected from Fethiye [14]. Bromotyrosine alkaloid, indole alkaloid, pyrrole

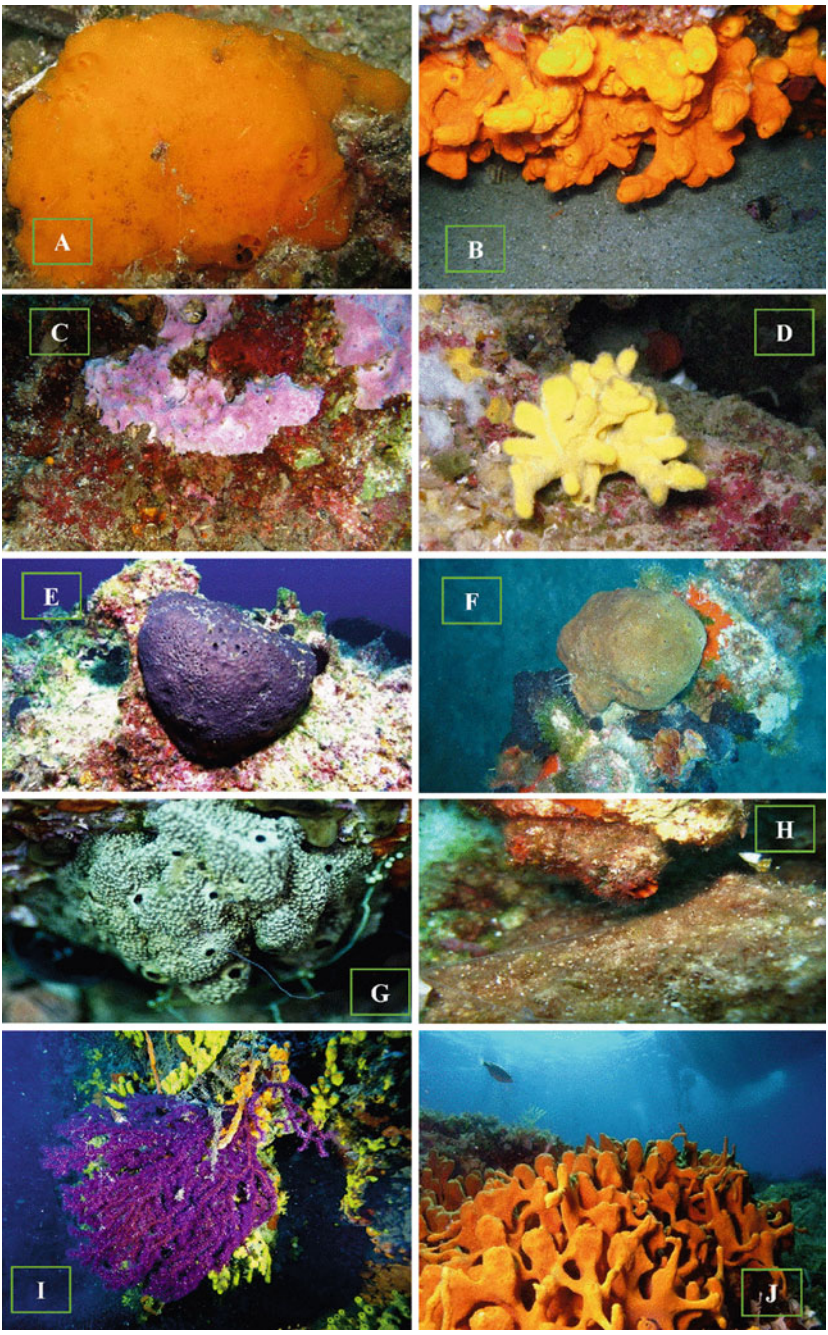


Fig. 1 Some of Turkish invertebrates which were studied for their secondary metabolites. (**a**) *Dictyonella incisa*; (**b**) *Agelas oroides*; (**c**) *Dysidea avara*; (**d**) *Axinella damicornis*; (**e**) *Sarcotragus spinulosus*; (**f**) *Ircinia oros*; (**g**) *Ircinia variabilis*; (**h**) *Microcosmus vulgaris*; (**i**) *Paramuricea clavata*; (**j**) *Aplysina aerophoba*

imidazole alkaloid, bromopyrrole alkaloid, and terpenes were detected by HPLC-DAD from *A. oroides* which were collected from Kemer [27].

2.2 *Dictyonella incisa*

Compound **S1** and **S2** isolated from *Dictyonella incisa* belonging to genus *Dictyonella* and family Dictyonellidae. This sponge was collected from Seferihisar, Turkey [10].

2.3 *Dysidea avara*

Dysidea avara species belong to *Dysidea* genus and Dysideidae family. Compounds **S6** and **S7** were isolated from *D. avara* from İbrice in the Aegean coast [12].

2.4 *Axinella* Species

Axinella species belong to *Axinella* genus and Axinellidae family. Compounds **S11** and **S12** were isolated from *Axinella cannabina* and *Axinella damicornis,* respectively [13]. Bromopyrrole type alkaloids (Purealidin R, Oroidin, Spongiacidin D, Hymenialdisin, Hymenidin, Stevensin, Aeroplysinin-1), indoline alkaloid (Communesin B), furanosester terpens (Fasciculatin), and terpens (Agelanesin A) were detected by HPLC-DAD from *Axinella polypoides* sponge species collected from Kemer [27].

2.5 *Sarcotragus* Species

Sarcotragus species belong to *Filifera* genus and Irciniidae family. Compounds **S15** and **S16** were isolated from *Sarcotragus spinulosus* which was collected from Antalya, Turkey [15]. Compounds **S19**, **S20**, and **S21** were isolated from *Sarcotragus muscarum* which was collected from Fethiye [14]. Bromopyrrole type alkaloids (Purealidin R, Oroidin, Spongiacidin D, Hymenialdisin, Hymenidin, Stevensin, Aeroplysinin-1), indole alkaloid (Communesin B), furanosester terpens (Fasciculatin), and terpens (Agelanesin A) were detected by HPLC-DAD from *Sarcotragus spinulosus* sponge species which was collected from Kemer [27].

2.6 *Ircinia* Species

Ircinia species belong to genus *Hircinia* and Irciniidae family. Compounds **S40** and **S44** were isolated from *Ircinia sp*., which was collected from Bodrum,

Turkey [20]. Four known terpenoids, **S39–S42**, were isolated from *I. spimulosa* [19]. Furanoterpenoids (polyprenyl-hydroquinones), furospongins, and furospinosulins (polyprenyl-furans) were isolated from sponges *I. spinulosa, I. muscarum* which were collected from the Mediterranean Sea [17]. Fasciculatin and heptaprenylhydroquinone were isolated from *I. variabilis* (Mersin) and *I. fasciculata* (Fethiye), respectively [14]. Bromopyrrole, indole alkaloids from *I. oros* and *I. variabilis* were also determined by HPLC-DAD [27].

2.7 *Spongia officinalis*

Spongia species belong to *Spongia* genus and Spongiidae family. *Spongia* sp. was collected from Bodrum and investigated. The compounds obtained as a result were **S30, S37, S38, S42, S45,** and **S46** [20]. Known terpenoids, **S33–38** and a mixture of furospongin-3- and 4, **S47** were isolated from *Spongia officinalis* [19].

Compounds **S31** and **S32** were isolated from *S. officinalis*. This sponge species was collected from Bodrum, Turkey [18]. Furanoterpenoids (polyprenyl-hydroquinones), furospongins, and furospinosulins (polyprenyl-furans) were also isolated from this sponge [17].

2.8 *Microcosmus vulgaris*

Microcosmus vulgaris belongs to genus *Microcosmus* of Pyuridae family. *Microcosmus vulgaris* was collected from İzmir, Agean Sea. From this tunicate, **S48** was isolated and stearic acid, palmitic acid, and myristic acid were detected in its butanol fraction. Palmitic acid has high ratio in the butanol fractions (50%) [21].

2.9 *Paramuricea clavata*

Paramuricea clavata belongs to genus *Paramuricea* and Plexauridae family. From Ayvalık- Agean Sea, this Turkish soft coral, *P. clavata* was collected and Compounds **S49** and **S50** were isolated [22].

2.10 *Anemonia viridis*

Anemonia viridis belongs to genus *Anemonia* of the Actiniidae family. Sea anemone, *A. viridis* was collected from Bodrum and from this species, three fatty acid ethyl esters **S51–S53** were isolated [23].

3 Bioactivity of Turkish Marine Invertebrates

Antimicrobial activity of *Agelas Oroides, Axinella damicornis, Ircinia spinulosa, Ircinia fasciculata, Dysidea avara, Aplysina aerophoba, Spongia agaricina, Parazoanthus axinella, Spongia officinalis, Dictyonella incisa, Halocynthia papillosa, Cladocora caespitose, Cerianthus membranaceus, Ascidiella aspersa, Eunicella cavolinii*, and *Styela clava* species was examined in different studies (Table 2). Antioxidant activity of *Agelas Oroides, Axinella damicornis, Ircinia spinulosa, Ircinia fasciculata, Dysidea avara, Axinella cannabina, Chondrilla nucula, Sarcotragus sp., Axinella verrucose, Axinella cannabina, Ciocallypta carbolloi, Petrocia ficiformis, Dictyonella incisa, Anemonia viridis*, and *Paramuricea clavate* species was screened using three different methods (Table 3). Cytotoxicity activity and tyrosinase inhibitor activity of just eleven species, *Parazoanthus axinella, Halocynthia papillosa, Cladocora caespitose, Cerianthus membranaceus, Eunicella cavoliniid, Ascidiella aspersa, Styela clava, Axinella sp., Dictyonella incisa, Anemonia viridis*, and *Paramuricea clavata* were investigated (Table 4). Although Turkey has a rich source of marine species, there are only few studies conducted so far especially on secondary metabolites derived from marine invertebrates and their bioactivities.

4 Marine Derived Fungi

In this section, the studies concerning the phytochemistry and bioactivity of marine fungal species have been reviewed.

Aspergillus carneus was isolated from inner tissues of the marine sponge *Agelas oroides* (Aliağa İzmir). Compounds **F1–F17** were isolated from this fungi strain [32]. *Penicillium sp.* was isolated from fresh water sediment sample (Selinos River) and compounds **F18–F31** were isolated from this fungi strain [33]. *Spirastrella cunctatrix* which collected from Kömür Port from which *Penicillium atrovenetum* was isolated and meroterpenoid **F32** was obtained from this strain. This compound showed antineuroinflammator activity [34]. *Arthrinium* sp. was obtained from *Sarcotragus muscarum* (Mersin) and 20 compounds **F33–F52** were isolated from *Arthrinium* sp. [35]. *Dictyonella incisa* (Seferihisar) was also collected and from this sponge *Trichoderma saturnisporum* was isolated. Sorbicillinoid-based compounds **F53–F60** were isolated from this fungus [36]. Sixteen Lactone derivatives **F61–F76** were isolated from *Talaromyces rugulosus*. This fungi species was isolated from *Axinella cannabina* (Sığaçık-İzmir) [37]. Phenolic bisabolene **F77–F89** were obtained from marine derived *Aspergillus* sp. isolated from Mediterranean sponge *Ircinia oros* (Ayvalık of Aegean Sea) [38]. The marine derived fungi, *Rhinocladiella* sp., *Clonostachys* sp., and *Engyodontium album* were obtained from *Ircinia oros, Axinella polypoides*, and *Ircinia variabilis* (Ayvalık of Aegean Sea), respectively. Chromone, isocumarin, and indole alkaloid derivatives **F90–F96** were isolated from these fungal strains [39]. Fusarielin analogue **F97** and **F98–F104** were derived from *Penicillium* sp. obtained from *Ircinia oros* (Kemer of Antalya) [40]. Some of isolated

Table 2 Antimicrobial activity of Turkish marine invertebrates

Sponge species	Gram-negative	Gram-positive	Yeast	Fungi	Reference
Agelas Oroides **Location:** Kemer, Hatay, Ayvalık	*Escherichia coli* ATCC 35218 (MIC: 4–16 μg/mL); *Pseudomonas aeruginosa* ATCC 10145(MIC: 2–8 μg/mL); *Proteus mirabilis* ATCC 7002 (MIC: 8–16 μg/mL); *Klebsiella pneumoniae* RSKK 574(MIC: 8–16 μg/mL); *Acinetobacter baumannii* RSKK 02026(MIC: 16–32 μg/mL)	*Staphylococcus aureus* ATCC 25923 (MIC: 0.5–1 μg/mL); *Enterococcus faecalis* ATCC 29212 (MIC: 4–16 μg/mL); *Basilus subtilis* ATCC 6633 (MIC: 16–32 μg/mL)	*Candida albicans* ATCC 10231 and *Candida parapsilosis* (MIC: 16 μg/mL)	–	[11]
Axinella damicornis **Location:** Kemer, Hatay, Ayvalık	*Escherichia coli* ATCC 35218 (MIC: 4–16 μg/mL); *Pseudomonas aeruginosa* ATCC 10145(MIC: 2–8 μg/mL); *Proteus mirabilis* ATCC 7002 (MIC: 4–16 μg/mL); *Klebsiella pneumoniae* RSKK 574(MIC: 4–16 μg/mL); *Acinetobacter baumannii* RSKK 02026(MIC: 8–32 μg/mL)	*Staphylococcus aureus* ATCC 25923 (MIC: 0.5–2 μg/mL); *Enterococcus faecalis* ATCC 29212 (MIC: 16 μg/mL); *Basilus subtilis* ATCC 6633 (MIC: 16–32 μg/mL)	*Candida albicans* ATCC 10231 and *Candida parapsilosis* (MIC: 16 μg/mL)	–	[11]
Ircinia spinulosa **Location:** Kemer, Hatay	*Escherichia coli* ATCC 35218 (MIC: 4–16 μg/mL); *Pseudomonas aeruginosa* ATCC 10145(MIC: 2–8 μg/mL); *Proteus mirabilis* ATCC 7002(MIC: 4–16 μg/mL); *Klebsiella pneumoniae* RSKK 574(MIC: 16 μg/mL); *Acinetobacter baumannii* RSKK 02026(MIC: 16–32 μg/mL)	*Staphylococcus aureus* ATCC 25923 (MIC: 0.5–2 μg/mL); *Enterococcus faecalis* ATCC 29212 (MIC: 8–16 μg/mL); *Basilus subtilis* ATCC 6633 (MIC: 8–32 μg/mL)	*Candida albicans* ATCC 10231 and *Candida parapsilosis* (MIC: 16 μg/mL)	–	[11]
Ircinia fasciculata **Location:** Kemer, Hatay	*Escherichia coli* ATCC 35218 (MIC: 2–8 μg/mL); *Pseudomonas*	*Staphylococcus aureus* ATCC 25923 (MIC: 1–2 μg/mL);	*Candida albicans* ATCC 10231 and *Candida*	–	[11]

(*continued*)

Table 2 (continued)

Sponge species	Gram-negative	Gram-positive	Yeast	Fungi	Reference
	aeruginosa ATCC 10145(MIC: 2–4 μg/mL); *Proteus mirabilis* ATCC 7002 (MIC: 4 μg/mL); *Klebsiella pneumoniae* RSKK 574(MIC: 8–16 μg/mL); *Acinetobacter baumannii* RSKK 02026(MIC: 16 μg/mL)	*Enterococcus faecalis* ATCC 29212 (MIC: 8–16 μg/mL); *Basilus subtilis* ATCC 6633 (MIC: 16 μg/mL)	*parapsilosis* (MIC: 16 μg/mL)		
Dysidea avara **Location:** Ayvalık	*Escherichia coli* ATCC 35218 (MIC: 16 μg/mL); *Pseudomonas aeruginosa* ATCC 10145(MIC: 8 μg/mL); *Proteus mirabilis* ATCC 7002(MIC: 8 μg/mL); *Klebsiella pneumoniae* RSKK 574(MIC: 8 μg/mL); *Acinetobacter baumannii* RSKK 02026(MIC: 32 μg/mL)	*Staphylococcus aureus* ATCC 25923 (MIC: 2 μg/mL); *Enterococcus faecalis* ATCC 29212 (MIC: 16 μg/mL); *Basilus subtilis* ATCC 6633 (MIC: 32 μg/mL)	*Candida albicans* ATCC 10231 and *Candida parapsilosis* (MIC: 16 μg/mL)	–	[11]
Aplysina aerophoba **Location:** Saros	*Escherichia coli* ATCC 25902 (IZ: 12.6 mm); Klebsiella pneumoniae (IZ: 14.3 mm)	*Bacillus cereus* ATCC 7064 (IZ: 14 mm); *Bacillus subtilis* ATCC 6633 (IZ: 16.3 mm); *Staphylococcus epidermidis* ATCC 122228 (IZ: 19.6 mm); *Staphylococcus aureus* ATCC 6538 (IZ: 17.3 mm); *Enterococcus faecalis* (IZ: 14.3 mm)	*Candida albicans* ATCC 10239 (IZ: 23.6 mm)	*Penicillium rugulosum* (IZ: 7.6 mm); *Penicillium jenseii* (IZ: 9.3 mm)	[28]
Spongia agaricina **Location:** Saros	*Escherichia coli* ATCC 25902 (IZ: 7.3 mm); Klebsiella pneumoniae (IZ: 8.3 mm)	*Bacillus cereus* ATCC 7064 (IZ: 10 mm); *Bacillus subtilis* ATCC 6633 (IZ: 9.3 mm); *Staphylococcus epidermidis* ATCC 122228	*Candida albicans* ATCC 10239 (IZ: 20.6 mm)	*Aspergillus niger* (IZ: 8.3 mm); *Aspergillus fumigatus* var. *elipticus* (IZ: 8.6 mm); *Aspergillus flavus*	[28]

(*continued*)

Table 2 (continued)

Sponge species	Gram-negative	Gram-positive	Yeast	Fungi	Reference
		(IZ: 13.6 mm); *Staphylococcus aureus* ATCC 6538 (IZ: 10 mm)		(IZ: 19.6 mm); *Aspergillus canditus* (IZ: 15.3 mm); Penicillium granulatum (IZ: 20 mm); *Penicillium rugulosum* (IZ: 11 mm); *Penicillium jenseii* (IZ: 9 mm)	
Spongia officinalis **Location:** Saros	–	*Bacillus subtilis* ATCC 6633 (IZ: 7 mm); *Staphylococcus aureus* ATCC 6538 (IZ: 7.6 mm)	–	*Aspergillus canditus* (IZ: 12.6 mm); *Penicillium rugulosum* (IZ: 11.6 mm)	[28]
Dictyonella incisa **Location:** Seferihisar	–	*Staphylococcus aureus* ATCC 43300 (MIC: 62.5 μg/mL); *Staphylococcus epidermidis* ATCC 12228 (MIC: 62.5 μg/mL)	*Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019 (MIC: 500 μg/mL)	–	[10]
Parazoanthus axinella **Location:** Kesan	*Salmonella typhimurium* ATCC 14028 (MIC: 32 μg/mL); *Pseudomonas aeruginosa* ATCC 27853 (MIC: 32 μg/mL)	*Staphylococcus aureus* JCSC 4744 (MIC: 32 μg/mL); *Enterococcus faecium* ATCC 6057 (MIC: 8 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 32 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 8 μg/mL); *Candida krusei* ATCC 6258 (MIC: 128 μg/mL)	–	[29]
Halocynthia papillosa **Location:** Kesan	*Klebsiella pneumoniae* CDC 529 (MIC: 16 μg/mL); *Salmonella typhimurium* ATCC 14028 (MIC: 64 μg/mL); *Pseudomonas aeruginosa* ATCC 27853 (MIC: 128 μg/mL)	*Enterococcus faecium* ATCC 6057 (MIC: 64 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 2 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 16 μg/mL); *Candida krusei* ATCC 6258 (MIC: 32 μg/mL)	–	[29]

(*continued*)

Table 2 (continued)

Sponge species	Gram-negative	Gram-positive	Yeast	Fungi	Reference
Cladocora caespitose **Location:** Ayvalık	–	*Staphylococcus aureus* JCSC 4744 (MIC: 64 μg/mL); *Enterococcus faecium* ATCC 6057 (MIC: 0.25 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 1 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 64 μg/mL)	–	[29]
Cerianthus membranaceus **Location:** Ayvalık	*Salmonella typhimurium* ATCC 14028 (MIC: 64 μg/mL); *Pseudomonas aeruginosa* ATCC 27853 (MIC: 32 μg/mL)	*Enterococcus faecium* ATCC 6057 (MIC: 64 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 64 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 64 μg/mL); *Candida krusei* ATCC 6258 (MIC: 128 μg/mL)	–	[29]
Eunicella cavolinii **Location:** Ayvalık	*Klebsiella pneumoniae* CDC 529 (MIC: 64 μg/mL); *Salmonella typhimurium* ATCC 14028 (MIC: 64 μg/mL); *Pseudomonas aeruginosa* ATCC 27853 (MIC: 32 μg/mL)	*Staphylococcus aureus* JCSC 4744 (MIC: 128 μg/mL); *Enterococcus faecium* ATCC 6057 (MIC: 0.25 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 1 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 1 μg/mL); *Candida krusei* ATCC 6258 (MIC: 4 μg/mL)	–	[29]
Ascidiella aspersa **Location:** Golcuk	*Klebsiella pneumoniae* CDC 529 (MIC: 64 μg/mL); *Salmonella typhimurium* ATCC 14028 (MIC: 31 μg/mL)	*Staphylococcus aureus* JCSC 4744 (MIC: 128 μg/mL); *Enterococcus faecium* ATCC 6057 (MIC: 1 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 4 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 2 μg/mL); *Candida krusei* ATCC 6258 (MIC: 2 μg/mL)	–	[29]
Styela clava **Location:** Golcuk	–	*Enterococcus faecium* ATCC 6057 (MIC: 4 μg/mL); *Streptococcus pneumoniae* ATCC 6303 (MIC: 32 μg/mL)	*Candida parapsilosis* ATCC 22019 (MIC: 16 μg/mL); *Candida krusei* ATCC 6258 (MIC: 32 μg/mL)	–	[29]

Table 3 Antioxidant activity of Turkish marine invertebrates

Sponge species	DPPH	Superoxide radical scavenging	Nitric oxide radical scavenging	Location	Reference
Agelas Oroides	In 2000 μg/mL concentration (8.7–47.6%); In 800 μg/mL (9.3–73.8%)	In 800 μg/mL (20.4–32.4%)	In 800 μg/mL (32.4–41%)	Kemer, Hatay, Ayvalık	[11, 30]
Axinella damicornis	In 2000 μg/mL concentration (12.8–38.2%); In 800 μg/mL (32–70.9%)	In 800 μg/mL (32.1–47.3–73.8%)	In 800 μg/mL (35.5–52.2%)	Kemer, Hatay, Ayvalık	[11, 30]
Ircinia spinulosa	In 2000 μg/mL concentration (14.2–17.1%); In 800 μg/mL (9.8–14.2%)	In 800 μg/mL (18.9–24.5–73.8%)	In 800 μg/mL (8.8–41.3%)	Kemer, Hatay	[11, 30]
Ircinia fasciculata	In 2000 μg/mL concentration (7.3–39.2%); In 800 μg/mL (9.9–21.8%)	In 800 μg/mL (16.3–44.4–73.8%)	In 800 μg/mL (38%)	Kemer, Kas	[11, 30]
Dysidea avara	In 2000 μg/mL concentration (15.3%); In 800 μg/mL (20.3–91.8%)	In 800 μg/mL (26.7–89.1–73.8%)	In 800 μg/mL (35–39.1%)	Ayvalık, Kemer	[11, 30]
Axinella cannabina	In 800 μg/mL (25.1–46.9%)	–		Ayvalık, Hatay	[30]
Chondrilla nucula	In 800 μg/mL (12.5%)	–	In 800 μg/mL (38.8%)	Guvercinlik	[30]
Sarcotragus sp.	In 800 μg/mL (21.7%)	–	In 800 μg/mL (12.7%)	Turgut Reis	[30]
Axinella verrucosa	In 800 μg/mL (83.9%)	–	In 800 μg/mL (38.2%)	Turgut Reis	[30]
Axinella cannabina	In 800 μg/mL (25.1%)	In 800 μg/mL (20.4–47.5%)	In 800 μg/mL (39–44.7%)	Hatay, Ayvalık	[30]
Ciocallypta carbolloi	In 800 μg/mL (3.8%)		In 800 μg/mL (73.5%)	Kas	[30]
Petrocia ficiformis	–	In 800 μg/mL (4.9%)	In 800 μg/mL (17.3%)	Kas	[30]
Dictyonella incisa	–	In 800 μg/mL (24.6%)	–	Seferihisar	[10]
Anemonia viridis	–	In 800 μg/mL (20%)	–	Bodrum	[23]
Paramuricea clavata	IC_{50}: 231.2 μg/mL	IC_{50}: 228.9 μg/mL	IC_{50}: 243.8 μg/mL	Ayvalık	[22]

Table 4 Cytotoxic and tyrosinase inhibitory activity of Turkish marine invertebrates

Sponge species	Cytotoxic activity	Tyrosinase inhibitory activity	Location	Reference
Parazoanthus axinella	Hep-2 (IC_{50}: 230.1 μg/mL)	IC_{50}: 97.7	Kesan	[29]
Halocynthia papillosa	Hep-2 (IC_{50}: 75.8 μg/mL)	IC_{50}: 94.9	Kesan	[29]
Cladocora caespitose	Hep-2 (IC_{50}: 72.6 μg/mL)	IC_{50}: 196.4	Ayvalık	[29]
Cerianthus membranaceus	Hep-2 (IC_{50}: 10.9 μg/mL)	IC_{50}: 72.6	Ayvalık	[29]
Eunicella cavolinii	Hep-2 (IC_{50}: 68.8 μg/mL)	IC_{50}: 53.3	Ayvalık	[29]
Ascidiella aspersa	Hep-2 (IC_{50}: 17.5 μg/mL)	IC_{50}:199.5	Golcuk	[29]
Styela clava	Hep-2 (IC_{50}: 86.5 μg/mL)	IC_{50}: 85.1	Golcuk	[29]
Axinella sp.	B16 melanoma	–	Eastern Mediterranean	[31]
Dictyonella incisa	HCT 116, Hep-2	–	Seferihisar	[10]
Anemonia viridis	Hep-2 (IC_{50}: 120.1 μg/mL)	IC_{50}: 81.2	Bodrum	[23]
Paramuricea clavata	Hep-2 (IC_{50}: 58.4 μg/mL)	IC_{50}: 73.7	Ayvalık	[22]

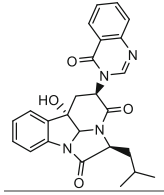
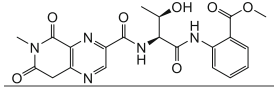
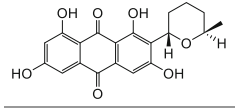
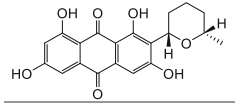
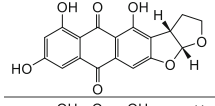
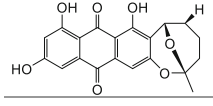
compounds were tested for their antimicrobial activities and compounds **F17, F9,** and **F1** showed high cytotoxicity activity against mouse lymphoma cell line (L5178Y) at IC_{50}: 0.2, 0.3, and 0.4 μM, respectively. Among all isolated compounds, **F86** showed antituberculosis activity against *Mycobacterium tuberculosis* (MIC: 100 μM) (Table 5).

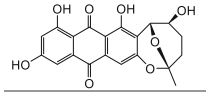
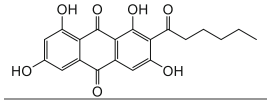
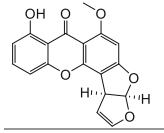
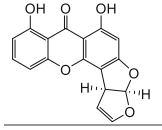
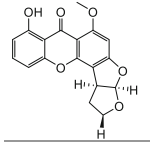
5 Conclusion

Marine macro- and microorganisms are rich sources of novel, unique, and biologically active compounds. The number of identified marine natural compounds increases each year. Many of these metabolites are high-valued and important commercial products for the pharmaceutical, cosmetic, and biomaterial sectors. Undoubtedly, these marine resources in every aspect create a huge economic prospect for the World.

Research on pharmaceutical science with emphasis on natural sources is one of the most important drug discovery strategies. Turkey is a country rich in marine and terrestrial resources and hence could be regarded an important geographical area to fulfill these objectives. The role of sponges in Turkey's economy dates back to Ottoman Empire. Until 1841, the world's sponges derived materials were supplied by Turkey. The world's pharmaceutical market has reached 1.10 trillion USD in

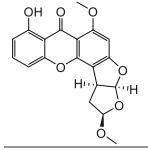
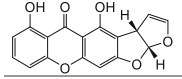
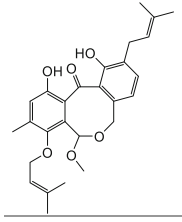
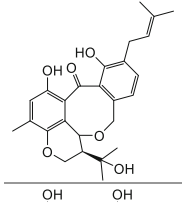
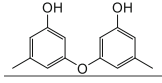
Table 5 Secondary metabolites from Turkish marine derived fungi

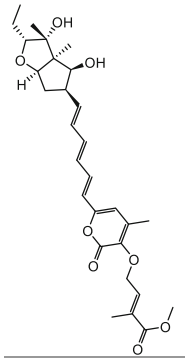
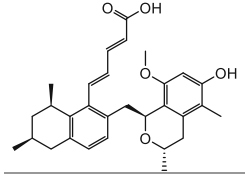
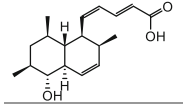
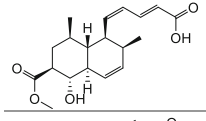
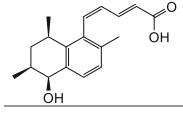
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	Propylchaetominine **F1**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}:0.4 μM)	*Agelas oroides*	[32]
	Isoterrelumamide A **F2**	*Aspergillus carneus*		*Agelas oroides*	[32]
	5′- *epi*-averufanin **F3**	*Aspergillus carneus*	Staphylococcus aureus ATCC700699 (MIC: 4.63 μg/mL); Enterococcus faecium ATCC 35667 (MIC: 9.3 μg/mL)	*Agelas oroides*	[32]
	Averufanin **F4**	*Aspergillus carneus*		*Agelas oroides*	[32]
	Versicolorin C **F5**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}:20 μM)	*Agelas oroides*	[32]
	Averufin **F6**	*Aspergillus carneus*		*Agelas oroides*	[32]

	Nidurufin **F7**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}:9 μM)	*Agelas oroides*	[32]
	Norsolorinic acid **F8**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}:25 μM)	*Agelas oroides*	[32]
	Sterigmatocystin **F9**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}:0.3 μM)	*Agelas oroides*	[32]
	*O*-demethylsterigmatocystin, **F10**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}:10 μM)	*Agelas oroides*	[32]
	Dihydrosterigmatocystin **F11**	*Aspergillus carneus*		*Agelas oroides*	[32]

(*continued*)

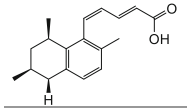
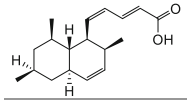
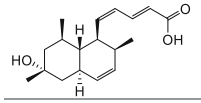
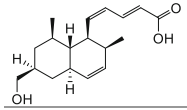
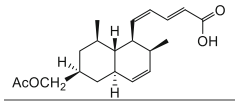
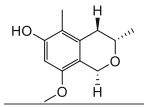
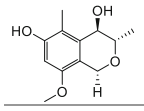
Table 5 (continued)

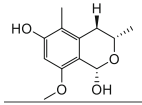
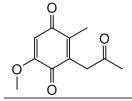
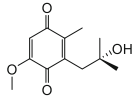
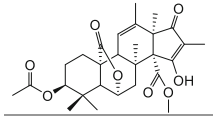
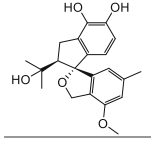
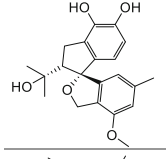
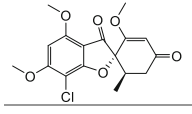
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	Oxisterigmatocystin C**F12**	*Aspergillus carneus*		*Agelas oroides*	[32]
	Sterigmatin **F13**	*Aspergillus carneus*		*Agelas oroides*	[32]
	25-*O*-methylarugosin A **F14**	*Aspergillus carneus*		*Agelas oroides*	[32]
	Arugosin C **F15**	*Aspergillus carneus*		*Agelas oroides*	[32]
	Diorcinol **F16**	*Aspergillus carneus*		*Agelas oroides*	[32]

	Asteltoxin E **F17**	*Aspergillus carneus*	Mouse lymphoma cell line L5178Y (IC_{50}: 0.2 μM)	*Agelas oroides*	[32]
	Penitanzchroman **F18**	*Penicillium sp.*		Sediment	[33]
	Tanzawaic acids Y **F19**	*Penicillium sp.*		Sediment	[33]
	Tanzawaic acids Z **F20**	*Penicillium sp.*		Sediment	[33]
	Arohynapene A **F21**	*Penicillium sp.*		Sediment	[33]

(*continued*)

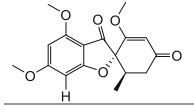
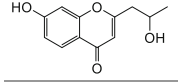
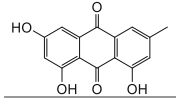
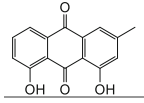
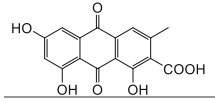
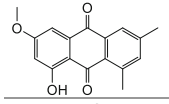
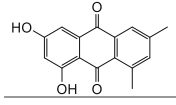
Table 5 (continued)

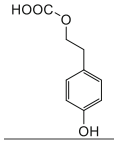
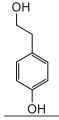
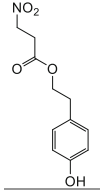
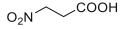
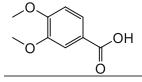
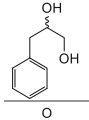
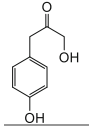
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	Tanzawaic acid A **F22**	*Penicillium sp.*		Sediment	[33]
	Tanzawaic acid B **F23**	*Penicillium sp.*	Staphylococcus aureus ATCC 700699 (MIC: 13.7 μg/mL)	Sediment	[33]
	Tanzawaic acid E **F24**	*Penicillium sp.*		Sediment	[33]
	Tanzawaic acid M **F25**	*Penicillium sp.*		Sediment	[33]
	Tanzawaic acid N **F26**	*Penicillium sp.*		Sediment	[33]
	(3S)-6-hydroxy-8-methoxy-3,5-dimethylisochroman **F27**	*Penicillium sp.*		Sediment	[33]
	(3S,4R)-6-hydroxy-8-methoxy-3,5-dimethylisochromanolm **F28**	*Penicillium sp.*		Sediment	[33]

	(1S,3S)-1,6-dihydroxy-3,5-dimethyl-8-methoxyisochroman **F29**	*Penicillium sp.*		Sediment	[33]
	Anserinones A **F30**	*Penicillium sp.*	Mouse lymphoma cell line L5178Y (IC_{50}: 27.4 μM)	Sediment	[33]
	Anserinones B **F31**	*Penicillium sp.*	*Staphylococcus aureus* ATCC 29213 (MIC: 10.5 μg/mL) Mouse lymphoma cell line L5178Y (IC_{50}: 20.9 μM)	Sediment	[33]
	Citreohybridonol **F32**	*Penicillium atrovenetum*		*Spirastrella cunctatrix*	[34]
	Spiroarthrinols A **F33**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Spiroarthrinols B **F34**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Griseofulvin **F35**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]

(*continued*)

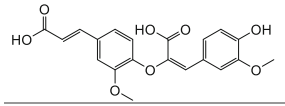
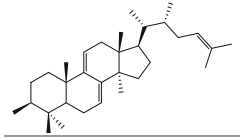
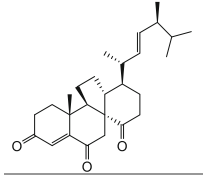
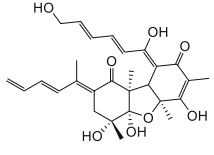
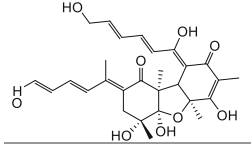
Table 5 (continued)

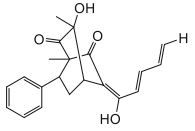
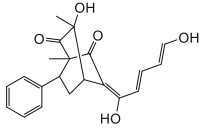
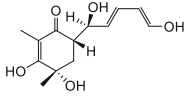
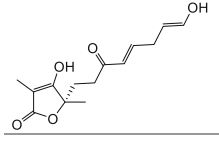
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	Dechlorogriseofulvin **F36**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	7-hydroxy-2-(2-hydroxypropyl)-5-methylchromone **F37**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Emodin **F38**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Chrysophanol **F39**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Endocrocin **F40**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	8-dihydroxy-6-methoxy-8-methylxanthon **F41**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Norlichexanthone **F42**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]

	4-hydroxyphenylethylacetat **F43**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	2-(4-hydroxyethylphenol **F44**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Phomonitroester **F45**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	3-nitropropionic acid **F46**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	3,4-dimethoxybenzoic acid **F47**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	3-phenylpropane-1,2-dio **F48**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	4-hydroxyphenylacetic acid **F49**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]

(*continued*)

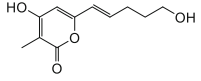
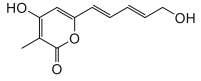
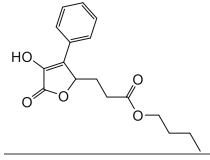
Table 5 (continued)

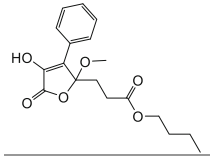
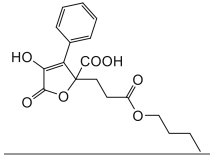
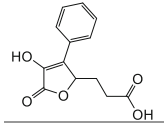
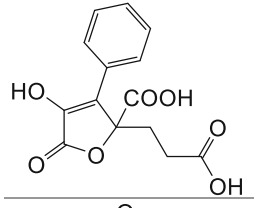
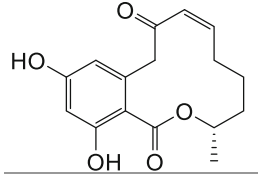
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	8-*O*-4 dehydrodiferulicacid **F50**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	3*β*,22-dihydroxylanosta-7,9 (11),24-triene **F51**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Dankasterone A **F52**	*Arthrinium sp.*		*Sarcotragus muscarum*	[35]
	Saturnispols A **F53**	*Trichoderma saturnisporum*	*Staphylococcusaureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococciaureus* (MIC: >64 μg/mL), *Bacillus subtilis* (MIC: >64 μg/mL), *Pseudomonas aeruginosa* (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)	*Dictyonella incisa*	[36]
	Saturnispols B **F54**	*Trichoderma saturnisporum*	*Staphylococcusaureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococciaureus* (MIC: >64 μg/mL), *Bacillus subtilis* (MIC: >64 μg/mL), *Pseudomonas*	*Dictyonella incisa*	[36]

			aeruginosa (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)		
	Saturnispols C **F55**	*Trichoderma saturnisporum*	*Staphylococcus aureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococci aureus* (MIC: >64 μg/mL), *Bacillus subtilis* (MIC: >64 μg/mL), *Pseudomonas aeruginosa* (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)	*Dictyonella incisa*	[36]
	Saturnispols D **F56**	*Trichoderma saturnisporum*	*Staphylococcus aureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococci aureus* (MIC: >64 μg/mL), *Bacillus subtilis* (MIC: >64 μg/mL), *Pseudomonas aeruginosa* (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)	*Dictyonella incisa*	[36]
	Saturnispols E **F57**	*Trichoderma saturnisporum*	*Staphylococcus aureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococci aureus* (MIC: >64 μg/mL), *Bacillus subtilis* (MIC: >64 μg/mL), *Pseudomonas aeruginosa* (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)	*Dictyonella incisa*	[36]
	Saturnispols F **F58**	*Trichoderma saturnisporum*	*Staphylococcus aureus* (MIC: 3.32 μg/mL), vancomycin-resistant *Enterococci aureus* (MIC: 1.63 μg/mL), *Bacillus subtilis* (MIC: 6.65 μg/mL),	*Dictyonella incisa*	[36]

(*continued*)

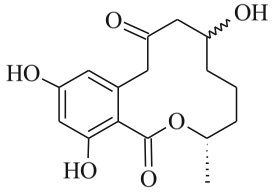
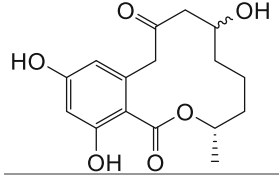
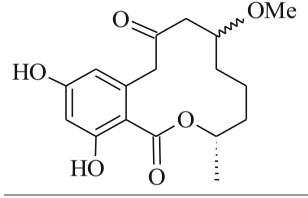
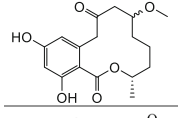
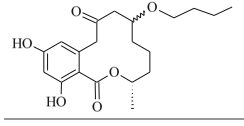
Table 5 (continued)

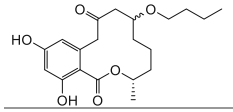
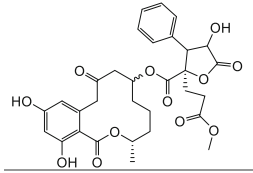
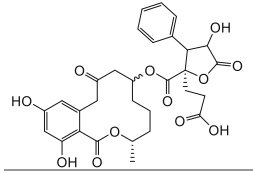
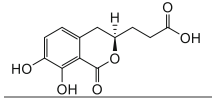
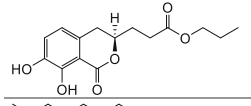
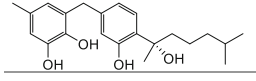
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
			Pseudomonas aeruginosa (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: 6.65 μg/mL)		
	Saturnispols G **F59**	*Trichoderma saturnisporum*	*Staphylococcus aureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococci aureus* (MIC: >64 μg/mL), *Bacillus subtilis* (MIC: >64 μg/mL), *Pseudomonas aeruginosa* (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)	*Dictyonella incisa*	[36]
	Saturnispols H **F60**	*Trichoderma saturnisporum*	*Staphylococcus aureus* (MIC: >64 μg/mL), vancomycin-resistant *Enterococci aureus* (MIC: 12.9 μg/mL), *Bacillus subtilis* (MIC: 12.9 μg/mL), *Pseudomonas aeruginosa* (MIC: >64 μg/mL), *Klebsiella Pneumoniae* (MIC: >64 μg/mL)	*Dictyonella incisa*	[36]
	Lactone acid n-butyl ester **F61**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]

	4-Methoxylactone acid n-butyl ester **F62**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Lactone diacid 7-O-n-butyl ester **F63**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Lactone diacid **F64**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Butenolide lactone diacid **F65**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	(3R)-*cis*-resorcylide **F66**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]

(*continued*)

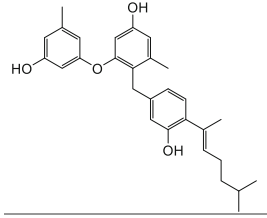
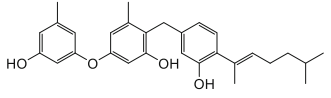
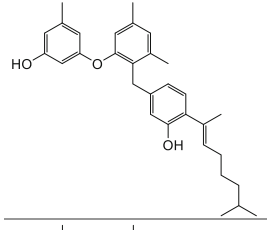
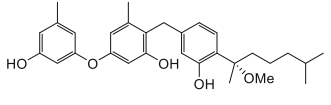
Table 5 (continued)

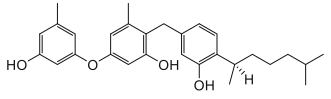
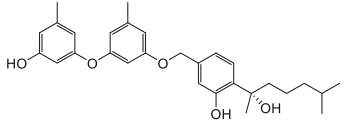
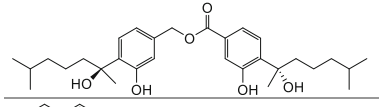
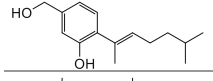
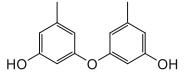
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	(3R,7R)-7-hydroxyresorcylide **F67**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	(3R,7S)-7-hydroxyresorcylide **F68**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	(3R,7R)-7-methoxyresorcylide **F69**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	(3R,7S)-7-methoxyresorcylide **F70**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	(3S,7S)-7-*O*-n-butylresorcylide **F71**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]

	(3S,7R)-7-*O*-n-butylresorcylide **F72**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Talarodilactones A **F73**	*Talaromyces rugulosus*	Mouse lymphoma cell line L5178Y (IC_{50}: 3.9 μM)	*Axinella cannabina*	[37]
	Talarodilactones B **F74**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Talumarin A **F75**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Talumarin B **F76**	*Talaromyces rugulosus*		*Axinella cannabina*	[37]
	Asperchondols A **F77**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC25923 (MIC: 50 μM)	*Chondrilla nucula*	[38]

(*continued*)

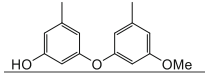
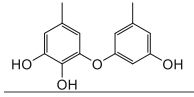
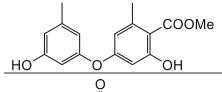
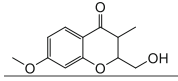
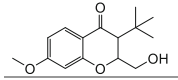
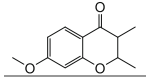
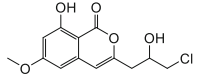
Table 5 (continued)

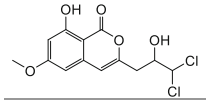
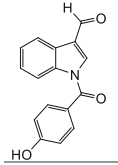
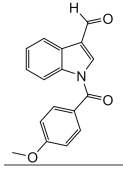
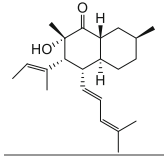
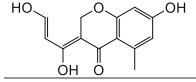
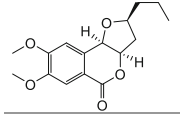
Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	Asperchondols B **F78**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC25923 and ATCC700699 (MIC: 25 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299 (MIC: 25 μM), *Enterococcus faecium* ATCC35667 and ATCC700221 (MIC: 25 μM)	*Chondrilla nucula*	[38]
	Expansols D **F79**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC25923 and ATCC700699 (MIC: 50 and 25 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299 (MIC: 12.5 μM), *Enterococcus faecium* ATCC35667 and ATCC700221 (MIC: 12.5 μM)	*Chondrilla nucula*	[38]
	Expansols F **F80**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC25923 and ATCC700699 (MIC: 50 and 25 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299 (MIC: 12.5 μM), *Enterococcus faecium* ATCC35667 and ATCC700221 (MIC: 12.5 μM)	*Chondrilla nucula*	[38]
	Expansols A **F81**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC700699 (MIC: 50 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299 (MIC: 50 and 25 μM),	*Chondrilla nucula*	[38]

			Enterococcus faecium ATCC35667 and ATCC700221 (MIC: 25 and12.5 μM)		
	Expansols B **F82**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC25923 and ATCC700699 (MIC: 100 and 50 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299 (MIC: 25 and 12.5 μM), *Enterococcus faecium* ATCC35667 and ATCC700221 (MIC: 12.5 μM)	*Chondrilla nucula*	[38]
	Peniciaculins A **F83**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC700699 (MIC: 50 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299 (MIC: 25 and 12.5 μM), *Enterococcus faecium* ATCC35667 and ATCC700221 (MIC: 12.5 μM)	*Chondrilla nucula*	[38]
	Peniciaculins B **F84**	*Aspergillus sp.*		*Chondrilla nucula*	[38]
	Aspergillusene A **F85**	*Aspergillus sp.*		*Chondrilla nucula*	[38]
	Diphenyl ethers diorcinol **F86**	*Aspergillus sp.*	*Staphylococcus aureus* ATCC25923 and ATCC700699 (MIC: 50 and 25 μM), *Enterococcus faecalis* ATCC29212 and ATCC51299	*Chondrilla nucula*	[38]

(*continued*)

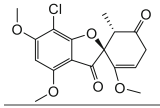
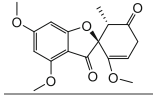
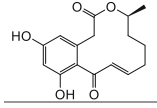
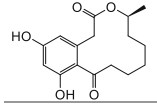
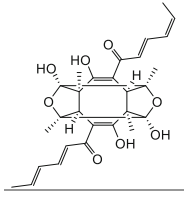
Table 5 (continued)

Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
			(MIC: 100 μM), *Enterococcus faecium* ATCC35667 and ATCC700221 (MIC: 50 and 25 μM), *Mycobacterium tubercu*losis (MIC: 100 μM)		
	Cordyols E **F87**	*Aspergillus sp.*		*Chondrilla nucula*	[38]
	Cordyols C **F88**	*Aspergillus sp.*		*Chondrilla nucula*	[38]
	4-methoxycarbonyldiorcinol **F89**	*Aspergillus sp.*		*Chondrilla nucula*	[38]
	2-hydroxymethyl-3-methyl-7-methoxychromone **F90**	*Rhinocladiella sp.*		*Ircinia Oros*	[39]
	2-hydroxymethyl-3-*tert*-butyl-7-methoxychromone **F91**	*Rhinocladiella sp.*		*Ircinia oros*	[39]
	2,3-dimethyl-7-methoxychromone **F92**	*Rhinocladiella sp.*		*Ircinia oros*	[39]
	3-(3-chloro-2-hydroxypropyl)-8-hydroxy-6-methoxy-isochromen-1-one **F93**	*Clonostachys sp.*		*Axinella polypoides*	[39]

	Dichlorodiaportin **F94**	*Clonostachys sp.*		*Axinella polypoides*	[39]
	1-(4-hydroxybenzoyl)indole-3-carbaldehyde **F95**	*Engyodontium album*		*Ircinia variabilis*	[39]
	1-(4-methoxybenzoyl)indole-3-carbaldehyde **F96**	*Engyodontium album*		*Ircinia variabilis*	[39]
	Fusarielin analogue **F97**	*Penicillium sp.*		*Ircinia oros*	[40]
	Norlichexanthone **F98**	*Penicillium sp.*		*Ircinia oros*	[40]
	Monocerin **F99**	*Penicillium sp.*	Mouse lymphoma cell line L5178Y (IC_{50}: 8.4 μM)	*Ircinia oros*	[40]

(*continued*)

Table 5 (continued)

Structures	Name of compounds	Fungi strains	Bioactivity	Host origin	Reference
	Griseofulvin **F100**	*Penicillium sp.*		*Ircinia oros*	[40]
	Dechlorogriseofulvin **F101**	*Penicillium sp.*		*Ircinia oros*	[40]
	Dehydrocurvularin **F102**	*Penicillium sp.*	Mouse lymphoma cell line L5178Y (IC_{50}: 4.7 μM)	*Ircinia oros*	[40]
	Curvularin **F103**	*Penicillium sp.*		*Ircinia oros*	[40]
	Trichodimerol **F104**	*Penicillium sp.*		*Ircinia oros*	[40]

2017. Holding the 17th rank in 2017, Turkey has confirmed its importance standing in the pharmaceutical industry.

Although Turkey has a long coastline and extensive marine ecosystem with great biodiversity, there are limited researches on marine bioactive compounds. Turkey's seas have tremendous potential for the discovery, development, and marketing of new marine biological products. For progressing on this important issue and using these resources for economical purposes, there is a need to develop an effective collaboration between universities and the pharmaceutical industry.

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Endophytes of *Nothapodytes nimmoniana* (J. Graham) Mabb.

5

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Abstract

Endophytes are excellent source of bioactive natural products. Fungal and bacterial endophytes have been isolated from medicinal plant *Nothapodytes nimmoniana*, and majority of them have the capability of accumulating camptothecin (CPT) similar to host plant. *Entrophospora infrequens*, *Neurospora crassa, Fusarium oxysporum, Colletotrichum fructicola,* and *Corynespora cassiicola* are some of the fungal isolates which have been cultured by following submerged fermentation and solid-state fermentation methods for the production of CPT. Here we presented the detailed account of various endophytes isolated from *Nothapodytes nimmoniana* and recent research developments made in this area.

Keywords

Axenic cultures · Bioactive compounds · Camptothecin · Endophytes · Fermentation · Secondary metabolites

Abbreviations

9-MCPT	9-methoxycamptothecin
A-549	Adenocarcinomic human alveolar basal epithelial cells (lung cancer cell line)
BEB	Beef extract broth
BOD	Biological oxygen demand
CCD	Central composite design
CDB	Czapek dox broth
COD	Chemical oxygen demand
CPT	Camptothecin
FDA	US Food and Drug Administration
HEP-2	Human epithelial type 2 (liver cancer cell line)
HPLC	High performance liquid chromatography
LC/MS	Liquid chromatography/mass spectroscopy
MEB	Malt extract broth
MS/MS	Mass spectroscopy/mass spectroscopy
OVCAR-2	Human epithelial carcinoma cell line of the ovary (ovarian cancer cell line)
PDB	Potato dextrose broth
RSM	Response surface methodology
SB	Sabouraud broth
SSF	Solid-state fermentation
TDS	Total dissolved solids
TSS	Total soluble solids
YEB	Yeast extract broth

1 Introduction

1.1 *Nothapodytes nimmoniana* and Its Chemical Constituents

Nothapodytes nimmoniana (J. Graham) Mabb. (syn. *Nothapodytes foetida* (Wight) Sleumer; *Mappia foetida* (Wight) Miers.) is an important medicinal tree belonging to family Icacinaceae (Fig. 1a), and it is popularly known as "Stinking tree" due to the foul smell it emits especially during flowering and fruiting. *Nothapodytes nimmoniana* is distributed in Indian subcontinent especially in the Western Ghats, Eastern Plateau, and Northeastern regions of India. This species is also reported from Sri Lanka, Myanmar, Indonesia, and Thailand [1]. Different species of *Nothapodytes*, namely, *Nothapodytes obtusifolia*, *Nothapodytes montana,* and *Nothapodytes pittosproides*, are found distributed in China, Thailand, Sumatra, Java, Sumbawa, and Indonesia. Various breeding systems have been reported in *Nothapodytes nimmoniana* including male, female, hermaphrodite (Fig. 1b, c), monoecious, andromonoecious, gynomonoecious, and trimonoecious. The tree flowers during June to August; the early flowering types are dioecious, whereas late flowering types are monoecious and hermaphrodite [2]. The fruit looks like "Jambul fruit" (Fig. 1d, e) and ripens during November to December.

The major constituents of *Nothapodytes nimmoniana* are alkaloid camptothecin (CPT) and 9-methoxycamptothecin (9-MCPT; Fig. 2), which are popular anticancer drugs [3]. This plant also contains (+)-1-hydroxypinoresinol, ω-hydroxypropioguaiacone, ρ-hydroxybenzaldehyde, scopoletin, uracil, thymine, sitosterol, sitosterol-β-D-glucoside, 3β-hydroxy-stigmast-5-en-7-one, stigmast-5-en-3β,7α-diol, 6β-hydroxystigmast-4-en-3-one, sitost-4-en-3-one, linoleic acid, trigonelline, and pumiloside. Among these, scopoletin, camptothecin, and 9-methoxycamptothecin were reported to have cytotoxic activities [4]. CPT is considered as one of the most promising drugs to cure various types of cancers, and it has exhibited broad spectrum of antitumor activities in vitro and in vivo systems [5]. CPT and various isomers in the presence of topoisomerase-I responsible for DNA damage by binding to and stabilizing a covalent DNA-topoisomerase-I complex in which one strand of DNA is broken [6]. However, CPT itself was not suitable for clinical applications due to its poor water solubility and certain side effects [7]. Nevertheless, some semisynthetic CPT derivatives such as topotecan and irinotecan (Fig. 3) were approved by the US Food and Drug Administration (FDA) for treating lung cancer, colorectal cancer, and ovarian cancer [8]. CPT was first recognized from *Camptotheca acuminata* (Chinese happy tree; Nyssaceae) [6], and later CPT was isolated from *Camptotheca lowreyana* and *Camptotheca yunnanensis* (Nyssaceae) [9], *Ervatamia heyneana* (Apocynaceae) [10], *Merrilliodendron megacarpum* (Icacinaceae) [11], *Mostuea brunonis* (Gelsemiaceae) [12], *Nothapodytes nimmoniana* (syn. *Nothapodytes foetida*, Icacinaceae) [3, 13–16], *Ophiorrhiza mungos* (Rubiaceae) [17], *Ophiorrhiza pumila* [18], and *Ophiorrhiza rugosa* [19] (Table 1). Even though CPT has been reported in several species, *Camptotheca acuminata* and *Nothapodytes nimmoniana* are considered as main source for CPT accumulation. In *Nothapodytes nimmoniana*, CPT content was quantified in different parts of the plant including stem wood, stem bark, root wood, root bark, shoot,

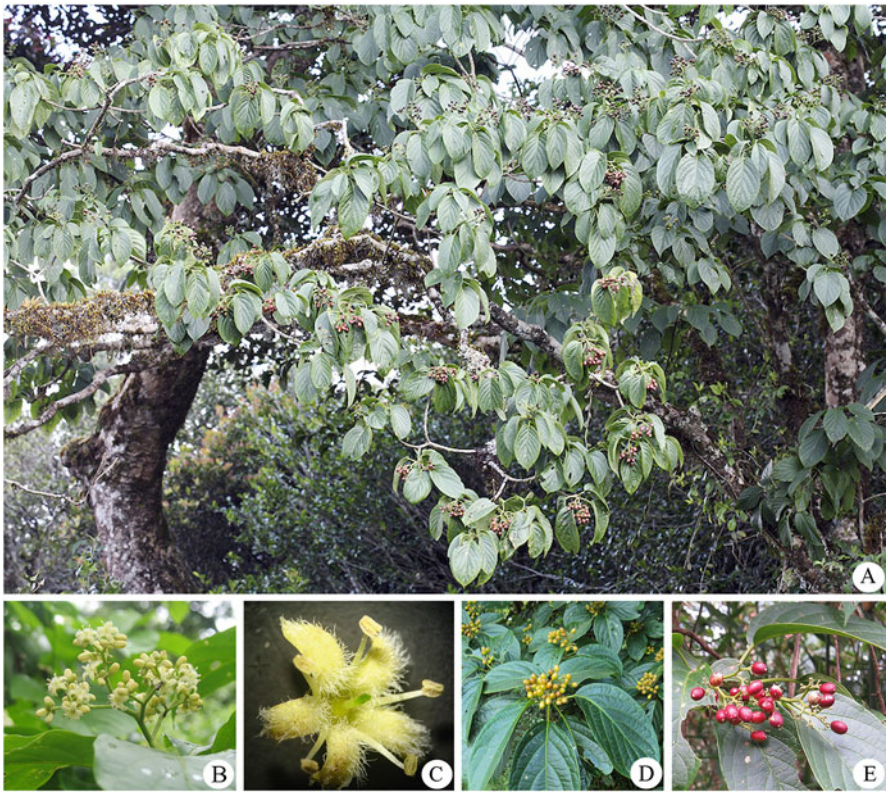

Fig. 1 *Nothapodytes nimmoniana*. (**a**) Habit, (**b**) flowering twig, (**c**) bisexual flower, (**d**) twig-bearing immature fruits, (**e**) twig-bearing mature fruits

leaves, and whole plant (Table 1). Among these, stem bark (0.23–0.30%) and root bark (0.33–0.77%) are promising source of CPT [3, 15].

2 Endophytes Isolated from *Nothapodytes nimmoniana*

Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissues without causing any symptoms/diseases which are known to occur ubiquitously in plants. Endophytes have been shown to enhance plant's ability to tolerate abiotic and biotic stresses existing in mutuality with host plants [20, 21]. Many of the endophytic species have been shown to produce a number of important secondary metabolites which are having antimicrobial and pharmacological importance including anticancer, antidiabetic, and immunosuppressant activities [20–23]. Many of the bioactive compounds produced by endophytes are similar to the compounds produced by the host plants, suggesting that the endophytes could potentially serve an alternative source of plant bioactive compounds [20–23]. Various endophytes including bacterial and fungal

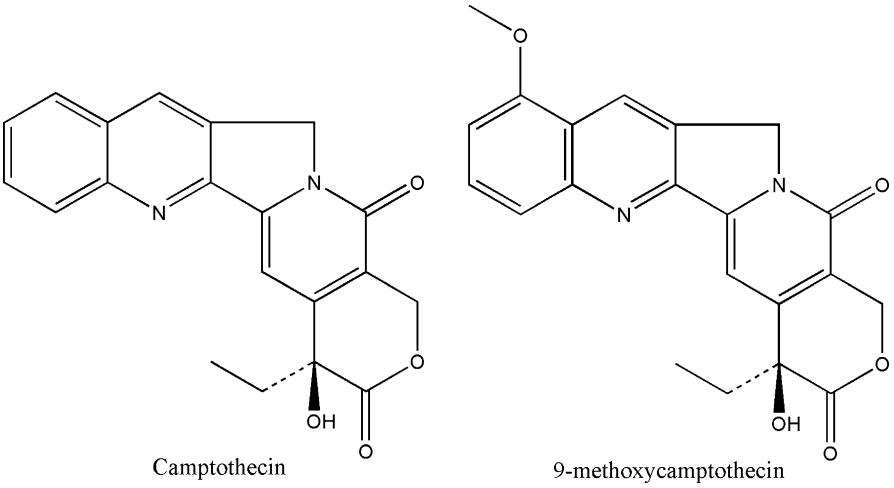

Fig. 2 Chemical structure of camptothecin and 9-methoxycamptothecin

species have been isolated from various parts of *Nothapodytes nimmoniana* as well as from rhizosphere (Table 2). Patil et al. [24] isolated *Azotobacter* sp., *Acidomonas* sp., and *Bacillus* species from rhizosphere of *Nothapodytes nimmoniana*. Various fungal endophytes were isolated from bark, twigs, stem, leaves, and flower tissues of *Nothapodytes nimmoniana* including *Aspergillus niger*, *A. flavus*, *A. terreus*, *Alternaria* sp., *Botryosphaeria parva, B. rhodina*, *Botryosphaeria* sp., *Choanephora mappiae*, *Chaetomium globosum*, *Chaetomium* sp., *Cladosporium oxysporum*, *Colletotrichum fructicola*, *C. globosum*, *C. gloeosporioides*, *Colletotrichum* sp., *Corynespora cassiicola*, *Corynespora* sp., *Daldinia eschscholzii*, *Daldinia* sp., *Dendryphiella siatra*, *Diaporthe conorum*, *Diaporthe* sp., *Entrophospora infrequens*, *Fusarium beomiforme, F. equiseti, F. moniliforme*, *F. oxysporum*, *F. oxysporum* kolhapuriensis, *F. sacchari*, *F. solani*, *F. subglutinans*, *F. verticillioides*, *Fusarium* sp., *Galactomyces* sp., *Gibberella intermedia*, *G. sacchari*, *G. moniliformis*, *Glomerella cingulata*, *Humicola* sp., *Hypocrea lixii*, *Hypoxylon fragiforme*, *Hypoxylon* sp., *Irpex lacteus*, *Lasiodiplodia pseudotheobromae*, *L. theobromae*, *Nectria haematococca*, *N. rigidiuscula*, *Nectria* sp., *Nigrospora* sp., *Penicillium* sp., *Phomopsis* sp., *Rhizoctonia* sp., *Sordariomycetes* sp., *Trichoderma album, Trichothecium roseum*, *Verticillium* sp., and *Xylaria* sp. [25–36] (Table 2).

3 Camptothecin Production by Endophytes of *Nothapodytes nimmoniana*

Microbial fermentation is an alternative for the production of useful bioactive compounds from endophytes of medicinal plants. The culture medium for cultivation of endophytes is fairly simple and inexpensive, largely consisting of industrial

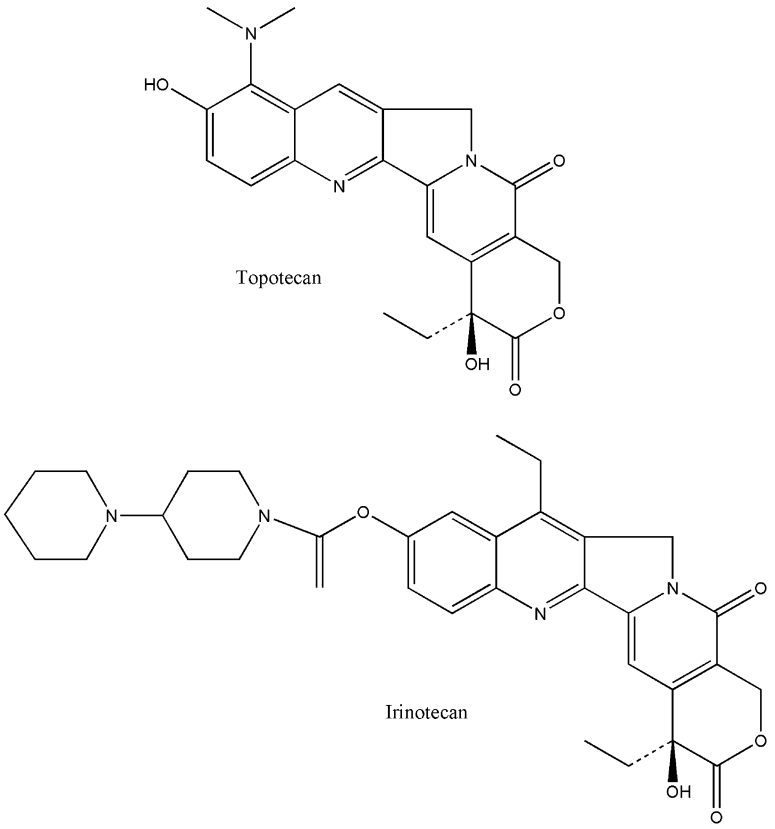

Fig. 3 Camptothecin derivatives: topotecan, irinotecan

products/wastes like corn steep liquor, molasses, sour whey, and others [20]. Both submerged fermentation and solid-state fermentation methods have been followed for cultivation of endophytes of *Nothapodytes nimmoniana.* Various strategies such as optimization of fermentation parameters, strain improvement, and co-cultivation have been followed. Research efforts made for the production of camptothecin by various endophytes of *Nothapodytes nimmoniana* are presented in the following sections.

3.1 Camptothecin Production by *Entrophospora infrequens*

Entrophospora infrequens isolated from inner bark of *Nothapodytes nimmoniana* was reported to possess camptothecin in detectable quantities [25]. Puri et al. [25] could able to culture the fungus in synthetic liquid medium (Sabouraud broth containing 4% dextrose, 1% peptone) in shake flasks and bench-scale fermentation

Table 1 Camptothecin content in various plant species

Plant species	Different plant parts	Camptothecin content (percent dry weight)	References
Camptotheca acuminata	Young leaves	0.24–0.50%	[9, 42]
	Bark	0.18–0.20%	
	Seeds	0.30%	
Camptotheca lowreyana	Young leaves	0.39–0.55%	[9]
	Old leaves	0.09–0.11%	
Camptotheca yunnanensis	Young leaves	0.25–0.44%	[9]
	Old leaves	0.05%	
Ervatamia heyneana	Stem wood and bark	0.13%	[10]
Merrilliodendron megacarpum	Leaves and stem	0.05%	[11]
Mostuea brunonis	Whole plant	0.01%	[12]
Nothapodytes nimmoniana (=*N. foetida*)	Stem wood	0.14–0.24%	[13, 15]
	Stem bark	0.23–0.30%	[3, 15]
	Root wood	0.18%	[15]
	Root bark	0.33–0.77%	[15]
	Shoot	0.07%	[14]
	Leaves	0.08%	[15]
	Whole plant	0.04%	[16]
Ophiorrhiza mungos	Whole plant	0.001%	[17]
Ophiorrhiza pumila	Young roots	0.10%	[18]
Ophiorrhiza rugosa	Whole plant	0.03–0.10%	[19]

conditions. Cultures could able to accumulate camptothecin which was detected by chromatographic and spectroscopic methods. The compound isolated from fungal cultures showed in vitro cytotoxicity against human cancer cell lines such as A-549 (lung cancer), HEP-2 (liver cancer), and OVCAR-5 (ovarian cancer). They could also raise pure cultures and preserved the fungus after lyophilization as well as by cryopreservation at −70 °C. Amna et al. [26] conducted experiments on production of camptothecin by cultivation of endophytic fungi *Entrophospora infrequens* in shake flasks and bioreactors and established optimal parameters affecting biomass and CPT production (Table 3). In shake flasks, the growth period of the culture extended to 6 days following incubation and peaked on day 7. No sporulation was recorded in submerged cultures. The fungal mass of 2.30 ± 0.43 g/l was recorded on day 7. CPT accumulation in the cell mass was evident by 24 h and reached optimum at 96 h (0.575 ± 0.031 mg/100 g dry mass of mycelia); thereafter decline in the content was recorded. In bioreactor cultures (5 l and 18 l batch bioreactor cultures), the growth of mycelium begins 2–3 h after inoculation and peaked at 96 h in the 18 l bioreactor cultures. The optimal parameters for the growth of *Entrophospora infrequens* and production CPT in bioreactor cultures were temperature of 28 ± 2 °C, initial medium pH of 5.6, aeration rate of 1 vvm, and agitation

Table 2 Endophytes isolated from different parts of *Nothapodytes nimmoniana*

Endophytes	Tissues from which isolation was done	References
Bacterial endophytes		
Azotobacter sp.	Rhizosphere	[24]
Acidomonas sp.		
Bacillus sp.		
Fungal endophytes		
Entrophospora infrequens	Bark and twigs	[25, 26]
Neurospora crassa	Seeds	[27]
Nodulisporium sp.		[37]
Aspergillus flavus	Inner bark	[28]
Aspergillus mappiae		
Aspergillus niger		
Choanephora mappiae		
Curvularia lunata		
Dendryphiella siatra		
Fusarium oxysporum		
Humicola sp.		
Nigrospora sp.		
Penicillium sp.		
Rhizoctonia sp.		
Trichothecium roseum		
Verticillium sp.		
Botryosphaeria parva	Inner bark	[29]
Diaporthe conorum		
Fusarium oxysporum		
Fusarium sacchari		
Fusarium solani		
Fusarium subglutinans		
Fusarium verticillioides		
Galactomyces sp.		
Irpex lacteus		
Phomopsis sp.		
Cladosporium oxysporum	Stem, pedicel and flower tissue segments	[30]
Corynespora sp.		
Fusarium moniliforme		
Fusarium oxysporum	Leaves	[31]
Colletotrichum fructicola	Stem and leaves	[32, 34]
Corynespora cassiicola		
Fusarium oxysporum kolhapuriensis		
Botryosphaeria rhodina		[35]

(*continued*)

Table 2 (continued)

Endophytes	Tissues from which isolation was done	References
Alternaria sp.		[36]
Aspergillus terreus		
Botryosphaeria parva		
Botryosphaeria rhodina		
Botryosphaeria sp.		
Chaetomium globosum		
Chaetomium sp.		
Colletotrichum gloeosporioides		
Colletotrichum sp.		
Corynespora cassiicola		
Daldinia eschscholzii		
Daldinia sp.		
Diaporthe sp.		
Fusarium beomiforme		
Fusarium chlamydosporum		
Fusarium equiseti		
Fusarium oxysporum		
Fusarium solani		
Fusarium subglutinans		
Fusarium sp.		
Gibberella intermedia		
Gibberella moniliformis		
Gibberella sacchari		
Glomerella cingulata		
Hypocrea lixii		
Hypoxylon fragiforme		
Hypoxylon sp.		
Irpex lacteus		
Lasiodiplodia pseudotheobromae		
Lasiodiplodia theobromae		
Nectria haematococca		
Nectria rigidiuscula		
Nectria sp.		
Nigrospora sp.		
Nodulisporium sp.		
Penicillium pinophilum		
Phalemonium sp.		
Phanerochaete tuberculata		
Phomopsis sp.		
Sordariomycetes sp.		
Trichoderma album		
Xylaria sp.		
Xylariaceae		

Table 3 Parameters controlling biomass and camptothecin accumulation by *Entrophospora infrequens* mycelia cultures

Parameter	Shake flask cultures	Bioreactor cultures (18 l)
Inoculum	Spores (10^5 spores/ml)	Spores (10^5 spores/ml)
Medium	Sabouraud medium	Sabouraud medium
Cultivation time	216 h	120 h
Mode of fermentation	Batch cultures	Batch cultures
Working volume	100 ml	5–18 l
Temperature	28 ± 2 °C	28 ± 2 °C
Vessel pressure	–	2 lbs
Aeration rate	–	1 vvm
Antifoam agent used	–	Silicon oil
Agitation (revolutions/min)	200–220	200–220

After Amna et al. [26]

rate of 200–220 rpm. The highest CPT content in bioreactor culture was 4.96 ± 0.73 mg/100 g dry mycelium at 96 h and was eightfold higher than respective content in the flask-scale cultures (0.575 ± 0.031 mg/100 g dry mycelium). These studies indicate that the endophyte *Entrophospora infrequens* may be potential organism for the production of CPT by adopting fermentation technology.

3.2 Camptothecin Production by *Neurospora crassa*

Rehman et al. [27] isolated another endophytic fungus *Neurospora crassa* from *Nothapodytes nimmoniana*. The endophyte typically possesses 3–4 μm in diameter, which spreads as a white mat on solid Sabouraud medium containing peptone (1%), dextrose (4%), and agar (1.5%) within 5–7 days. Sporulation could not be observed in the endophytic fungus even after a long period of inoculation. The cultures showed small beadlike uniform growth in liquid media. Rehman et al. [27] could able to grow the endophyte in Sabouraud broth consisting of dextrose (4%) and peptone (1%) in 500 ml Erlenmeyer flask each containing 100 ml liquid broth for a period of 10 days at 28 ± 2 °C, on an incubatory shaker at 220 rpm. The growth kinetics of endophyte exhibited an exponential increase in weight of mycelia up to 7 days of incubation. Production of camptothecin was observed on 168 h through HPLC analysis followed by LC/MS and MS/MS. The biological activity of camptothecin produced by endophyte was tested against human cancer lines (A-549 for lung cancer, OVCAR-5 for ovarian cancer) against authentic camptothecin, which resulted in comparable activities.

3.3 Camptothecin Production by *Nodulisporium* sp.

Rehman et al. [37] isolated endophyte *Nodulisporium* sp. from inner bark of *Nothapodytes nimmoniana*, and this fungus had thin hyphae ranging between 2.5

and 6.4 μm in diameter. It produced abundant verticillately branched conidiophores. Authors could able to grow the fungus on Sabouraud broth media in 5–7 days which could produce camptothecin (5.5 μg CPT/g dry weight of mycelia) on day 4. Rehman et al. [37] also established 5–18 l capacity airlift bioreactors, and growth of mycelium was observed to begin 4–5 h after inoculation; the highest growth was recorded on day 6 in the 18 l bioreactor, and a maximum CPT of 45 μg CPT/g dry weight of mycelia at 120 h of fermentation was recorded.

3.4 Camptothecin Production by *Fusarium oxysporum* NFX06

Musavi et al. [31] conducted a study for the production of CPT from an endophytic fungus *Fusarium oxysporum* NFX06 isolated from *Nothapodytes nimmoniana* and applied response surface methodology (RSM) based on central composite design (CCD) to construct a model to describe the effect of substrate concentration. Three independent variables (namely, dextrose, peptone, and magnesium sulfate) were successfully employed to study the yield of CPT under submerged fermentation. The maximum yield of CPT obtained from central composite design was about 598.0 ng/g biomass. The model-validated optimum predicted CPT yield and the experimental CPT yield from biomass were found to be 628.08 ng/g and 610.09 ng/g at the concentrations of 42.64 g/l dextrose, 9.23 g/l peptone, and 0.26 g/l magnesium sulfate, respectively.

3.5 Camptothecin Production by *Fusarium oxysporum* kolhapuriensis Using Whey (Sour Whey)

Whey is an abundant dairy waste, and it was used as a component of medium for camptothecin production by *Fusarium oxysporum* kolhapuriensis, a novel endophytic fungus isolated from *Nothapodytes nimmoniana* [32]. They have made efforts to optimize the media and process conditions. Initially, Bhalkar et al. [32] have investigated parameters like medium and culture conditions such as synthetic medium (Sabouraud medium), complex supplements like whey, yeast extract, beef extract, malt extract, pH, incubation temperature, agitation rate (revolution per minute), and incubation period on camptothecin production. Four most influencing factors, namely, whey, malt extract, incubation period, and temperature, were further considered for optimization using response surface methodology (RSM). Authors have generated three-dimensional response surface plots/contour plots to observe the interactive effect of variables for CPT production. Central composite design (CCD) was used to optimize the complex medium and culture conditions for CPT production. These results showed that every individual factor such as temperature, whey, incubation period, and malt extract had independent effect on CPT production. Authors have applied statistical model and regression equation to validate their results by taking optimum values of temperature (30 °C), whey (70%), incubation period (6 days), and malt extract (2%) of the experiments.

The effective concentrations of four influencing factors have resulted in the predicted CPT yield of 283.03 mg/l, while the experimental yield was found to be 284 mg/l. These results suggested that optimized culture conditions are useful in enhanced production of bioactive compounds during fermentation of endophytic fungi.

3.6 Camptothecin Production by *Fusarium oxysporum* kolhapuriensis by Solid-State Fermentation of Soybean Waste

Submerged fermentation usually involves higher energy inputs, selection, and utilization of specific bioreactor for biomass production. Further, it involves various time- and energy-consuming procedures like separation of biomass, washing, extraction, purification, and analysis of product. On the other hand, solid-state fermentation (SSF) has several advantages over submerged fermentation process such as lower energy requirements, reduced water requirement which minimizes risk of contamination, higher levels of aeration, and cheaper and simpler media requirements due to nutrient-rich complex substrates [38]. Materials obtained from agro-industrial waste have been utilized successfully for cultivation of microorganisms for the production of food, pharmaceutical, and industrially important products. Experiments were conducted by Bhalkar et al. [33] to produce camptothecin by solid-state fermentation using agro-industrial waste. Initially they carried out experiments by using agriculture and agro-industrial waste materials including soybean meal, soybean husk, rice bran, maize bran, gram bran, and sugarcane bagasse at flask level by inoculating equal fungal inoculum (5.4×10^5 spores per g dry substrate), to presterilized substrate (100 mg) containing 60% moisture content maintained using distilled water. These flasks were incubated at static conditions at 30 °C for 30 days. Different parameters were checked for each type of substrate that could support maximum production of CPT. Process parameters such as incubation period (from 1 to 30 days), temperature (from 20, 25, 30, and 35 to 40 °C), moisture content (30%, 40%, 50%, 60%, and 70%), and pH (4, 5, 6, 7, and 8 pH units) were optimized for maximum CPT yield. The results obtained by them suggest that all the substrates used in their study could be applied for CPT production by *Fusarium oxysporum* kolhapuriensis. However, wheat bran, gram bran, and maize bran showed significant CPT yield, while soybean husks exhibited lowest yield, and highest yield was obtained with soybean meal substrate. They also showed that total protein content of all the substrates including whey liquid and protein values in soybean meal (41.8%), wheat bran (17.2%), gram bran (13.4%), maize bran (11.6%), rice bran (4.3%), soybean husk (8.4%), rice straw (2.4%), and whey liquid (0.8%) and the higher protein availability in the substrate could favor enhanced CPT production by the fungus. Bhalkar et al. [33] conducted experiments on optimization of culture conditions for SSF and studied role of factors such as temperature, pH, moisture content, and incubation days for CPT production by fungus using soybean meal as substrate. Experimental evidences of Bhalkar et al. [33] on growth kinetics showed

that there was a lag phase for the first 4-5 days and exponential phase from 6-7 days and remained constant till 14 day (stationary phase) at initial arbitrarily chosen values as temperature 30 °C, pH 5, and moisture content 50%. Their results on effect of varying moisture contents showed that reducing the moisture content below 60% (w/w) caused drying of the substrate and hampered the production thereafter, while increasing the moisture level above 60% (w/w) also reduced the production of CPT. The optimized pH value of source (whey or distilled water) used for maintaining optimum moisture content was to be between 5 and 6 pH units, and the whey with an initial pH of 5 favors the growth of fungus. The effect of incubation temperature exhibits maximum production of CPT by the fungus at 30 °C below which it reduced CPT production drastically and did not differ significantly at 35 °C and 40 °C. The effect of whey proteins and other protein supplement experiments revealed that the use of amino acid mixture, peptone, malt extract, beef extract, and whey liquid did not affect the CPT production significantly as compared to whey concentrate. Addition of 4% whey concentrate powder which was supplied to soybean meal substrate yielded a maximum CPT of about 128 mg/100 g dry weight of substrate.

Bhalkar et al. [33] designed and established a bench-scale upflow column bioreactor for continuous production of CPT from *Fusarium oxysporum* kolhapuriensis. The length of upflow column bioreactor was 50 cm with an inner diameter of 2.5 cm, and it was filled with cultivated soybean meal substrate. Pieces of sterilized rice straw were inserted between the stacked substrates to act as barriers that prevent complete outflowing of the substrate upon passing the liquid medium through the column (Fig. 4). Sterile whey (100% concentration) was continuously pumped upward through a sterile pipe (05 cm inner diameter) into the lower opening of the column which passed through entire substrate in upward direction and was then collected from the collector pipe connected at the upper outlet. Glass wool was placed tightly at both the openings of the column which prevented any substrate material to ooze out from the pipelines. In the bioreactor, they used soybean meal substrate (500 g) which was supplemented with whey concentrate powder (4%, w/w) and moistened with whey (60% moisture content). Fungal culture (2.45×10^6 spores per g dry substrate) was inoculated to the sterilized substrate under aseptic conditions and incubated for 7 days at 30 °C. The feeding rate of the liquid medium was maintained with the help of a peristaltic pump initially set at a rate of 20 ml/h. Stable and optimum production of CPT was checked at different feeding rates ranging from 10 to 40 ml/h initially for a period of 24 h. The optimum feeding rate for the bioreactor was determined by considering the best CPT yield with lowest values of environmental parameters such as total soluble solids (TSS), total dissolved solids (TDS), biological oxygen demand (BOD), and chemical oxygen demand (COD) of the utilized whey. Their results showed that a flow rate of 20 ml/h was the best, and optimal CPT production (128 mg/l) was achieved in the fractions collected at 48 h of incubation. Continuous production of CPT was achieved by endophytic fungus using the SSF of agro-industrial waste “soybean meal” and “whey.”

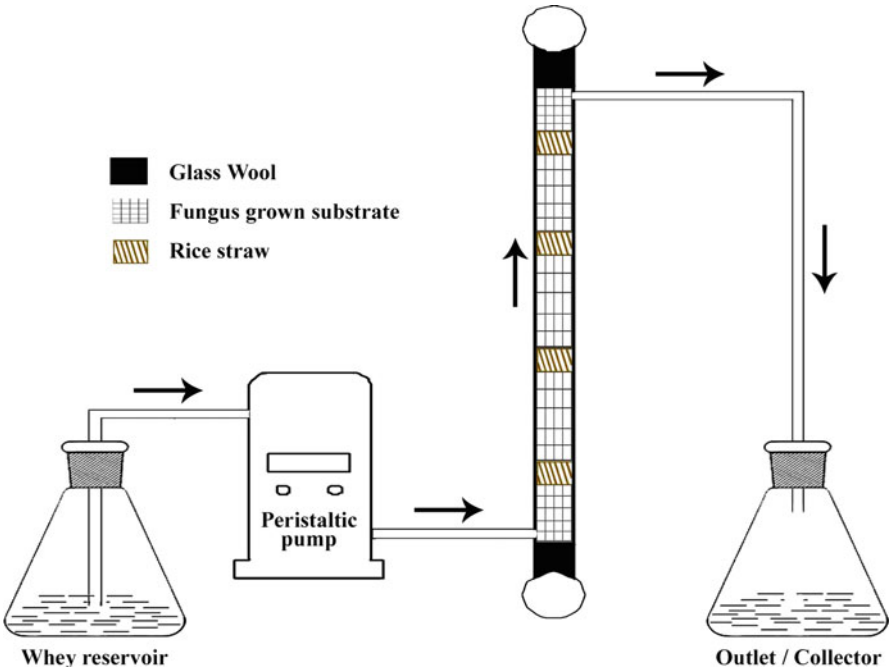

Fig. 4 Schematic representation of column bioreactor

3.7 Camptothecin Production by *Colletotrichum fructicola* SUK1 and *Corynespora cassiicola* SUK2 by Mixed Fermentation

Various strategies have been adopted for the production of bioactive compounds from endophytes including manipulation of culture medium and culture conditions, elicitation, metabolic engineering, and epigenetic modulation [20]. Mixed fermentation is one such strategy which was applied for production of antibacterial polyketide enacyloxin by Watanabe et al. [39], and they cocultured *Gluconobacter* sp. W-315 with the fungi *Neurospora crassa* or *Aspergillus oryzae* and reported enhanced accumulation of polyketide enacyloxin. Mixed fermentation was initially used for identification of certain novel secondary metabolites and later successfully used for increasing productivity of the microorganisms [40]. Bhalkar et al. [34] isolated seven endophytes (six fungal and one bacterial) from leaf and stem segments of *Nothapodytes nimmoniana*, and only three of the fungal isolates [isolate 1, isolate 5 (F1), and isolate 6 (F2)] showed CPT production; these were used for molecular characterization and identification of the strain and used for mixed fermentation experiments (Table 4). They cultivated endophytic fungi individually (monoculture) as well as together in the same confined media (mixed fermentation) using Sabouraud broth (SB), potato dextrose broth (PDB), Czapek dox broth (CDB), malt extract broth (MEB), yeast extract broth (YEB), beef extract broth (BEB), and whey

Table 4 Monoculture and mixed fermentation of fungal endophytes isolated from *Nothapodytes nimmoniana*

Fungal endophyte	CPT detection by HPTLC	CPT quantification by HPLC (mg/l)	CPT structure confirmation by LCMS (m/z)
Isolate 1 (*Fusarium oxysporum*)	Detected	90	349.1
Isolate 2 (*Fusarium* sp.)	Scarcely detected	Not detected	Not detected
Isolate 3	Not detected	Not detected	Not detected
Isolate 4	Not detected	Not detected	Not detected
Isolate 5 (F1)	Detected	35	349.1
Isolate 6 (F2)	Detected	70	349.1
Mixed fermentation			
Isolate 1 + isolate 5	Detected	32	348.8
Isolate 1 + isolate 6	Detected	45	349.1
Isolate 1 + isolate 5 + Isolate 6	Detected	91	349.1
Isolate 5 + isolate 6	Detected	145	349.1

After Bhalkar et al. [34]

liquid (20–100%). They also tested factors such as temperature (ranging from 20 to 40 °C), pH (ranging from 2 to 12), agitation speed (ranging from 50 to 200 revolutions per minute), and concentration of whey (ranging from 20% to 100%) on CPT production by monocultures as well as mixed fermentation (Table 5). Further, they tested for most influencing parameters for mixed fermentation which were considered for further process optimization by response surface methodology. Bhalkar et al. [34] reported that mixed fermentation was superior to monoculture of endophytic fungus for CPT production (Table 4). Among the different combinations of mixed fermentation of isolates, the mixed fermentation of isolate 5 (F1) and isolate 6 (F2) yielded higher CPT amount than any other combination (Table 4). Since mixed culture of F1 and F2 isolates showed intriguing increase and consistent CPT levels, they used these two fungal isolates for optimization of process parameters. Among the different media used, maximum biomass and CPT yield was exhibited by mixed fermentation when whey (70%) was used. The other optimized conditions were incubation temperature of 30 °C, agitation rate of 100 rpm, and pH of 6 (Table 5). After 15 days of incubation, the individual monoculture F1 exhibited maximum CPT production. F2 monoculture exhibited maximum yield of CPT after 20 days of incubation; however, the period was significantly reduced to 7 days in case of mixed culture. CPT production by individual monocultures of the two fungal species F1 and F2 using best optimized culture medium was 33 mg/l and 69 mg/l, respectively, while their mixed fermentation under the same defined conditions yielded 146 mg/l. This study paves way to the application of mixed fermentation for the production of endophyte-based bioactive compounds.

Table 5 Optimized culture parameters to achieve maximum CPT yield by monoculture and mixed fermentation of fungal endophytes isolated from *Nothapodytes nimmoniana*

Parameter studied	Fungus F1	Fungus F2	Mixed fermentation of F1+F2
Media type	Whey	Whey	Whey
Concentration of whey (%)	100	100	70
Temperature (°C)	30	35	30
pH (units)	6	6	6
Incubation period (d)	15	20	7
Agitation rate (rpm)	100	100	100
Optimized CPT yield (mg/l)[a]	33	69	146

After Bhalkar et al. [34]
[a]Values denote average of three experimental data

4 Constraints Associated with Long-Term Cultures of Endophytes of *Nothapodytes nimmoniana*

4.1 Attenuation of Camptothecin Production by Endophytes over Successive Generations

Endophytic species of medicinal plants have been shown to produce a number of secondary metabolites, and many of these metabolites are closely similar to those produced by respective host plants [20–23]. However, a major problem associated with commercial exploitation of endophytic species for the production of secondary metabolites is attenuation of production of secondary metabolites during culture. Such attenuation of CPT production and negative relation between fungal biomass and CPT content in endophytic fungal strains (25 endophytic fungal isolates) isolated from *Nothapodytes nimmoniana* have been reported by Gurudatt et al. [29]. Similarly, Bhalkar et al. [32] reported the attenuation of CPT production from *Fusarium oxysporum* kolhapuriensis, a fungal endophyte isolated from *N. nimmoniana*. A considerable decrease in the CPT productivity by successive generations of the fungus was recorded (Table 6). First-generation fungal culture yielded up to 283.3 mg/l of CPT using the optimized culture conditions, while second-generation subculture grown under same conditions produced 198 mg/l of CPT, which was attenuated to the lowest level of 33 μg/l of CPT in the eighth generation (Table 6). Such studies have led the researchers to hypothesize that endophytes might have possessed few genes and not complete set of genes which are responsible for CPT biosynthesis [41].

4.2 Restoration of Camptothecin Production in Attenuated Endophytes

Attenuation has been a major difficulty in realizing the potential of endophytic fungi as an alternative source of plant secondary metabolites. However, recent

Table 6 Camptothecin production by *Fusarium oxysporum* kolhapuriensis (fungal isolate from *Nothapodytes nimmoniana*) over successive generation

Subculture generation	Camptothecin content (mg/l)[a]
First	283 ± 0.27
Second	198 ± 0.12
Third	102 ± 0.87
Fourth	46 ± 0.54
Fifth	0.138 ± 0.24
Sixth	0.260 ± 0.12
Seventh	0.56 ± 0.18
Eighth	0.033 ± 0.16

After Bhalkar et al. [32]
Fungal cultures were established 70% (v/v) acid whey + 2% (w/v) malt extract; incubation temperature of 30 °C; incubation period for 6 days
[a]Values of CPT are mean of five replicates; standard error calculated by GraphPad InStat3 software

studies have shown that attenuated endophytic fungi that are re-inoculated into host plants are capable of synthesizing significant amount of CPT than the attenuated fungi [35]. Similarly, attenuated fungus cultured in the presence of 5-azacytidine, a DNA methyltransferase inhibitor, had an enhanced CPT content compared to untreated attenuated fungus [35]. These studies indicated that attenuation of CPT production in endophytic fungi could in principle be reversed by eliciting some signals from plants tissues, most likely that which prevents the methylation or silencing of the genes responsible for CPT biosynthesis.

5 Conclusions

Endophytes of medicinal plants are rich sources of bioactive secondary metabolites. Recent literature suggests that various endophytes that have been isolated from *Nothapodytes nimmoniana* can synthesize camptothecin like that of host plant. Fungal isolates from *Nothapodytes nimmoniana* have been cultured under axenic monoculture conditions for the production of CPT. Various researchers have developed suitable axenic culture methods and optimized culture conditions for accumulation of CPT in endophytes. However, a major constraint of axenic cultures of endophytes is attenuation of production of the metabolite over subculture generations. Most recent research efforts have also depicted restoration of camptothecin production in attenuated endophytic fungus, on re-inoculation into host plant as well as treatment with DNA methyltransferase inhibitors. It is necessary to identify, characterize, and select out superior isolates which can synthesize higher amounts of camptothecin, which are resilient to culture conditions and consistent in accumulating CPT over several subculture cycles. It is essential to understand the biosynthesis of camptothecin among endophytes and to identify host and endophyte

relationship for CPT accumulation. It is also necessary to develop appropriate fermentation technology for suitable, high yielding isolates for the production of CPT.

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Endophytes of *Ginseng*

6

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Contents

Abstract

Ginseng (*Panax ginseng* C. A. Meyer) is a well-known medicinal plant which is used as a tonic in oriental medicine. Ginsenosides are the most important secondary metabolites of ginseng which have pharmacological effects including anticancer,

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antidiabetic, immunomodulatory, neuroprotective, hepatoprotective, and anti-stress properties. Bacterial and fungal endophytes associated with ginseng plants have been isolated, characterized from its natural distribution range. Endophytes of ginseng showed tissue or organ, age, genotype, and geographical location specificity with their distribution and abundance. *Bacillus*, *Burkholderia*, *Lysinibacillus*, *Micrococcus*, *Paenibacillus*, and *Pseudomonas* are major bacterial genera isolated from ginseng. *Alternaria*, *Colletotrichum*, *Entrophospora*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Phoma*, *Setophoma*, *Verticillium*, and *Xylaria* are the most frequent fungal genera isolated from ginseng. Majority of ginseng endophytes depicted many of biological activities such as plant growth promotion, antimicrobial, antitumor, ginsenoside biosynthesis, and biotransformation activities. In this chapter we presented the recent progress made in the area of biology of ginseng endophytes.

Keywords
Bioactive compounds · Biotransformation · Endophytes · Ginseng · Ginsenosides · Saponins · Secondary metabolites

Abbreviations

A-549	Adenocarcinomic human alveolar basal epithelial cells (lung cancer cell line)
B-16	Murine tumor cell line
CF	Colonization frequency
CFU	Colony-forming units
HepG2	Human epithelial type G2 (liver cancer cell line)
HPLC	High-performance liquid chromatography
IAA	Indole-3-acetic acid
IC50	50% inhibition concentration
L-1210	Leukemia 1210
L-929	Mouse fibroblast cell line
LC/MS	Liquid chromatography/mass spectroscopy
MK-1	Human epithelial cell line
MKN45	Human gastric cancer cell line
MS/MS	Mass spectroscopy/mass spectroscopy
OVCAR-2	Human epithelial carcinoma cell line of the ovary (ovarian cancer cell line)
PDB	Potato dextrose broth

1 Introduction

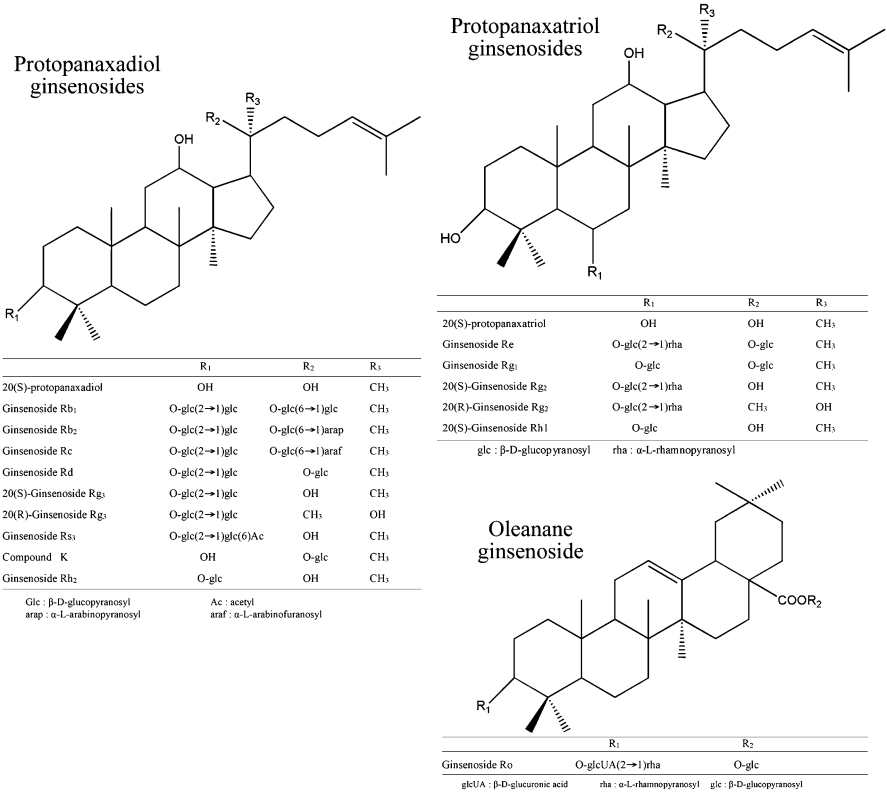
1.1 Ginseng, Its Active Ingredients and Uses

Panax ginseng which is commonly known as "Korean ginseng or Asian ginseng" is a popular medicinal plant used for thousands of years in Russia, China, Korea, and Japan as an adaptogen or as tonic to boast the immune system. It is also major

ingredient of Western herbal preparations [1, 2]. Ginseng belongs to the Araliaceae family and is indigenous to Eastern Russia (Siberian region), China, and Korea. The wild plant is nearly extinct due to both excessive collections from the wild for medicinal purposes and destruction of natural habitat [3]. It is cultivated in Korea, China, and Japan for export and used as a medicinal herb. Ginseng is a shade-loving, deciduous perennial plant with five fingered leaves, tiny white flowers, yellow or red berries, and a yellowish-brown root. The root of ginseng is utilized medicinally, although active compounds are present in all parts of the plant. The root of ginseng is a thick cylindrical structure that resembles a humanlike form, which is responsible for its name in Chinese, "Jen Shen" (man root, Fig. 1). The word *Panax* is formed from two Greek words, "pan" (all) and "akos" (cure), based on reputed use of the plant in traditional Chinese medicine as a "panacea" [4].

Major constituents of *Panax ginseng* are triterpenoid glycosides or saponins, commonly referred to as "ginsenosides." Other active ingredients such as amino acids, alkaloids, phenols, proteins, polypeptides, polysaccharides, fatty acids, vitamin B1, and vitamin B2 are also abundant in all parts of the plant [2]. Total ginsenosides vary depending on the species, growing environment, and extraction method, for example, 6-year-old *Panax ginseng* roots from the Tonghua district of China have higher ginsenosides, on dry weight basis, than roots from the Jilin district (6.4% vs 4.4%, respectively). In addition, total ginsenosides in cultivated ginseng

Fig. 1 Root of *Panax ginseng*



	R_1	R_2	R_3
20(S)-protopanaxadiol	OH	OH	CH_3
Ginsenoside Rb_1	O-glc(2→1)glc	O-glc(6→1)glc	CH_3
Ginsenoside Rb_2	O-glc(2→1)glc	O-glc(6→1)arap	CH_3
Ginsenoside Rc	O-glc(2→1)glc	O-glc(6→1)araf	CH_3
Ginsenoside Rd	O-glc(2→1)glc	O-glc	CH_3
20(S)-Ginsenoside Rg_3	O-glc(2→1)glc	OH	CH_3
20(R)-Ginsenoside Rg_3	O-glc(2→1)glc	CH_3	OH
Ginsenoside Rs_3	O-glc(2→1)glc(6)Ac	OH	CH_3
Compound K	OH	O-glc	CH_3
Ginsenoside Rh_2	O-glc	OH	CH_3

Glc : β-D-glucopyranosyl
arap : α-L-arabinopyranosyl
Ac : acetyl
araf : α-L-arabinofuranosyl

	R_1	R_2	R_3
20(S)-protopanaxatriol	OH	OH	CH_3
Ginsenoside Re	O-glc(2→1)rha	O-glc	CH_3
Ginsenoside Rg_1	O-glc	O-glc	CH_3
20(S)-Ginsenoside Rg_2	O-glc(2→1)rha	OH	CH_3
20(R)-Ginsenoside Rg_2	O-glc(2→1)rha	CH_3	OH
20(S)-Ginsenoside Rh1	O-glc	OH	CH_3

glc : β-D-glucopyranosyl rha : α-L-rhamnopyranosyl

	R_1	R_2
Ginsenoside Ro	O-glcUA(2→1)rha	O-glc

glcUA : β-D-glucuronic acid rha : α-L-rhamnopyranosyl glc : β-D-glucopyranosyl

Fig. 2 Chemical structures of various ginsenosides

per unit of root dry weight increase with the age of the roots. Ginsenosides are classified into three categories based on their structure, namely, Rb group (protopanaxadiols, including Rb1, Rb2, Rc, Rd, etc.), the Rg groups (protopanaxatriols, including Rg1, Re, Rf, Rg2, etc.), and Ro group (oleanolic acid) (Fig. 2) [5, 6]. Pharmacological effects of ginseng have been demonstrated in cancer, diabetes mellitus, cardiovascular system, immune system, and central nervous system including anti-stress and antioxidant activity [5]. More recently ginseng has been used as functional food, and it is also used in cosmetic and beverage industry [6–9].

2 Endophytes Isolated from Ginseng

Endophytes are the bacterial and fungal species that reside inside the living tissues of healthy plants. Endophytes occur in all known plant species; they live symbiotically with their host plants and produce a large number of chemical compounds that provide protection and survivability of the hosts [10]. Bacterial and fungal endophytes colonize either locally or systematically in inter- or intracellular locations [11]

and have several positive effects on plants, including promotion of growth, nutrient uptake, and tolerance to abiotic and biotic stresses [12]. Recent studies have shown that endophytes of many medicinal plants produce bioactive compounds that can be used as raw material for pharmaceutical, cosmetic, and fragrance industries [13]. Some of the bacterial and fungal endophytes synthesize unique secondary metabolites including alkaloids, steroids, terpenoids, isocaumarin derivatives, quinines, flavonoids, and lignans that are used as antimicrobials, antibiotics, immunosuppressants, and anticancer compounds [14–16]. Thus, isolation of endophytes of medicinal plants and their axenic cultivation had commercial potential in the fields of agriculture, pharmacy, medicine, and cosmetic-related industries. Extensive research has been carried out on isolation; characterization of endophytes from various tissues/organs of *Panax ginseng* and biological activity of bacterial and fungal endophytes have been reported (Tables 1 and 2).

2.1 Diversity of Endophytic Bacteria in Ginseng

Many investigations were carried out on isolation and identification of bacterial endophytes from stem, leaves, and roots of ginseng [17–21]. Both Gram-positive and Gram-negative bacterial endophytes have been isolated including *Agrobacterium tumefaciens* strain C58, *Agrobacterium tumefaciens* strain ISSDS, *Arthrobacte*r sp., *Bacillus acidiceler* strain CBD, *Bacillus altitudinis*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus cereus* strain DS16, *Bacillus flexus*, *Bacillus flexus* strain L2S2, *Bacillus megaterium*, *Bacillus pumilus* strain CT13, *Bacillus pumilus* strain HN005, *Bacillus pseudomycoides*, *Bacillus sphaericus*, *Bacillus subtilis* strain GH 38, *Bacillus* sp., *Bacillus thuringiensis* serovar kurstaki, *Bacillus thuringiensis*, *Burkholderia* sp., *Erwinia persicinus*, *Lysinibacillus sphaericus* C3-41, *Microbacterium hydrocarbonoxydans*, *Microbacterium phyllosphaerae*, *Micrococcus luteus* strain 164, *Paenibacillus glucanolyticus*, *Paenibacillus polymyxa*, *Pantoea agglomerans*, *Pantoea ananatis*, *Pectobacterium carotovorum*, *Pseudoclavibacter helvolus*, *Pseudomonas poae*, *Pseudomonas marginalis* strain ATCC 10844 T, *Pseudomonas* sp., *Rahnella* sp., *Serratia plymuthica*, *Staphylococcus epidermidis* strain DS14, *Staphylococcus epidermidis* strain KL-096, *Staphylococcus epidermidis* strain RW35, *Staphylococcus epidermidis* S09, *Staphylococcus epidermidis* TMPSB-D, *Staphylococcus pasteuri* CV5, *Staphylococcus* sp. RP22, *Stenotrophomonas maltophilia* strain LMG 20578, *Stenotrophomonas maltophilia* HK40 (Table 1).

2.2 Diversity of Endophytic Fungi in Ginseng

Fungal endophytes of ginseng have been isolated from both field cultivated plants and naturally distributed plants in mountains of Korea and China (Table 2) [22–25, 28, 30]. Efforts have been made to investigate the geographical distribution of fungal endophytes, their ecological associations with plant populations, cultivar

Table 1 Bacterial endophytes isolated from different parts of *Panax ginseng* C.A. Meyer

Endophytes	Tissues from which isolation was done	References
Arthrobacter sp.	Roots	[17]
Bacillus cereus		
Bacillus flexus		
Bacillus megaterium		
Bacillus sphaericus		
Bacillus sp.		
Erwinia persicinus		
Kocuria carniphila		
Microbacterium hydrocarbonoxydans		
Microbacterium phyllosphaerae		
Paenibacillus polymyxa		
Pantoea agglomerans		
Pantoea ananatis		
Pectobacterium carotovorum		
Pseudoclavibacter helvolus		
Pseudomonas poae		
Pseudomonas sp.		
Rahnella sp.		
Serratia plymuthica		
Agrobacterium tumefaciens strain C58	Stem bark	[18]
Agrobacterium tumefaciens strain ISSDS		
Bacillus acidiceler strain CBD		
Bacillus amyloliquefaciens		
Bacillus cereus strain DS16		
Bacillus flexus strain L2S2		
Bacillus megaterium strain EJH-7		
Bacillus megaterium		
Bacillus pumilus strain CT13		
Bacillus pumilus strain HN005		
Bacillus pseudomycoides		
Bacillus subtilis strain GH38		
Bacillus subtilis strain SC2-4-1		
Bacillus subtilis strain QD434		
Bacillus subtilis		
Bacillus thuringiensis LDC-391		
Bacillus thuringiensis LDC-415		
Bacillus thuringiensis serovar kurstaki		
Bacillus thuringiensis		
Lysinibacillus sphaericus C3-41		
Microbacterium phyllosphaerae		
Micrococcus luteus strain 164		
Paenibacillus glucanolyticus		

(*continued*)

Table 1 (continued)

Endophytes	Tissues from which isolation was done	References
Pseudomonas marginalis strain ATCC 10844 T		
Staphylococcus epidermidis strain DS14		
Staphylococcus epidermidis strain KL-096		
Staphylococcus epidermidis strain RW35		
Staphylococcus epidermidis S09		
Staphylococcus epidermidis TMPSB-D		
Staphylococcus pasteuri CV5		
Staphylococcus sp. RP22		
Stenotrophomonas maltophilia strain LMG 20578		
Stenotrophomonas maltophilia HK40		
Paenibacillus polymyxa	Leaves	[19]
Bacillus altitudinis	Roots	[20]
Burkholderia sp.	Roots	[21]

specificity, tissue or organ specificity, and age-dependent distribution [22–25]. Park et al. [22] isolated fungal endophytes in *Panax ginseng* cultivars, namely, Chungpoong, Yunpoong, and Gumpoong cultivated in Korea. Overall, 38 fungal endophytes were isolated which fall into three groups, namely, *Fusarium*, *Phoma*, and *Setophoma*. The most dominant fungal endophyte was *Phoma* (65.8%) in all the three cultivars, and the percentage of colonization frequency of *Phoma radicina* was 80%, 52.9%, and 75% in Chungpoong, Yunpoong, and Gumpoong cultivars, respectively. Park et al. [23] isolated endophytic fungi from root, stem, petiole, leaf, and flower stalk of 3- and 4-year-old ginseng plants cultivated in Korea. The *Alternaria*, *Colletotrichum*, and *Phoma* were most frequently isolated endophytes followed by *Entrophospora*, *Fusarium*, and *Xylaria* among 127 isolates. *Phoma radicina* and *Fusarium solani* were most frequently isolated species colonizing the tissues of 3- and 4-year-old ginseng plants. They reported that colonization frequency (CF) of endophytes was dependent on the age and tissues examined: the CFs of the roots and stems in the 3-year-old ginseng were higher than the CF of tissues in the 4-year-old plants. In contrast, higher CF's were observed in the leaves and petioles of 4-year-old plants, and endophytic fungi in the flower stalks were detected only in the 4-year-old plants. These results suggest that endophytic fungi in ginseng plants were distributed differently depending on the age and tissues. Park et al. [24] conducted a study to examine the diversity of fungal endophytes obtained from different ages (endophytes were isolated from 1-, 2-, 3- to 4-year-old ginseng roots) of *Panax ginseng* cultivated in Korea. Their results showed that ginseng roots that were 1-, 2-, 3-, and 4-year-old were colonized by 2, 6, 8, and 5 species of fungal endophytes, respectively. While *Phoma radicina* was the most frequent fungal endophyte in 2-, 3-, and 4-year-old ginseng roots, *Fusarium solani* was the dominant endophyte in 1-year-old ginseng roots (Table 3). Such results suggest that a variety

Table 2 Fungal endophytes isolated from different parts of *Panax ginseng* C.A. Meyer

Endophytes	Tissues from which isolation was done	References
Paecilomyces sp.	Roots	[27]
Colletotrichum pisi	Roots	[22]
Fusarium oxysporum		
Phoma radicina		
Setophoma terrestris		
Alternaria arborescens	Roots, stem, petiole, flower stalk	[23]
Alternaria alternata		
Aureobasidium sp.		
Botryosphaeria dothidea		
Cladosporium sphaerospermum		
Colletotrichum ignotum		
Colletotrichum pisi		
Coprinellus radians		
Coprinopsis cinerea		
Entrophospora sp.		
Eutypella scoparia		
Fusarium proliferatum		
Fusarium solani		
Monacrosporium microscaphoides		
Nectria haematococca		
Nemania diffusa		
Phomopsis sp.		
Pythium sylvaticum		
Stachybotrys cylindrospora		
Trichoderma harzianum		
Valsa ambiens		
Xylaria sp.		
Colletotrichum panacicola	Roots	[24]
Colletotrichum pisi		
Cylindrocarpon destructans		
Fusarium acuminatum		
Fusarium oxysporum		
Fusarium solani		
Leptodontidium orchidicola		
Phoma radicina		
Trichoderma citrinoviride		
Aspergillus fumigatus	Roots	[30]
Aspergillus protuberus		
Aspergillus sydowii		
Cladosporium cladosporioides		
Cladosporium silenes		
Cladosporium sp.		
Engyodontium album		

(*continued*)

Table 2 (continued)

Endophytes	Tissues from which isolation was done	References
Fusarium oxysporum		
Fusarium proliferatum		
Fusarium solani		
Fusarium sp.		
Nectria haematococca		
Nectria radicicola		
Paraphoma chrysanthemicola		
Penicillium guttulosum		
Penicillium menonorum		
Penicillium simplicissimum		
Penicillium sp.		
Verticillium psalliotae		
Verticillium sp.		
Penicillium janthinellum Yuan-27	Roots	[29]
Penicillium melinii Yuan-25		
Alternaria sp.	Leaves, stem, roots	[25]
Bjerkandera sp.		
Ceratobasidium sp.		
Ceriporia sp.		
Fusarium sp.		
Geomyces sp.		
Penicillium sp.		
Hydnochaete sp.		
Irpex sp.		
Peniophora sp.		
Mortierella sp.		
Mucor sp.		
Phoma sp.		
Phomopsis sp.		
Resinicium sp.		
Umbelopsis sp.		
Zygorhynchus sp.		

of fungal endophytes were distributed depending on the age of the ginseng plants. In another study Park et al. [25] have isolated 129 species of fungal endophytes from root, stem, and leaves of mountain ginseng. The fungal endophytes belonged to *Ascomycota* (81.7%), *Basidiomycota* (7.08%), and *Zygomycota* (10%), and few were unknown. Most of the isolates belonged to *Alternaria*, *Bjerkandera*, *Ceratobasidium*, *Ceriporia*, *Fusarium*, *Geomyces*, *Hydnochaete*, *Irpex*, *Mortierella*, *Mucor*, *Penicillium*, *Peniophora*, *Phoma*, *Phomopsis*, *Resinicium*, *Trichoderma*, *Umbelopsis*, and *Zygorhynchus* genera. They also recorded diversity of fungal endophytes within tissues, and the highest number of different fungal endophytes

Table 3 Percentage of dominant endophytes isolated from 1-, 2-, 3-, to 4- year-old ginseng roots in Korea

Age (year)	Dominant endophyte (DE)	DE(%)[a]
1	*Fusarium solani*	60.0
2	*Phoma radicina*	37.5
3	*Phoma radicina*	38.5
4	*Phoma radicina*	52.5

After Park et al. [24]

[a]The percent of DE (DE%) was calculated as follows: DE%=(Nt/Ni)× 100, where Ni=number of each isolated fungus; Nt=total isolated number of fungi

was recorded in root tissues (70 isolates), followed by the stem (54 isolates) and the leaf (48 isolates). These results depict that plant-endophyte association largely depends on hosts and environmental factors and geographical location.

3 Biological Activities of Endophytes Isolated from Ginseng

Many of the endophytes isolated from medicinal and other plants have the potential to synthesize various secondary metabolites which possess various biological activities such as antimicrobial, cytotoxic, and antitumor activities [26]. Occasionally, endophytes produce host plant secondary metabolites with therapeutic potential including paclitaxel, podophyllotoxin, camptothecin, hypericin, emodin, and azadirachtin [26]. Various researchers have isolated both bacterial and fungal endophytes from ginseng which have demonstrated different biological activities including antifungal activity, plant growth-promoting activity, stimulation of ginseng biosynthesis and conversion of native ginsenosides into bioactive ginsenosides, and antitumor activity, and an account of such investigations is presented in the following sections.

3.1 Biological Activity of Bacterial Endophytes

3.1.1 Antifungal Activity

Cho et al. [17] demonstrated the cellulase, xylanase, pectinase, and protease activities among the bacterial endophytes (*Arthrobacter*, *Bacillus*, *Kocuria*, *Paenibacillus*, *Pseudomonas*) isolated from *Panax ginseng*. Cho et al. [17] also studied in vitro antibiotic activity of ginseng endophytic bacteria against fungal phytopathogens, namely, *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora ultimum*, and *Phytophthora polymyxa*. Three bacterial isolates *Bacillus* sp., *Paenibacillus polymyxa*, and *Pseudomonas poae* are reported to have antifungal activity against phytophathogenic microorganisms.

3.1.2 Plant Growth-Promoting Activity

Vendan et al. [18] studied endophytic bacterial isolates of ginseng for their phosphate solubilizing ability by culturing bacterial endophytes in a medium containing tricalcium phosphate with glucose as sole source of carbon (Table 4). *Lysinibacillus fusiformis* showed higher solubilization of mineral phosphate (0.39 mm), whereas *Bacillus cereus* (0.38 mm) and *B. megaterium* (0.35 mm) depicted moderate solubilization of calcium phosphate. Vendan et al. [18] also recorded synthesis of indole-3-acetic acid (IAA) by endophytes in nutrient broth supplemented with tryptophan as precursor. The isolates *Micrococcus luteus*, *Lysinibacillus fusiformis*, and *Bacillus cereus* produced higher amounts of IAA of 13.93 μg/ml, 7.23 μg/ml, and 4.61 μg/ml, respectively (Table 4). Microorganisms produce and secrete siderophores to sequester iron, and *Bacillus cereus*, *B. flexus*, *B. megaterium*, *Lysinibacillus fusiformis*, *L. sphaericus*, *Microbacterium phyllosphaerae*, and *Micrococcus luteus* were reported to exhibit siderophore activity (Table 4) [18].

In another study, Gao et al. [19] isolated *Paenibacillus polymyxa* from *Panax ginseng* plants, and they showed that inoculation of field cultivated ginseng plants with *Paenibacillus polymyxa* by foliar application combined with irrigation enhanced the growth, reduced morbidity, and increased plant ginsenoside concentrations in the field experiments. They conducted field experiments at Jilin Agricultural University, China, from 2011 to 2013. The average weights of 1-, 2-, 3- and 4-year-old ginseng plants were 0.5 ± 0.02 g, 1.3 ± 0.05 g, 3.9 ± 0.1 g, and 17.6± g, respectively. Plants were grown under the same environmental conditions, and the planting distance was 10 cm. Bacteria were grown in potato dextrose broth (PDB) for 48 h on orbital shaker (160 rpm at 28 °C), and cells were harvested by centrifugation at 3000 rpm for 10 min at 4 °C; pellets were resuspended in sterile water (0.8%) at about 10^8 colony-forming units (CFU)/ml. Ginseng plants, aged 1, 2, 3, and 4 years old, were divided into control and treatment groups. The controls were treated with distilled water, while the treatment group were inoculated (a) ~10^8 CFU/ml at 50 ml/m^2 sprayed over the entire plant, (b) irrigation ~10^8 CFU/ml at 50 ml/m^2, and (c) combination of both (~10^8 CFU/ml at 50 ml/m^2 spraying and irrigation). They conducted field experiments 1 month per year and was repeated three times over 3 years. During spraying, the soil surface and other plants were covered with plastic to prevent contamination. Growth parameters and morbidity for each plant were recorded upon harvest. Gao et al. [19] reported maximum increase in height and weight and lowest morbidity in plants treated with *Paenibacillus polymyxa* by foliar application combined with irrigation at all ages. On average, the heights of 1-, 2-, 3-, and 4-year-old plants were 38.44%, 35.24%, 41.90%, and 24.00% higher, respectively, than those of controls of the same age, while average weights in the treatment group were 31.64%, 58.87%, 46.70%, and 18.6% greater, respectively, than the controls. Similarly, morbidity in *Paenibacillus polymyxa*-treated 1- to 4-year-old plants was 13.64%, 17.67%, 21/67%, and 27.34% lower, respectively, than in ginseng treated with sterile water. The total ginsenoside concentration in 1- to

Table 4 Phosphate solubilization, indole-3-acetic acid, and siderophore production ability of ginseng endophytic bacterial isolates

Endophytic bacteria	Diameter of phosphate solubilization zone (mm)	IAA produced (μg/ml)	Siderophore production – color change
Agrobacterium tumefaciens strain C58	0.00	0.31	–
Bacillus amyloliquefaciens	0.25	0.31	–
Bacillus cereus strain DS16	0.38	4.61	+
Bacillus flexus strain L2S2	0.29	2.04	+
Bacillus megaterium strain EJH-7	0.35	1.78	+
Bacillus pseudomycoides	0.00	0.00	–
Bacillus pumilus strain CT13	0.23	0.52	–
Bacillus subtilis strain SC2-4-1	0.29	0.52	–
Bacillus thuringiensis serovar kurstaki	0.00	0.00	–
Lysinibacillus fusiformis strain X-9	0.39	7.23	+
Lysinibacillus sphaericus C3-41	0.00	2.30	+
Microbacterium phyllosphaerae	0.31	2.46	+
Micrococcus luteus strain 164	0.32	13.93	+
Paenibacillus glucanolyticus	0.00	2.04	–
Pseudomonas marginalis strain ATCC 10844T	0.00	0.00	–
Staphylococcus epidermidis strain RW35	0.00	0.84	–
Staphylococcus pasteuri CV5	0.00	1.57	–
Stenotrophomonas maltophilia strain LMG 20578	0.00	0.00	–

+ indicates siderophore production; – indicates siderophore nonproduction
After Vendan et al. [18]

4-year-old ginseng plants treated with *Paenibacillus polymyxa* were 36.63%, 44.52%, 67.96%, and 79.44% higher, respectively, than in control plants (Table 5). In 4-year-old groups, the concentration of Rc ginsenoside was 54.24% lower than the control; that of Rd ginsenoside was 308.01% higher. These results indicate that *Paenibacillus polymyxa* can be effectively used to enhance the yield and quality of ginseng plant.

Table 5 Effect of *Paenibacillus polymyxa* inoculation by a combination of foliar application and irrigation on ginsenoside concentration in *Panax ginseng* C. A. Meyer of different ages

Age (years)		Ginsenoside[a]									
		Rg_1	Re	Rf	Rb_1	Rg_2	Rc	Rb_2	Rb_3	Rd	Rt
1	C	2.507 ± 0.111	1.515 ± 0.118	0.384 ± 0.051	3.806 ± 0.162	0.115 ± 0.092	2.099 ± 0.170	1.253 ± 0.113	0.296 ± 0.017	0.986 ± 0.091	12.161 ± 1.169
	T	3.065 ± 0.225[b]	2.202 ± 0.216[b]	0.951 ± 0.047[c]	3.779 ± 0.313[b]	0.270 ± 0.014	0.509 ± 0.040[c]	2.081 ± 0.179[c]	1.121 ± 0.010[c]	3.663 ± 0.201[c]	16.641 ± 1.243[b]
2	C	2.701 ± 0.221	1.639 ± 0.124	0.515 ± 0.044	3.231 ± 0.310	0.163 ± 0.009	2.437 ± 0.171	1.370 ± 0.089	0.228 ± 0.021	1.382 ± 0.106	13.666 ± 1.145
	T	3.821 ± 0.114[c]	2.862 ± 0.079[c]	1.041 ± 0.056[c]	4.524 ± 0.334[c]	0.277 ± 0.016[c]	1.605 ± 0.079[c]	2.139 ± 0.107[c]	0.355 ± 0.032[c]	3.127 ± 0.206[c]	19.751 ± 1.789[b]
3	C	3.051 ± 0.220	1.736 ± 0.079	0.746 ± 0.052	3.418 ± 0.217	0.181 ± 0.011	2.736 ± 0.146	1.511 ± 0.109[b]	0.243 ± 0.017	1.396 ± 0.112	15.018 ± 1.230
	T	4.335 ± 0.247[c]	3.345 ± 0.148[c]	1.382 ± 0.109[c]	5.801 ± 0.308[c]	0.366 ± 0.024[c]	1.231 ± 0.106[c]	1.959 ± 0.179[b]	0.404 ± 0.031[c]	6.497 ± 0.417[c]	25.235 ± 2.232[c]
4	C	3.105 ± 0.158	2.147 ± 0.216	0.883 ± 0.054	3.689 ± 0.239	0.213 ± 0.017	3.145 ± 0.272	1.774 ± 0.134	0.286 ± 0.015	1.603 ± 0156	16.845 ± 1.301
	T	5.572 ± 0.365[c]	3.953 ± 0.247[c]	1.635 ± 0.131[c]	6.936 ± 0.512[c]	0.420 ± 0.033[c]	1.476 ± 0.104	1.068 ± 0.106[c]	0.535 ± 0.047[c]	8.643 ± 0.688[c]	30238 ± 2.253[c]

C Control group for ginsenoside concentration in *Panax ginseng* C.A. Meyer at different ages, *T* Treatment groups for combination of foliar application and irrigation on ginsenoside concentration in *Panax ginseng* C.A. Meyer at different ages

After Gao et al. [19]

[a]Concentration of ginsenosides (mg/kg DW). *DW* Dry weight, *Rt* sum of nine ginsenosides ($Rt=Rg_1+Re+Rf+Rb_1+Rg_2+Rc+Rb_2+Rb_3+Rd$)

[b]The significantly different values between treatments and control groups at $P < 0.05$ (Duncan's test)

[c]The very significantly different values between treatments and control groups at $P < 0.05$ (Duncan's test)

3.1.3 Stimulation of Ginsenoside Biosynthesis

Song et al. [20] isolated *Bacillus altitudinis*, a bacterial endophyte from ginseng, and studied the effect of *Bacillus altitudinis* (used as elicitor) with ginseng adventitious root cultures. They raised the ginseng adventitious root cultures and treated the adventitious root cultures with different volumes of 0, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0 ml of endophyte suspension (*Bacillus altitudinis*); then cultures were maintained further for 12 days, and root growth and ginsenoside content were measured. Their results revealed that after 6 days of elicitation with a 10 ml of *Bacillus altitudinis*, the total ginsenoside content was 2.026 mg/g dry weight which was four times more than that in unchallenged/untreated roots. This report demonstrates that the endophytic bacterium, *Bacillus altitudinis*, is an effective elicitor and can increase the ginseng adventitious root growth and ginsenoside concentration.

3.1.4 Biotransformation of Ginsenoside Rb1 to Ginsenoside Rg3

Panax ginseng is possessing Rb group ginsenoside (protopanaxadiols) including Rb1, Rb2, Rc, Rd, Rg1, and others along with Rg group ginsenosides (protopanaxatriols) [5]. Minor ginsenoside monomers, such as F2, Rh2, Rg3, and compound K (CK), in particular have greater antitumor activity than that the major monomers [5]. The minor ginsenoside monomers are not naturally found in ginseng roots, and various transformation methods, including chemical methods such as hydrolysis, alkaline hydrolysis, heat treatment, enzymatic conversion, and microbial conversion, have been used for the conversion of major ginsenosides to minor ginsenosides. However, the chemical conversion methods could cause side reactions of epimerization, hydration, and hydroxylation. Heat treatment degrades the other active minor ginsenosides by randomly hydrolyzing glycosidic bonds that can remove the other pharmaceutical activities of ginseng. Enzymatic conversion by the appropriate sugar hydrolysis of a specific position is desirable for the production of an active minor ginsenosides; however, extraction and separation of the enzyme is complex process. Therefore, microbial conversion is a very good method, and in that the reaction conditions are simple and relative low cost, have strong specificity, and generate very few by-products. Recently, research has been carried out in this direction and reports are available on successful use of bacterial endophytes of ginseng for conversion of major ginsenosides to minor ginsenosides. Fu et al. [21] have isolated a β-glucosidase-producing endophytic bacterium *Burkholderia* sp. from *Panax ginseng* and used for the transformation of the major protopanaxadiol ginsenoside Rb1 to minor ginsenoside Rg3. Fu et al. [21] used Esculin-PDB for isolation of β-glucosidase-producing endophytic bacteria from ginseng and selected ten β-glucosidase-producing bacteria, out of which *Burkholderia* sp. showed the highest activity in conversion of ginsenoside Rb1 to ginsenoside Rg3. They grew the endophyte bacteria in PDB at 30 °C. A mixture with the same volume of ginsenoside Rb1 (1.0 mg/ml) and a suspension of the strain that reached logarithmic phase were incubated at 30 °C with gentle shaking at 150 rpm. The phosphate/citrate buffer of pH 7.0 and temperature of 30 °C were found to be optimal for effective biotransformation activity. Ginsenoside Rb1 was gradually hydrolyzed by *Burkholderia* sp. and converted into ginsenoside Rd, and ginsenoside

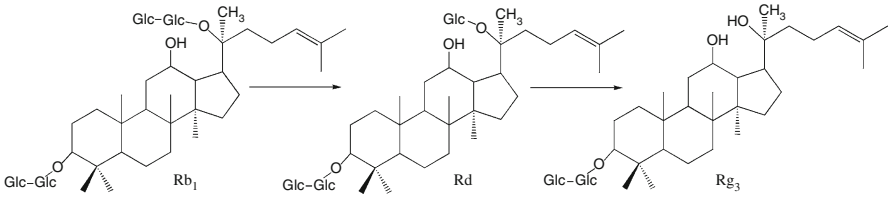


Fig. 3 Conversion of Rb1 into Rg3 ginsenoside by biotransformation

Rd yield was highest after 8 h of incubation; subsequently ginsenoside Rd was converted into ginsenoside Rg3 (Fig. 3). After 5–15 h of incubation, the reactant (ginsenoside Rb1) and intermediate (ginsenoside Rd) were gradually hydrolyzed to ginsenoside Rg3. The reaction time was 15 h and the maximum conversion rate of ginsenoside Rg3 was 98%. These investigations have great potential to be applied in the synthesis of the minor ginsenoside Rg3 in the pharmaceutical industry.

3.2 Biological Activity of Fungal Endophytes

Metabolites isolated from ginseng fungal endophytes have depicted antimicrobial activities against microbial pathogens. Xu et al. [27] isolated 51 compounds of ginseng plant and 38 compounds of *Paecilomyces* sp. through GC/MS analysis of ether extract. The ether extract of ginseng consisted mainly decalin, 2-methylene-5,5,8a-trimethyl-1-2,5-dimethoxybenzyl-4aα (27.26%), retinal (12.68%), ±-trans-nerolidol (3.96%), falcarinol (2.87%), and β-panasinsene (1%). In the ether extract of *Paecilomyces* sp., the main constituents were indane-1,3-dione,2(3,4-dimethoxy-benzyllideno) (40.01%), androst-2-en-4-one, 17-(tetrahydropyran-3-yl)oxy (7.76%), xanthatin (4.41%), isotanshinone II (3.03%), and falcarinol (1.38%). Among these compounds falcarinol is common chemical both in the extracts of ginseng (2.87%) and *Paecilomyces* sp. (1.38%); these findings depict that ginseng endophytes produced similar metabolites as its host. Xu et al. [27] tested in vitro anti-*Pyricularia oryzae* activity of ether extract of *Paecilomyces* sp. and ginseng, and their results showed that minimum inhibitory concentration values from *Paecilomyces* sp. and ginseng extract were 7.8 and 125 μg/ml, respectively. Xu et al. [27] assessed antifungal activity of ginseng as well as *Paecilomyces* sp. ether extracts against four human pathogenic fungi: *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Aspergillus fumigatus*, and results showed the extracts of ginseng and *Paecilomyces* sp. antifungal activities against tested organisms (Table 6). In another study Park et al. [28] screened many endophytic fungi of *Panax ginseng* for antimicrobial activity against ginseng pathogens and reported very good biocontrol activity against tested ginseng pathogens. Four fungal endophytes, namely, *Colletotrichum pisi*, *Fusarium oxysporum*, *Fusarium solani*, and *Phoma terrestris*, were assessed for their chemical components by GC-MS, and their results revealed that 3-furanacetic acid,4-hexyl-2,5-dihydro-2,5-dioxo

(85.00%) in *Colletotrichum pisi*, phthalic acid (14.31%) and erucylamide (10.95%) in *Fusarium oxysporum*, 4(15)-aromadendren-12,5alpha-olide (12.13%), phthalic acid (9.86%) in *Fusarium solani*, and N-amino-3-hydroxy-6-methoxyphthalimide (32.17%) and 5H-dibenz [B,F] azepine (7.12%) in *Phoma terrestris* were major components (Table 7). Park et al. [28] conducted disc diffusion and the fermentation broth assays to test the antimicrobial activity of *Colletotrichum pisi*, *Fusarium oxysporum*, *Fusarium solani*, *Phoma terrestris* isolated from ginseng on five fungal pathogens *Alternaria panax*, *Botrytis cinerea*, *Colletotrichum panacicola*, *Rhizoctonia solani*, and *Phytophthora cactorum* (Table 8). Among the tested endophytes, Phoma terrestris showed better antimicrobial activity ranging from 52.2% to 82.5%

Table 6 The in vitro antifungal activity of the ether extracts of ginseng and the *Paecilomyces* sp. [MIC_{80} (μg/ml)]

Test organisms	Ginseng	*Paecilomyces* sp.	Amphotericin B (positive control)
Candida albicans	16	8	1
Cryptococcus neoformans	64	1	0.25
Trichophyton rubrum	>64	4	4
Aspergillus fumigatus	>64	>64	1

After Xu et al. [27]

Table 7 Major chemical composition of ginseng fungal isolates

Isolate	Chemical compounds	Content (%)
Colletotrichum pisi	3-furanacetic acid, 4-hexyl-2,5dihydro-2,5-dioxo	85.00
	Aceto vanillin	6.14
Fusarium oxysporum	Phthalic acid	14.31
	Erucylamide	10.95
	3,3-dimethyl-3,4,7,12-tetrahydrobenzo[a] anthracene-7,12-dione	6.69
	2-hydroxymethyl-4-isopropyloxy-5,7-dimethoxynapthalene	2.43
Fusarium solani	4(15)-armadendren-12,5-alph-olide	12.13
	Phthalic acid	9.86
	6-pentyl-5,6-dihydro-2H-pyran-2-one	9.15
	13-alpha-scopadulanol	8.43
	Erucylamide	5.79
	Malettinin A	5.61
Phoma terrestris	N-amino-3-hydroxy-6-methxyphthalimide	32.17
	5H-dibenz [B,F] azepine	7.12
	3-methyl(6)(2,4) thiophenophane	4.31
	2-phenylindole	3.95
	5-(methozycarbonloxy)pent-3-yn-2-ol	3.90
	5-hydroxydodecanic acid lactone	3.89

After Park et al. [28]

in the disc diffusion test and 30.6–80.2% in the fermentation broth test (Table 8). These reports indicate the antimicrobial potential of metabolites extracted from the ginseng endophytic fungi, and these metabolites can be used as alternatives to chemicals for biocontrol.

3.2.1 Antitumor Activity

Several researchers have shown the antitumor activity of fungal endophytes of ginseng. Xu et al. [27] demonstrated the cytotoxic activity of extracts from ginseng and *Paecilomyces* sp. (fungal endophyte of ginseng) against tumor cell lines MKN45, LOVO, HepG2, and HL-60 (Table 9). They reported that IC50 values of the ether extract of ginseng against four human cell lines were 18.7, 9.2, 72.18, and 17.98 μg/ml, and the values of the ether extracts of *Paecilomyces* sp. were 7.85, 12.33, 67.26, and 8.67 μg/ml, respectively (Table 9). The antitumor activity of ether extracts of *Paecilomyces* sp. was better than the extracts of the ginseng. Zheng et al. [29] isolated two strains of endophytic fungi, *Penicillium melinii* Yuan-25 and *Penicillium janthinellum* Yuan-27 from the roots of *Panax ginseng*; further they cultured these isolates by fermentation and isolated novel benzaldehyde derivatives

Table 8 Radial growth inhibition of endophytic fungi using disk diffusion and fermentation broth tests against ginseng pathogens

	Alternaria panax		*Botrytis cinerea*		*Colletotrichum panacicola*		*Rhizoctonia solani*		*Phytophthora cactorum*	
Isolate	DD (%)[a]	FB (%)[b]	DD (%)	FB (%)	DD (%)	FB (%)	DD (%)	FB (%)	DD (%)	FB (%)
Colletotrichum pisi	nt[c]	nt	na[d]	na	36.1 (3.9)	25.4 (2.5)	nt	nt	nt	nt
Fusarium oxysporum	nt	nt	nt	nt	nt	nt	45 (2.6)	69.2 (6.0)	64 (1.4)	71.6 (1.7)
Fusarium solani	nt	nt	nt	nt	nt	nt	47 (2.1)	90.5 (0)	nt	nt
Phoma terrestris	58.7 (2.7)	65 (0.9)	59 (0.8)	30.6 (1.7)	56.1 (1.2)	42.8 (1.6)	52.2 (7.9)	72.1 (4.3)	82.5 (2.1)	80.2 (2.1)

Values are given as the means (SE) of 4 replications
After Park et al. [28]
[a]DD (%) Percentage of radial growth inhibition in dark diffusion
[b]FB (%) Percentage of radial growth inhibition in fermentation broth
[c]nt Not determined
[d]*na* Not active

Table 9 The in vitro antitumor activity of the ether extracts of ginseng and the *Paecilomyces* sp. [IC_{50} (μg/ml)]

Cell lines	Ginseng	*Paecilomyces* sp.	Doxorubicin (positive control)
MKN45	18.7	7.85	0.0622
LOVO	9.2	12.33	0.0125
HepG2	72.18	67.26	0.0350
HL-60	17.98	8.67	<0.001

After Xu et al. [27]

ginsenocin and other compounds, methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate, 3,4,5-trimethyl-1,2-benzenediol, penicillic acid, mannitol, ergosterol, ergosterol peroxide from *Penicillium melinii* Yuan-25, and brefeldin-A from *Penicillium janthinellum* Yuan-27 (Fig. 4). All the isolated compounds were evaluated for their cytotoxicity against six human cancer cell lines, viz., MKN45, LOVO, A549, MDA-MB-435, HepG2, and HL-60. They reported that brefeldin-A was the most cytotoxic constituent against all the tested cell lines with IC50 values <0.12 μg/ml. Ginsenocin and penicillic acid also reported to be potent antitumor compounds with IC50 values ranging from 0.49 to 7.46 μg/ml. These results suggest that fungal endophytes of *Panax ginseng* are a promising natural source of potential antitumor agents.

3.2.2 Ginsenoside Biosynthesis Capability

Wu et al. [30] studied the diversity of endophytic fungi from root of *Panax ginseng* (natural population grown in China) and reported their saponin yield capabilities. They isolated 38 isolates which belong to genera *Aspergillus*, *Cladosporium*, *Engyodontium*, *Fusarium*, *Penicillium*, *Plectosphaerella*, *Nectria*, and *Verticillium*. Among these *Aspergillus* sp., *Fusarium* sp., and *Verticillium* sp. exhibited saponin biosynthesis capabilities; Rb2 ginsenoside was detected in *Aspergillus* sp., *Fusarium* sp., and Rc ginsenoside in *Verticillium* sp. These isolates could be good candidates for further studies on their capacity to produce ginsenosides.

4 Endophytes from Heterologous Species Involved in Transformation of Ginsenoside Monomers

Protopanaxadiols and protopanaxatriols are the major ginsenosides of ginseng and are responsible for varied pharmaceutical properties including antitumor, antiaging, blood vessel softening, anti-inflammatory and hepatoprotective activities [5]. Diglycosylated ginsenosides (minor ginsenosides Rh1, Rh2, F2, Rg2, and Compound K) are more pharmaceutically active than major glycosylated ginsenosides such as Rb1, Rb2, Rc, Rd, Rg1, and others, because of the smaller size, higher bioavailability, and better permeability across the cell membrane [31]. However, minor ginsenosides are not present in natural ginseng plant, or they are present in very smaller quantities. Therefore, the study of converting major ginsenosides to minor ginsenosides is of great significance. Various methods such as heating, hydrolysis by acid/alkali, enzymatic treatment, and microbial transformation (biotransformation) have been followed. Among these, biotransformation was reported to have high specificity, low cost, selective, and environmental friendly method. Biotransformation is defined as chemical reactions catalyzed by various enzymes of various microbial systems, which can be alternative for biosynthesis of minor ginsenosides. Recently, Cui et al. [32] isolated β-glucosidase-producing endophyte from *Platycodon grandiflorum* (balloon flower, family: Campanulaceae, heterologous species) cultivated in Yanji, China, and identified endophyte as *Luteibacter* sp., which showed a strong ability to major ginsenoside monomers

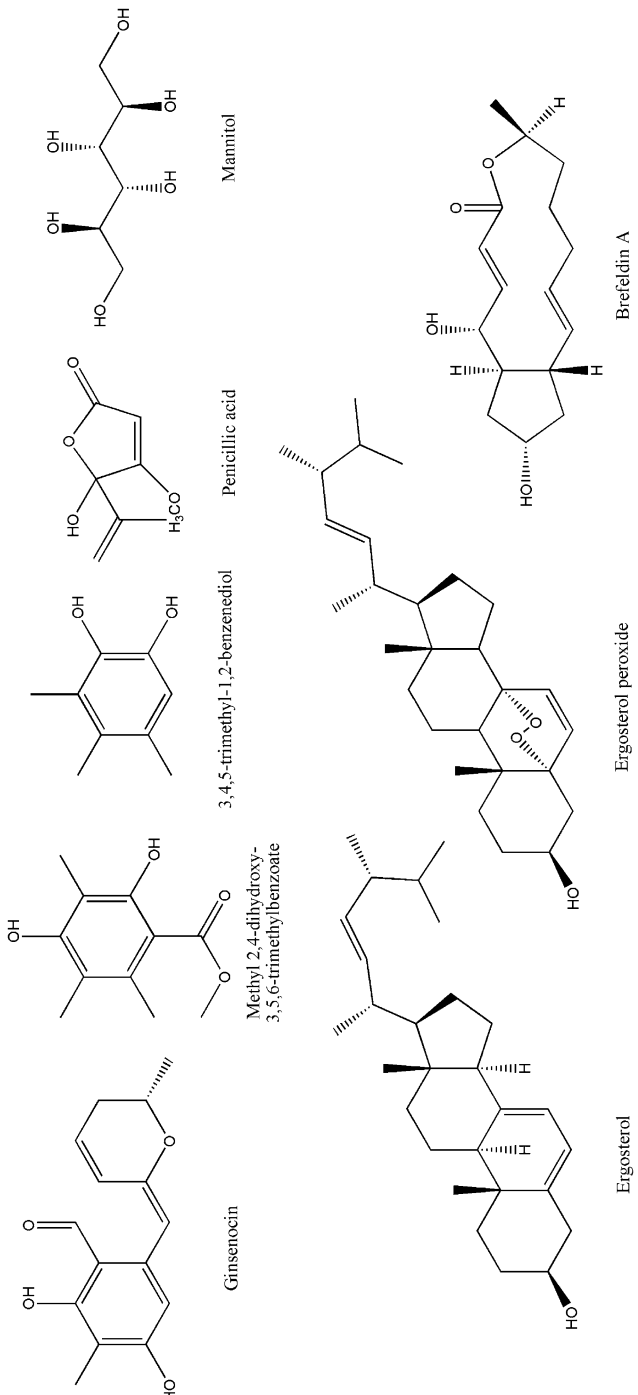


Fig. 4 Structures of cytotoxic compounds isolated from ginseng endophytes, *Penicillium melinii* and *Penicillium janthinellum*

Rb1, Rb2, Rc, Rd, and Rg1 into minor ginsenosides F2, CK, and Rh1. Cui et al. [32] demonstrated that under the optimized fermentation conditions, viz., 3% v/v *Luteibacter* sp., suspension was cultured in 100 ml lysogeny liquid broth medium (set at pH 4) in 250 ml Erlenmeyer's flask containing 1.5 g ginseng total saponins for period of 10 days in a shaking incubator (30 °C, 150 rpm). They compared the change of saponin content before and after fermentation by HPLC analysis, the content of protopanaxadiol-type major saponins (Rb1, Rb2, Rc, and Rd) and protopanaxatriol-type major saponins (Rg1 and Re) was decreased, and Rb1 was almost completely transformed after 10 days of incubation. At the same time, a significant increase in the content of minor ginsenosides F2, CK, and Rh1 and major ginsenosides Rb1, Rb2, Rc, Rd, and Rg1 was observed. They also worked out the transformation pathways of major ginsenosides by endophyte *Luteibacter* sp. and pathways were as follows: Rb1 → Rd → F2 → CK; Rb2 → C-O → CK; Rc → C-Mc1 → C-Mc → CK; and Rg1 → Rh1. The maximum production rate of ginsenosides F2 and CK reached 94.53% and 66.34%, respectively. These results indicate that the endophyte *Luteibacter* sp. which was isolated from heterologous species (*Platycodon grandiflorum*) would be a potential microbial source for conversion of major abundant ginsenosides into minor pharmaceutically rich ginsenosides, and such results are of great importance to pharmaceutical industry.

5 Conclusions

Tremendous research efforts have been made in *Panax ginseng* on isolation, characterization of endophytes, and identification of bioactive compounds from both host and endophytes and assessment of their biological activities. Various species of bacterial and fungal endophytes have been isolated from the cultivated and naturally occurring populations of *Panax ginseng* both from Korea and China. Bacterial and fungal endophytes of ginseng have shown plant growth promotion, antimicrobial, antitumor/anticancer activities and also illustrated promotion of ginsenoside biosynthesis, biotransformation of major ginsenosides into pharmaceutically active ginsenosides. There is scope for isolation of superior bacterial and fungal endophytes which have biological activities. Further, research effort should be made to select out superior endophytic strains which have saponin biosynthetic ability and play a major role in biotransformation of major ginsenosides into pharmaceutically active ginsenosides.

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Endophytism in Zingiberaceae: Elucidation of Beneficial Impact

7

Avijit Chakraborty, Subrata Kundu, Swapna Mukherjee, and Biswajit Ghosh

Contents

Abstract

Endophytism is a unique relationship between plant and endosymbiotic microorganism wherein the microbes colonize within plant tissues without producing any disease etiology. Various groups of endophytes isolated from different medicinal plants are extremely significant in this respect for their ability to synthesize novel bioactive compounds as well as for the modulation of productivity. Endophytes also play various crucial roles in growth, biotic and abiotic stress tolerance, and adaptation. With the implementation of "state-of-the-art" technologies in molecular biology, the specific identification of associated microorganism as well as their relationship with corresponding host plants has been explicitly deciphered in

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recent years. Zingiberaceae, generally recognized as ginger family, comprises of rhizomatous medicinal and aromatic plants and is characterized by the presence of plethora of bioactive compounds along with volatile oils. They are widely cultivated in tropical and subtropical regions of Asia. This chapter aims to explore the endophytic relationship between medicinally important species of Zingiberaceae and the corresponding microbes, for improved production of imminent natural products and their role in protection of host plants from pathogens as well as in stress tolerance, thus helping the plants, indirectly, to grow better.

Keywords
Endophytism · Zingiberaceae · Plant natural products · Antimicrobial activity · Plant growth-promoting bacteria · Medicinal plants

1 Introduction

Endophytism is an exclusive relationship between plant and bacterial or fungal microorganism wherein the microbes colonize within healthy plant tissues without producing any disease symptom. This complex association can be either mutualistic or antagonistic and is almost ubiquitous among most of the plants examined till date. The term "endophyte" was first coined about 150 years ago for pathogenic fungi infecting the plants [1]. It originates from Greek, "endo" denoting within and "phyte" meaning plant. Since the introduction of the term, many scientists have been involved to redefine it. Galippe was the first scientist to hypothesize that numerous vegetable plants host microbes within it, and these microbes are originated from soil [2]. Although scientist di Vestea agreed with this postulate, most of the renowned scientists including Pasteur, Chamberland, Fernbach, and Laurent were against Galippe's proposal and established that the plants are free of any kind of microbes [3, 4]. However, it is well established that the plants generally host a wide range of phylogenetically distinct endophytes in various organs [5] and that almost all of these microbes are derived from the soil environment [4, 6–8]. Nevertheless, endophytes were considered as "any microorganism if it can be isolated or extracted from inside surface of disinfected plant tissue and it does not seemingly harm the plant" [9]. Conversely, due to the lack of suitable techniques for removal of nucleic acids after sterilization of plant surfaces, this definition appeared to be less suitable for non-cultured species upon the exclamation of molecular biology techniques in endophyte research. Therefore, the improved definition of endophytes was proposed by Coombs and Franco as "ubiquitous colonizers of the interior tissues of host plants and can constitute a range of different relationships such as symbiotic, mutualistic, and commensalistic where they do not usually cause any substantial morphological changes and disease symptoms" [10].

The Zingiberaceae family includes about 52 genera and more than 1300 species that are dispersed throughout tropical Africa, Asia, and the America. This family is enriched with aromatic perennial herbs with creeping horizontal or tuberous

rhizomes, and many species are economically important as ornamental plants, spices, or folk medicine. It also includes vital groups of medicinal plants with volatile essential oils and oleoresins of export quality. The secondary metabolites extracted from different genera of Zingiberaceae including *Curcuma*, *Kaempferia*, *Hedychium*, *Amomum*, *Zingiber*, *Alpinia*, and *Elettaria* have antimicrobial, anti-arthritic, antioxidant, anticancer, anti-inflammatory, and antidiabetic properties. The essential oil of the rhizome of *Kaempferia galanga* has been reported to constitute over 54 components including derivatives of cyclohexene oxide and diterpenes that have insecticidal properties [11–14]. Saponin, an essential bioactive compound synthesized by the plant species *Costus speciosus*, was found to have anti-conidial germinal effect on the most effective pathogen, i.e., *Botrytis cinerea* and *Alternaria* sp. [15]. *Alpinia* is the genus under Zingiberaceae family and an important medicinal herb that stimulates digestion, blood purification, and antifungal activity [16–19]. *Zingiber officinale*, an important aromatic medicinal plant, contains essential oil with versatile biological potential including antirhinoviral activity [20, 21]. The root of *Hedychium spicatum* is useful in asthma, pains, inflammations, foul breath, vomiting, bronchitis, and "tridosha" diseases of the blood [22]. *Kaempferia angustifolia* is an aromatic medicinally important rhizomatous plant and has potential in the treatment of fever, cold, coughs, diarrhea, stomach ache, and dysentery [23, 24].

The endophytes associated with different family members of Zingiberaceae play pivotal roles in growth, development, fitness, as well as induction of several bioactive secondary metabolites. There are several such reports on the existence and beneficial roles of diverse endophytic microorganism within different genera of Zingiberaceae. To the best of our knowledge, limited cumulative information are available regarding the endosymbiotic microorganisms associated with the entire family of Zingiberaceae. The comprehensive information on endophytes provides understanding into the complex nature of the microbiome connected with Zingiberaceae family. Therefore, the objective of the present chapter was to explore different types of endophytes and their multipartite interactions with host plants along with favorable impression within the entire family of Zingiberaceae.

2 Decoding the Molecular Interaction in Endophytism

Knowingly, a complex interaction exists between host and endophytes although suitable methods to study *in planta* mechanisms are unavailable. Only limited reports are there elucidating comprehensive mechanisms of plant-endophyte interactions [25–27]. The phenomenon of endophytism primarily depends upon the genotype of plant and the corresponding strain of endophyte [28, 29]. It has been reported that chemoperception systems within the plants sense the existence of secretory molecules from endophytes [30]. This interaction activates a cascade of signal transduction pathway that induces the expression of defense-related genes within the host plants [29]. Thus, endophytism produces reactive oxygen species (ROS) within the plants and stimulates the synthesis of antioxidant

enzymes [31, 32]. Nevertheless, some endophytes also modulates host metabolism by altering the nutrient uptake and homeostasis [25, 33]. During endophytism, the magnitude of induction of pathogenesis-related genes in the absence of pathogenic determinants is relatively lower [34]. However, these are the preliminary research works on the mechanistic feature of endophytism; comprehensive molecular events can be explored through the application of "omics" technologies including metagenomics, metabolomics, and transcriptomics. Thus cumulative approaches have increased potential for analyzing total microbial community in endophytic relationship with host plants. Nevertheless, functional assay of the whole microbiome also offers novel opportunity to explore biogeochemical environments, multifaceted ecosystems related to host organisms, metabolism, and interactions between them. These aforementioned systematic approaches accompanied with progressive computational tools (system biology) are the crucial tactics to elucidate comprehensive biochemical and environmental interactions happening within host plants and associated microbial community.

3 Impact of Endophytic Microorganisms on Plants

The endophytes can induce growth of the plants by fixing atmospheric nitrogen, producing phytohormones, regulating the growth of phytopathogens by accumulating secondary metabolites or through enhanced uptake of minerals [6, 35, 36]. Beneficial properties of different types of endophytes were represented in Fig. 1. The magnitude of growth enhancement by endophytic community is highly dependent on the soil that encourages colonization and compatibility between plant and endophytes [37–39]. Furthermore, endophytes produce various bioactive compounds including benopyranones, alkaloids, flavonoids, chinones, phenolics, steroids, and terpenoids that have immense potential in medicine and agrochemical industries as well as in biotechnological applications [40–44]. The endophytes also play pivotal role in the maintenance of soil nutrients and make them accessible to each component of the ecosystem. They have the potential to degrade complex compounds into simpler compatible form that can be assimilated by plants [45]. Nevertheless, endophytes secrete several enzymes including amylase, pectinase, cellulase, lipase, and proteinase that are associated with biodegradation and hydrolytic processes during plant-pathogen interaction as well as for the biodegradation of litter of the host plant [46, 47].

4 Diversity of Endophytes Associated with the Family Zingiberaceae

The endophytic organisms associated with Zingiberaceae family have been isolated from different parts including midrib segment leaf, meristem, roots, stem, leaf blade, and petiole. They are generally isolated by surface sterilization followed by culturing from crushed tissue extract or through culturing of plant tissues on

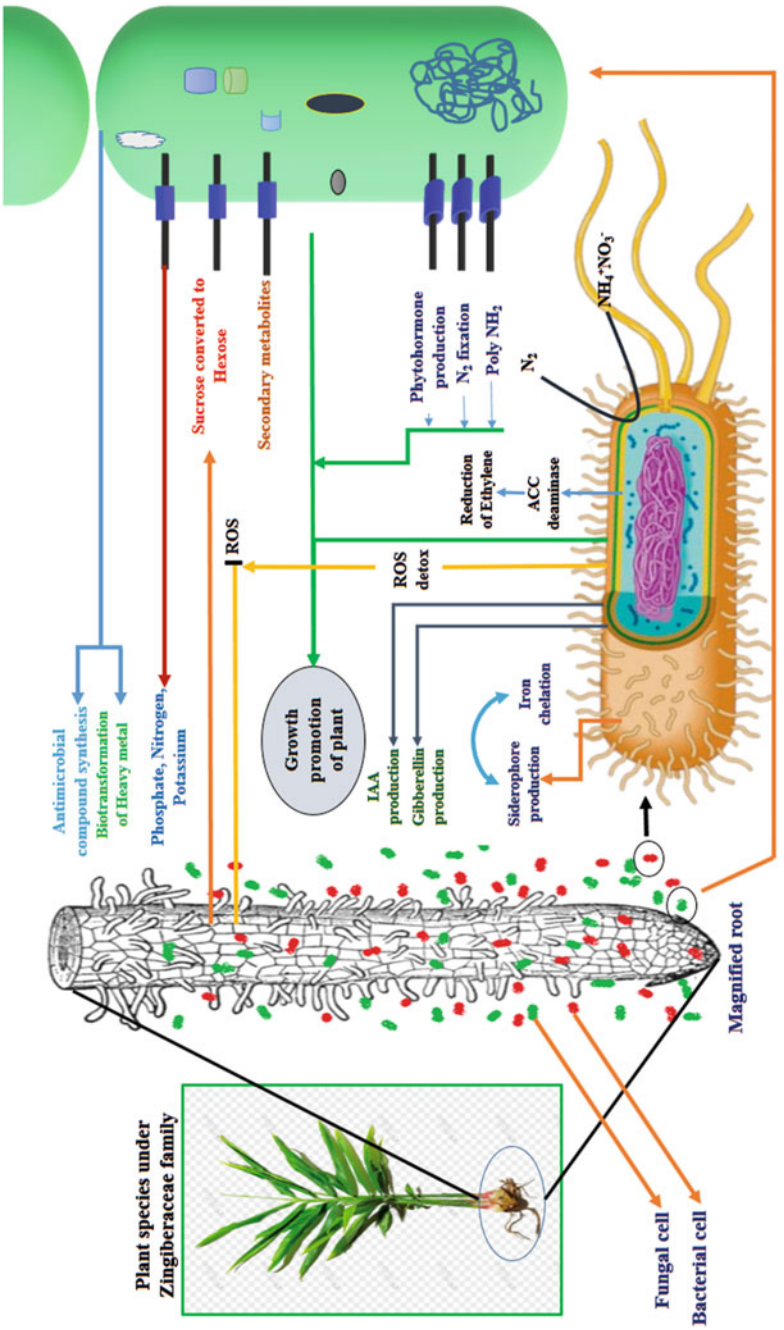

Fig. 1 Schematic representation of beneficial impact of different types of endophytes

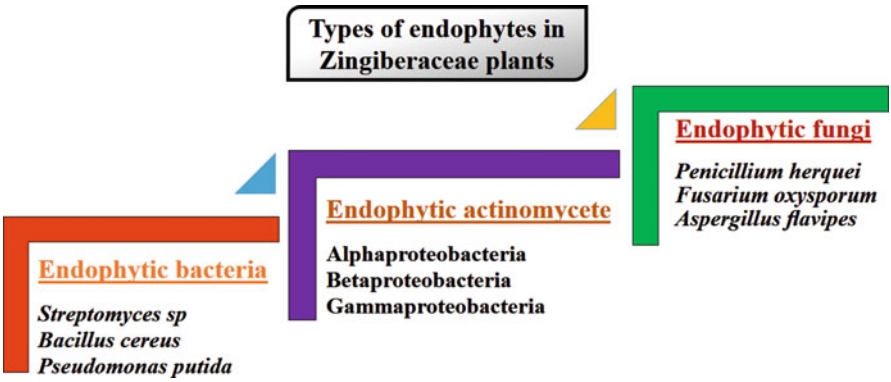

Fig. 2 Different types of endophytes associated with Zingiberaceae family

suitable media [48, 49]. The identification of endophytes was executed based on morphological characteristics corroborated with biochemical tests. The modern molecular biological techniques including ribosomal DNA internal transcribed spacer (ITS) sequence analysis have also been explored in identification of microorganisms as well as their phylogenetic relationships. The endophytes associated with Zingiberaceae are highly diverse (Fig. 2). Different types of endophytes including fungus and bacteria within Zingiberaceae family are represented in Table 1. Fourteen bacterial endophytes, isolated from the rhizome of *Curcuma longa*, were characterized through 16S rRNA sequence analysis [50]. In another study, 11 endophytic fungal strains have been reported from *Curcuma longa* plant [51]. Different parts of the plant including leaf, rhizome, root, and stem of *Zingiber officinale* Rosc., an important plant with medicinal properties, are also associated with various types of endophytes [52–54]. Different types of endophytic bacteria were also identified from *Alpinia galanga*, another important member of this family [55–57].

5 Advantageous Imprint Within the Zingiberaceae Family due to Endophytism

The diverse groups of endophytes associated with Zingiberaceae family are responsible for different types of advantageous functions including growth promotion, synthesis of unique medicinally important bioactive compounds, biocontrol agent, antimicrobial activity, and protection against different types of biotic stress (Fig. 3).

5.1 Plant Growth Promotion by Endophytes

The microorganisms that are capable of providing different types of benefits to the plants are known as plant growth-promoting microorganism [58]. Among different

Table 1 Diversity of endophytes associated with the plants belonging to the family Zingiberaceae

Plant	Associated endophytes		Reference
	Bacteria	Fungi	
Alpinia galanga	*Streptomyces* sp. Tc022; *Nocardia*; *Microbispora*; *Micromonospora*; *Streptomyces* sp. LJK109		[55–57]
Alpinia officinarum		*Marasmius* sp.; *Penicillium herquei*; *Fusarium oxysporum*; *Colletotrichum siamense*; *Pestalotiopsis* sp.; *Sebacina vermifera*; *Exserohilum* sp.; *Mycoleptodiscus* sp.; *Mycoleptodiscus indicus*; *Scopulariopsis hibernica*; *Meyerozyma guilliermondii*; *Exserohilum* sp.	[92]
Alpinia malaccensis		*Aspergillus flavipes*; *Cladosporium* sp.; *Cladosporium oxysporum*; *Colletotrichum boninense*; *Exophiala* sp.; *Exophiala lecanii-corni*; *Guignardia mangiferae*; *Penicillium citrinum*; *Pyricularia costina*; *Ochroconis gallopava*; *Colletotrichum cliviae*; *Colletotrichum gloeosporioides*; *Diaporthe gardeniae*	[93]
Boesenbergia rotunda	*Streptomyces* sp.; *Asanoa endophytica* sp.		[85, 86]
Curcuma longa	*Bacillus cereus*; *Bacillus thuringiensis*; *Bacillus* spp.; *Bacillus pumilus*; *Pseudomonas putida*; *Clavibacter Michiganensis*; *Stenotrophomonas maltophilia*; *Bacillus safensis*; *Brevibacterium halotolerans*; *Bacillus pumilus*	*Arthrobotrys foliicola*; *Cochliobolus kusanoi*; *Daldinia eschscholzii*; *Fusarium oxysporum*; *Fusarium proliferatum*; *Fusarium solani*; *Fusarium verticillioides*; *Phaeosphaeria ammophilae*; *Phanerochaete chrysosporium*	[50, 51, 94]
Curcuma heyneana	*Agrobacterium larrymoorei*; *Sphingomonas* sp.; *Herbaspirillum* sp.; *Sphingomonas paucimobilis*; *Agrobacterium tumefaciens*; *Bacillus aerophilus*; *Enterobacter asburiae*; *Brevundimonas* sp.; *Chromobacterium aquaticum*; *Enterobacter cancerogenus*;		[95]

(*continued*)

Table 1 (continued)

Plant	Associated endophytes		Reference
	Bacteria	Fungi	
	Microbacterium testaceum; *Enterobacter asburiae*; *Curtobacterium plantarum*; *Microbacterium laevaniformans*; *Rhizobium alamii*; *Agrobacterium larrymoorei*		
Curcuma xanthorrhiza		*Fusarium* cf. *oxysporum*; *Fusarium* cf. *solani*; *Eupenicillium* sp.; *Actinomycetes*; *Xylaria* sp.	[96, 97]
Hedychium coronarium		*Trichoderma* sp.; *Mycelia sterilia*; *Penicillium* sp.; *Alternaria* sp.; *Penicillium* sp.; *Fusarium* sp.; *Aspergillus* sp.; *Bipolaris* sp.; *Nigrospora* sp.	[93]
Hedychium acuminatum		*Fusarium oxysporum*; *Rhizoctonia* sp.; *F. solani*; *Colletotrichum alienum*; *C. aotearoa*; *Aspergillus parasiticus*; *Hansfordia biophila*; *F. semitectum*; *C. coccodes*; *C. gloeosporioides*	[84]
Hedychium flavescens		*Colletotrichum* sp.; *Fusarium* sp.; *Bipolaris* sp.; *Pithomyces* sp.; *Mucor* sp.; *Alternaria* sp.; *Mycelia sterilia*; *Rhizopus* sp.; *Fusarium* sp.; *Cladosporium* sp.	[93]
Hornstedtia conica		*Aspergillus flavipes*; *Cladosporium* sp.; *Colletotrichum boninense*; *Diaporthe* sp.; *Diaporthe anacardii*; *Exophiala* sp.; *Exophiala lecanii-corni*; *Penicillium citrinum*; *Pyricularia costina*; *Arthrinium malaysianum*; *Colletotrichum cliviae*; *Sydowiellaceae*; *Ochroconis gallopava*	[93]
Kaempferia rotunda	*Actinobacteria*		[96]
Stahlianthus campanulatus	*Nonomuraea stahlianthi*		[98]
Zingiber officinale	*Streptomyces aureofaciens*	*Acremonium macroclavatum*; *Cochliobolus geniculatus*;	[52–54]

(*continued*)

Table 1 (continued)

Plant	Associated endophytes		Reference
	Bacteria	Fungi	
		Colletotrichum gloeosporioides; *Curvularia affinis*; *Fusarium oxysporum*; *Fusarium solani*; *Glomerella cingulata*; *Lecanicillium kalimantanense*; *Leiosphaerella lycopodina*; *Myrothecium verrucaria*; *Neonectria punicea*; *Periconia macrospinosa*; *Rhizopycnis vagum*; *Talaromyces assiutensis*; *Ascomycota* sp.; *Fusarium* sp.; *Gliocladiopsis*; *Acremonium furcatum*; *Trichothecium* sp.; *Colletotrichum crassipes*; *Aspergillus niger*; *Phlebia* sp.; *Fusarium oxysporum*; *Earliella scabrosa*; *Pseudolagarobasidium* sp.; *Cerrena* sp.	
Zingiber montanum	*Pseudomonas baetica*		[99]

types of microorganism, several genera of bacteria are proficient to encourage plant growth through plentiful independent or interconnected pathways [4]. The endophytic bacteria, associated with Zingiberaceae family, can stimulate growth by synthesizing phytohormones, modulating the uptake of minerals from soil, maintaining iron homeostasis, and increasing availability of phosphorus (Table 2).

5.1.1 Production of Phytohormone Indole-3-Acetic Acid

The plant growth-promoting phytohormone indole-3-acetic acid (IAA) is responsible for the induction of root growth in plant. It has been reported that the bacteria including *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus pumilus*, *Pseudomonas putida*, and *Clavibacter michiganensis* isolated from *Curcuma longa* are capable of synthesizing IAA in associative condition with the plant [59]. In another study, IAA producing bacteria including *Pseudomonas aeruginosa*, *Enterobacter* sp., and *Acinetobacter* sp. were also isolated from *C. longa* [60]. The bacteria of *Paenibacillus* sp., found in *C. longa* rhizome, also have the ability to synthesize IAA and have been reported to promote the growth of the plant species [61]. The endophytic bacteria of *Pseudomonas* sp. isolated from *Z. officinale* were also found responsible for the production of IAA [62]. Nineteen endophytic bacteria, including *P. putida*, *L. adecarboxylata*,

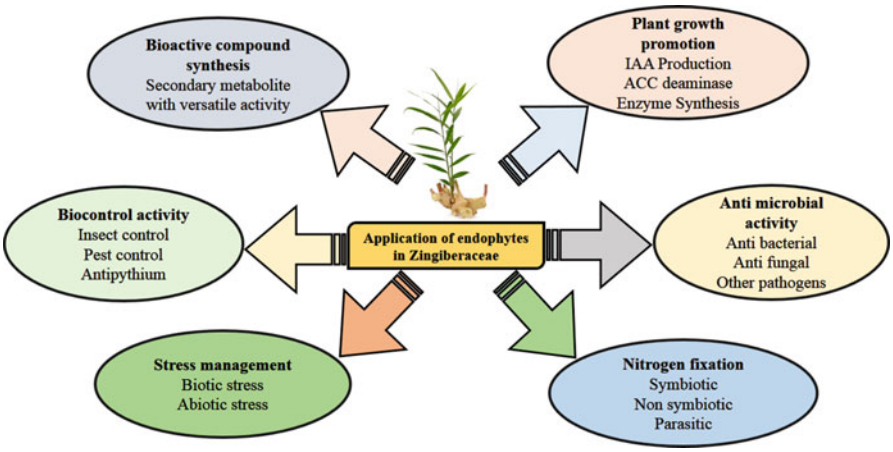

Fig. 3 Advantageous roles of endophytes within Zingiberaceae family

S. nematodiphila, *Pantoea ananatis*, *A. pasteurianus*, *S. maltophilia*, *A. trotsa*, and *A. larrymoorei*, were reported to be able to produce IAA and could promote the growth in ginger plant [63]. In another study, about 57 endophytic bacteria, isolated from ginger rhizome and identified through 16S rDNA PCR-RFLP fingerprinting, 14 bacteria of genera including *Agrobacterium*, *Ochrobactrum*, *Bacillus*, *Serratia*, *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Enterobacter*, and *Tetrathiobacter* were found to be capable of synthesizing IAA [64]. Another study showed that one of the *Klebsiella* spp., present in the rhizome of the same plant, also helps in the production of the important hormone IAA [65].

5.1.2 Maintenance of Iron Homeostasis Through Production of Siderophore

Iron acts as an essential element for the growth of all living organisms due to its diverse biological activities including electron transfer, oxygen metabolism, enzymatic reactions, etc. [66]. Under low bioavailability condition, different types of microorganisms follow some specific iron uptake strategies such as the production of siderophores [67, 68]. The extracellular chelating compounds, siderophores, are divided into four major categories: rhodotorulic acid, fusarinines, coprogens, and ferrichromes. Although its primary function is to convert soil iron into a soluble form under iron starvation, its complex forming ability with other essential elements of siderophores is also reported [67, 68]. It has also been recommended that these compounds play pivotal role in the induction of systemic resistance against bacterial, fungal, and viral diseases [69–71]. The endophytic bacteria *Pseudomonas putida* were able to synthesize siderophore in the plant *C. longa* and could be able to provide protection against other pathogenic microbes [59]. In another study, it has been reported that the synthesis of siderophore protects turmeric against rot and leaf

Table 2 Plant growth-promoting activity of endophytes associated with Zingiberaceae family

Plant Name	Associated Endophytes		PGPR activity	Reference
	Bacteria	Fungi		
Alpinia malaccensis		*Aspergillus flavipes*; *Cladosporium* sp.; *Cladosporium oxysporum*; *Colletotrichum boninense*; *Exophiala* sp.; *Exophiala lecanii-corni*; *Guignardia mangiferae*; *Penicillium citrinum*; *Pyricularia costina*; *Ochroconis gallopava*; *Colletotrichum cliviae*; *Colletotrichum gloeosporioides*; *Diaporthe gardenia*	Amylase, cellulase, pectinase, and asparaginase activity	[93]
Amomum siamense		*Eupenicillium crustaceum*; *Fusarium* spp.; *Glomerella* spp.; *Phomopsis* spp.; *Phyllosticta* spp.; *Pyricularia* spp.; *Talaromyces flavus*; *Xylariaceae taxa*; *Mycelia sterilia*	Absorption of soil nutrients including phosphorus	[75]
Curcuma longa	*Stenotrophomonas maltophilia*; *Bacillus safensis*; *Brevibacterium halotolerans*; *Bacillus pumilus*		Nitrate reduction, H_2S production, starch hydrolysis, IAA production, phosphorus solubilization, siderophore production,	[59]
Curcuma heyneana	*Agrobacterium larrymoorei*; *Sphingomonas* sp.; *Herbaspirillum* sp.; *Sphingomonas paucimobilis*; *Agrobacterium*		Nitrogen fixation	[95]

(*continued*)

Table 2 (continued)

Plant Name	Associated Endophytes		PGPR activity	Reference
	Bacteria	Fungi		
	tumefaciens; *Bacillus aerophilus*; *Enterobacter asburiae*; *Brevundimonas* sp.; *Chromobacterium aquaticum*; *Enterobacter cancerogenus*; *Microbacterium testaceum*; *Enterobacter asburiae*; *Curtobacterium plantarum*; *Microbacterium laevaniformans*; *Rhizobium alamii*; *Agrobacterium larrymoorei*			
Hedychium coronarium		*Trichoderma* sp.; *Mycelia sterilia*; *Penicillium* sp.; *Mycelia sterilia*; *Alternaria* sp.; *Penicillium* sp.; *Fusarium* sp.; *Aspergillus* sp.; *Bipolaris* sp.; *Nigrospora* sp.	Amylase, cellulase, pectinase, and asparaginase activity	[93]
Hedychium flavescens		*Colletotrichum* sp.; *Fusarium* sp.; *Bipolaris* sp.; *Pithomyces* sp.; *Mucor* sp.; *Alternaria* sp.; *Mycelia sterilia*; *Rhizopus* sp.; *Fusarium* sp.; *Cladosporium* sp.	amylase, cellulase, pectinase, and asparaginase activity	[93]
Stahlianthus campanulatus	*Nonomuraea stahlianthi* sp.		Nitrate reduction, carbon utilization	[98]

blight diseases caused by *Pythium aphanidermatum* (Edson) Fitzp. and *Rhizoctonia solani* Kuhn. [72]. Siderophore production, in the root of *Zingiber officinale* by different species of *Pseudomonas* and *Stenotrophomonas*, has also been reported by Jasim et al. 2014 [62].

5.1.3 Phosphate Solubilization

Insoluble phosphate is often present in the soil, but plant is unable to take up this form of phosphate. The soluble form is most important mineral for the growth of the plants and enhances crop productivity [73, 74]. Bacterial and fungal endophytes, associated with plant root, can help to solubilize the phosphate and convert insoluble phosphate to the common soluble form, so that plant can take up easily [64]. *Bacillus cereus*, *Bacillus* sp., *Bacillus pumilus*, and *Pseudomonas putida*, present in the roots and associated area of the important medicinal *C. longa*, promote the growth of the plant by inducing availability of phosphate [50, 59]. It has also been reported that the strain of *Klebsiella* sp., present in the rhizome of the turmeric plant, can also promote the growth of the plant by transforming insoluble phosphate into soluble form [65]. The endophytes associated with *Amomum siamense* were found to help the host plant in absorption of soil nutrients, such as phosphorus and other essential molecules that can stimulate the growth of the plant [75].

5.1.4 ACC Deaminase Production

It is well accepted that the plant growth-promoting bacteria (PGPB) that produce the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase facilitates plant growth by lowering the plant ethylene levels that, when present in high concentrations, can be detrimental to plants. The enzyme ACC deaminase actually catalyzes the cleavage of 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor, into ammonia and α-ketobutyrate. Thus, the ACC deaminase-producing bacteria help the plant growth by reducing a portion of ethylene biosynthesis. Many members of the genera *Azospirillum*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Burkholderia*, *Ralstonia*, *Pseudomonas*, and *Enterobacter*, along with various endophytic strains, produce ACC deaminase [76]. Some strains of *Pseudomonas* sp., found in association with *Zingiber officinale*, can produce ACC deaminase and hence can suppress the effect of the hormone ethylene and can enhance the growth of the plant [62]. Pseudomonas-related production of ACC deaminase is also reported by another group of researchers [77]. Ethylene-mediated plant growth inhibition decreases with the decrease of the level of ACC and ethylene. Endophytic microbes increase the plant growth by residing inside the host plants with these capabilities and can promote the growth of the host plant by reducing ethylene-inducing stresses [7, 78].

5.2 Synthesis of Bioactive Compounds

Bioactive natural products represent the class of compounds synthesized by living organisms with high therapeutic value. These compounds are secondary metabolites and produced mostly by plants and microbes – alone or in association with each other. Different endophytic microbes including fungi, bacteria, and actinomycetes produce varied types of bioactive compounds like terpenoids, alkaloids, flavonoids, steroids, peptides, quinols, and phenols. These bioactive secondary metabolites, produced by endophytes, are usually used by plants for defense against abiotic and

biotic stresses. Nevertheless, a plethora of these compounds has been confirmed to be valuable for novel drug synthesis in pharmaceutical industry. It has been reported that hundreds of these natural products serve as important sources of anticancer, antioxidant, antibacterial, antidiabetic, antifungal, immunosuppressive, insecticidal, and antiviral agents [79–81]. It is also established that these secondary metabolites are associated with cell signaling, alleviation of biotic and abiotic stress, and establishment of symbiosis [82]. Recently, it has also been reported that apart from the production of secondary metabolites, endophytes are also able to modulate the secondary metabolism within their host plant [83]. There are reports of production of several important secondary metabolites by various endophytic microorganisms in different members of Zingiberaceae (Table 3; Fig. 4). In the medicinal plant *Alpinia galanga*, the important secondary metabolites including kaempferol, scutellarin, umbelliferone, and actinomycin D were found to be synthesized by different endophytic bacteria [55, 56]. In another plant, *Hedychium accuminatum*, 11 endophytic fungi were found to be responsible for the production of various metabolites including alkaloids, flavonoids, terpenoids, and tannins [84]. The endophytic species of *Streptomyces* (strain BO-07), isolated from the root tissue of *Boesenbergia rotunda*, was found to contain two biphenyls, 3′-hydroxy-5-methoxy-3,4-methylenedioxybiphenyl and 3′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxybiphenyl, with antibacterial, antioxidant, and anticancer activities [85]. Fifteen fungal strains including the genera of *Acremonium*, *Gliocladiopsis*, *Fusarium*, *Colletotrichum*, *Aspergillus*, *Phlebia*, *Earliella*, and *Pseudolagarobasidium* were found to be associated with *Z. officinale* [53]. The fungal bodies extracted from this plant were subjected to metabolite profiling and found to contain many bioactive compounds including tyrosol, dehydromevalonic lactone, ergone, *N*-aminopyrrolidine, benzene acetic acid, linoleic acid, oleic acid, myristic acid, n-hexadecanoic acid, palmitic acid, and methyl ester. In another report, *Streptomyces aureofaciens* CMUAc130 was isolated from *Z. officinale* root [54], and compounds like 5,7-dimethoxy-4-p-methoxylphenylcoumarin and 5,7-dimethoxy-4-phenylcoumarin, with antifungal activity, were isolated from the bacterial extract. The bacterial strain of *Streptomyces* BT01, isolated from *Boesenbergia rotunda* (L.), was identified through 16S rDNA sequencing [86]. Two important flavonoids with antibacterial activity, namely, 7-methoxy-3,3′,4′,6-tetrahydroxyflavone and 2′,7-dihydroxy-4′,5′-dimethoxyisoflavone, were identified by thin-layer chromatography. The secondary metabolites like cyclo-(L-tryptophanyl-L-prolyl) (3S, 8aR)-3-(1H-indol-3ylmethyl) hexahydropyrrolo [1, 2-a] pyrazine-1, 4-dione, CAP 2,2-dichloro-*N*-[(1R, 2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl]-acetamide (diketopiperazine cyclo-(tryptophanyl-prolyl), and chloramphenicol, isolated from *Streptomyces* sp., an endophytic association of rhizomatous root of *Zingiber spectabile* [87], showed anti-methicillin resistance against *S. aureus* activity.

Several endophytic bacteria are capable of synthesizing a vast range of uncharacterized secondary metabolites. There are limited information regarding the amount and conditions under which these metabolites are produced. The revolution in the field of genomics along with expansion of modern analytical techniques will definitely hasten the detection of such cryptic compounds and will assist to

Table 3 Endophyte associated with the plants belonging to family Zingiberaceae and their bioactive compounds

Plant	Associated Endophytes		Active compound	Reference
	Bacteria	Fungi		
Alpinia galanga	*Streptomyces* sp. Tc022; *Nocardia*; *Microbispora*; *Micromonospora*; *Streptomyces* sp. LJK109		Kaempferol, scutellarin, umbelliferone, ctinomycin D	[55, 56]
Boesenbergia rotunda	Strain BO-07 *Streptomyces* sp.		3′-Hydroxy-5-methoxy-3,4-methylenedioxybiphenyl and 3′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxybiphenyl	[85]
Curcuma xanthorrhiza		*Fusarium* cf. *oxysporum*; *Fusarium* cf. *solani*; *Eupenicillium* sp.; *Actinomycetes*; *Xylaria* sp.	$C_{17}H_{16}O$, xylarugosin, and resacetophenone	[97]
Hedychium acuminatum		*Fusarium oxysporum*; *Rhizoctonia* sp.; *F. solani*; *Colletotrichum alienum*; *C. aotearoa*; *Aspergillus parasiticus*; *Hansfordia biophila*; *F. semitectum*; *C. coccodes*; *C. gloeosporioides*	Secondary metabolite	[84]
Zingiber officinale		*Ascomycota* sp.; *Fusarium* sp.; *Gliocladiopsis*; *Acremonium furcatum*; *Trichothecium* sp.; *Colletotrichum crassipes*; *Aspergillus niger*; *Phlebia* sp.; *Fusarium oxysporum*; *Earliella scabrosa*; *Pseudolagarobasidium* sp.; *Cerrena* sp.	Tyrosol, benzene acetic acid, ergone, dehydromevalonic lactone, N-aminopyrrolidine, and many bioactive fatty acids and their derivatives which included linoleic acid, oleic acid, myristic acid, n-hexadecanoic acid, palmitic acid methyl ester, and methyl linoleate	[53]
Zingiber officinale	*Streptomyces aureofaciens*		5,7-Dimethoxy-4-phenylcoumarin (51b), 5,6-dimethoxy-4-(p-methoxyphenyl)	[55]

(*continued*)

Table 3 (continued)

Plant	Associated Endophytes		Active compound	Reference
	Bacteria	Fungi		
			coumarin (51c) vanillin 3-methoxy-4-hydroxytoluene	
Zingiber spectabile	*Streptomyces omiyaensis* NBRC 13449T		prolyl (3S, 8aR)-3-(1H-indol-3-ylmethyl) hexahydropyrrolo [1, 2-a], pyrazine-1, 4-dione CAP 2,2-dichloro-*N*-[(1R, 2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl]-acetamide	[87]

explore the wide spectrum of secondary metabolites with novel chemical structures encoded by the genomes of endophytic microorganisms of the entire Zingiberaceae family.

5.3 Antimicrobial Activity of Endophytes

The diverse groups of endophytes isolated from plants of Zingiberaceae family are recognized for their antimicrobial properties (Table 4). The crude extract of bacterial strain of *Streptomyces* sp. LJK109, isolated from root tissues of *Alpinia galanga* [88], showed antifungal potential against pathogenic fungi *Alternaria porri*, *Colletotrichum gloeosporioides*, *Colletotrichum musae*, *Curvularia* sp., *Drechslera* sp., *Exserohilum* sp., *Fusarium oxysporum*, *Verticillium* sp., and *Sclerotium rolfsii*. The endophytic fungi, isolated and identified via internal transcribed spacer (ITS) region of ribosomal DNA from *Curcuma longa*, have antibacterial potential against histamine-producing bacteria *Morganella morganii* [51]. Several endophytic actinomycetes were isolated from *Zingiber officinale* rhizome among which Nocardiopsis sp. ZoA1 was found to be highly active against phytopathogen and human clinical pathogens [89].

5.4 Biocontrol Activity of Endophytes

Some of the microbes often prevent the deleterious effects of the potent plant pathogens including bacteria, fungi, and nematodes. This is considered as biocontrol activity. In agriculture, biocontrol microorganisms have appeared as harmless replacements of chemical pesticides. They also cause indirect promotion of plant growth by limiting the damage caused by their phytopathogens. The endophytes

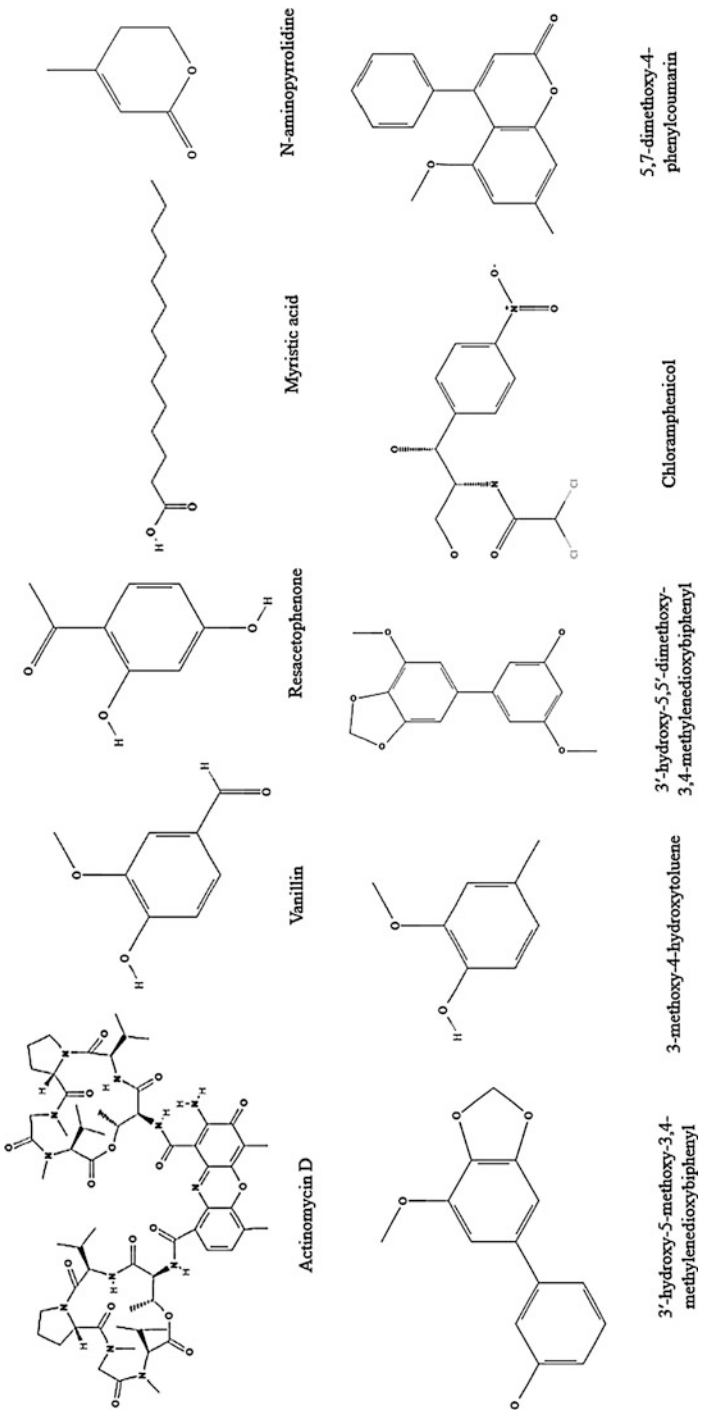

Fig. 4 The chemical structure of different secondary metabolites associated with various endophytic microorganisms

Table 4 Antimicrobial activity of endophytes associated with plants belonging to family Zingiberaceae

Plant	Associated Endophytes		Antimicrobial activity	Reference
	Bacteria	Fungi		
Alpinia galanga	*Streptomyces* sp. Tc022; Nocardia; Microbispora; Micromonospora; *Streptomyces* sp. LJK109		Antifungal activity	[56, 88]
Boesenbergia rotunda	*Streptomyces* sp.; *Asanoa endophytica* sp.		Antibacterial activity	[86]
Curcuma longa	*Bacillus cereus*; *Bacillus thuringiensis*; *Bacillus* sp.; *Bacillus pumilus*; *Pseudomonas putida*; *Clavibacter Michiganensis*; *Stenotrophomonas maltophilia*; *Bacillus safensis*; *Brevibacterium halotolerans*; *Bacillus pumilus*	*Arthrobotrys foliicola*; *Cochliobolus kusanoi*; *Daldinia eschscholzii*; *Fusarium oxysporum*; *Fusarium proliferatum*; *Fusarium solani*; *Fusarium verticillioides*; *Phaeosphaeria ammophilae*; *Phanerochaete chrysosporium*	Antibacterial and antifungal activity	[51, 94]
Curcuma xanthorrhiza		*Fusarium* cf. *oxysporum*; *Fusarium* cf. *solani*; *Eupenicillium* sp.; *Actinomycetes*; *Xylaria* sp.;	Antibacterial activity	[97]
Kaempferia rotunda	*Actinobacteria*		Antibacterial activity	[96]

associated with different members of Zingiberaceae can efficiently remove plant pathogens (Table 5). There is a potent pathogen *Colletotrichum gloeosporioides* that causes leaf spot on turmeric and is responsible for major economic damage in cultivation of turmeric. It has been reported that about 200 endophytic fungal strains, isolated from *Curcuma longa*, have antagonistic effect on this pathogen [90]. In another study, five endophytic fungi isolated from turmeric have been found to have biocontrol activity against *Morganella morganii* [51]. Thirty-one endophytes from the rhizome of *C. longa*, isolated and identified by morphological and ITS analysis and by rDNA sequencing, were found to be active against *Pythium aphanidermatum* (Edson) Fitzp, the causal agent of turf rot blights [72]. In another report, 154 endophytes were identified from turmeric with anti-*Pythium* activity [91]. The endophytic strain isolated from *Zingiber officinale* was very much effective

Table 5 Biocontrol activity of endophytes associated with Zingiberaceae family

Plant	Associated endophytes		Effects	Reference
	Fungi	Bacteria		
Alpinia galanga		*Streptomyces* sp. Tc022	Effective against pathogen *Colletotrichum musae* and *Candida albicans*	[55]
Amomum siamense	*Eupenicillium crustaceum*; *Fusarium* spp.; *Glomerella* spp.; *Phomopsis* spp.; *Phyllosticta* spp.; *Pyricularia* spp.; *Talaromyces flavus*; *Xylariaceae taxa*; *Mycelia sterilia*		Protect from insect attack	[75]
Curcuma longa	*Trichoderma harzianum*		Antagonistic activity against *Pythium aphanidermatum*	[72]
		Bacillus cereus	Inhibit growth of *Rhizoctonia solani*	
	Arthrobotrys foliicola; *Cochliobolus kusanoi*; *Daldinia eschscholzii*; *Fusarium oxysporum*; *Fusarium proliferatum*; *Fusarium solani*; *Fusarium verticillioides*; *Phanerochaete chrysosporium*, *Phaeosphaeria ammophilae*		Inhibit growth of the histamine-producing bacteria	[51]
	Fungal isolates		Leaf spot disease	[90]
		Bacillus; *Pseudomonas*; *Klebsiella*; *Citrobacter*	*Pythium aphanidematum*	[91]
Zingiber officinale	*Acremonium macroclavatum*; *Cochliobolus geniculatus*; *Colletotrichum gloeosporioides*; *Curvularia affinis*; *Fusarium oxysporum*; *Fusarium solani*; *Glomerella cingulata*; *Lecanicillium kalimantanense*; *Leiosphaerella lycopodina*; *Myrothecium*		Inhibitory activity against *F. oxysporum*	[52]

(*continued*)

Table 5 (continued)

Plant	Associated endophytes		Effects	Reference
	Fungi	Bacteria		
	verrucaria; *Neonectria punicea*; *Periconia macrospinosa*; *Rhizopycnis vagum*, *Talaromyces assiutensis*			
	Ascomycota sp.; *Fusarium* sp.; *Gliocladiopsis*; *Acremonium furcatum*; *Trichothecium* sp.; *Colletotrichum crassipes*; *Aspergillus niger*; *Phlebia* sp.; *Fusarium oxysporum*; *Earliella scabrosa*; Pseudolagarobasidium sp.; Cerrena sp.		Anti-*Pythium* activity	[53]

against *Fusarium oxysporum*, a potent phytopathogen that causes excessive loss in agriculture [52]. Streptomyces sp. Tc022, associated with root of *Alpinia galanga*, showed inhibitory effect on the fungal pathogens, *Colletotrichum musae* and *Candida albicans* [55].

6 Conclusion

Comprehensive studies on endophytism in different important members of Zingiberaceae suggest that endophytic microorganisms could find a direct application on the improvement of productivity and protection of plants against biotic and abiotic stresses. The endophytes isolated from different members of this family can also be considered as a novel source of bioactive molecules. Several endophytes of this family are potential source of secondary metabolites that have enormous industrial and pharmaceutical applications. However, *in planta* synthesis of these medicinally important natural products is very much challenging due to involvement of multiple genes and complex nature of biosynthetic pathways. Consequently, identification and isolation of specific genes in endophyte genome associated with the synthesis of bioactive molecule and subsequent metabolic engineering can ultimately result in enhanced production of the desired metabolites, at industrial level. Therefore, the identification of holobiont (host and its associated microorganisms) is a major and fascinating field of research that reveals symbiotic relationship between plant and microbes. The involvement of contemporary molecular biological tools and improved techniques of isolation along with an application of efficient genomic data mining techniques and identification of uncharacterized endophytes within Zingiberaceae family, for the purpose of comprehensive analysis

of endophytic microorganisms and plant holobiome, is quite an achievable target. Nevertheless, implementation of multidisciplinary "omics" science and methods is inevitable to decipher the endophytism within Zingiberaceae family for human welfare. These approaches, corroborated with the system biology, will definitely explore the comprehensive physiological and biochemical processes associated with the host-endophyte symbiotic relationship, stress tolerance displayed by endophytes, and their pivotal role in growth promotion.

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Part II

Biotechnology for Identification of Endophytes Using Conventional and Molecular Tools

Identification and Determination of Characteristics of Endophytes from Rice Plants

8

Hadis Yousefi and N. Hasanzadeh

Contents

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Abstract

Rice is the staple food of more than half of the world's population. It is considered the oldest and the most important crop throughout the world, especially in Asia. Considerable agricultural areas are under cultivation of rice in the world. Studies on beneficial microbial interactions that lead to plant health and development are significantly increasing. Local and systemic colonizing microorganisms of plant tissues that have beneficial effects, such as increasing access to food or suppressing pathogens, are parts of endophytic populations. In this article, we tried to highlight the recent studies about identification and determination of characteristics of endophytes from various rice cultivars in the world. Numerous evidence show that rice plant harbors beneficial bacterial endophytic communities. These endophytes have many capabilities including plant growth-promoting activity, plant protection against biotic and abiotic stresses, and synergistic interactions with root-colonizing bacteria, which, in turn, are all in the direction of sustainable agriculture for sustainable agriculture development.

Keywords

Endophytic bacteria · Rice · Physiological characteristics · Molecular identification · Plant growth-promoting potential · Antagonistic activity

1 Introduction

1.1 Definition of Endophytes

Endophytes are certain bacteria and fungi which are localized within plant tissues without damaging their hosts. According to the existing reports, microbial endophytes are present in almost all host plant tissues, including meristem tissues [1, 2].

2 Endophytes in Seeds

The existence of endophytes inside the seeds was first introduced by Baker et al. [3]. Numerous research was done thereafter. The extensive research results showed that endophytic bacteria in oocyte tissues (several plants) [4] are present at all rice seed maturity stages [5] and matured seeds' endosphere [6]. Further, it was found that a set of varied endophytes can be derived from the plant tissues that were previously thought to be germ-free because of the callus tissue of the plants. The endophytic population of the callus tissue were consisted of 11 bacteria and 17 fungi (ascomyctes) taxa [7].

In addition, a set of seed-borne endophytes have been traced in seeds that have endured for hundreds of seed generations. These have different mode of actions. In a typical study of seed-bore endophytes, it was shown that *Pseudomonas* sp. SENDO 2, *Acinetobacter* sp. SENDO 1, and *Bacillus* sp. SENDO 6 improved cactus carotene growth by dissolving rock minerals [8]. They concluded that the selected endophytes have long-term interactions with their host and can affect the

boundaries of evolution, human, and environmental selection [9]. Based on these features of seed-borne endophytes in the plant tissues, they may have more and more essential functions than those already known [10].

It is well documented that bacterial population within a plant is clearly susceptible to changes in the plant physiology [11]. The factors that modify the physiology of the plant are growth stage, soil type, and agricultural management regime, and even bacterial density makes significant changes in endophytic structures. And of course, it should not be forgotten that desired endophytes may grow even under adverse conditions in the host plant [12].

The term "a desired endophyte" is referred to as microorganisms that successfully colonize the plant tissues and can stimulate the plant physiology. It is either chosen selectively or succeeded, which in either case leads to maintaining a good relationship with the plant [13]. For the majority of bacterial endophytes, their function or their ecosystem is unknown within their host plants. However, certain bacterial endophytes may actively affect the host physiology as a result of the production of phytohormones and/or modulation of host ethylene levels.

Other functions of plant growth-enhancing endophytes include N2 fixation, mineral phosphate solubility, nutrient supply, photosynthetic activity enhancement, plant defense system induction, antibiotic production, and heavy metal biotransformation and biodegradation of organic pollutants that can be due to the factor islands of compatibility [14].

3 The Effect of Environmental Conditions, Soil, and Water on Endophytes' Yield

Hardoim et al. [10] performed a comprehensive analysis of the bacterial endophytes of rice seeds by evaluating dependent and independent parts on the cultivation of a bacterial population in two consecutive generations of rice seeds. They also considered abiotic conditions in their experiments to understand the effect of environmental factors on the bacterial endophyte population. They used two soil types (neutral and low pH) and two irrigation regimes (flooded and unflooded). They came to this conclusion that endophytic population in the plant tissue is affected by soil type and irrigation regime. Because of these, during the development of seedlings, rice stem tissue had high bacterial endophytic concentration and richness than root tissues. On the contrary, the bacterial endophytes of the seed were very vulnerable with a gradual growth of the plant. A number of them became predominant and some were suppressed. However, rice seeds' endophytes were generally compatible with the plant tissue and quickly colonized rice branches.

4 Bacterial Variety in Rice Seeds

This phenomenon is really amazing about rice seeds. In short, rice seeds are a place for a variety of bacteria that can become desired endophytes in response to the plant's physiological status. Some organisms may even spread to the rhizosphere and surrounding soil (Table 1). So, they interact directly with the microbial populations

Table 1 List of rice endophytes isolated by culture-dependent techniques

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
Achromobacter xylosoxidans	–	Stem or root	Vietnam	[60]
Acidovorax facilis	Super basmati	Rice grains	Pakistan	[61]
A. facilis	KSK-133	Rice grains	Pakistan	[61]
A. facilis	Kasur	Rice grains	Pakistan	[61]
A. oryzae	–	Stem or root	Phu Yen province, Vietnam	[62]
Acidovorax sp.	Basmati-385	Rice grains	Pakistan	[61]
Acidovorax sp.	Kainat	Rice grains	Pakistan	[61]
Acidovorax sp.	Kinuhikari	Endophyte	Japan	[5]
Acidovorax avenae	Kinuhikari	Seed inside	Japan	[63]
A. temperans	Kainat	Rice grains	Pakistan	[61]
Acinetobacter	–	Root	Thailand	[64]
Acinetobacter	–	Stem	Thailand	[64]
Acinetobacter calcoaceticus	–	Stem or root	Phu Yen province, Vietnam	[62]
A. junii	Kasur	Rice grains	Pakistan	[61]
A. junii	Basmati 86	Rice grains	Pakistan	[61]
A. radioresistens	–	Stem or root	Vietnam	[60]
Acinetobacter sp.	Basmati-2000x33797-1	Rice grains	Pakistan	[61]
Acinetobacter sp.	–	Stem or root	Phu Yen province, Vietnam	[62]
Acinetobacter soli	–	Stem or root	Phu Yen province, Vietnam	[62]
Aeromonas	–	Stem	Thailand	[64]
Aeromonas diversa	–	Root	Venezuela	[65]
A. enteropelogenes	–	Stem or root	Phu Yen province, Vietnam	[62]
A. hydrophila subsp. *hydrophila*	DANAC SD20A	Root	Venezuela	[51]
A. veronii	DANAC SD20A	Root	Venezuela	[51]
Agrobacterium larrymoorei	Kinuhikari	Seed surface	Japan	[5]
A. larrymoorei	Kinuhikari	Seed surface	Japan	[63]
A. larrymoorei	Kinuhikari	Leaf surface	Japan	[63]
Agrobacterium sp.	–	Root	Venezuela	[65]
Anaeromyxobacter sp.	Nipponbare	Root	Japan	[67]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
Aurantimonas altamirensis	Kinuhikari	Leaf surface	Japan	[63]
Aurantimonas altamirensis	Kinuhikari	Leaf inside	Japan	[63]
Aureobacterium liquefaciens	Super basmati	Rice grains	Pakistan	[61]
A. liquefaciens	Basmati-198	Rice grains	Pakistan	[61]
Aureobacterium sp.	Basmati-385	Rice grains	Pakistan	[61]
Aureobacterium sp.	Basmati 86	Rice grains	Pakistan	[61]
A. testaceum	*O. rufipogon* Wl989	Stem	Japan	[67]
Azospirillum amazonense	*O. alta* WOO18	Stem	Japan	[67]
A. amazonense	–	Stem or root	Phu Yen province, Vietnam	[62]
A. brasilense	*O. rufipogon* W1989	Stems	Japan	[68]
A. brasilense	*O. sativa*	Root		[69]
A. caulinodans	*O. sativa*	Root		[69]
Azoarcus indigens	*O. sativa*	Root		[69]
Azospirillum irakense	Kinuhikari	Root inside	Japan	[63]
A. lipoferum	*O. sativa*	Root		[69]
A. lipoferum	*O. sativa* Nipponbare	Stems	Japan	[68]
A. lipoferum	*O. sativa* Kasalath	Stems	Japan	[68]
A. lipoferum	*O. glandiglumis* W1194	Stems	Japan	[68]
Azoarcus sp.	*O. minuta*	Root		[69]
Azoarcus sp.	*O. officinalis*	Root		[69]
Azoarcus sp.	*O. sativa*	Root		[69]
Bacillaceae bacterium NR184	Basmati	Root	Kenya	[70]
Bacillus	–	Root	Thailand	[64]
Bacillus	–	Stem	Thailand	[64]
B. altitudinis	DANAC SD20A	Root	Venezuela	[51]
B. amyloliquefaciens	–	Root	Venezuela	[65]
B. aryabhattai	–	Stem or root	Phu Yen province, Vietnam	[62]
B. aryabhattai	Japonica	–	Republic of Korea	[37]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
B. cereus	–	Stem or root	Phu Yen province, Vietnam	[62]
B. cereus	Nipponbare	Seed	Japan	[71]
B. circulans	–	Stem or root	Vietnam	[60]
B. firmus	Kinuhikari	Inside of root	Japan	[6]
B. fusiformis	Kinuhikari	Inside of root	Japan	[6]
B. gibsonii	Kinuhikari	Leaf inside	Japan	[63]
B. koreensis	–	Stem or root	Phu Yen province, Vietnam	[62]
B. luciferensis	Kinuhikari	Root inside	Japan	[63]
B. megaterium	Kinuhikari	Leaf surface	Japan	[63]
B. megaterium	Kinuhikari	Root inside	Japan	[63]
B. megaterium	Japonica	–	Republic of Korea	[37]
B. megaterium	–	Stem or root	Phu Yen province, Vietnam	[62]
B. megaterium	Basmati	Root	Kenya	[70]
B. methylotrophicus	–	Stem or root	Vietnam	[60]
B. methylotrophicus	–	Stem or root	Phu Yen province, Vietnam	[62]
B. nealsonii	–	Stem or root	Phu Yen province, Vietnam	[62]
B. pumilus	Kinuhikari	Endophyte	Japan	[5]
B. pumilus	Kinuhikari	Seed inside	Japan	[63]
B. pumilus	Kinuhikari	Leaf surface	Japan	[63]
B. pumilus	Kinuhikari	Root inside	Japan	[63]
B. pumilus	–	Root	Venezuela	[65]
B. pumilus	Kinuhikari	Inside of remain of seed	Japan	[6]
B. pumilus	Kinuhikari	Agar medium for rice growth	Japan	[6]
B. pumilus	–	Stem or root	Phu Yen province, Vietnam	[62]
Bacillus sp.	IC32	Rice seeds	South Korea	[35]
Bacillus sp.	–	Stem or root	Phu Yen province, Vietnam	[62]
Bacillus sp.	Binam	Root	Iran	[59]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
B. subtilis	Kinuhikari	Seed surface	Japan	[5]
B. subtilis	Kinuhikari	Endophyte	Japan	[5]
B. subtilis	Kinuhikari	Seed surface	Japan	[63]
B. subtilis	Kinuhikari	Leaf surface	Japan	[63]
B. subtilis	Kinuhikari	Seed inside	Japan	[63]
B. subtilis	Binam	Stem	Iran	[59]
B. subtilis	Japonica	–	Republic of Korea	[37]
B. subtilis	–	Stem or root	Vietnam	[60]
B. subtilis	–	Stem or root	Phu Yen province, Vietnam	[62]
B. thuringiensis	Basmati	Root	Kenya	[70]
B. velezensis	Pionero 2010 FL	Root	Venezuela	[51]
Bradyrhizobium sp.	Nipponbare	Root	Japan	[66]
Bradyrhizobium japonicum	Nipponbare	Root	Japan	[66]
B. elkanii	Kinuhikari	Root inside	Japan	[63]
B. japonicum	Kinuhikari	Root inside	Japan	[63]
Brevibacillus agri	Kinuhikari	Root inside	Japan	[63]
Burkholderia	–	Stem	Thailand	[64]
B. cepacia	*O. sativa*	Root	India	[72]
B. glumae	Basmati 86	Rice grains	Pakistan	[61]
B. glumae	KSK-133	Rice grains	Pakistan	[61]
B. glumae	Basmati-198	Rice grains	Pakistan	[61]
B. glumae	Kasur	Rice grains	Pakistan	[61]
B. glumae	Stg 567989	Rice grains	Pakistan	[61]
B. glumae	Basmati-2000x33797-1	Rice grains	Pakistan	[61]
B. kururiensis	Kinuhikari	Root inside	Japan	[63]
B. kururiensis	–	Stem or root	Phu Yen province, Vietnam	[62]
B. pseudomallei	Super basmati	Rice grains	Pakistan	[61]
B. pseudomallei	Basmati-2000x1053-2-2	Rice grains	Pakistan	[61]
Burkholderia sp.	*O. sativa*	Root		[69]
Burkholderia sp.	Basmati-385	Rice grains	Pakistan	[61]
Burkholderia vietnamiensis	–	Stem or root	Phu Yen province, Vietnam	[62]
B. vietnamiensis	–	Stem or root	Vietnam	[60]
Caulobacter crescentus	Kinuhikari	Inside of shoot	Japan	[6]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
C. crescentus	Kinuhikari	Root inside	Japan	[63]
Cellulomonas sp.	Kouhsar	Leaf inside	Iran	[59]
Chryseobacterium	–	Stem	Thailand	[64]
Chryseobacterium	–	Root	Thailand	[64]
C. gleum	–	Stem or root	Phu Yen province, Vietnam	[62]
C. indologenes	–	Stem or root	Phu Yen province, Vietnam	[62]
C. indologenes	–	Stem or root	Phu Yen province, Vietnam	[62]
C. kwangyangense	–	Stem or root	Phu Yen province, Vietnam	[62]
C. taichungense	Kinuhikari	Root inside	Japan	[63]
Citrobacter	–	Root	Thailand	[64]
Citrobacter diversus	Basmati-2000x1053-2-2	Rice grains	Pakistan	[61]
Citrobacter sp.	Super basmati	Rice grains	Pakistan	[61]
Curtobacterium	–	Stem	Thailand	[64]
C. citrum	*O. sativa* Bu-24	Stem	Japan	[67]
C. citrum	*O. eichingeri* Wl521	Stem	Japan	[67]
C. citrum	*O. longiglumis* Wl228	Stem	Japan	[67]
C. citrum	*O. rufipogon* Wl989	Stem	Japan	[67]
C. citrum	IC37	Rice seeds	South Korea	[37]
Curtobacterium citreum/ Flaccumfaciens/ pusillum	Kinuhikari	Seed inside	Japan	[63]
Curtobacterium citreum/ Flaccumfaciens/ pusillum	Kinuhikari	Leaf surface	Japan	[63]
Curtobacterium citreum/ Flaccumfaciens/ pusillum	Kinuhikari	Seed surface	Japan	[63]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
Curtobacterium flaccumfaciens pv. *basellae*	Kinuhikari	Seed surface	Japan	[5]
Curtobacterium flaccumfaciens pv. *basellae*	Kinuhikari	Endophyte	Japan	[5]
C. flaccumfaciens pv. *basellae*	Kinuhikari	Endophyte	Japan	[5]
Curtobacterium sp.	APO	Seed endophyte	Netherlands	[10]
Curtobacterium sp.	APO	Leaf endophyte	Netherlands	[10]
Curtobacterium sp.	APO	Leaf surface	Netherlands	[10]
Cronobacter	–	Root	Thailand	[64]
Corynebacterium aquaticum	*O. punctata* Wl564	Stem	Japan	[67]
Cytophagales str. MBIC4147	*O. sativa* Bu-24	Stem	Japan	[67]
Diaphorobacter nitroreducens	Kinuhikari	Leaf surface	Japan	[63]
Delftia tsuruhatensis	–	Root	Venezuela	[65]
Delftia lacustris	DANAC SD20A	Root	Venezuela	[51]
Endophytic bacterium C03	Basmati	Root	Kenya	[70]
Endophytic bacterium HA04	Basmati	Root	Kenya	[70]
Enterobacter	IC32	Rice seeds	South Korea	[35]
Enterobacter aerogenes	–	Stem or root	Phu Yen province, Vietnam	[62]
Enterobacter cancerogenus	*O. rufipogon* W1989	Stems		[68]
E. cancerogenus	–	Stem or root	Phu Yen province, Vietnam	[62]
E. asburiae	–	Stem or root	Vietnam	[60]
E. asburiae	Basmati-385	Rice grains	Pakistan	[61]
E. asburiae	Basmati-198	Rice grains	Pakistan	[61]
E. asburiae	Basmati-2000x33797-1	Rice grains	Pakistan	[61]
E. cloacae	Kinuhikari	Root inside	Japan	[63]
E. cloacae	–	Stem or root	Vietnam	[60]
E. cloacae	Stg 567989	Rice grains	Pakistan	[61]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
E. cloacae	–	Stem or root	Phu Yen province, Vietnam	[62]
E. cloacae	Basmati	Root	Kenya	[70]
E. hormaechei	–	Stem or root	Phu Yen province, Vietnam	[62]
E. ludwigii	Kinuhikari	Root inside	Japan	[63]
E. ludwigii	Basmati	Root	Kenya	[70]
E. kobei	–	Stem or root	Vietnam	[60]
E. sacchari	–	Stem or root	Phu Yen province, Vietnam	[62]
Enterobacter sp.	–	Stem or root	Phu Yen province, Vietnam	[62]
Enterobacter sp.	Basmati	Root	Kenya	[70]
Enterobacter sp.	Kainat	Rice grains	Pakistan	[61]
Enterobacter sp.	Basmati-2000x1053-2-2	Rice grains	Pakistan	[61]
Enterobacter sp.	Khazar	Root	Iran	[59]
Enterobacter xiangfangensis	–	Stem or root	Vietnam	[60]
Erwinia soli	–	Stem or root	Phu Yen province, Vietnam	[62]
Exiguobacterium acetylicum	–	Stem or root	Phu Yen province, Vietnam	[62]
Fictibacillus	–	Stem	Thailand	[64]
Fictibacillus	–	Root	Thailand	[64]
Flavobacterium	–	Root	Thailand	[64]
Flavobacterium gleum	*O. alta* WOO17	Stem	Japan	[67]
Flavobacterium sp.	IR29	Seed endosphere	South Korea	[35]
Flavobacterium sp.	FL478	Seed endosphere	South Korea	[35]
Flavobacterium sp.	IC27	Seed endosphere	South Korea	[35]
Flavobacterium sp.	IC31	Seed endosphere	South Korea	[35]
Flavobacterium sp.	IC32	Seed endosphere	South Korea	[35]
Flavobacterium sp.	IC37	Seed endosphere	South Korea	[35]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
Flavobacterium sp.	APO	Paddy soil	Netherlands	[10]
Gallionella sp.	*O. nivara*	Root		[69]
Halobacillus	–	Root	Thailand	[64]
Herbaspirillum huttiense	IC32	Rice seeds	South Korea	[35]
H. huttiense	–	Stem or root	Phu Yen province, Vietnam	[62]
H. rubrisubalbicans	–	Stem or root	Phu Yen province, Vietnam	[62]
H. rubrisubalbicans	*O. barthii* W1407	Stems	Japan	[68]
H. seropedicae	*O. officinalis* W0012	Stems	Japan	[68]
H. seropedicae	*O. rufipogon* W1989	Stems	Japan	[68]
H. seropedicae	*O. meridionalis* WI627	Seed	Japan	[67]
Herbaspirillum sp.	*O. sativa*	Root		[69]
Hyphomicrobium facilis	Kinuhikari	Leaf surface	Japan	[63]
Hyphomicrobium sulfonivorans	Kinuhikari	Root inside	Japan	[63]
Hyphomicrobium sp.	Nipponbare	Root	Japan	[66]
Ideonella dechloratans	*O. sativa* SC41	Stems	Japan	[68]
I. dechloratans	*O. sativa* Kasalath	Stems	Japan	[68]
I. dechloratans	*O. sativa* SC41	Stems	Japan	[68]
Klebsiella	–	Stem	Thailand	[64]
Klebsiella sp.	*O. granulata*	Root		[69]
Klebsiella sp.	–	Stem or root	Phu Yen province, Vietnam	[62]
K. oxytoca	*O. sativa* Bu-24	Seed	Japan	[67]
K. oxytoca	–	Sem or root	Phu Yen province, Vietnam	[62]
K. pneumoniae	Japonica	–	Republic of Korea	[37]
K. pneumonia	*O. sativa*	Root		[69]
K. pneumoniae	–	Stem or root	Phu Yen province, Vietnam	[62]
Kluyvera sp.	Basmati-198	Rice grains	Pakistan	[61]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
Kosakonia sp.	IC27	Rice seeds	South Korea	[35]
Kosakonia sp.	IC31	Rice seeds	South Korea	[35]
Kosakonia sp.	IC32	Rice seeds	South Korea	[35]
Kocuria palustris	Kinuhikari	Inside of remain of seed	Japan	[6]
Kurthia sibirica	KSK-133	Rice grains	Pakistan	[61]
Kurthia sp.	Stg 567989	Rice grains	Pakistan	[61]
Kurthia zopfii	Kainat	Rice grains	Pakistan	[61]
Lactococcus	–	Stem	Thailand	[64]
Lysinibacillus	–	Stem	Thailand	[64]
Methanobacterium ivanovii	Nipponbare	Root	Japan	[66]
Methanobacterium sp.	Nipponbare	Root	Japan	[66]
M. acetivorans	Nipponbare	Root	Japan	[66]
M. aquaticum	Kinuhikari	Endophyte	Japan	[5]
M. aquaticum	Kinuhikari	Seed surface	Japan	[5]
M. aquaticum	Kinuhikari	Seed surface	Japan	[63]
M. aquaticum	Kinuhikari	Seed inside	Japan	[63]
M. aquaticum	Kinuhikari	Leaf surface	Japan	[63]
M. aquaticum	Kinuhikari	Leaf inside	Japan	[63]
M. fujisawaense/	Kinuhikari	Leaf surface	Japan	[63]
Radiotolerans	Kinuhikari		Japan	[63]
M. fujisawaense/	Kinuhikari	Leaf inside	Japan	[63]
Radiotolerans	Kinuhikari		Japan	[63]
M. fujisawaense	Kinuhikari	Inside of shoot	Japan	[6]
M. fujisawaense	Kinuhikari	Inside of remain of seed	Japan	[6]
M. fujisawaense	Kinuhikari	Surface on Seedling	Japan	[6]
M. nodulans	Nipponbare	Root	Japan	[66]
M. organophilum	Nipponbare	Root	Japan	[66]
M. radiotolerans	Nipponbare	Root	Japan	[66]
M. radiotolerans	Kinuhikari	Inside of shoot	Japan	[6]
M. radiotolerans	Kinuhikari	Inside of remain of seed	Japan	[6]
M. radiotolerans	Kinuhikari	Inside of root	Japan	[6]
M. radiotolerans	Kinuhikari	Surface on seedling	Japan	[6]
M. radiotolerans	Kinuhikari	Agar medium for rice growth	Japan	[6]
Methylobacterium sp.	*O. sativa* SC-41	Stem	Japan	[67]
Methylobacterium sp.	*O. longiglumis* WI228	Stem	Japan	[67]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
Methylobacterium sp.	*O. brachyantha* W0656	Stem	Japan	[67]
Methylobacterium sp.	*O. sativa* 1-10 I	Stem	Japan	[67]
Methylobacterium sp.	*O. sativa* Bu-24	Stem	Japan	[67]
Methylobacterium sp.	*O. sativa* SC-41	Leaf sheath	Japan	[67]
Methylobacterium sp.	*O. latifolia* WOO19	Stem	Japan	[67]
Methylobacterium sp.	*O. longiglumis* WI220	Stem	Japan	[67]
Methylobacterium sp.	*O. rufipogon* WI989	Stem	Japan	[67]
Methylobacterium sp.	*O. minuta* W1318	Stem	Japan	[67]
Methylobacterium sp.	*O. rufipogon* WI964	Stem	Japan	[67]
Methylobacterium sp.	*O. meridionalis* WI627	Seed	Japan	[67]
Methylocapsa acidiphila	Kinuhikari	Root inside	Japan	[63]
Methylocella silvestris	Nipponbare	Root	Japan	[66]
Methylocystis sp.	Nipponbare	Root	Japan	[66]
Methylosinus sp.	Nipponbare	Root	Japan	[66]
Methylosinus sporium	Nipponbare	Root	Japan	[66]
Methylosinus trichosporium	Nipponbare	Root	Japan	[66]
Microbacterium	–	Stem	Thailand	[64]
Microbacterium binotii	Japonica	–	Republic of Korea	[37]
Microbacterium sp.	*O. officinalis* WOOl2	Stem	Japan	[67]
Microbacterium sp.	IR29	Rice seeds	South Korea	[35]
Microbacterium sp.	FL478	Rice seeds	South Korea	[35]
Microbacterium sp.	IC31	Rice seeds	South Korea	[35]
Microbacterium sp.	IC37	Rice seeds	South Korea	[35]
Microbacterium sp.	APO	Leaf surface	Netherlands	[10]
Microbacterium sp.	APO	Paddy soil	Netherlands	[10]
Microbacterium testaceum	Kinuhikari	Leaf surface	Japan	[63]
Microbacterium trichotecenolyticum	Japonica	–	Republic of Korea	[37]
Micrococcus	–	Root	Thailand	[64]
Micrococcus luteus	Kinuhikari	Endophyte	Japan	[5]
M. luteus	Kinuhikari	Seed surface	Japan	[5]
M. luteus	Kinuhikari	Seed surface	Japan	[63]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
M. luteus	Kinuhikari	Seed inside	Japan	[63]
M. luteus	Kinuhikari	Leaf surface	Japan	[63]
M. luteus	Kinuhikari	Root inside	Japan	[63]
M. luteus	Basmati	Root	Kenya	[70]
M. luteus	Kinuhikari	Inside of shoot	Japan	[6]
M. luteus	Kinuhikari	Inside of shoot	Japan	[6]
Mitsuaria chitosanitabida	–	Stem or root	Phu Yen province, Vietnam	[62]
Mucilaginibacter	–	Stem	Thailand	[64]
Mycobacterium petroleophilum	Kinuhikari	Root inside	Japan	[63]
Mycobacterium sp.	APO	Root endosphere	Netherlands	[10]
Novosphingobium	–	Root	Thailand	[64]
Novosphingobium	–	Stem	Thailand	[64]
Novosphingobium subarcticum	Kinuhikari	Leaf surface	Japan	[63]
Ochrobactrum	–	Stem	Thailand	[64]
Ochrobactrum sp.	*O. sativa*	Root		[69]
Ochrobactrum sp.	APO	Paddy field	Netherlands	[10]
Pantoea	IR29	Rice seeds	South Korea	[35]
Pantoea	FL478	Rice seeds	South Korea	[35]
Pantoea	IC31	Rice seeds	South Korea	[35]
Pantoea	–	Stem	Thailand	[64]
Pantoea agglomerans	–	Stem or root	Phu Yen province, Vietnam	[62]
P. ananatis	Kinuhikari	Endophyte	Japan	[5]
P. ananatis	Kinuhikari	Seed inside	Japan	[63]
P. ananatis	Kinuhikari	Leaf inside	Japan	[63]
P. ananatis	Kinuhikari	Inside of shoot	Japan	[6]
P. ananatis	Kinuhikari	Inside of remain of seed	Japan	[6]
P. ananatis	Kinuhikari	Surface on seedling	Japan	[6]
P. ananatis	Kinuhikari	Agar medium for rice growth	Japan	[6]
P. ananatis	Nipponbare	Seed	Japan	[71]
P. ananatis	–	Stem or root	Phu Yen province, Vietnam	[62]
P. ananatis	Hashemi	Leaf inside	Iran	[59]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
P. ananatis	*O. alta* WOO 17	Seed	Japan	[67]
Pantoea calida	–	Stem or root	Phu Yen province, Vietnam	[62]
Pantoea cypripedii	–	Stem or root	Phu Yen province, Vietnam	[62]
Pantoea sp.	–	Stem or root	Phu Yen province, Vietnam	[62]
Pantoea sp.	Hashemi	Leaf inside	Iran	[59]
Paenibacillus	–	Root	Thailand	[64]
P. alve	Kinuhikari	Root inside	Japan	[63]
P. amylolyticus	Kinuhikari	Seed inside	Japan	[63]
P. amylolyticus	Kinuhikari	Endophyte	Japan	[5]
P. hunanensis	–	Stem or root	Phu Yen province, Vietnam	[62]
P. kribbensis	Japonica	–	Republic of Korea	[37]
Paenibacillus sp.	FL478	Rice seeds	South Korea	[35]
Paenibacillus sp.	IC32	Rice seeds	South Korea	[35]
Paenibacillus sp.	APO	Paddy field	Netherlands	[10]
Pedobacter	–	Root	Thailand	[64]
Pseudacidovorax	–	Stem	Thailand	[64]
Pseudomonas	–	Root	Thailand	[64]
Pseudomonas	–	Stem	Thailand	[64]
P. aeruginosa	–	Root	Venezuela	[65]
P. chengduensis	Pionero 2010 FL	Root	Venezuela	[51]
P. fluorescens	–	Root	Venezuela	[65]
P. fluorescens	Basmati	Root	Kenya	[70]
P. fulva	Binam	Leaf inside	Iran	[59]
P. geniculata	–	Stem or root	Phu Yen province, Vietnam	[62]
P. gessardii	Pionero 2010 FL	Root	Venezuela	[51]
P. helmanticensis	DANAC SD20A	Root	Venezuela	[51]
P. hibiscicola	–	Stem or root	Phu Yen province, Vietnam	[62]
P. jessenii	–	Root	Venezuela	[65]
P. mendocina	–	Root	Venezuela	[65]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
P. oleovorans subsp. *oleovorans*	Pionero 2010 FL	Root	Venezuela	[51]
P. oryzihabitans	Hashemi	Leaf inside	Iran	[59]
P. pseudoalcaligenes	–	Root	Venezuela	[65]
Pseudomonas putida	–	Stem or root	Phu Yen province, Vietnam	[62]
Pseudomonas putida	Basmati	Root	Kenya	[70]
Pseudomonas putida	Tarom hashemi	Root	Iran	[59]
Pseudomonas sp.	APO	Rhizosphere	Netherlands	[10]
Pseudomonas sp.	–	Stem or root	Phu Yen province, Vietnam	[62]
Pseudomonas sp.	–	Root	Venezuela	[65]
Rhizobium etli	Nipponbare	Root	Japan	[66]
R. larrymoorei	FL478	Rice seeds	South Korea	[35]
R. leguminosarum	*O. sativa*	Root	Egypt	[73]
R. leguminosarum	*O. sativa*	Root	India	[72]
R. loti	Kinuhikari	Root inside	Japan	[63]
R. oryziradicis	DANAC SD20A	Root	Venezuela	[51]
Rhodopseudomonas palustris	*O. ridleyi* WOOOl	Stem	Japan	[67]
R. palustris	Nipponbare	Root	Japan	[66]
Roseateles depolymerans	Kinuhikari	Root inside	Japan	[63]
Serratia fonticola	–	Root	Venezuela	[65]
S. glossinae	DANAC SD20A	Root	Venezuela	[51]
S. marcescens	*O. sativa*	Root	Philippines	[74]
S. marcescens	*O. sativa*	Stem	Philippines	[74]
Sphingomonas	–	Root	Thailand	[64]
Sphingomonas	–	Stem	Thailand	[64]
Sphingomonas adhaesiva	*O. rufipogon* W1964	s	Japan	[67]
S. echinoides	Nipponbare	Seed	Japan	[71]
S. echinoides	Kinuhikari		Japan	[63]
S. melonis	Kinuhikari	Endophyte	Japan	[5]
S. melonis	Kinuhikari	Seed inside	Japan	[63]
S. melonis	Kinuhikari	Leaf inside	Japan	[63]
S. melonis	Kinuhikari	Leaf surface	Japan	[63]
S. parapaucimobilis	Nipponbare	Seed	Japan	[71]
S. paucimobilis	*O. sativa*	Root		[69]
S. paucimobilis	Kinuhikari	Leaf surface	Japan	[63]
S. paucimobilis	Kinuhikari	Leaf surface	Japan	[63]
S. phyllosphaerae	Kinuhikari	Leaf surface	Japan	[63]

(*continued*)

Table 1 (continued)

Bacterial endophytes	Rice (*O. sativa*) host cultivars	Colonized plant compartments	Geographical location	References
S. pituitosa	Kinuhikari	Leaf surface	Japan	[63]
S. sanguinis	–	Stem or root	Phu Yen province, Vietnam	[62]
S. yabuuchiae	Kinuhikari	Endophyte	Japan	[5]
S. yabuuchiae	Kinuhikari	Seed surface	Japan	[5]
S. yabuuchiae	Kinuhikari	Seed surface	Japan	[63]
S. yabuuchiae	Kinuhikari	Seed inside	Japan	[63]
S. yabuuchiae	Kinuhikari	Leaf inside	Japan	[63]
S. yabuuchiae	Kinuhikari	Leaf surface	Japan	[63]
Staphylococcus	–	Root	Thailand	[64]
Staphylococcus	–	Stem	Thailand	[64]
Staphylococcus arlettae	–	Stem or root	Phu Yen province, Vietnam	[62]
Stenotrophomonas maltophilia	Kinuhikari	Seed surface	Japan	[5]
S. maltophilia	Kinuhikari	Seed surface	Japan	[63]
S. maltophilia	Kinuhikari	Leaf inside	Japan	[63]
S. maltophilia	–	Stem or root	Vietnam	[60]
Streptomyces lateritius/	Kinuhikari	Leaf inside	Japan	[63]
Venezuelae			Japan	[63]
Uncultured bacterium	Nipponbare	Root	Japan	[66]
Uncultured *Methylocystis* sp.	Nipponbare	Root	Japan	[66]
Uncultured *Sphingomonas* clone	APO	Root endosphere	Netherlands	[10]
Uncultured *Stenotrophomonas* clone	APO	Root endosphere	Netherlands	[10]
Xanthobacter agilis	Stg 567989	Rice grains	Pakistan	[61]
Xanthobacter agilis	KSK-133	Rice grains	Pakistan	[61]
Xanthobacter flavus	Kasur	Rice grains	Pakistan	[61]
Xanthomonas axonopodis	Kinuhikari	Leaf surface	Japan	[63]
Xanthobacter sp.	Basmati-2000x33797-1	Rice grains	Pakistan	[61]
Xanthomonas sp.	IR29	Rice seeds	South Korea	[35]
Xanthomonas sp.	FL478	Rice seeds	South Korea	[35]
Xanthomonas sp.	IC31	Rice seeds	South Korea	[35]
Xanthomonas sp.	IC32	Rice seeds	South Korea	[35]
Xanthomonas translucens pv. *poae*	Kinuhikari	Endophyte	Japan	[5]
X. translucens pv. *poae*	Kinuhikari	Seed surface	Japan	[5]
X. translucens	Kinuhikari	Seed surface	Japan	[63]
X. translucens	Kinuhikari	Seed inside	Japan	[63]

of the soil [15]. Evaluations of the endophytic population of matured roots of cultivated rice in field soil showed that the members of the genus *Enterobacter* had the highest frequency and genetic diversity of isolated bacteria [16]. Two strains of *Enterobacter* sp. REICA_142 and REICA_082 have improving plant growth properties such as N2 fixation, solubility of mineral phosphate, and ACC production [16]. Different strains of *Pseudomonas oryzihabitans* were capable of decomposing mineral phosphate [17], producing IAA, siderophores, and N2 fixation [18]. Another bacterium that resembled *Pseudomonas oryzihabitans* was able to colonize the host plant widely in the neutral pH soil [10]. The other species was *Pseudomonas* sp. strain R6, a species similar to *Pseudomonas protegens* $CHA0^T$ that widely protected the plant and produced antimicrobial compounds 2,4 diacetylphloroglucinol and pyoluteorin [10, 19]. Obviously, bacterial populations are selected by the host seeds and vary in diversity and function depending on the soil conditions. For example, rice grown in neutral pH soils had dominant bacteria of *Pseudomonas oryzihabitans* and *Rhizobium radiobacter*. On the other hand, in acidic soil, bacterial growth such as *Enterobacter*-like strain REICA_082 and *Dyella ginsengisoli* was evident [10]. The role of commensal bacteria is unknown in rice plants. But in case of *Dyella ginsengisoli* isolated from Ginseng fields in South Korea [20], the bacterium could dissolve the mineral phosphate and convert into glucanase, enhanced rapseed root length upto 145% [21].

5 Methods for Identification and Characterization of Rice Bacterial Endophytes

5.1 Phenotypic Characteristics

The phenotypic characteristics such as color, texture and pigmentation, cell shape and size, motility, aerobic and anaerobic growth, temperature tolerance, pH and salt, and numerous biochemical tests are very important in initial diagnosis of the bacteria.

6 Biochemical Tests

Biochemical experiments are important to determine the type of enzyme that bacteria produce. These enzymes have a large variety, each of which is evaluated for the purpose of determining a bacterial trait in vitro. Some of these enzymes used for identification and functioning of endophytic bacteria consist of phosphatase, nitrate reductase and nitrogenase, citrate lyase and urease, cellulase, catalase, amylase and protease, and tests such as siderophore production, chitin hydrolysis, and C/N preferences [22–31].

7 Numerical Taxonomy

Cluster analysis of isolates is performed based on phenotypic traits within a binary matrix using software NTSYSpc® and UPGMA as grouping algorithm and Jaccard as correlation coefficient [32].

In the table below, several phenotypic traits of rain-fed rice bacteria in Sarado region of Brazil were shown (Table 2). As specified in the table, all isolates were able to use sucrose and malic acid as carbon source. Also, 45% and 35% of isolates were able to use fructose and myo-inositol, respectively.

In an analysis based on the combination of morphological characteristics, enzymatic activity, and the use of carbon source and antibiosis, isolates were classified into six clusters with similarity of 60% (Fig. 1). This indicates a high variation in enzymatic activities among 20 isolates studied (Table 3).

8 Molecular Analyses

8.1 16S rRNA Gene Sequencing Analysis

The first and most important method for early detection of strains is molecular method of 16S rRNA gene replication. The replication of the above molecule is carried out with several primers by performing PCR. The sequencing of 16S rRNA replicated gene and its blast in the nucleotide database (www.ncbi.nlm.nih.gov) is crucial for confirming the basic identification based on phenotypic traits. The bacterial 16S rRNA-based phylogenic relationships can be determined with various softwares such as CLUSTAL W, and its evolutionary analysis can be done with the help of software MEGA [33]. The study of the endophytic population of rice seeds using two successive generations based on 16S rRNA gene showed that the isolated endophytes included nine genus members in *Alpha*- and *Gammaproteobacteria*, *Flavobacteria*, *Bacilli*, and *Actinobacteria* classes [10]. Strains close to *Stenotrophomonas maltophilia* (R2 and R8), *Mycobacterium abscessus* (R1 and R5), and *Ochrobactrum* spp. (R3 – *O. tritici* and R12 – *O. grignonense*) were observed within both seed generations. The first-generation seeds had the highest density of endophytic population per gram of fresh rice seed weight ($3.5\ 10^5$ CFU g^{-1} FW), while in the second generation, this amount was reduced to $4.5\ 10^3$ CFU g^{-1}FW.

Pseudomonas sp. (JN110435), Uncultured *Stenotrophomonas* clone SHCB1148 (JN110437), *Curtobacterium* sp. (JN110438), *Flavobacterium* sp. (JN110440), *Ochrobactrum* sp. (JN110441), *Microbacterium* sp. (JN110444), and *Curtobacterium* sp. (JN110445) strains from rice seed endosphere in terms of similarity to the sequence of gene 16S rRNA are very close to the bacteria isolated

Table 2 Metabolic characteristics of endophytic isolates obtained from rice plants

Evaluations	Isolates																			
	S2	S4	S5	S6	S6A	S8A	S8B	S17	S22	S25	S26	S29	S32	S35	S37	S39	S41	S63	S97	S105
Carbon sources																				
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Fructose	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	+
Mannitol	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	+	–	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+
Mio-inositol	+	+	+	–	–	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–
Arabinose	–	–	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+
Maleic acid	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–
Nicotinic acid	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Malic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Enzyme activity																				
Cellulase	+	–	+	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	+	+
Amylase	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+
Protease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phosphatase	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+
Urease	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–
Nitrogenase	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Reductase nitrate	–	+	–	+	+	+	+	–	+	+	+	–	+	+	+	–	–	–	+	+
Citratelyase	–	+	–	–	–	+	–	+	+	–	+	+	–	+	–	–	–	+	+	–
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

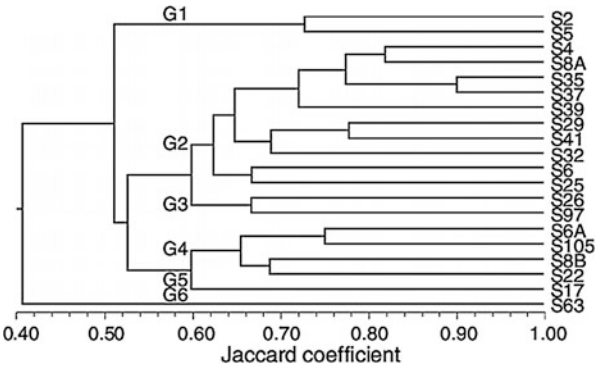

Fig. 1 Consensus dendrogram obtained by combining the morphological, enzymatic activity, carbon source use, and antibiosis data among 20 endophytic isolates obtained from upland rice plants. Dendrogram was generated by the algorithm UPGMA and similarity matrix obtained from the use of Jaccard coefficient

from Phytosphere, rhizosphere, and rice field soil. This suggests that the above bacteria fit well with rice niche [10].

Phylogenetic analysis of 16S rRNA gene sequence performed for 11 isolates showed that all of them belong to the genus *Bacillus* (Fig. 2). The presence of *Bacillus* genus related to rice from Brazil has also been reported [34]. But never the species of *B. amyloliquefaciens* has been reported [32].

8.2 Identification and Determination of Isolated Genetic Diversity by BOX-PCR Method

The results of a study conducted on PGPR endophytic bacteria in rice seed using 16S rRNA and BOX-PCR methods showed that most of them belonged to *Microbacterium*, *Flavobacterium*, *Xanthomonas*, *Kosakonia*, and *Paenibacillus* reference strains. But there was a chance that there might be genetic differences between subspecies. The reason is the formation of sub-clusters among their own groups [35].

8.3 Identification of PCR Bands by PCR-DGGE Method

For this purpose, denaturing gradient electrophoresis gel is used. The product obtained from the replication of 16S rDNA bacterial or 18S rDNA fungal genes is distinguished in three dimensions by the above method. The advantage of this method is to demonstrate the role of the soil in determining the type of bacteria desired. For example, rice grown in neutral soil was suitable for *Pseudomonas oryzihabitans* and *Rhizobium radiobacter* growth increase, while two bacteria *Enterobacter*-like and *Dyella ginsengisoli* predominated in soils with lower pH

Table 3 Solubilization index (SI) of endophytic isolates obtained from rice plants for inorganic phosphate assay in Pikovskaya medium. Mean values of three replicates

Isolate	Solubilization index (%)
S2	133.86 (±16.9)
S4	126.94 (±13.7)
S5	133.60 (±14.5)
S6	130.56 (±4.8)
S6A	116.78 (±9.8)
S8A	119.63 (±2.8)
S8B	0
S17	117.10 (±7)
S22	131.02 (±7.9)
S25	129.17 (±19)
S26	114.95 (±4.3)
S29	123.15 (±11.2)
S32	119.39 (±10)
S35	120.20 (±6.1)
S37	117.78 (±1.9
S39	116.06 (±5.3)
S41	128.52 (±5.7)
S63	0
S97	116.74 (±8.4)
S105	126.52 (±9.1)

levels. Seed-borne *Stenotrophomonas maltophilia* was also the only bacterial endophyte that was present in both soil types. In these seed-based studies by PCR-DGGE method, it was found that about 45% of the bacterial population present in the first generation of seed was also found in the second generation [10] (Fig. 3).

9 Antagonistic Tests

A dual culture test is widely used as one of the in vitro tests for early screening of biological control factors [36].

The antagonistic effects of N_2 fixation endophyte bacteria in vitro against soil pathogen fungi of *F. oxysporum* and *R. solani* showed that isolates *Klebsiella pneumoniae* (GU373625), *Bacillus subtilis* (AY030331), and *Microbacterium trichotecenolyticum* (EU714362) were the most effective inhibitory bacteria against the two abovementioned fungi with forming inhibition zone [37] (Fig. 4).

10 Production of Proteases

Another important tool for the growth-promoting bacteria is their ability to produce protease enzymes. Protease production by bacteria has been studied frequently. It is also important to evaluate the production of protease in industrial applications as well as understand the activities that occur in the soil environment. According to Oliveira et al. (2006), the bacteria with a protease enzyme production index (PEI) of

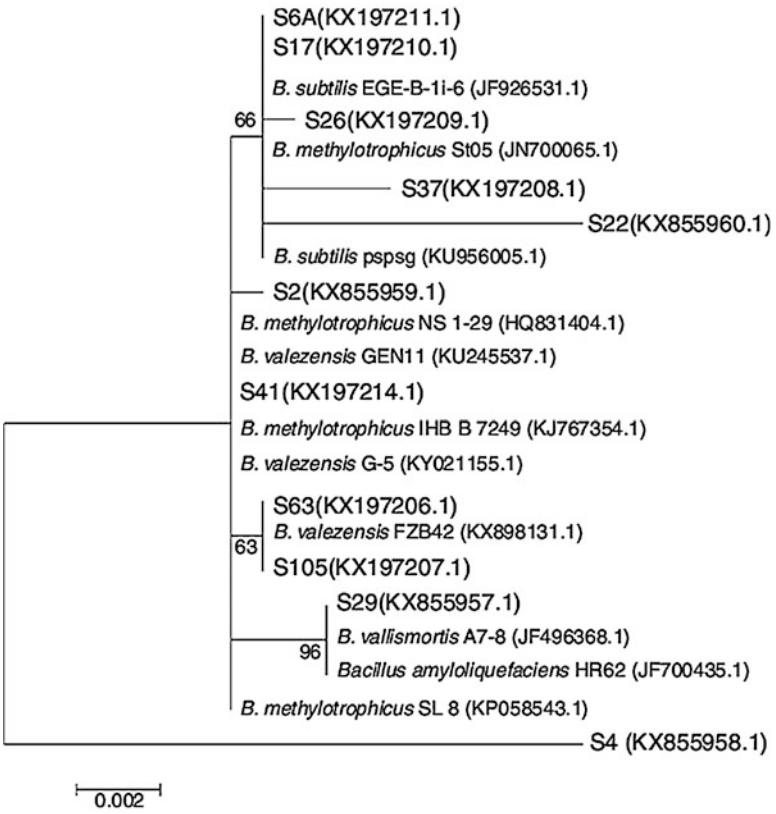

Fig. 2 Maximum likelihood phylogeny of the 16S rRNA gene showing the relationships among endophytic isolates obtained from rice plants (in bold) with *Bacillus* sp. reference strains. GenBank accession numbers are shown in parentheses. Bar, 2 nt substitutions per 1000 nt

about $\geq$2 are good producers of protease. In this regard, the production index of protease enzyme in isolate S6 of rice and *Bacillus megaterium* and *Corynebacterium renale* bacteria isolated from *Jacaranda decurrens* plants was about 3, 3.1, and 4.3, respectively [32, 38].

We can use the enzyme diversity of endophytic bacteria to develop a strategy to use these selected microorganisms as bio-fertilizers and plant growth promoter to improve rice production [32].

11 Siderophore Production

The production of siderophore is a distinctive representative of *Pseudomonas* various strains. *Microbacterium* sp. isolated from different rice cultivars was not able to produce siderophore. Also, siderophore production was negative in representatives of *Bacilli* and *Actinobacteria*. In contrast, *Pantoea* sp., *Xanthomonas* sp., and *Kosakonia* sp. strains have the ability to produce siderophore [35].

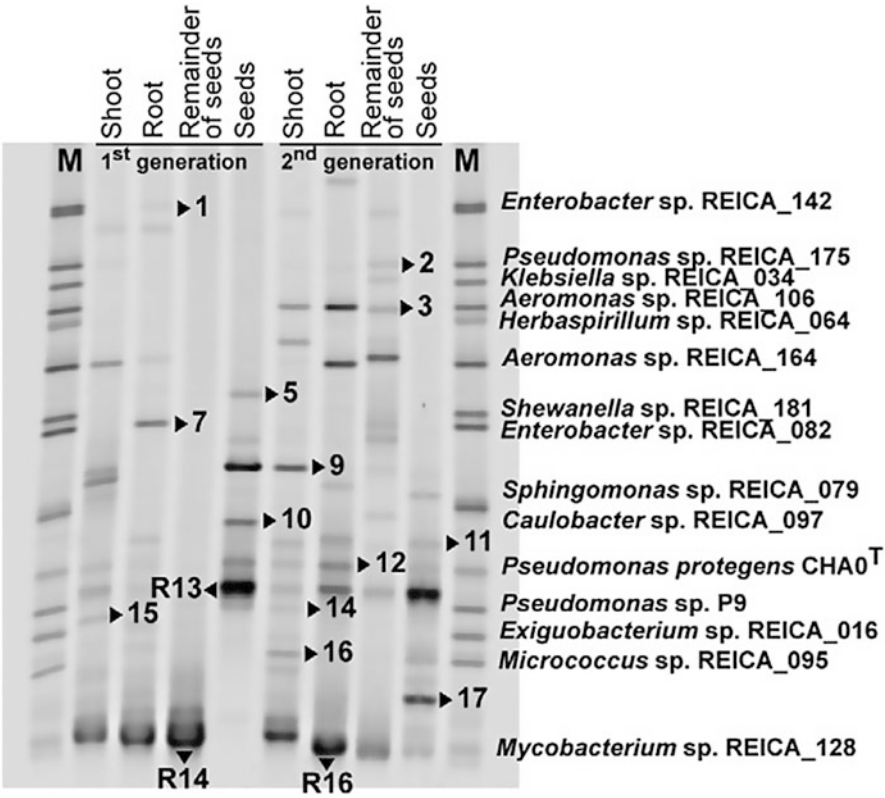

Fig. 3 Dynamics of rice endophytes as revealed by PCR-DGGE profiles of seed, 3- and 5-week-old rice plants. Rice endophyte PCR-DGGE patterns of surface-sterilized dehulled seeds and 5-day-old shoot, root, and remainder of the seeds from two consecutive generations are shown

12 Ethylene Production

Flavobacterium sp. was able to produce large amounts of IAA in the presence of tryptophan up to 10–20 $\mu g ml^{-1}$. Also, *Kosakonia* sp., *Pantoea* spp., and *Xanthomonas* sp. strains were able to produce less amounts of IAA than *Flavobacterium* sp. (3–5 $\mu g ml^{-1}$). The important point in producing IAA is its dependence on osmotic tolerance at a concentration of 1.2 M sucrose.

13 Determination of PGP Traits of Endophytes and Colonization of Plants

Useful endophytic bacteria directly or indirectly play an important role in the plant growth and development [39]. The accelerated growth of plants by the production of IAA by endophytes is very prominent. Rice seeds are reserves of bacterial

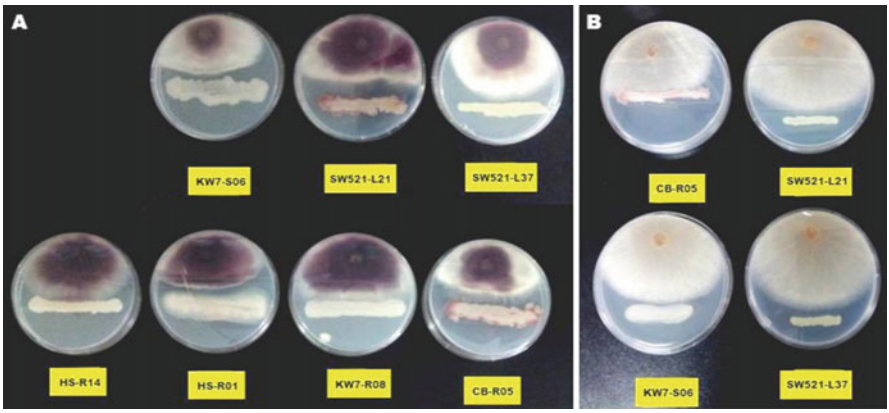

Fig. 4 Antifungal activities of endophytic diazotrophic bacteria against *Fusarium oxysporum* and *Rhizoctonia solani*: (A) Seven isolates showed the highest antifungal activity in the *F. oxyporum*, and (B) four isolates showed the highest antifungal activity in the *R. solani*. All endophytic diazotrophic bacterial isolates showed antifungal activities against *F. oxyporum*. All endophytic diazotrophic bacterial isolates showed antifungal activities against *R. solani*, except for two strains (HS-S05, KW7-R08; data not shown). In particular, KW7-S06, CB-R05, and SW521-L21 showed highest antifungal activities on both fungal isolates. These antifungal activity tests were repeated three times ($n = 3$)

endophytes with PGP traits that help seed germination and early development. Almost all bacteria with ACCD activity isolated from rice endosphere are able to improve growth during the initial development of seedlings with *Microbacterium* sp. IC37-16 and significantly increase germination and growth parameters. In a study on 15 dominant bacterial endophytes isolated from Venezuelan rice with IAA production power, IAA hormone was the main auxin in plants that in fact controls root architecture by improving nutrient uptake [40–42]. Recently, two *Bacillus* strains with E1101 and E2315 codes were identified that did not affect the early growth of rice seedlings, but after 1 month, the growth of treated plants increased positively. *Rhizobium* sp. E2321 is another accelerating bacterium that has the highest positive effect on the seedling growth rate. The above strain has several characteristics of PGP in vitro. *Serratia* sp. E2309 was the only bacterial inoculum that increased the early growth of rice seedling and the whole plant. Other *Serratia* spp. are more or less considered as PGP strains [43–45]. The isolates such as *Delftia* sp. E2330 and *Pseudomonas* spp. were ineffective on early seedling growth, but finally increased the plant growth. Among endophytes, *Pseudomonas* spp. have the highest frequency in terms of endorhizospheres [6, 7, 46, 47].

Apart from the bacteria that produce ACCD, phytohormone IAA is a vital phytostimulator for bacteria. IAA, the most commonly produced auxin, is a signaling molecule in both plants and microorganisms, which essentially serves as an interactive signaling molecule in the interaction between the plant and microbe [48]. The production of IAA is one of the prominent features of rice seed endophytes, as well as other endophytes. Many isolates in characterizing endophytic competence

and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of rice are able to produce IAA in different amounts [35]. Observations of Kim et al. [49] show that plants are cloned with a large number of IAA-producing bacteria. Inoculation of plants with IAA-producing PGPB causes changes in the root architecture stimulating root hair, increasing the number and length of lateral and primary roots, and finally enhancing the root surface for absorption of minerals and secretion and infiltration [40, 50]. *Flavobacterium* sp. IR29-16 and other *Flavobacterium* sp. strains in this regard can stimulate the plant growth significantly, especially along with the plant roots through the production of IAA [35] (Table 4).

14 Formulation

Microorganisms do not act as individuals (individual and unique), but as a variable microbial population dynamically interact with each other and mechanisms such as quorum sensing at the cell level and act jointly. These relationships are sometimes as the same genus, a family, and/or at the level of the members of the two families. Such interactions affect the microbial population phenotypes [51]. For PGPR bacteria, this is important because, at the same time, they should compete with the microbial factors in rhizosphere and have the ability to accelerate the growth of the host plant [52]. Therefore, it is expected that these bacteria have all the capabilities, including coexistence with rhizobacteria and tolerance of various environmental conditions [53]. But in some cases, this does not occur, and the results of the greenhouse experiments are not fully consistent with the field conditions. Therefore, due to intense competition in the field soil and lack of knowledge of unknown competitors, root colonization is not well done, which in some cases leads to the cease of the normal growth of treated plants [54, 55]. Therefore, two important factors play a key role in the formulation: (1) optimal formulation using several antagonists and (2) selection of isolates from the plant's rhizosphere. From this perspective, the design of simplified microbial populations has recently been considered as a priority for the use of plant microbiome in sustainable agriculture [56], and this approach has been considered in *Arabidopsis* [57] and maize [58].

15 The Role of Cultivars in Determining Bacterial Microbiota

The results of various studies have shown that each plant with a cultivar of a plant species is its own microbiota site, which has evolved over the centuries. Today, in order to investigate more precisely, the interaction between PGPRs and host plants and the achievement of a desired transgenic commercial product are considered in three categories: (1) PGPR2 bacteria, (2) soil quality, and (3) host plants. Soil factor is important because it is the basis for the establishment of holobiome (Fig. 5). The plant holobiome means the host plant and all the associated microorganisms. Naturally, plants have the ability to select/absorb some microbial

Table 4 Comparison of plant growth-promoting traits and physiological activities of different bacterial endophytes in rice plants during 2001–2018

PGP trait or putative endophytic trait	[68]	[71]	[75]	[6]	[76]
Total isolates tested	11	26	263	78	20
Catalase	ND	26(%100)	3(%3.29)	ND	13 (65%)
Cellulase	10(%91)	ND	ND	ND	8 (40%)
Pectinase	11(%100)	ND	ND	ND	ND
Motility	11(%100)	26(%100)	4(%4.39)	58 (74%)	12 (60%)
Oxidase	ND	18 (69%)	4(%4.39)	ND	14 (70%)
IAA	ND	ND	ND	ND	9 (45%)
ACCD	ND	ND	ND	ND	14 (70%)
Siderophore	ND	ND	ND	ND	12 (60%)
Phosphate solubilization	ND	ND	ND	ND	10 (50%)
Nitrogen fixation	ND	ND	91(%34.6)	ND	4 (20%)
Spore formation	ND	19 (73%)	ND	ND	ND
Salinity tolerance (6% NaCl and higher)	ND	ND	ND	ND	ND
Osmotic tolerance (0.6 M sucrose)	ND	ND	ND	54 (69%)	ND
Osmotic tolerance (1.2 M sucrose)	ND	ND	ND	ND	ND
Amylase	ND	ND	ND	10 (13%)	2 (10%)
Host plant	Rice	Rice	Rice	Rice	Rice
PGP trait or putative endophytic trait	[70]	[37]	[62]	[60]	[61]
Total isolates tested	73	12	561	160	22
Catalase	73(%100)	ND	ND	ND	4(%18)
Cellulase	ND	ND	ND	ND	ND
Pectinase	ND	ND	ND	ND	ND
Motility	ND	ND	73(%13)	ND	ND
Oxidase	ND	ND	ND	ND	11(%50)
IAA	10(%14)	10(%83)	73(%13)	60(%37.5)	4(%18)
ACCD	ND	ND	ND	ND	ND
Siderophore	ND	6(%50)	ND	ND	ND
Phosphate solubilization	67(%92)	4(%33)	73(%13)	60(%37.5)	ND
Nitrogen fixation	73(%100)	12(%100)	73(%13)	60(%37.5)	ND
Spore formation	ND	ND	ND	ND	ND
Salinity tolerance (6% NaCl and higher)	ND	ND	ND	ND	ND
Osmotic tolerance (0.6 M sucrose)	ND	ND	ND	ND	ND
Osmotic tolerance (1.2 M sucrose)	ND	ND	ND	ND	ND
Amylase	ND	ND	ND	ND	ND
Host plant	Rice	Rice	Rice	Rice	Rice

(*continued*)

Table 4 (continued)

PGP trait or putative endophytic trait	[64]	[35]	[65]	[32]	[51]
Total isolates tested	12	49	87	20	15
Catalase	ND	49(%100)	ND	20(%100)	ND
Cellulase	ND	45 (92%)	ND	ND	ND
Pectinase	ND	47 (96%)	ND	ND	ND
Motility	ND	20 (41%)	9(60%)	ND	9(60%)
Oxidase	ND	35 (71%)	ND	ND	ND
IAA	2(%16.6)	49 (100%)	35(%40.2)	ND	15(%100)
ACCD	2(%16.6)	5 (10%)	2(%5.7)	ND	2(%13.3)
Siderophore	2(%16.6)	32 (65%)	ND	ND	ND
Phosphate solubilization	ND	36 (73%)	7(%20)	18(%75)	7(%46.6)
Nitrogen fixation	5(%41.6)	16 (33%)	4(%11.4)	18(%90)	5(%33.3)
Spore formation	ND	ND	ND	ND	ND
Salinity tolerance (6% NaCl and higher)	ND	33 (67%)	ND	ND	ND
Osmotic tolerance (0.6 M sucrose)	ND	49 (100%)	ND	ND	ND
Osmotic tolerance (1.2 M sucrose)	ND	47 (96%)	ND	ND	ND
Amylase	ND	ND	ND	18(%90)	ND
Host plant	Rice	Rice	Rice	Rice	Rice

ND not determine

consortiums, while some microbes are more suitable for colonization of the endoriososphere [51].

16 Conclusion

One of the most invaluable results on PGPRs of most crops is the recognition of the identity of their microbiota, mechanism of action, and, finally, optimal formulation for sustainable agriculture. As mentioned above, the choice of microbial consortiums for each plant is important. Therefore, in order to optimize the bacterial mix in an optimal formulation, we should study and evaluate carefully rates of growth, metabolism, and antagonism of each bacterial isolate together and separately [51]. The next step is to use modern technologies such as biotechnology and nanotechnology to produce effective transgenic products with better compliance with the plant root and high root colonization capability through competition with soil-based microbes. Fortunately, extensive research has been carried out on rice, and given the importance of the host, further research is needed on the formulation of dominant endophytic strains for most rice cultivars around the world.

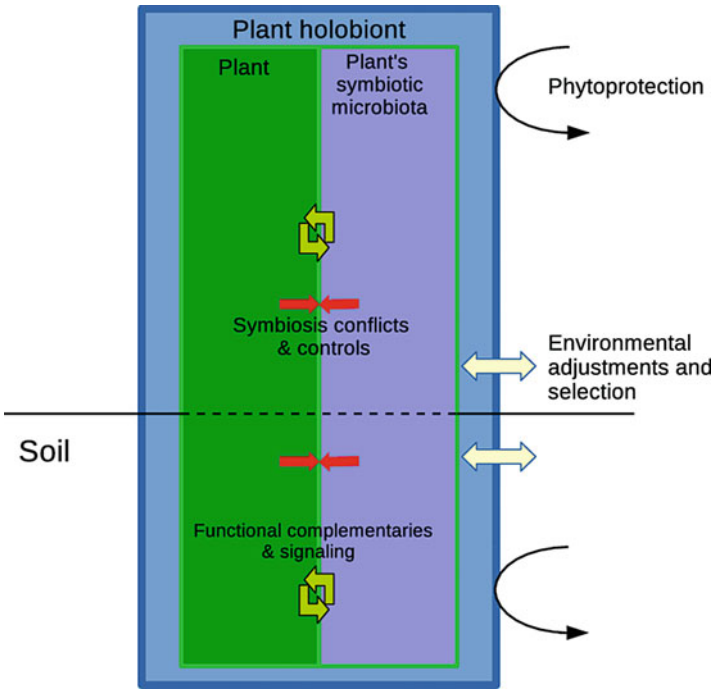

Fig. 5 Scheme of the plant holobiont and related key interaction aspects both in terms of evolution and functioning [77]

Note

Considering the harmful and pathogenic endophytic bacteria is useful in the mass of bacteria. In the authors' research, a number of these agents have been mentioned including *Pseudomonas oryzihabitans*, *P. fulva*, *Pantoea ananatis*, *Pantoea* sp., and *Cellulomonas* sp. [59].

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Unraveling Plant-Endophyte Interactions: An Omics Insight

9

Enketeswara Subudhi, Rajesh Kumar Sahoo, Suchanda Dey, Aradhana Das, and Kalpana Sahoo

Contents

Abstract

Plants are home to a wide assemblage of nonpathogenic microbial community belonging to different phyla, bacteria, fungi, actinomycetes and viruses, the collective term for which is called endophyte. These endosymbiotic individuals exhibit endophytism principally by assisting in vigor and endurance to host plant and protect them from biotic (pathogenic infections) and abiotic stress (water, heat, nutrient, salinity, and herbivory). In return, these endosymbionts receive energy in the form of carbon from the host tissue. Colonization of endophyte in the internal tissues has been reported almost in every plant examined so far either

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in intercellular or intracellular mode. The form of relationships established with the host plant may be mutualistic, symbiotic, commensalistic, and trophobiotic. These are either rhizospheric or phyllospheric in origin. To establish such mutualistic relationships between plants and endophytes, certain chemical signals play important role in inducing production of the enhanced amount of secondary metabolites in host plant tissues. These novel metabolites act as a very good source of stress relievers to host and protect from grazing animals. The renewed interest in endophyte is due to the biotechnological relevance of these signal molecules as these have been used as a good source for production of biochemical compounds of industrial importance more specifically in agriculture and medicine. Additionally, their capacity to decontaminate soil bacteria and bring in soil fertility invites huge application in phytoremediation. However, the physiology, biochemistry, and genetics behind such complex interactions, exchange of chemical signals, and their production (the endophytism of plan-microbiome) are still half-understood. With the advent of new efficient analytical technology in molecular biology and genomics, the basic information on the existing diversity, phylogenetic lineage, evolution, and ecophysiological information about these endophytes has been tried to understand. However, the functional gene expression, posttranslational modifications, and protein turnover under various environmental circumstances are only revealed through transcriptome and proteomics analysis. Soon, high-throughput next-generation sequencing technology has remarkably changed the whole scenario of solving the intricate issues entangled with the complexity underlying endophytism. Sequencing of the whole genome of individuals following cultivable method (genomics), multiple host plants and their microbiome (comparative genomics), non-cultivable methods (metagenomics and metatranscriptomics), and microarray has been proved to be potential approaches to unravel the truth behind the plant-endophyte interactions. The present script deals with scopes, prospects, and outcomes of use of these "omics tools" to understand the deep insight into the mechanism of plant host infestation, biological reason behind the mutualism between host and endophytes, exchange of biochemical compounds, enhanced production of secondary metabolite, and host plant ecology.

Keywords
Endophytes · Genomics · Metagenomics · Metatranscriptomics · Metaproteogenomics · Endophytism

Abbreviations

BLAST	Basic local alignment search tool
BLAT	BLAST-like alignment tool
Bp	Base pairs
Brenda	Braunschweig enzyme database
CAMERA	Community cyberinfrastructure for advanced microbial ecology research and analysis
COGs	Clusters of orthologous groups
DGGE	Denaturing gradient gel electrophoresis

Gbp	Giga base pairs
ITS	Intertranscribing regions
KEGG	Kyoto encyclopedia of genes and genomes
LSU	Large subunit
LTQ	Linear trap quadrupole
MALDI	Matrix-assisted laser desorption/ionization
MALDI ToF	Matrix-assisted laser desorption/ionization time of flight
Mbp	Mega base pair
MEGAN	MEtaGenome ANalyzer
MetAMOS	Open source and modular metagenomic assembly and analysis pipeline
MG-RAST	Metagenomic rapid annotations using subsystems technology
MS	Mass spectroscopy
NCBI	National center for biotechnology information
NGS	Next-generation sequencing
NOGs	Non-supervised orthologous groups
NR	Negative regulatory domain
Pfam	Protein families
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
PRINTS	Protein fingerprints
Q-ToF	Quadruple time-of-flight mass spectrometer
RDP	Ribosomal database project
SMART	Simple modular architecture research tool
SRTINGS	Search tool for the retrieval of interacting genes/proteins
SSU	Small subunit

1 Introduction

The compelling interest in endophytes has been for the secondary metabolites they are able to produce. These molecules of natural origin generally hold over-abundance of beneficial properties useful as a source of potential drug [1, 2]. With time, molecules with desired bioactivities have been identified and isolated from a large number of endophytes from a list of medicinal plants which are yet to be explored for their large-scale commercial production. Accumulation of these secondary metabolites, nutrients, and hormones might have been produced in host plants associated with endophytes in response to the biotic and abiotic stress or due to some unknown reason during such mutualism exhibited by the endophytes [3, 4].

Therefore, understanding the whole science behind the establishment of endophytism is the prime effort to be taken so as to utilize the incredible potential of these high valued molecules produced by endophytes having potential applications in pharmaceutical, food, agriculture, and medical industry. So far, attempts are taken to establish their identity and diversity and to unravel the metabolite potential. But, there has been a paradigm shift among the scientific community toward

understanding the physiology, biochemistry, and the genetics behind the plant-endophyte relationship of several ecological niches.

Endophytes are basically bacteria or fungi which reside as intercellular or intracellular in rhizospheric or phyllospheric tissues of the host plant under symbiosis or commensal type of association. Horizontally transmitted endophytes, the most ubiquitous fungal endophyte, inhabiting major plants studied for their potential production of bioactive molecules, have been subjected to unanswered questions on interactions of endophytes with their plant hosts, phytophagous insects, and other fungi. The present review highlights the possible role of modern omics-based methods in understanding the gray areas of endophytism and their potential exploration in different avenues of biotechnology.

With the advent of new efficient analytical technology in molecular biology and genomics, the basic information on the existing diversity, phylogenetic lineage, evolution, and ecophysiological information about these endophytes has been understood [5]. Although the genomic study provides the information on molecular machinery and functional expression is only revealed through transcriptome analysis under various environmental circumstances without any information on posttranslational modifications and protein turnover, etc. proteomics deals with the study of functional gene expression products. Alone, the transcriptomic or proteomic study is incomplete in interpretation in absence of genomic information. Moreover, supplementing the information generated from the metagenomic study with those of metatranscriptomics and metaproteomics may help to find detailed intricacies involved in the establishment of endophytism. All these techniques although self-sufficient are inter-reliant, and thus the information obtained from individual method or technique is the accompaniment to each other. Thus, the combinatorial approach of analyzing the data produced from various recent "omics" tools will help in resolving the enigma existing in the endophyte-host relationship. Genome sequencing options, metagenomics and metatranscriptomics, have increased the perspective of analyzing the microbial community. These meta-omics methodologies explore the community having the genes, transcripts, and proteins from millions of microbes and provide a scope to analyze their biochemical functions as well as systems-level microbial interactions. Functional assays involving whole community analysis in addition to metagenomics and metatranscriptomics offer new avenues to understand biogeochemical environments, complex ecosystems involving host organisms, their metabolism, and the possible interactions among them. These meta-omics studies characteristically aim to recognize a panel of microorganisms, genes, their variants, and metabolic pathways of the microbial community inhabiting an uncultivated sample. These abovementioned analytical methods complemented with advanced computational tools (systems biology science) are the key approaches to understanding significant biochemical and environmental interactions occurring in a community. Thus, we have described here the current skills, recent technological advances, and unresolved challenges involved in the functional analysis of microbial community.

2 Conventional Techniques Used in Endophyte Studies

2.1 Direct Observation Method

This is the most common, simple, and preliminary method of observing the endophyte harboring the living host plant tissue directly under light or electron microscope. This method reveals the limited morphological features of infested microorganism inside the intercellular and rarely intracellular tissue of the plant which is generally restricted to the hyphal structure or the shape of the bacteria. It excludes isolation of endophyte in vitro and further possible characterization. Since it cannot provide any information about taxonomically distinguishable features like spores/endospores/conidia or spore-producing structures, this method can hardly be used for understanding the phylogenetic identification and biodiversity analysis of the endophyte [6, 7].

2.2 Cultivation-Dependent Method

Typical methodology of isolation and cultivation of endophyte through in vitro culture-dependent techniques involves the following few steps (Fig. 1): (i) surface sterilization of host plant tissue infested with endophyte adopting different protocols [8], (ii) isolation of endophyte grown out of the incubated plant sample on suitable media, (iii) manipulation of cultivation and incubation parameters to promote sporulation, and finally (iv) identification through morphological, microscopic, and biochemical analysis [9–13]. This cultivation-dependent method has been followed across the globe since it is one of the rapid effective methods of isolation of endophytes from the plant tissue under changeable parameters during the whole process of sterilization, inoculation, and incubation in artificial culture media. Cultivation and characterization of endophyte isolates have been inevitable not only to understand the population structure and species diversity [13–17] but also to unravel the physiology behind its role in plant growth and protection through the production of secondary metabolic compounds [18–21].

Reports reveal that the enhanced recovery of endophyte from the host plant using the smaller size of tissue incubated [22] or whole leaves instead of leaf disk [13, 23]. However, retrieval and growth of the higher amount of endophyte without spores (sterile isolates) add problem in detailed characterization and identification as no taxonomic units have been assigned based on limited morphological features. This urges implementation of different means to promote sporulation or production of the fruiting body. Guo and his coworkers could enhance the rate of sporulation from 48% to 59.5% by incubating the palm leaf tissue onto media surface and again to 83.5% though longer incubation of isolates for 3 months onto pieces of petiole of the leaves [24]. It has been also observed that some of the endophyte species of a community might be suppressed by fast-growing isolates in vitro due to competition for nutrients in artificial media.

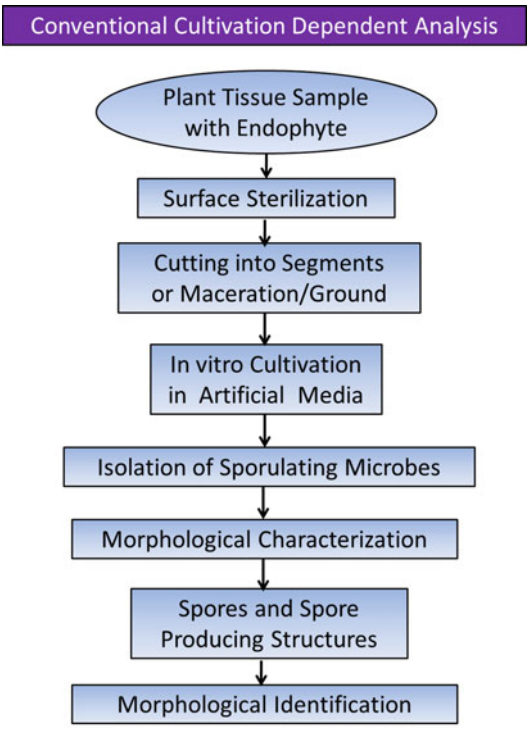

Fig. 1 Flow chart showing the cultivation-dependent analysis of endophyte, Source: [65]

However cultivation-dependent method has been subjected to methodological shortcomings and technical biases. Characterization, more specifically sporulation of endophyte, gets affected by the techniques followed for sterilization, the conditions maintained for incubation, and the type of media used. The adaptability of the plant type, the tissue size, their number, and the endophyte community to the overall procedure of isolation also bring in limitations in revealing the facts and features about the harboring endophyte in the host tissue.

3 Omics Intervention in Endophyte Studies

3.1 Genomic Analysis by the Cultivation-Dependent Method

In absence of omics-based analytical methods, different isolates obtained from conventional in vitro cultivation procedures having similarities in morphological (color and texture of colonies) and growth characteristics had been named as different "morphotypes" and were designated as "Mycelia sterilia" (where the sporulation could not be obtained). But these morphotypes could not be accepted as units of the taxon to classify and establish the diversity existing within them and failed as the criteria to establish phylogenetic lineage [13, 16, 25, 26]. With the

intervention of molecular techniques, the bottlenecks that generally cropped up through traditional protocols for identification and diversity analysis could, however, be overcome.

Molecular identification of sporulating and non-sporulating endophytes basing on DNA markers like ITS, 23S, and 18S for fungus and 16S for bacteria may be the suitable solution in detecting the diversity existing in the community. Using ITS marker, 19 non-sporulating morphotypes of *L. chinensis* could be identified and grouped into three genera *Mycosphaerella*, *Xylaria*, and *Diaporthe* [16]. Similarly, 221, 74, and 18 non-sporulating fungal endophytes were grouped into 37, 64, and 3 taxa, respectively [13, 25, 26]. González and Tello in Spain could assign taxonomic identifier at the level of genus and species for non-sporulating *Vitis vinifera* employing ITS sequences [27]. In this way, these DNA marker-based molecular analyses will not only help to assign a taxonomic place for the community present in phyllosphere and rhizosphere but also understand the species diversity existing within them. ITS analysis supported with the morphological information became the preferred practice specifically for understanding biodiversity among the isolates present in host tissues and their ecology [28, 29]. As uses of 18S and 28S genes are generally employed to find out the higher taxonomic level (order and suborder) for endophytic fungi, these genes are analyzed in supplementation to ITS marker study which reveals the taxonomic lineage at a lower level (genus and species) and to detect novelty. Morakotkarn and his associate could identify 71 strains from host bamboos belonging to *Phyllostachys* and *Sasa* species under *Sordariomycetes* and *Dothideomycetes* order by employing ITS and 18S, respectively [30]. Similar protocols were followed by other workers for taxonomic diversity analysis of *Theobroma cacao* and *Pinus halepensis*, respectively [31, 32].

The abovementioned cultivable methods and techniques have been limited to identification of only those isolates that could be cultivable in artificial media, the establishment of their novelty, and the understanding of diversity existing among the community. Nevertheless, outcome of these protocols fails to throw sufficient light on deciphering the relationship between the host plant and the endophyte and the molecular basing of intricacies behind endophytism probably because these methods do not truly encourage the growth of all the members of endophyte present in the community in the plant tissue in a defined artificial media in vitro (cultivation-dependent method).

3.2 Metagenomic Analysis by Cultivation-Independent Method

Metagenomics is the genomic analysis of total DNA of all the members of the microbial community in an environment which is otherwise called as community genomics or environmental genomics bypassing the detection and in vitro cultivation of every single organism present in any microbiome. Metabolic implications and factors associated with host-endophyte interactions, due to non-cultivable microbes whose population is reported to be much higher (90–99%) in any environmental sample than the in vitro cultivable isolates, can thus be better realized

following this protocol [33]. The size of metagenomic DNA (DNA of the entire microbiome present in the sample) is generally of huge size and warrants a fast and efficient high-throughput method to handle and analyze the large-sized genome and suitable pipelines and software to translate into understandable information. Next-generation sequencing (NGS) is the most recent intervention for metagenomic analysis which brings in the unrecorded unprecedented information of the microorganisms present in any host-endophyte association much beyond the data generated from individual cultivable taxa. It is further supported by several numbers of tools that make fat data into information explicable to the analyzer.

With the recent discovery and intervention of alternate omics tools since the last two decades, the above inherent disadvantages of culturable methods can be overcome where total community genomic DNA of the sample (both host plant and the endophyte) is subjected to molecular analysis. Non-cultivable or cultivation-independent methods involve a sequence of molecular reaction steps as shown in Fig. 2: (i) community DNA (genomic DNA of host plant and all the members of endophyte present) isolation; (ii) ITS, 28S, and 18S gene amplification for fungal and 16S for bacterial endophyte; (iii) electrophoretic separation and excision of bands generated from DGGE (denaturing gradient gel electrophoresis); (iv) cloning into vector and transforming into heterologous host *E.coli* DH5α and sequencing; and (v) phylogenetic analysis using NCBI database for identification of the taxa.

The outcome from the genome analysis through uncultivable method employing ITS could unravel novel taxa never been reported through cultivation-dependent method which are YJ4-61, YJ4-9, and YJ4-70 from *H. japonica* tissues [34], 1 unidentifiable clone from *L. chinensis* [35], and 14 novel taxonomic units from *Magnolia liliifera* [36]. The novelty attained by this method of exploring the endophyte community diversity could be possible due to the ability to overcome the technical biases of traditional protocols that did not allow scoring all the genomes present but could not be grown in vitro and the high-resolution ability of DGGE coupled with sequencing covering the whole genome.

3.3 Predicted Functional Analysis of Metagenome

In a sequence-based analysis, genomic information is assessed from microbes without culturing them and can be used to identify microorganisms and genes and compare organisms of different communities. Sequence-based metagenomics can also be used to establish the diversity, enumeration of bacterial species present in the sample, ecophysiological relationship with the microflora dwelling in it with prevailing physiochemical parameters of that environment, and predicted genes and metabolic pathways. Analyzing microbial diversity can provide valuable information at less cost of experimentation and also predict the metabolism prevailing and the ecology of microbes. Recent developments on different efficient cloning vectors, along with newer methods of DNA isolation and sequencing, have been possible to clone and express bigger-sized DNA into large-sized metagenomics clone library for functional analysis. Over the past 10 years, shotgun sequencing technology used in

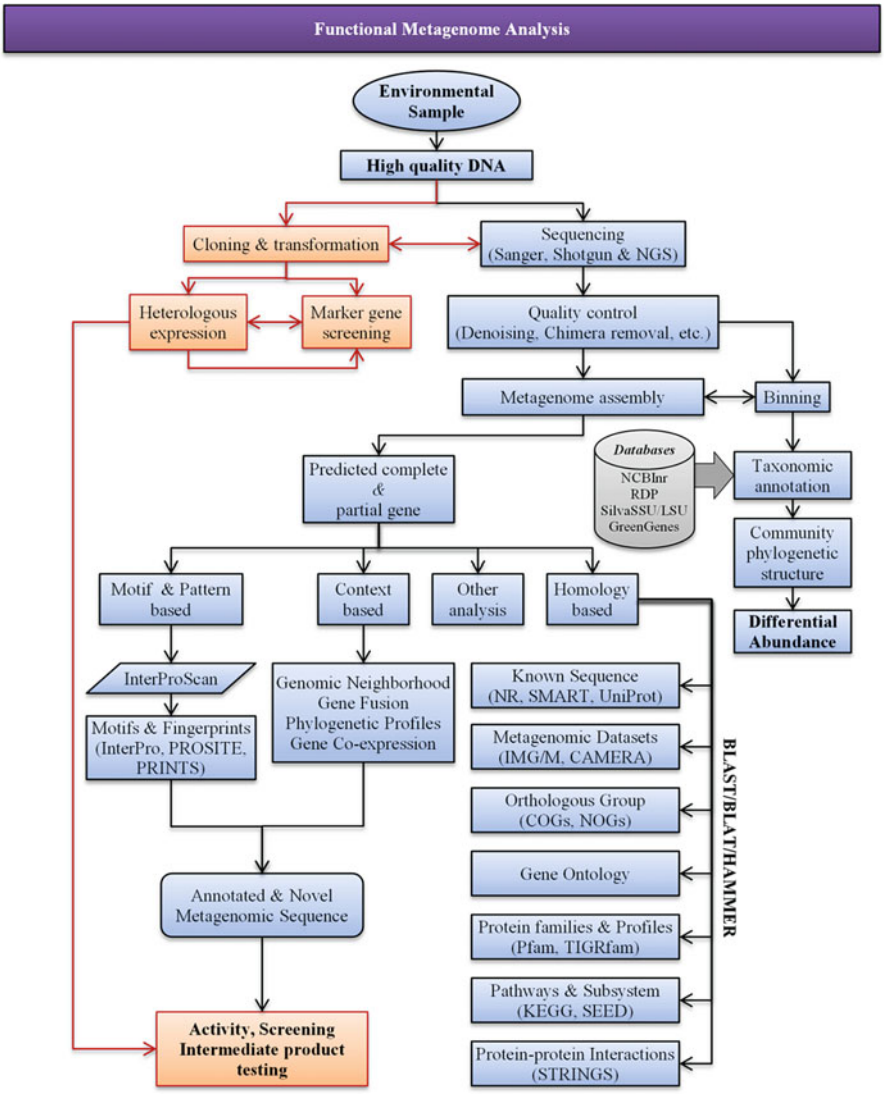

Fig. 2 Flow chart showing the cultivation-independent analysis (metagenomic and predicted functional) of endophyte, Source: [65]

metagenomics has gradually shifted from classical Sanger sequencing to NGS methods [37]. Although Sanger sequencing technology is the best sequencing technology because of its low error rate for sequencing maximum 30 Kb insert size [37], the main disadvantages are the labor-intensive cloning process and cost-intensive factor for giga base pair sequencing (approximately 400,000 USD) [37]. In next-generation sequencing technology, 454/Roche and Illumina/Solexa systems are

widely used for analyzing the sequence of the microbial community and functional analysis of metagenomic samples. The sequence reads generated from NGS methods are generally shorter than Sanger's sequencing read. In 454/Roche technology, average read length is 600–800 bp and produces ~500 Mbp in single run, whereas in Illumina/Solexa, the read length is 150–300 bp and produces ~6 Gbp in single run sequencing [37]. After NGS sequencing, post-sequencing analysis such as assembly, annotation, binning, ORF prediction, taxonomic profiling, and metabolic reconstruction is the most challenging step which decides the output of any metagenomic sample. Several bioinformatic tools and data storage pipelines have been developed to simplify the post-sequencing analysis, such as MEGAN [38], MG-RAST [39], GALAXY [40], CAMERA [13], and MetAMOS [41]. PICRUSt [42] and TAX4FUN [43] tools are used to analyze the predicted functional activity by using 16S rRNA gene sequences, the details of which presented in Fig. 2. However, so far as our knowledge goes, many attempts are taken to predict the functional genes and their possible activities from any community DNA of any endophytic niche.

3.4 Multigenomic Analysis

Whole genome analysis of an individual endophyte harboring a plant may not be able to completely establish their lifestyle, the kind of which may vary from mutualistic symbionts to commensalistic symbionts or saprotropism to biotropism. They can also behave as latent pathogens and latent saprotrophs [44]. Therefore, comparing the genome of isolates having endophyte association and the non-endophyte complement can help realize the controlling factors responsible for their adaptation to host plant, their evolutionary trajectory, and genetic basis of endophytism exhibited by them [45].

Endophyte adaptation, potential to promote the growth of host plant, as well as the tolerance to stress and production of protection metabolites could be understood from metagenomic analysis of rice plant root tissue associated with the endophyte [46]. Dinsdale and his groups presented the differential functional characters of nine endophyte microbiome following comparative metagenomic analysis [33]. Comparative genome analysis using Illumina platform for *Cadophora* sp. and *Periconia macrospinosa* with their 32 close relatives with different lifestyles could reveal the functional differences with respect to the presence of a number of genes for aquaporins, melanin synthesis, enzyme proteases, and lipases, despite their common origin. The insight into basic biological and evolutional understandings has been made available through comparative genomic study in several endophyte species, *M. bolleyi* (37), *P. subalpine* (29), *S. indica* (34), *X. heveae*, (31), *P. scopiformis* (33), and *C. trifolium* (35), originating from different habitats [47]. The detailed community diversity of fungal endophyte and its composition in a Japanese forest have been analyzed [48]. Large-scale functional characterization of fungal communities using 454 genomes employing metagenomic protocol could accumulate a surplus of information of ecophysiology of the endophyte community which reported the existence of fungi of both mycorrhizal and endophytic origin [49].

Since the metagenomic analysis has been recently the intervened approach in understanding endophytism as a whole, attention must be given in making the public database more furnished with genome/reference genome sequence information for the target plant/endophyte species. However, the intervention of proteomic analysis in supplementation with the metagenomic analysis in a non-cultivable approach can help further to understand the existing interaction of these two ecotypes.

3.5 Transcriptomics and Metatranscriptomic Analysis

Although whole genome analysis or metagenomic analysis could provide the existence of genes in a community, their functionality in terms of whether the gene is expressed in that particular environment could not be accounted which is very much key in realizing the endophytism in any endophyte-host plant association. The environmental parameters present in and around of any ecological niche determine the expression of a character in any organism irrespective of the presence of the gene that controls it. Therefore, understanding differentially expressed genes with respect to the altered environment through isolation and characterization of all the RNA present in a community (transcriptomics and metatranscriptomics) can be a better way of knowing the response of interacting endophyte species with the host and the environment. Comparative expression analysis of the transcriptome of plants with and without endophyte infestation and of endophytes in and outside of host can help to understand the interactive factors responsible for endophytism, production of secondary metabolites, and plant growth-promoting substances. Ambrose and Belanger and their associates successfully revealed the differential expression of 200 genes associated with host plant *Epichloe festucae* infested with endophyte named *Festuca rubra*. However, these transcriptome data correlated with the data generated from their respective genomes can complete the understanding about the facts [50].

In the metatranscriptomic analysis, the transcripts or RNAs are directly isolated from environment or community. This type of analysis brings the direct connection between the genetic makeup of the community and the respective functionality in situ through the profiling of the expressed transcripts and linking them with the prevailing ecophysiological conditions. Such metatranscriptomic analysis is accomplished by either cDNA clonal libraries derived from mRNA as given in Fig. 3.

Using dual RNA-sequencing technology for comparative transcriptional profiling, the differential regulation of genes meant for nutrient availability was observed in wheat roots infested with bacterial endophyte *A. brasilense* [51]. This helped him to interpret the basic mutualistic relationship existing between them. The occurrence of transcripts foreign to host soybean genome system through comparative metatranscriptomic analysis helped in tracing the infestation of endophytes and free-living microbes in different soybean host plants [52].

Although these recent methods of analyzing community RNA provide a considerable amount of information and insight, they are not free from limitations. First, extraction of RNA directly from an environmental sample is often problematic and

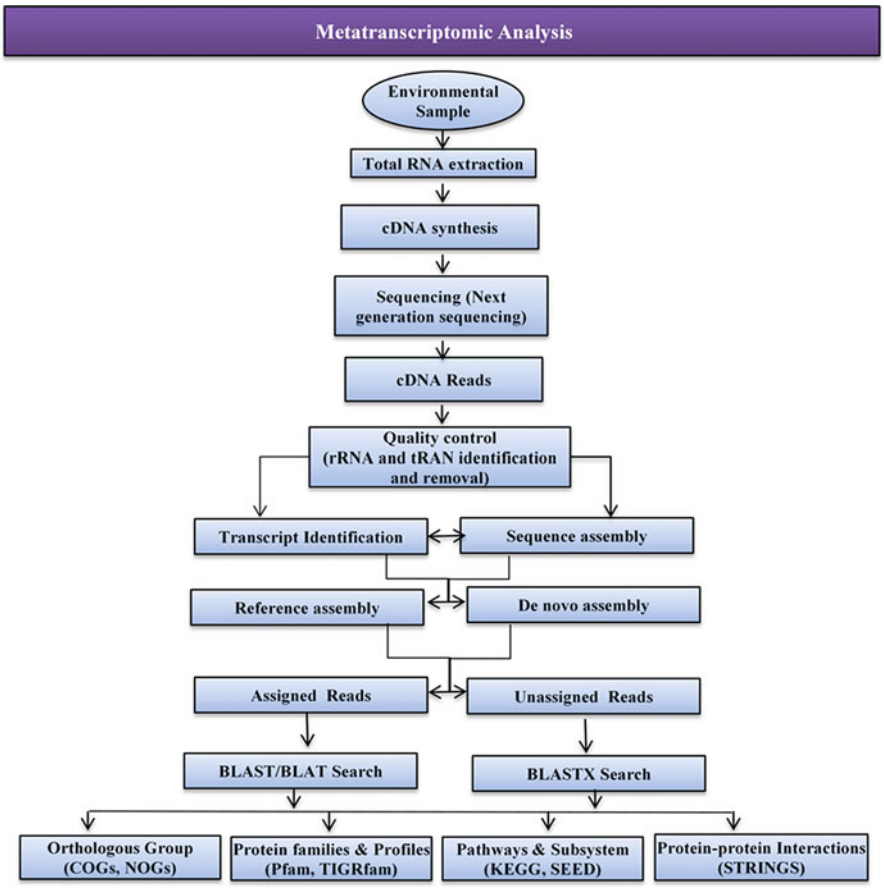

Fig. 3 Flow chart showing the metatranscriptomic analysis of endophyte, Source: [65]

the concentration is often low. For this reason, previous studies have used additional amplification steps to increase the concentration of initial transcripts [45, 53]. Second, separation of mRNA from the abundant non-mRNA (e.g., ribosomal or transfer RNA) is also problematic, and, as a result, the gene expression profile of the sample often remains limited. Consequently, the low gene expression profile may not result in statistically meaningful transcription patterns or may not provide sufficient coverage for most of the genes of a complex community. Thus, in earlier studies, the focus was only on the most dominant members present in a respective community.

3.6 Proteomics and Metaproteomic Analysis

Soon after the realization that genomic and metagenomic analysis is still unable to unravel the real-time in situ functional information about the community,

post-genomic analysis like proteomics and metaproteomics is gaining expedition. Proteomics involves the scale analysis of total proteins present in an organism, and on the other hand metaproteomics is basically the analysis of functional expression of the community genes and interpretation of activities at the time of sampling. Metaproteomics is the process of direct identification and assessment of the prevailing functionality of the microbial community of an environmental sample. It directly assesses the microbial functional profile. In addition, the developments of computing and bioinformatic tools provide a more solid source of protein identification [54].

The metaproteomic analyses include four important steps, the process flow of which is given in (Fig. 4): (i) extraction and purification concentration of protein; (ii) denaturation and reduction; (iii) protein separation, digestion, and analysis by MS; and (iv) protein identification basing on spectroscopic data [54]. In metaproteomics it is vital that the sample protein should be a characteristic one in terms of both quality and quality [55]. The first metaproteomic analysis conducted was the AMD biofilm system [56]. Metaproteomic analysis of endophytes has been either done by direct lysis method which involves extraction of total protein of the endosphere (the microenvironment where the plant and endophyte association is established) under different environmental conditions or comparative analysis of their fingerprinting two-dimensional gel electrophoresis to understand the effect of any parameter on secondary metabolite production, etc. [55]. On the other hand, the indirect method of lysis involves extraction of total protein of isolated endophytes subjected to different treatments or stress environments [57]. However, going another step further, similar protein analysis protocol may be followed for host plants with and without the association of endophytes in order to ascertain some particular proteins responsible for bringing in the possible interactions between the host and the endophyte. One such metaproteomic report in sugarcane associated with endophyte *Gluconacetobacter* reveals 78 differentially expressed proteins using mass spectrometry-based analysis.

The most common methodological bottlenecks in this type of analysis could be the on-site interference of large quantity of secondary metabolites and other cell contents (organic acids, lipids, and polysaccharides) present in the sample tissue. In addition, lack of sufficient amount of information on the microbial community from varied possible ecological niches to characterize these endophytes adds insufficiency of this technique. However, the metaproteomic study needs to be supplemented with its genomic information to make the analysis complete.

3.7 Metaproteogenomic Analysis

It is much well known to the scientific community that not all the genes present in any ecological niche (individual organism or community) are functional at any point of time under a specific environmental condition which makes the analysis of DNA, RNA, or protein (individual organism or community) incomplete in isolation. Metaproteogenomics is a study which deals with the combined exploration of

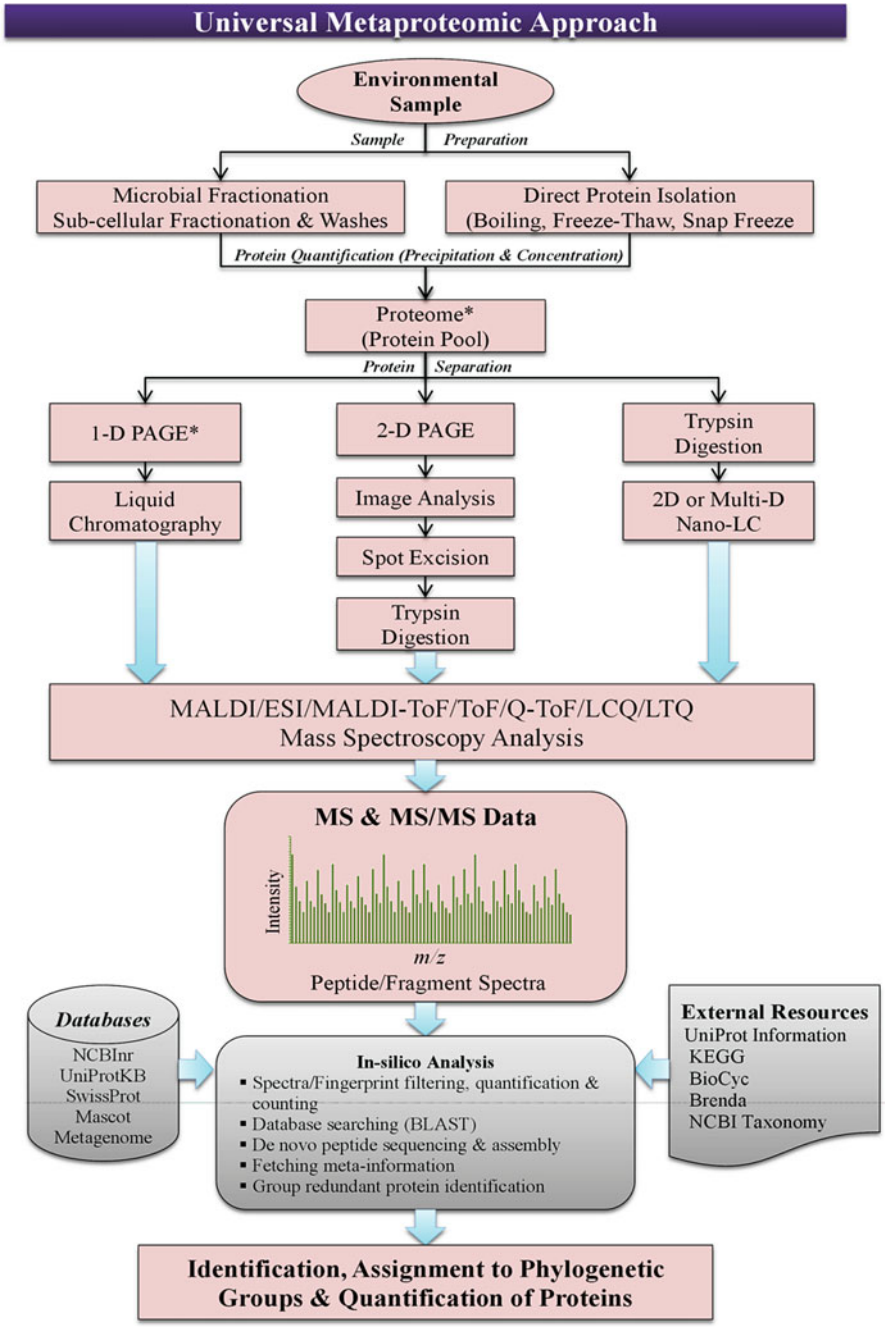

Fig. 4 Flow chart showing the metaproteomic analysis of endophyte, Source: [65]

metaproteome as well as metagenome of the same sample linking the genome and proteome of any environmental sample. One outstanding experimental analysis following metaproteogenomic approach has been done in rice where they analyzed the microbial communities of both rhizosphere and phyllosphere and reported that the expression of nifH genes was restricted to rhizosphere only, although present in both [58]. Similarly using metaproteomic approach, a group of workers could mine out certain distinctive traits that were restricted only to phyllospheric bacteria but in the rhizosphere [59]. This approach has the potentiality to correlate the genetic and functional diversity of any community. With time there has been the advent of newer tools and establishment of suitable specific proteogenomic pipelines which urges application of such techniques for more insight study of endosphere and endophytism. The functional proteins are involved in establishing the plant-endophyte interactions, the endophyte protein secretion systems and their identification [60, 61].

3.8 Microarray-Based Analysis

Microarray is basically a laboratory tool where two-dimensional ordered array of microscopic amount of DNA of entire genome of any organism is immobilized onto a solid surface (slide/chip/membrane) so as to measure the simultaneous expressions of all these genes/genetic material or to genotype (polymorphism and mutation) multiple regions of entire genome together. Microarray-based analysis has been attempted to understand the mystery lying behind the endophytism, gene profiling and expression studies of endophytes, unravelling the facts behind the possible interaction between the host plant and the associated endophytes. The advantage of the use of Symbiosis Chip in this technique has been the unique consecrations to study the expressional analysis of both the partners to understand the exchange of signals between them in terms of differential coordinated differential expressions [62]. He specially designed a dual-genome Symbiosis Chip to reveal the physiology behind the nodule development host legume plant *Medicago truncatula* and the bacterial host *Sinorhizobium meliloti* using its complete genome. Another advantage of this method is its ability to characterize an unknown species if the genome sequencing of its allied species is done by following genomic interspecies microarray hybridization technique [63]. One such successful accomplishment could be the efficient discovery of genes of unknown endophyte *K. pneumoniae* 342 by hybridizing its DNA associated with those of *Escherichia coli* K12. Thus this technique became popular and got very fast applications in endophyte genome analysis. Identification of genes in host plants responsible for initiation of endophyte infestations could be possible in *Epichloe-Neotyphodium* endophyte [64] and differential regulation of genes in *Arabidopsis-Pseudomonas* endophyte [25] through microarray studies of induced transcriptional changes. The limitations of this advanced technique are restricted access to the specific gene profiling databases and absence of a specific reference.

4 Conclusion

Profound knowledge of endophytism is inevitable to utilize the enormous potential of endophytes for human welfare in many different valuable means employing multidisciplinary omics science and techniques. This will sure help the better realization of the establishment of such symbiosis between plant and endophyte, tolerance exhibited by endophytes, and their role in growth promotion of host plants. Omics study-based generation of information when supplemented with other disciplinary approaches related to systems biology, several myths behind the total physiological and biochemical processes involved in host-endophyte interaction can be busted and most expectedly predicted models can be established to further expedite the process of understanding. This can ultimately pave a path to sustainable bioprospecting through several biotechnological means.

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Isolation of Endophytes: The Gold Standard?

10

Binay Chaubey

Contents

Abstract

Endophytes live in the internal tissues of plants without causing any visible damage to their hosts. They provide many beneficial effects to their hosts which range from promoting the plant growth to providing protection against various biotic and abiotic stresses to the host. They have also been considered to play direct or indirect roles in the synthesis of various biomolecules obtained from their host. However, most of the endophytes isolated and characterized so far have been culture dependent, and their number has been very low. Culture-independent studies of endophytes include high-throughput assays like transcriptomics, proteomics, etc. These high-throughput assays have predicted much higher numbers of endophytes as compared to the culture-dependent studies. The high-throughput assays have helped in deciphering the phylogenetic analysis of the whole microbiome of the plant and indicated very strong and deeper role of

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the endophytes in the host than anticipated before. However, in the absence of any gold standard approach for isolation and proper characterization of these endophytes, the high-throughput omics-based assays remain isolated to the particular hosts only. Their true potential in agriculture or crop protection will not be utilized. Therefore, the complex interaction of endophytes with their hosts needs to be studied by combining the omics-based assays with the culture-dependent methods, which can actually provide the true study material and their appropriate analysis.

Keywords
Endophyte · Epiphyte · Phytohormones · Rhizosphere · Unculturable bacteria · 16S RNA · Pyrosequencing · Transcriptomics

1 Introduction

Interaction of plants with the microbes in their vicinity is inevitable, diverse, and a very complex phenomenon. It incorporates symbiosis, mutualism, commensalism, or pathogenic interactions. The major source of these interactions is the immediate rhizosphere and phylosphere. The plant–microbe interaction has been an interesting area of research where endophytes have been implicated for diverse roles. Endophytes are mainly the bacteria or fungi with few archaea and protists that live in the internal tissues of plants without causing any apparent disease or negative effects to their hosts [1–7]. Endophytes colonize intra- and intercellular spaces in the roots as well as the aerial parts of the host plant [3, 4]. Major information available on endophytes is obtained from fungal and bacterial groups, although algae if present inside are also considered endophytes. The close association between endophytes and the host and their range of interactions can be a parameter to study the coevolution [8].

The possibility of a plant growing in gnotobiotic ambience without any endophytic influence is almost negligible under natural environmental conditions [9]. In fact, the ability of the plants to fight with biotic and abiotic stresses will be severely compromised in the absence of endophytes [10]. Endophytes with their direct or indirect interactions with the host help in the growth of host plant [11, 12]. It has been, therefore, suggested that almost every species of plant on earth hosts one or more endophytes [13, 14]. Numerous studies have shown that certain medicinal properties of plants may be attributed directly or indirectly to the endophytes colonizing the respective plants [15]. Therefore, the role of endophytes in their respective hosts should be a matter of discreet study. However, it is important to note that many endophytic bacteria or fungi have also been isolated independently from the soil indicating that these organisms are not obligate endophytes. This puts forth few important questions: Why and how an organism becomes an endophyte if it can survive independently? What indispensable role endophytes play in the host, if any? Furthermore, is it advantageous to the bacteria to be endophyte; if so then what is the advantage that it gains as an endophyte and what is the mechanism?

2 Why Study Endophytes?

Endophytes have been implicated for their wide range of direct and indirect interactions with plants. They have been studied for their roles in promoting the plant growth, helping them fight with various biotic and abiotic stresses, source of various biomolecules, etc. These functions have the potential for their utilization in fields like agriculture, industry and medicine, etc. Therefore, increasing studies based on different aspects of endophytes have been emerging in the recent times.

3 Role of Endophytes in Plant Growth and Agriculture

Based on their ecological and metabolic preferences, the host plants sustain the endophytes which promote their growth [16–18]. Knowledge about interactions between endophytes and their hosts can help in developing new strategies for more productive and sustainable methods in agriculture. Endophytes facilitate the acquisition of essential metabolites or modulate the level of hormones within the host plant thereby directly influencing the plant growth [19]. Endophytes also impart their hosts the antagonistic strength against phytopathogens as their indirect impact on the host [20]. Plant growth-promoting bacteria (PGPB) help in acquiring the important nutrients like nitrogen, iron, and phosphorus. About 30–50% of nitrogen in crop fields is obtained by biological nitrogen fixation by soil microbes [21]. Metagenomic analysis of rice roots detected the genes involved in nitrogen cycling suggesting involvement of endophytic microbiome of rice in nitrification and ammonia oxidation processes [22]. *Azoarcus* sp., an obligate nitrogen-fixing endophyte, colonizes through lateral root tips by secreting cell wall-degrading enzyme endoglucanase and expresses nitrogenase (nif) genes inside the rice roots. Mutants with lower endoglucanase expression show poorer host colonization and lesser amount of fixed nitrogen [23]. Endophytic biomass also serves as a source of nitrogen after being directly digested in *Agave tequilana* [24]. Common endophytic genera like *Bacillus*, *Enterobacter*, *Staphylococcus*, *Pseudomonas*, *Methylobacterium*, *Micrococcus*, *Pantoea*, and *Rhizobium* promote plant growth by phosphate solubilization [25–27].

3.1 Phytohormone Production

Production of phytohormones which directly promote plant growth and improve their stress tolerance is reported from endophytic bacteria [28]. Genes encoding proteins for biosynthesis of plant hormones like indole acetic acid (IAA), cytokinins, or gibberellins (GAs) have been detected by metagenomic analysis of the plant microbiome [29–31]. In the metagenome of root gall of tomato, four pathways for the biosynthesis of IAA were detected [32]. Recently IAA and GA3 were isolated from *Streptomyces mutabilis* IA1 strain of Saharan soil, which reduced the progression and severity of *Fusarium culmorum* infection in wheat seedlings [33].

With increasing global population, there is consistent pressure on agriculture for increased crop yield. However, due to increase in global warming, drought, and other abiotic factors, the global crop production is facing greater stress than ever before [34]. Plants accumulate 1-aminocyclopropane-1-carboxylate (ACC) under these stresses. ACC is the immediate precursor of ethylene. It is synthesized in roots and distributed to the apical parts and converted to ethylene by ACC oxidase [35]. High ethylene levels in stressed plants often lead to reduced plant growth or even cell death [36]. Selected endophytes reduce the harmful environmental effects on plants by metabolizing the ACC by producing ACC deaminase [36–39].

3.2 Cold and Drought Stress Tolerance

Our understanding of endophytic bacteria-mediated improvements of plant resistance to abiotic stress is still in its infancy and based on culturable endophytic bacteria only. Inoculation of psychrotolerant strains *Pseudomonas vancouverensis* OB155 and *P. frederiksbergensis* OS261 in tomato significantly improved their tolerance to cold stress, and it also induced the cold-related stress genes of tomato [40]. Inoculation of *Burkholderia phytofirmans* strain PsJN in *Arabidopsis* promoted its growth and increased cell wall synthesis which improved resistance against cold stress [41]. Inoculation of *B. phytofirmans* PsJN also demonstrated several functionalities in potato plants [42]. There was upregulation of the genes involved in transcriptional regulation, cellular homeostasis, and ROS detoxification in drought-affected host plants. This indicates that endophytes could sense physiological changes in host and modulate its gene expression to adapt to the new environments.

3.3 Boosting Plant Nutrient Uptake

Endophytes also boost the uptake of important plant nutrients like iron, which is a very essential element for the plants but has limited bioavailability. Siderophores, produced by bacterial endophytes, chelate the iron in the soil and make it available to the host in soluble complexes, which also reduces the bioavailability of iron to the phytopathogens [43–46]. Quenching of quorum sensing is also used as antivirulence strategy by some endophytes [47]. Keeping in view the colonizing patterns and the close contact with the host plants, bacterial endophytes have good potential to act as biocontrol agents against phytopathogens in agriculture [19].

Several compounds produced by endophytic bacteria also provide promising protection against phytopathogens [48]. Treatment of cotton plant with the purified iturins increased their protection [49]. Many volatile organic compounds (VOCs) produced by endophytes enhance resistance against phytopathogens. VOC 2,3-butanediol-producing *Enterobacter aerogenes* when inoculated in maize is protected against the northern corn leaf blight disease caused by the fungus *Setosphaeria turcica* [50, 51].

These observations indicate that endophytic bacteria can be exploited in agriculture to improve the productivity as well as protect the plants from extreme biotic and abiotic stresses. However, in order to actually exploit these biocontrol traits and their potentials in agriculture, the identification of the endophytes and understanding about the mechanisms of their different cross talks in the host plant need to be studied. For this universal method for their isolation, identification and characterization are badly needed.

4 Isolation and Identification of Endophytes

Major challenge in endophyte study is their isolation apart from epiphytes and evaluation of their discreet endophytic characteristics. As most of the endophytes are commonly taken from rhizosphere, they are considered as subpopulation of rhizospheric microbial mass [52, 53]. So far, the most predominant and consistent endophytes have been reported from Classes α, β, and γ Proteobacteria of phylum Proteobacteria followed by the members of Firmicutes and Actinobacteria. Endophytes, although less frequently found classes such as Acidobacteria, Planctomycetes, Verrucomicrobia, and Bacteroidetes, have also been reported. The commonly found bacterial endophytic genera include *Bacillus*, *Enterobacter*, *Staphylococcus*, *Pseudomonas*, *Methylobacterium*, *Micrococcus*, *Pantoea*, and *Rhizobium* [52, 54–58].

Colonization of plant tissues by endophytes usually takes place through primary and lateral roots, root hair cells, and tissue wounds which emerge during plant growth; however, the obligate endophytes enter usually through seeds [59, 60]. Chemically diverse exudates from plant root attract bacteria for colonization [55]. Endophytes also enter the plants through apical routes like stomata, lenticels, etc. [61–63]. *Enterobacter asburiae* JM22 colonizes cotton plants by hydrolyzing cellulose in the plant cell wall [55]. However, *Herbaspirillum seropedicae*, another endophyte, colonize the plant tissues even without producing cell wall-degrading enzymes by an unknown mechanism [64, 65]. These studies indicate that almost all the plants host one or more endophytes. A plant without harboring endophytes would be possibly at disadvantage with respect to its growth as well as to deal with biotic and abiotic stresses. However, the effect of ecology on distribution of endophytes is poorly understood so far. The diversity of endophytic microorganisms is influenced by several factors, including the developmental stage of the host and its geographical origin and distribution [66], pH of soil and its moisture content, temperature and altitude, etc. [16, 67]. Therefore, there are many missing links in endophyte research which need serious consideration like effects of different environmental variables on endophyte, diversity of endophytes in multiple plant species, etc. before we can exploit the endophytes to their real potential.

Most of the endophyte isolation procedures from plants involve washing and surface sterilization of the plant parts using different optimized protocols. The plant parts are then either crushed or cut in small pieces and aseptically placed directly on the culture plates or in broth, followed by possible pure culture isolation [68–75].

Different types of bacterial culture media with various supplements have been used to isolate the endophytes. However, this conventional approach has not been able to isolate the majority of endophytes from plant microbiome, often referred as unculturable bacteria. This is certainly serious setback for endophyte based study particularly when the aim is to exploit the endophytes in agricultural production or as a source of biomolecules or protecting the crop plants from biotic and abiotic stresses. Clear understanding of the mechanisms of interactions of individual endophytes with their surrounding is important for their rational exploitation. The present endophyte research is confined to only culturable microbes, and minimal information is available on the unculturable endophytes or the total microbiome of the plant. This is important to point out that the endophytes maintain a complex and dynamic direct or indirect relation with their host as well as with other endophytes inside the host. The study and explanation of this complex and dynamic interaction is often missing from the endophyte research.

In recent times using whole-cell transcriptomics, pyrosequencing of 16S rRNA, and other high-throughput omics approaches, the complexity of the endophytic populations has been well realized. By pyrosequencing of 16S rRNA of endophytic population of tomato leaves, five phyla were identified which comprised of 90% Proteobacteria, 1.5% Actinobacteria, 1.4% Planctomycetes, 1.1% Verrucomicrobia, and 0.5% Acidobacteria [54]. Dynamics of endophytic diversity at different stages of growth of *Beta vulgaris* L was evaluated by detecting the operational taxonomic units (OTU) using PCR-based Illuminapyrosequencing. Maximum OTUs were detected at rosette and tuber formation stages [58]. Bredow et al. evaluated 16S rRNA gene using bioinformatics tools and evaluated the phylogenetic relations among the endophytes from the aerial parts and seeds of different crops [76].

The introduction of high-throughput assays will certainly generate vast amount of data on endophyte diversity which will help in better understanding of their interaction with the surrounding microbiome and ultimately their better exploitation in agriculture and crop protection. However, it is important to point out that different sequencing studies of whole-cell 16S rRNA gene often indicate higher numbers of possible endophytes in the plant than actually isolated by the culture method, although phylogenetic relations have been drawn using 16S rRNA gene sequencing or other high-throughput assays in several cases. Therefore, our present understanding about the host-endophyte relation is far from satisfactory unless majority of endophytes are cultured and studied in isolation with a possible simulation of their endophytic ecology.

5 What Makes an Endophyte?

The rhizosphere is a dynamic, highly complex and competitive microecosystem for microorganisms to survive. Endophyte, which is a subpopulation of this rhizosphere, can colonize the plant which possibly gives them better advantage over the other members of the rhizosphere to get more nutrients and survive more effectively. However, one fundamental question emerges. What makes a bacterium an

endophyte? So far we have no clear answer to this question, but it is well understood that the capacity of endophyte to colonize and survive in the host is a complex and multifactorial process. The mechanism of colonization of plant by endophyte has been studied to a great extent though. Efforts have been made to understand what makes a bacterium an endophyte by sequencing of phylogenetic marker genes and multiple high-throughput omics-based culture-independent assays. These studies indicate that plants form their endophytic microbiome, and roots play the role of gatekeeper in this process. To evaluate the role of genes involved in host colonization, *Azoarcus* sp. strain BH72 was exposed to the exudates from *Oryza sativa* cv. Nipponbare, and the whole-genome microarray was performed to analyze gene expression profile of the endophyte [77]. Expression of many genes possibly involved in rhizosphere competence was upregulated, and many others were downregulated. This result indicated the role of root exudates in inducing the colonization. However, the high-throughput assays on endosphere microbiome are often difficult due to poor abundance of bacterial DNA compared to plant DNA in non-root tissues. A non-biased culture-dependent enrichment of endophytic bacterial cells from plant tissues may circumvent this problem [78]. Although the potential of high-throughput omics-based methods is impeccable, its drawbacks to answer different questions like what decides the bacteria to be an endophyte and its relation with the host and the whole microbiome are also very well realized in the absence of a standard culture-dependent method. The required efforts on making culture-dependent studies have not been exploited to its strength so far. Therefore, there is an urgent need of a gold standard approach to culture and study the various aspects of endophytes. This will help in more structured utilization of endophytes to the benefit of crop production and protection, although the contradicting findings obtained so far indicate that for simulation of plant's endosphere microbiome for economic purposes, a lot of work still needs to be done.

6 Conclusion

Application of plant-associated endophytes or microbiome in increasing the agricultural production or using them in protecting the plants against various biotic and abiotic stresses is a promising future prospect. It is envisaged that bacterial endophytes can be used as substitutes for chemical fertilizers and pesticides on crops which may lead to a paradigm shift in future agricultural practices. However, this field is still in its infancy and full of various contradicting or incomplete results from different studies which indicate the challenges of studying endophytes. Among many unanswered questions, it is not yet known why the bacteria colonize to exert its beneficial effects on plants and to what extent the host is actually benefited by this. It is bewildering to find that *Acidobacteria*, *Gemmatimonadetes*, or *Archaea* which are so difficult to culture can survive as endophytes. Therefore, definitive and exhaustive knowledge base is needed for the future development of more rational approaches of biocontrol or plant growth-promoting endophytes.

It must be emphasized that culture-dependent methods have not lost their relevance as they are the only source of genuine indispensable study materials for identification and characterization of bacteria which can be a true gold standard for endophyte study. The use of gnotobiotic plants would allow the elucidation of the importance of the endosphere microbiota in plant growth and health. However, it is also true that we have no clue about the culture conditions of many microbes which are important members of the plant microbiome. Therefore, broad culture-dependent screening techniques including pyrosequencing, to determine the genotype-specific endophyte associations, need to be carried out in order to have a more profound understanding of the specific association(s) between plants and endophytes as well as between the various types of endophytes found within a single host plant. Future studies need to focus on answering many questions which are unanswered so far in endophyte research. The combination of high-throughput methods like metagenomics, proteomics, and metabolomics along with data mining will render more exhaustive and comprehensive picture of the endosphere microbiome in the future.

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Part III

Production of Useful Metabolites

Pharmaceutical Potential of Marine Fungal Endophytes

11

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Abstract

The marine environment is currently well explored as one of the most essential sources regarding to natural products in research, since organisms from oceans have exhibited exceptional biological, biochemical, and biosynthetic potential. Similarly, microorganisms' natural products represent a substantial area for novel therapeutic compounds search. Many reviews highlighted microbial metabolites as targets for discovery and development of new drugs, especially anticancer, antibiotics, antifungals, and antiparasitics among others. Marine fungal endophytes are therefore virtually unlimited sources of novel compounds with numerous potential therapeutic applications due to their immense diversity and proven ability to produce natural products of medicinal and pharmaceutical importance, thus inspiring researchers to further study them. This book chapter reviews some of the endophytic fungi isolated from marine sources that produce metabolites with various biological activities against human pathogenic microorganisms. The potential for the exploitation in the pharmaceutical industry and concerns are also discussed.

Keywords

Fungi · Metabolites · Biological activities · Industry · Medicine · Mangroves

1 Introduction

Microorganisms are present everywhere and are very versatile in all ecosystems around the globe. Studies demonstrated that 1% of all bacterial species and less than 5% of fungal species are currently known, and at least 10 million microbial species are unknown, remaining hidden in nature [1]. The marine environment is a huge untapped reservoir for fungal diversity. Jones et al. [2] estimated that there could be up to 10,000 species of marine fungi and currently more than 1100 species are documented [3]. These fungi have a wide distribution and recently a number of new species have been discovered [4–7]. Endophytes, a term introduced in 1866, are microorganisms that reside inter- and intracellularly in plants without causing harm to their host cells [8–11]. Endophytic fungi have been found in every plant species examined, and it is estimated that there are over one million endophytic fungi [12–16]. Interest in endophytes has increased immensely with the discovery of the endophytic fungus *Taxomyces andreanae*, from *Taxus brevifolia*, producing the billion dollar anticancer drug paclitaxel (taxol), a diterpinoid used in the treatment of breast and ovarian cancers. However, the industrial-scale production of taxol, although being a promising drug, is not meeting the demand of the world population with respect to the number of cancer patients dying from the disease. According to

the World Health Organization [17], between 2008 and 2030, the number of new cancer cases is expected to increase more than 80% in low-income countries, which is twice the rate expected in high-income countries (40%). This alarming fact posits scientists to explore new possibilities to synthesize the compound (taxol) from other sources.

Many *Taxus* species are recognized as good taxol producers, namely, *T. baccata*, *T. chinensis*, *T. cuspidata*, *T. media*, *T. floridana*, *T. canadensis*, *T. yunnanensis*, *T. mairei*, *T. sumatrana*, and *T. wallichiana* [18]. Interestingly, many non-*Taxus* species also yield taxol compound such as *Cardiospermum halicacabum*, *Citrus medica*, *Cupressus* sp., *Ginkgo biloba*, *Hibiscus rosa-sinensis*, *Taxodium distichum*, *Podocarpus* sp., *Torreya grandifolia*, *Terminalia arjuna*, and *Wollemia nobilis* [19]. Indeed, till date it has been found that taxol can be produced by some other endophytic fungi, namely, *Metarhizium anisopliae* and *Cladosporium cladosporioides* MD2 producing the compound of interest up to a level of 800 μg/L [20]. Recently, a study conducted by El-Sayed et al. (2018) has demonstrated that fungal endophytes, *Aspergillus terreus* EFB108, EFB59, and EFB14 isolated from the leaves and twigs of *Podocarpus gracilior* cork are potent taxol producers [21]. Moreover, El-Maali et al. (2018) proposed a faster and accurate isolation method of taxol from two endophytic fungi, namely, *Clasdosporium sphaerospermum* and *Metarizium anisoplae* yielding up to 30.36 and 116.373 μg/L, respectively [22].

More than two decades have elapsed since the notable ground breaking discovery of taxol happened, but importantly numerous bioactive molecules have also been isolated from various endophytic fungi [8, 23]. However, only a handful have been described, which means the opportunity to find new and targeted natural products from endophytic microorganisms in different niches and ecosystems is boundless [24] and fungi can be easily grown under routine culture techniques and hence the potential for discovering a virtually inexhaustible supply of metabolites is high. Additionally, modification of culture conditions alters biosynthetic pathways thus leading to the possibility of producing more novel derivatives [25]. Since endophytes are capable of producing compounds similar to their host plants, research on endophytes can help in the preservation of world's diminishing biodiversity [26].

2 Biodiversity and Taxonomy of Endophytes

Endophytic fungi comprise a highly diverse ecological and taxonomic group. An ecosystem having the greatest biodiversity is the one with the greatest number of endophytes and the most diverse microorganisms [26–28]. Tropical and temperate forests are considered to be the most diverse terrestrial ecosystems, with the greatest number and diversity of endophytic fungi since the plants residing in these regions are in a constant evolutionary race to survive [29–32]. For instance, the isolation of 418 endophyte morphospecies from 83 healthy leaves of *Heisteria concinna* and *Ouratea lucens* found in the tropical forest of central Panama demonstrated high diversity in these regions. High diversity is associated with the ability of endophytic

fungi to cope efficiently with environmental conditions due to exchange of information between them and the higher plant [31, 33]. The oceans, specifically deep-sea hydrothermal vents, mangrove forests, algae, and sponges, have recently been discovered to be an ecological niche with high diversity of endophytes [29, 34, 35]. Endophytes are also widely distributed in marine sources especially in sponges and algae with a higher ratio of new compounds to known compounds from algicolous fungi (3.1:1) as compared to sponge-derived fungi (1.4:1) [30, 36–38]. Endophytic fungi are therefore detected in all plants such as algae, mosses, ferns, and numerous angiosperms and gymnosperms including plants from tropical, temperate, and boreal forests, as well as those from extreme arctic, alpine, and xeric environments [36, 39]. Taxonomically, most of the endophytic fungi belong to the phylum *Ascomycota* and its associated anamorphs, while some species belong to the phyla *Basidiomycota* and *Zygomycota* [31]. The majority of these isolates belonged to ubiquitous genera (e.g., *Acremonium*, *Alternaria*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, *Fusarium*, *Geniculosporium*, *Phoma*, and *Pleospora*), but some genera are common in both tropical and temperate climates (e.g., *Fusarium*, *Phomopsis*, and *Phoma*) while members of the *Xylariaceace*, *Colletotrichum*, *Phyllosticta*, and *Pestalotiopsi*s predominate as endophytes in the tropics [33, 40].

3 Natural Products from Endophytes

Endophytic fungi open a new avenue for the quest of biologically active secondary metabolites. Various prominent and novel secondary metabolites from various phytochemical classes have been derived from endophytic fungi, namely, alkaloids, terpenoids, steroids, lactones, phenols, and isocoumarins among others [41]. In the past two decades, many valuable bioactive compounds with antimicrobial, antioxidant, antiviral, cytotoxic, immunosuppressive, and anticancer activities have been successfully discovered from the endophytic fungi [42–44]. Metabolites produced by endophytic fungi usually originate from biosynthetic pathways and include isoprenoid, polyketide, and amino acid and belong to structural groups, such as terpenoids, steroids, xanthones, quinines, phenols, isocoumarins, benzopyranones, tetralones, cytochalasins, and enniatins [45, 46]. The major supply of paclitaxel, a well-known and highly functionalized tetracyclic diterpenoid bioactive compound used as anticancer agent, from wild *Taxus* plants was problematic as it was unable to satisfy the growing demand of the market since they were produced in minute amount in these plants [42]. The promising approach to eradicate this problem was the successful discovery of a paclitaxel producing endophytic fungus *Taxomyces andreanae* isolated from the Pacific yew *Taxus brevifolia* in 1993. There are nowadays at least 19 genera of endophytic fungi (that is, *Alternaria*, *Aspergillus*, *Botryodiplodia*, *Botrytis*, *Cladosporium*, *Ectostroma*, *Fusarium*, *Metarhizium*, *Monochaetia*, *Mucor*, *Ozonium*, *Papulaspora*, *Periconia*, *Pestalotia*, *Pestalotiopsis*, *Phyllosticta*, *Pithomyces*, *Taxomyces*, and *Tubercularia*) which are capable of producing paclitaxel and its analogues, for instance, 10-deacetylbaccatin III and baccatin III among others [47–49]. Lovastatin, a potent inhibitor of HMG-CoA

reductase enzyme used as lipid lowering agent was produced as secondary metabolite by the fungi *Penicillium* sp., *Monascus ruber*, and *Aspergillus terreus.* It was recently found to be produced by the endophytic fungus *Aspergillus niger* which was isolated from the healthy tissues of *Taxus baccata* [50]. Another anticancer drug podophyllotoxin, an aryl tetralin lignin derivative, most commonly isolated from *Sinopodophyllum* plants show good antiviral and cytotoxic activities [41]. The fungus *Fusarium oxysporum*, isolated from the plant *Juniperus recurva*, produced the compound Podophyllotoxin up to a level of 28 μg/g of dry mass and showing activity in U-87 cell line [51]. In 2014, Huang et al. successfully isolated six fungi from the species *Sinopodophyllum hexandrum* (Royle) [52]. One fungal strain named TW5 was able to produce two metabolites, namely, kaempferol and podophyllotoxin. The yield of the latter compound was 49.3 μg/g of mycelia dry weight after 7 days of fermentation. The fungal endophyte *Pestalotiopsis fici* was isolated from branches of *Camellia sinensis* (L.) Kuntze grown in China. Seven new isoprenylated chromone derivatives, namely, pestaloficiols F-L were isolated. Among the new compounds, pestaloficiol J, K, and L exhibited cytotoxicity with IC_{50} values ranging between 8.7 and 99.3 μM for HeLa cell line [53]. Recently a study conducted by Katoch et al. (2018) isolated 27 endophytes from the plant species *Viola odorata* Linn. and the endophytes were evaluated for lipase inhibitory activity which results in an IC_{50} of <10 μg/mL [54]. It is reported that the extracts of *Aspergillus* sp. exhibited promising lipase activity with an IC_{50} value of 3.8 μg/mL. Interestingly, endophytic fungi also have the ability to increase the yield of key natural products by producing gene products that are responsible for inducing metabolites production. For instance, the genomic analysis of the fungus *Aspergillus nidulans* revealed the expression of terrequinone A which was further reported to generate up to 27 polyketides, 14 non-ribosomal peptides, one terpene, as well as two indole alkaloids [55]. Thus, realizing the capability of terrestrial endophytic fungi to produce diverse bioactive molecules, research is underway to isolate and screen microbes of diverse habitats and unique environment for discovery of novel metabolites. One such unexplored and less-studied microorganisms are the marine endophytic fungi [1, 56].

4 Marine-Derived Compounds from Endophytes

The marine environment is an extremely diverse reservoir of life, and across the range of organisms, there is a virtually untapped source of structurally unique natural products [57–59]. About 70% of earth's surface is covered with water and it comprises 500,000 live species divided into 30 different phyla. The oceans world over have a coastline of about 312,000 km and a volume of 137 $km^3 \times 106$ km^3 making it the largest ecosystem on earth [60]. Marine-derived fungi, which are obtained from various marine substrates such as fish, sponges, mangroves, and algae, has attracted considerable attention from chemists due to their outstanding capacities to produce active metabolites with pertinent biological activities [37, 59, 61, 62]. It is statistically reported that among the 272 new compounds

discovered from marine-derived fungi till 2002, 85% of them are produced by epi/endophytes [45]. Nevertheless, the first marine-derived product to gain approval as a drug was ziconotide, a non-narcotic analgesic isolated from the cone snail genus *Conus*, which is currently marketed as Prialt® [55]. Ziconotide works by binding to N-type calcium channels to block pain signal transmission to the brain through nerve cells in the spinal cord [63]. It is used to alleviate neuropathic pain associated with cancer and AIDS and also as systemic analgesics adjunctive therapies or intrathecal morphine [64, 65]. Since then, many clinical trials are being conducted by the marine preclinical pharmaceutical pipeline on several marine-derived metabolites (Table 1). Although terrestrial fungi have an extraordinary diversity of life, the greatest biodiversity is in the oceans particularly in the deep sea floor and coral reefs with 34 out of 36 phyla of life represented [68]. The genetic and metabolic diversity of marine fungi is reflected by their particular living conditions, salinity, nutrition, higher pressure, temperature variations, and competition with bacteria, viruses, and other fungi to which they have to adapt themselves during their evolution [69]. In addition, marine fungi live in close association with soft-bodied marine organisms (algae, seaweeds, and sponges) which lack obvious structural defense mechanisms, thus relying on chemical defense through production of bioactive secondary metabolites, either by themselves or by associated microflora, to survive in their extreme habitat [70, 71]. Consequently, marine-derived fungi have been recognized as resources for new biologically active secondary metabolites [72]. The emergence of multidrug resistant bacteria, the insufficient number of effective antibiotics against diverse bacterial species, and the urgent need to eradicate the problem of infectious diseases (IDs) and noncommunicable diseases (NCDs) has inspired researchers to discover novel compounds from endophytes [60, 73].

5 Antibacterial Agents

A new polyoxygenated decalin derivative, dehydroxychlorofusareilin B, obtained from extracts of *Aspergillus* species (family *Trichocomaceae*) which was isolated from the marine brown algae *Sargassum horneri* in Korea, was found to exhibit mild antibacterial activity against *Staphylococcus aureus (S. aureus)*, methicillin-resistant *S. aureus* (MRSA), and multidrug-resistant *S. aureus* [71]. Usually, structural compounds obtained from endophytes associated with brown algae are naphto and pyrone derivatives presenting antifungal and antioxidant activity, macrolides, and bicyclic lactones showing antimicrobial and antioxidant potential as well as cytotoxic ergosterolide derivates with an unusual pentalactone B-ring [74]. As an illustration, the marine fungi *Pestalotia* sp. and *Penicillium glabrum* isolated from the surface of the brown algae *Rosenvingea* sp. and *Sargassum thunbergii*, respectively, was able to produce antimicrobial substances with potent antibiotic activity [45]. When *Pestalotia* sp. was cultured in the presence of a bacterial antagonist, the fungus produced the potent antibiotic pestalone, which showed an MIC value of 37 ng/mL against MRSA and an MIC value of 78 ng/mL against vancomycin-resistant *Enterococcus faecium* (VREF) [38].

Table 1 List of marine-derived compounds under clinical development [66, 67]

Clinical status	Compound name	Structural class	Molecular target	Source	Disease
Approved	Cytarabine Ara-C	Nucleoside	DNA polymerase	Sponge	Cancer
	Ecteinascidin **Yondelis**®	Alkaloid	Soft tissue sarcoma	Tunicate	Cancer
	Eribulin mesylate	Macrolide	Microtubules	Sponge	Cancer
	Omega-3-acid ethyl esters **Lovaza**®	Omega-3-fatty acids	Triglyceride-synthesizing enzymes	Fish	Hypertriglyceridemia
	Trabectedin	Alkaloid	Minor groove of DNA	Tunicate	Cancer
	Vidarabine Ara-A	Nucleoside	Viral DNA polymerase	Sponge	Antiviral
	Ziconotide **Prialt**®	Peptide	N-type Ca channel	Snail	Pain
Phase III	Brentuximab vedotin	Antibody drug conjugate	CD30 and microtubules	Mollusk	Cancer
	Plitidepsin **Aplidin**®	Depsipeptide	Rac1 and JNK activation	Tunicate	Cancer
	Glembatumumab vedotin	Alkaloid	Transmembrane glycoprotein NMB	Snail	Cancer
Phase II	DMXBA	Alkaloid	α7 nicotinic acetylcholine receptor	Worm	Cognition & schizophrenia
	Elisidepsin **Irvalec**®	Depsipeptide	Plasma membrane fluid	Mollusk	Cancer
Phase I	Bryostatin 1	Polyketide	Protein kinase C	Bryozoa	Cancer
	Hemiasterlin	Tripeptide	Microtubules	Sponge	Cancer
	Pseudopterosins	Diterpene glycoside	Eicosanoid metabolism	Soft coral	Wound healing
	Floridosides	Glycolipid	Free radicals	Alga	Anti-inflammatory
	Contulakin G	Peptide	Neurotensin receptor	Marine cone snail	Anti-inflammatory

Jiang et al. (2018) recently isolated the endogenous fungus *Penicillium* sp. GD6 of the mangrove *Bruguiera gymnorhiza* (L.) Lam [75]. A compound 2-deoxy-sohirnone C was isolated from the fungus and tested against *S. aureus* resulting in an MIC value of 80 μg/mL. Another mangrove-derived fungus is *Penicillium citrinum* HL-5126 isolated from the species *Bruguiera sexangula* var. *rhynchopetala.* A novel chlorinated metabolite 20-acetoxy-7-chlorocitreorosein was identified from the ethyl acetate extract of the fungi and tested against *Vibrio parahaemolyticus* resulting with a MIC value of 10 μM [76]. Endophytic fungi and the labyrinthulid *Aplanochytrium minutum* were also successfully isolated from *Sargassum cinereum* and *Padina tetrastomatica* [77]. Extracts of *Fasciatispora nypae*, a marine fungus isolated from Mangrove plant, had the widest range of antimicrobial activity against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli (E.coli)*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* [59]. The salt water culture of an unidentified fungus obtained from a *Haliclona* sp. sponge was shown to produce several new hirsutane sesquiterpenes hirsutanols A and *ent*-gloeosteretriol which exhibited mild antibiotic activity against *Bacillus subtilis*. Hirsutanols were previously isolated from the terrestrial fungus *Coriolus consors*, but a seawater-based culture of *C. consors* produced hirsutanol D demonstrating that the use of seawater with terrestrial fungi can yield new metabolites [38]. Penicilactone, obtained from extracts of *Penicillium* sp. isolated from *Annella* sea fan species exhibited potent antifungal and antibacterial activity against *Microsporum gypseum* and MRSA with MIC values of 228.57 mM and >700 mM, respectively [71]. Marine fungi belonging to the genus *Aspergillus*, *Pencillium*, and *Fusarium*, isolated from mangrove forest were tested for antibacterial activity with *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and for anti-mycobacterial activity against *Mycobacterium tuberculosis* H37 RV. These fungal compounds showed best antibacterial and antimycobacterial activities when extracted with *n*-hexane, methanol, and ethyl acetate [78]. The endophytic *Chloridium* sp. isolated from the surface-treated root tissues of *Azadirachta indica* A. Juss was reported to have promising antibacterial activity against *E.coli* and *Bacillus* sp. with the production of a highly functionalized naphthaquinone javanicin [12].

A marine fungus, *Xylaria psidii* KT30, isolated from the red seaweed, *Kappaphycus alvarezii*, exhibited significant antibacterial activities against *Bacillus subtilis* and *S. aureus* with the respective inhibition zones of 8 ± 0.57 mm and 7 ± 0.57 mm [79]. [80] successfully isolated *Cladosporium cladosporioides* from the seaweed, *Sargassum wightii* [80]. This compound proved to be a potential candidate for future cost-effective antimicrobial treatment. The aqueous extract (50 mg/mL) showed inhibition activity against *S. aureus*, *S. epidermidis*, *Bacillus subtilis*, *E. coli*, and *Aspergillus niger* with inhibition zones of 6.5 ± 0.11, 5.0 ± 0.50, 6.0 ± 0.90, 3.0 ± 0.10, and 3.0 ± 0.10 mm, respectively.

Recent studies described cultivable fungal community associated with jellyfish, Nemopilema *nomurai.* Yue et al. (2015) findings reported a total of seven morphotypes isolated, which were assigned into four genera *Aspergillus*, *Cladosporium*, *Purpureocillium*, and *Tilletiopsis* from two main phyla (Ascomycota

and Basidiomycota) [81]. Ethyl acetate was used as solvent for the antimicrobial assays. The antimicrobial results showed that all of the 13 EtOAc extracts displayed different levels of antibacterial activity, three extracts from the genera *Aspergillus*, *Tilletiopsis*, and *Cladosporium* of which exhibited strong to significant antibacterial activity to the bacterial pathogens *Staphylococcus aureus* and *Salmonella enterica*.

6 Marine Fungi as Antiparasitic, Antifungal, and Antiviral Agents

Green algae are known to have a high fungal colonizing rate and are promising sources for bioactive compounds. For instance, a novel polyketide ascosalipyrrolidinone-A was isolated from the marine fungus *Ascochyta salicornia* associated with the green marine alga *Ulva* species. This compound was reported to possess antiplasmodial activity and thus could be used in treating Malaria [77]. Metabolites isolated from endophytes derived from marine green algae usually possess bicyclical structures with oxygen and aromatic rings demonstrating cytotoxicity, antiprotozoal, and antimicrobial activities [74]. Fermentation of *Ascochyta salicornia*, an obligate marine fungus associated with green algae *Ulva* sp., resulted in the production of a diverse array of metabolites including two new unusual tetramic acid derivatives ascosalipyrrolidinones A and B, as well as the new pyrone ascosalipyrone. Ascosalipyrrolidinone A displayed antiplasmodial activity against *Plasmodium falciparum*, antimicrobial activity, and also inhibited the tyrosine kinase p56 [38]. The polyketide citrinin, produced by *Penicillium janthinellum* a fungus from the fruit of *Melia azedarach*, was found to inhibit 100% *Leishmania mexicana* at a concentration of 40 μg/mL [31].

Extracts of *Aspergillus niger* isolated from the marine brown alga *Colpomenia sinuosa*, synthesised Asperamide A, a sphingolipid which displayed activity against *Candida albicans*. Additionally, two novel cyclic hexapeptides containing both anthranilic acid and dehydroamino acid units, sclerotides A and B were isolated from the marine-derived halotolerant *Aspergillus sclerotiorum* and showed antifungal and antibacterial activity [82]. The endangered tree *Torreya taxifolia* harbored *Pestalotiopsis microspora* which exhibit antifungal activity associated with pestaloside, an aromatic glucoside, and two pyrones: pestalopyrone and hydroxypestalopyrone [27]. *Penicillium* sp. from marine sea fan yielded the macrolide (+)-brefeldin which showed antifungal activity against *Microsporum gypseum* with MIC value of 228.57 mM [71]. *Bostrychia tenella* (family Ceramiales), a Brazilian marine seaweed harbored about 45 endophytic microorganisms, were evaluated for their antifungal properties showing positive activities [83].

Cytonic A and B are two novel compounds, recently isolated from *Cytonaema* sp. and were reported to be novel human cytomegalovirus protease inhibitors. Hinnuliquinone was also isolated from an EF inhabiting the leaves of Oak trees. It was identified as being a potent inhibitor of HIV-1 protease [84]. An endophytic fungus *Pestalotiopsis theae* of an unidentified tree on Jianfeng Mountain, China, was capable of producing Pestalotheol C with anti-HIV properties [33]. Interestingly,

Liu et al. (2017) isolated seven compounds from the endophyte *Treptomyces* sp. OUCMDZ-3434 from green algae, *Enteromorpha prolifera* among which three of them, namely, ailupemycin J, *R*-, and *S*-wailupemycin K and 5-deoxyenterocin showed moderate anti-H1N1 activity with percentage inhibition 47.8%, 42.5%, and 60.6% at 50 μg/mL [85].

7 Antioxidant Agents

As part of the ongoing efforts towards finding novel antioxidants from natural resources, fungal endophytes were investigated to be potential sources of antioxidants [86]. The momentousness of compounds bearing antioxidant activity lays in the fact that they are highly effective against damage caused by reactive oxygen species (ROSs) and oxygen-derived free radicals, which contribute to a variety of pathological effects [87]. The anti-inflammatory, anti-mutagenic, antiviral and anti-artherosclerotic activities of antioxidants have been offering propitious therapy for prevention and treatment of ROS-associated diseases as cancer, cardiovascular diseases, diabetes mellitus, Alzheimer, and Pakinson diseases [73]. *Penicillium roquefortii*, *Aspergillus candidus*, *Mortierella* sp., and *Emericella falconensis* are known to be producers of natural antioxidants. Extracts of *Acremonium* sp. were recently analyzed and were found to produce two novel hydroquinone derivatives which showed significant antioxidant activity [88]. Extracts from South Indian green alga *Ulva reticulata* were found to have neuroprotective effects by inhibiting both acetyl and butyrylcholinesterases. They were compared to agents currently approved for Alzheimer's disease treatment and were found to be very potent [89]. The marine fungus *Halorosellinia oceanica* was isolated from Mai Po mangrove in Hong Kong and produced a new sesquiterpenoid which displayed a unique mechanism of bio-oxidation by biotransforming 1, 2, 3, 4-tetrathydronaphthalene into four oxidative products without disrupting the activated alicyclic skeleton [90].

A novel isocoumarin, desmethyldichlorodiaportintone was isolated from the endophytic fungus *Ascomycota* sp. CYSK-4 originating from a mangrove plant, *Pluchea indica* (L.) Less. The compound was evaluated for its anti-inflammatory property and displayed IC50 value of 15.8 μM against nitric oxide production in LPS-induced RAW 264.7 cells [90]. Polysaccharides obtained from the marine fungus *Penicillium* sp. showed significant antioxidant properties against superoxide and hydroxyl radicals [89]. *Curvularia tuberculata*, an algicolous marine fungus exhibited good antioxidant properties with 62.15% of inhibition in total reducing power assay and 11.69% of inhibition in hydroxyl radical scavenging assay [91]. Moderate radical scavenging activity was observed with chaetopyranin, isotetrahydroauroglaucin, and erythroglaucin which are benzaldehyde secondary metabolites produced by the endophytic fungi *Chaetomium globosum*, isolated from the inner tissue of the marine red alga *Polysiphonia urceolata* [92]. Parasitenone was produced by the marine fungus *Aspergillus parasiticus* and possessed free radical scavenging activity. This fungus was isolated from the red alga *Carpopeltis cornea* [88].

Pyrenocine A, produced by the marine-derived fungus *Penicillium paxilli* was recently found to possess anti-inflammatory activity [93]. Anti-inflammatory activity of extracts is often associated with polyphenols metabolites. For instance, in-vivo anti-inflammatory activity which resulted in significant inhibition of asthmatic reactions was observed in polyphenolic extracts from red algae *Laurencia undulata.* Additionally, the antioxidant activity in phenolic compounds from the marine algae *Halimeda monile* and *Porphyra haitanensis* indicate the potent sources for isolation of metabolites from endophytes [89]. Plants are also screened for antioxidant compounds derived from endophytic fungi, since the discovery of pestacin and isopestacin, isolated from *Pestalotiopsis microspore*, residing in *Terminalia morobensis.* Similarly, phenolic acids and flavonoids have been isolated from endophytic fungi. For instance, extracts of *Xylaria* sp. from *Ginkgo biloba* exhibited strong antioxidant activity [73, 84]. Recently Liu et al. (2018) isolated the fungus *Ascomycota* sp. SK2YWS-L endogenous to the mangrove plant *Kandelia candel* [94]. The fungus produced the enantiomers (+)- and (−)-ascomindone D that were known to possess potential anti-inflammatory properties with IC50 values of 17.0 and 17.1 μM, respectively.

8 Cytotoxic Agents

According to the World Health Organization (WHO) Global Burden of Disease report in the year 2004, cancers occupied the third place on the list of deadliest diseases in the world [12]. It is crucial to find curative measures which hold no loopholes and act accurately and precisely to curb cancer [95]. The leptosin family of dimeric diketopiperazines is the largest classes of cytotoxic fungal metabolites. The producing fungus, *Leptosphaeria* sp., was isolated from the marine alga *Sargassum tortile* and produced lepsosins which exert antitumor activity [38]. Extracts of endophytic fungi isolated from *Sargassum* sp. yielded two ring lactones among which Lasidiplodin displayed strong anti-leukemic and potato microtuber-inducing activities [90]. Makaluvamine A is a pyrroloquinoline, principally isolated from the sponge *Zyzzya fuliginosa* and is known to have potent anticancer activity via inhibiting topoisomerase II which is important for DNA replication [95]. Several classes of terpenes, diterpenes, monoterpenes, and polyoxygenated compounds with antibacterial, antifungal, and anticancer properties have been discovered from endophytes isolated from red algae [74]. As example, the diterpene compound (+)-epiepoxydon with activity against human cancer cell lines was obtained from the fungal extracts of *Apiospora montagnei* isolated from the red alga *Polysiphonia violacea* [96]. Anti-cancer alkaloids were also derived from *Penicillium citrinum* extracts isolated from the red alga *Actinotrichia fragilis* [77]. Endophytes isolated from green algae are promising sources of cytotoxic compounds. *Fusarium* sp. isolated from the green alga *Codium fragile* produced tetrapeptide anticancer metabolite [77]. Chemical analysis of the fungus *Aspergillus versicolor*, isolated from the green alga *Penicillus capitalus*, revealed the presence of four new sesquiterpenoid nitrobenzoyl esters belonging to the cinnamolide class of drimane

sesquiterpenes. These compounds were responsible of all the HCT-116 colon carcinoma cell cytotoxicity [97]. Verrucarin A, a compound isolated from the broth of marine fungus *Myrothecium roridum*, was investigated to significantly inhibit interleukin-8 production from human leukemia cells by a mechanism that involved inhibition of the activation of the mitogen-activated kinases c-JUN and p38 [78].

Recently, in a study conducted by Rajivgandhi et al. (2018), an endophytic actinomycete identified as *Streptomyces coeruleorubidus* GRG 4 (KY457708) was found to possess potential cytotoxic activity against A549 lung cancer cells [98]. This result was linked to the chemical compound, Bis (2-ethylhexyl) phthalate (BEP), isolated from the strain. The cytotoxic test of the isolated compound showed complete inhibition against the cancer cells at 100 μg/ml during 24 h treatment. Five new phenolic polyketides, namely, 3-*O*-methylwailupemycin G (1), wailupemycin J (2), *R*- and *S*-wailupemycin K (3 and 4), and wailupemycin L (5) and two known compounds, enterocin and 5-deoxyenterocin, were identified in the endophyte *Streptomyces* sp. OUCMDZ-3434 cohabitating the green algae, *Enteromorpha prolifera*. Results showed that compound (4) exhibited cytotoxic activity on the HeLa cell with IC50 of 8.2 μM [85].

The endophytic fungal strain was isolated from the roots of the mangrove plant *Excoecaria agallocha* (Euphorbiaceae) growing in Wenchang, Hainan, China. The compounds were evaluated for their cytotoxicity against A549 and HL-60 cell lines. Compound 56 exhibited moderate cytotoxic activity against HL-60 cell line with an IC50 value of 15.7 μM, while 57 showed pronounced activity against both cell lines with IC50 values of 1.9 and 5.4 μM, respectively [99]. Chemical investigation of the mangrove-derived endophytic fungus *Fusarium* sp., isolated from fresh stems of the mangrove tree *Kandelia candel* (Rhizophoraceae), yielded a new isoflavone, 5-*O*-methyl-2′-methoxy-3′-methylalpinumisoflavone (60), together with four known compounds. Compound 60 was tested for its cytotoxic activity against HEp-2 and HepG2 cell lines. The compound inhibited the growth of both cancer cell lines with IC50 values of 4 and 11 μM, respectively [100].

Furthermore, the endophyte *Diaporthe phaseolorum* SKS019 was isolated from the mangrove plant *Acanthus ilicifolius* L. Through phytochemical screening, the compound 5-deoxybostrycoidin was identified and subjected to cytotoxic screening. Indeed the compound showed good activity against the human cancer cell lines, namely, MDA-MB-435 and NCI-H460 with IC50 values of 5.32 and 6.57 μM, respectively [101].

Cultures of *Chaetomium globosum* isolated from the marine green alga *Ulva pertusa* (Ulvaceae) collected at the Qingdao coastline, China, yielded seven new cytochalasan derivatives, cytoglobosins A–G (61–67). The cytotoxicity of cytoglobosins A–E and G (61–65 and 67) was evaluated against P388, A549, and KB cancer cell lines, where only cytoglobosins C (63) and D (64) exhibited cytotoxic activity toward the A549 cancer cell line with IC_{50} values of 2.26 and 2.55 μM, respectively, whereas the remaining compounds showed no activity (IC_{50} >10 μM). Chemical investigation of *Aspergillus ochraceus*, isolated from the marine brown alga *Sargassum kjellmanianum (Sargassaceae)*, afforded a rare 7-nor-ergosteroid possessing an unusual pentalactone B-ring system, 7-nor-ergosterolide (68).

The compounds were tested for their cytotoxic activities against NCI-H460, SMMC-7721, SW1990, DU145, HepG2, Hela, and MCF-7 cancer cell lines. Compound 68 showed selective cytotoxic activity against the NCI-H460, SMMC-7721, and SW1990 human cancer cell lines with IC_{50} values of 12.0, 16.9, and 67.6 μM, respectively.

Mohamed et al. (2009) reported on a new group of natural products isolated from a marine-derived fungal strain classified as a *Phoma* sp., which was isolated from the Caribbean marine sponge *Ectyplasia perox* (Raspailiidae) [102]. The compounds isolated include the new epoxyphomalins, namely, epoxyphomalin C (77), D (78), and E (79), together with the known congeners epoxyphomalins A and B. The cytotoxicity of compounds 77–79 was investigated using a monolayer cell survival and proliferation assay and a panel of 36 human tumor cell lines. D (78) exhibited a mean IC_{50} value of 6.12 μM and showed selectivity toward PC3M ($IC_{50} = 0.72$ μM) and BXF 1218 L ($IC_{50} = 1.43$ μM) tumor cell lines [103].

Spartinoxide (97), an enantiomer of the known compound A82775C (98), was isolated from marine-derived *Phaeosphaeria spartinae*, together with two known metabolites. The fungal strain was isolated from an algal sample belonging to the genus *Ceramium* (Ceramiaceae) which was collected from the North Sea, Büsum, Germany. When tested against the enzymes human leukocyte elastase (HLE), trypsin, acetylcholinesterase, and cholesterolesterase, Spartinoxide (97) and the known 4-hydroxy-3-prenyl-benzoic acid (99) showed potent inhibition of HLE with IC_{50} values of 6.5 and 8.1 μM, respectively. HLE is involved in the migration of neutrophiles from blood to tissues, and its excessive activity may cause diseases such as pulmonary emphysema, rheumatoid arthritis, and cystic fibrosis [104].

In 2013, Teiten et al. reported that altersolanol A, a natural anthraquinone derivative originally isolated from the endophytic fungus *Stemphylium globuliferum*, showed cytotoxic, cytostatic, anti-inflammatory, and anti-migrative activity against human cancer cell lines (chronic myeloid K562 leukemia and A549 lung cancer cells) in a dose-dependent manner [105]. Interestingly, this compound did not affect the viability of noncancerous cells. Results clearly demonstrated that altersolanol A induces cell death by apoptosis through the cleavage of caspase-3 and -9, and through the decrease of antiapoptotic protein expression. Acetylation of altersolanol A did not improve activity, whereas other altersolanol derivatives such as tetrahydroaltersolanol B and ampelanol (one of the carbonyl group reduced and some hydroxyl substituents removed) were inactive in comparison.

Alterporriol L, a new bianthraquinone derivative, was isolated from endophytic marine fungus *Alternaria* sp. ZJ9-6B [106]. The fungus was isolated from the mangroves fruits and the extracts were tested for their cytotoxic activity and anticancer mechanisms for breast cancer cells lines. Moreover, the alterporriol L could induce cancer cell apoptosis or necrosis, through the destruction of the mitochondria confirmed the positive effect of the extracts, thus a new potential tool against cancer.

9 Antidiabetic

The World Health Organization reported that there are 387 million people living with diabetes worldwide in 2014, and the number would increase to 592 million in 2035 (IDF, 6th Ed). In the past decade, several research groups have focused on the exploration of new bioactive metabolites from mangrove endophytic fungi collected from the South China. Recently, a chemical investigation of the mangrove-derived fungus *Aspergillus* sp. 16-5B, from the leaves of *Sonneratia apetala*, had led to the isolation and characterization of four new compounds. All compounds were evaluated for their α-glucosidase inhibitory activities. Details of the isolation, structural elucidation, as well as evaluation of the biological activity of these compounds are reported herein. Chemical investigation of *Aspergillus* sp. 16-5B, a marine endophytic fungus isolated from the leaves of *Sonneratia apetala*, led to the discovery of six compounds (1–6), including new compound aspergifuranone (1), two new pairs of enantiomers of isocoumarin derivatives (±) 2 and (±) 3, (R)-3-demethylpurpurester A (4) and pestaphthalides A (6). Compounds 1, 2, and 6 exhibited more potent inhibitory effects against α-glucosidase activity than the clinical α-glucosidase inhibitor acarbose. The phenolic polyketide, 3-*O*-methylwailupemycin G, isolated from *Streptomyces* sp. OUCMDZ-3434 of the green algae *Enteromorpha prolifera* showed good activity with IC50 value 0.86 mM compared to the positive control, acarbose with IC50 1.12 mM [85]. The endophytic fungus *Aspergillus versicolor* SYSU-SKS025 endogenous to the mangrove species *Excoecaria agallocha* L. produced the metabolite 7-deoxy-7, 14-didehydrosydonol. The latter exhibited significant activity against α-glucosidase resulting with an IC50 value of 12.5 μg/mL [107]. One new isopimarane diterpene (1), together with two known compounds, 11-deoxydiaporthein A (2) and iso-pimara-8(14),15-diene (3) were isolated from the culture of *Epicoccum* sp., which was associated with *Apostichopus japonicus*. In the bioactivity assay, both Compounds 1 and 2 exhibited α-glucosidase inhibitory activity with IC50 values of 4.6 ± 0.1 and 11.9 ± 0.4 μM, respectively.

Aquastatin A is a novel therapy for type II diabetes and obesity. It has been produced by the endophytic fungi *Cosmospora* sp. collected at inter-tidal sediment. It competitively and selectively inhibits protein tyrosine phosphatases which are a group of enzymes responsible for modulating tyrosine phosphorylation-dependent cellular events [38]. Insulin mimetic was isolated from the endophytic fungus *Pseudomassaria* sp., which resides in an African rainforest. This new insulin mimetic had the advantage of lowering blood glucose level without being destroyed in the digestive tract and can thus be considered as a new therapy for diabetes [27].

10 Miscellaneous Agents

EF isolated from terrestrial or marine plants are thus renowned to produce secondary metabolites of various activities (Table 2).

Table 2 Bioactive compounds of secondary metabolites from endophytic fungi

Metabolite	Fungus	Sources	Properties
7-deacetoxyyanuthone A	ZSDS1-F7 isolated from the sponge *P. fusca*	Sponge	Antitubercular activities
Aigialomycins D	*Aigialus parvus*	Mangroves	Antiplasmodial and cytotoxic activities
Anicequol	ZSDS1-F7 isolated from the sponge *P. fusca*	Sponge	Cytotoxic and anticancer activities
Balticolid	*Ascomycetous 222*	Marine origin	Antiviral
Cajaninstilbene acid	*Fusarium* sp.	*Cajanuscajan*	Antioxidant
Cephalosorolides H and I	*Penicillium* sp.	Red alga *Polysiphonia urceolata*	Xanthine oxidase and steroid dehydrogenase inhibitor
Cercosporin	*Mycosphaerella* sp.	*Psychotria horizontalis*	Anti-parasitic
Chaetominedione	*Chaetomium* sp.	Marine alga	Enzyme inhibition
Circumdatin I	*Exophiala* sp.	Sponge *Halichondria panicea*	Treatment of CNS disorder
Circundatin B	*Aspergillus ostianus*	Marine origin	Antibacterial
Cladosporin, epiepotormin, phyllostine	*Penicillium* sp.	Brown alga *Fucus spiralis*	Antibacterial
Colletotric acid	*Colletotrichum gloeosporioides*	*Artemisia mongolica*	Antibacterial
Corollosporine	*Corospora maritima*	Driftwood	Antibacterial
Cytochalasin U and H	*Geniculosporium* sp.	Red alga *Polysiphonia* sp.	Antibacterial
Dreschslerin E	*Drechslera dematioidea*	Red alga *Liagora viscida*	Antimalarial against *P.falciparum*
Fusarielin E	*Fusarium* sp.	Marine origin	Antifungal
Graphislactone A	*Cephalosporium* sp.	*Trachelospermum jasminoides*	Free radical scavenger
Hypothemycin	*Aigialus parvus*	Mangroves	Antiplasmodial and cytotoxic activities
Lepicoccone	*Epicoccum* sp.	Seaweed *Fucus vesiculosus*	Antioxidant
Paclitaxel	*Ozonium* sp.	*Taxus chinensis* var. *mairei*	Anti-cancer
Peribysin J	*Periconia byssoides*	Sea hare *Aplysia kurodai*	Cell adhesion inhibitor
Phomactin D	*Phoma* sp.	Crab shell *Chionoecetes opilio*	Inhibit platelet aggregation

(*continued*)

Table 2 (continued)

Metabolite	Fungus	Sources	Properties
Phosphorohydrazide thioate	*L. laevis*	Marine woody substrata	Cytotoxic activities
Siderin, arugosin C, vericulanol	*Aspergillus versicolor*	Green alga *Halimeda opuntia*	Inhibition of hepatitis C virus
Spartinoxide, spartinol C	*P. spartinae*	Marine alga *Ceramium* sp.	Inhibition of the enzyme human leukocyte elastase
Subglutinol A and B	*Fusarium subglutinans*	*Tolypocladium wilfordii*	Immunosuppressive
Vinblastine	*Fusarium oxysporum*	*Catharanthus roseus*	Anti-cancer
Viriditoxin	*Paecilomyces variotii*	Mangrove plant	Antibacterial
Xestodecalactone B	*Penicillium* c.f. *montanense*	Sponge *Xestospongia exigua*	Antifungal
Zopfiellamide A and B	*Zopfiella latipes*	Marine *Zopfiella latipes*	Antifungal

[27, 33, 38, 45, 49, 78, 84, 88, 97, 108–113]

11 Source of Novel Molecules

Marine-derived fungi are an important source of secondary metabolites that can possess both unique structure and potent pharmaceutical activity. Three new alkaloids, brocaeloids A–C (1–3), containing C-2 reversed prenylation, were isolated from cultures of *Penicillium brocae* MA-192, an endophytic fungus obtained from the fresh leaves of the marine mangrove plant *Avicennia marina*. Brocaeloid B (2) showed lethality against brine shrimp (*Artemia salina*) with an LD50 value of 36.7 μM.

12 Conclusion and Future Work

There is a need to bring marine-derived drugs from the ocean to the pharmacy. The search for potential drugs derived from marine organisms should continue to tap more natural sources of medicinal compounds. Marine-derived endophytic fungi, as a novel and abundant microorganism resource owing to its special ability to produce similar compounds as that of their hosts, as well as other bioactive compounds, have been the focus of interest which have led to important drugs available from the pharmaceutical industry such as Prialt®, Yondelis®, Aplidin®, and Irvalec®. The preceding sections have highlighted the existing possibilities for exploiting endophytic fungi for the production of a plethora of known and novel bioactive

metabolites. There is great opportunity to find reliable and novel pharmaceutical leads in endophytic fungi which could be used to alleviate CDs and NCDs, especially those isolated from marine sources.

As elaborated along the length of this review article, results from many studies corroborated with countless endophytic species showing that marine organisms represent a potential platform for future pharmaceutical development. As presented in Table 1, there are some drugs under clinical development and a few have been approved as potential cancer drugs namely cytarabin Ara-C, Yondelis®, and trabectedin. However questions are still being raised concerning the sustainable use of fungi to extract potential drugs for medical use. And the major problem facing the future of endophytes is the decrease of rainforest and the limited amounts of biomass of most marine invertebrates available from wild stocks [27, 60]. A decrease in biodiversity certainly means a decrease in potential drugs. There is also a need to investigate cultural conditions of these fungi so that they can be grown in vitro and the potential to enable them to produce specific metabolites are maximized. Furthermore, there is a need to call for expertise of talented synthetic chemists, taxonomists, and pharmacologists who have the ability to explore structures to their full potential in order to optimize their ADME properties for future drug use [55, 60].

There is also a need to assess whether the beneficial medical properties of these compounds derived from these endophytes are substantiated. Although marine endophytes are potential sources of medicinal products, it is noteworthy to point out that any minute amount of derived product might contain a diverse array of chemicals that can contribute to other harmful side effects in humans. Which specific ones are really important, what are their mechanisms of action, and how do they interact with multiple molecular targets are questions that warrant further investigations. While there is a rush to adopt natural compounds for safety reasons, one needs to identify what are the levels of risks of other adverse effects compared to synthetic pharmaceuticals. Proper pharmacological evaluations of active ingredients need to be carried out. The methods of extraction and characterization of metabolites on a large scale and at reasonable cost also need further studies. Studies should focus on improving methods and clinical research on human subjects with more scientifically accurate experiments so that the effectiveness of these natural products can be assessed.

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Diversity of Plant Endophytic Volatile Organic Compound (VOC) and Their Potential Applications

12

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Contents

Abstract

Plant endophytes ranging from bacteria to fungi produce a diverse class of volatile organic compounds (VOCs) that are important for the development of symbiotic relation under highly competitive environment with the host. Not only that, they also play an important role in intra- and inter-kingdom signalling. Chemically, this gas-phase mixture may contain acids, alcohols, aldehydes, aromatics, esters, heterocycles, ketones, terpenes, thiols, and so forth. Several evidences suggested their potential use for sustainable crop production and industrial applications. Many VOCs have been reported with significant effects for antibiosis and growth promotion. They provide for an alternative to chemicals

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used to protect plants from pathogens and thus allow for better crop welfare. They also possess food and flavor properties which can be exploited in depth for food industries. Recent studies revealed that endophytes also produce diverse volatile hydrocarbons with fuel properties. They emit mixtures of volatile biofuel molecules comprising of alkanes, alkenes, acids, benzene derivatives, esters, etc. A vast diversity of endophytes are associated with plants for their ecology and fitness, and a systematic exploration of their VOCs will likely uncover novel use for their future utilization. In this chapter we highlight the nature and known or proposed functions of endophytic bacterial and fungal VOCs with a focus on the ones which have potential applications.

Keywords
Endophyte · Volatile organic compound (VOC) · Infochemicals · Biofuel · Plant growth promotion · Plant-microbe interaction

1 Introduction

Most organisms employ a network of signalling pathways to sense the environment and other organisms and to launch one or more specific molecular, cellular, or developmental changes. This signalling mechanism ensures cellular homeostasis, directs orderly growth and development, and controls behavior. In turn, many organisms also have evolved the ability to exploit these mechanisms in other organisms to benefit themselves or coordinate symbiosis [1–3]. Plant-associated microbes secrete various molecules that affect plant health both directly and indirectly [4]. They do so by (i) altering physical and chemical properties of their immediate surroundings to increase nutrient availability for themselves and associated plants (e.g., siderophores for iron acquisition and enzymes and metabolites to facilitate phosphorus acquisition), (ii) antagonizing pathogenic organisms (e.g., antibiotics and antimicrobial proteins), and (iii) priming host cells for subsequent colonization (e.g., modulation of external pH to facilitate pathogenesis and molecules that coordinate symbiosis or quorum sensing) [2, 3]. Molecules secreted by plants and microbes affect the structure and emergent properties of plant-associated microbial communities as well as the health of plants and soils [5]. Endophytes are microorganisms that spend part of their life cycle within plant tissues without causing any visible damage or eliciting any defense reaction in host plants [6, 7]. Endophytes exhibit a range of symbiotic relationship with their hosts as well as various life styles; for example, some of these interactions can be mutualistic in which the long-term relationship is beneficial to both partners [8]. In addition, some endophytes may only exhibit a mutualistic interaction for one plant species, but not for another [9].

Organisms ranging from microbes to animals to plants secrete volatile organic compounds (VOCs) that affect their environments and each other [10–15]. Biogenic VOCs exhibit certain common chemical and physical properties: they (i) belong to chemical classes such as alcohols, thiols, aldehydes, esters, terpenoids, and fatty acid

derivatives, (ii) are usually lipophilic, and (iii) have low molecular weights. The isoprenoids produced by plants are perhaps the best known biogenic VOCs. Numerous factors influence the release of VOCs from different biogenic sources including the population of producing species, substrates, temperature, radiation, associations with other organisms, types of ecosystem, and general climate.

The VOC emitting endophytes benefit their host in various aspects, for example, activity against plant pathogens [16], enhancement of host survival in desert habitats [17], inhibition of seed germination and thereby supporting the host in its competition with other plants [18, 19], and involvement in repelling or attracting insects [20–23].

2 Ecological Role of VOCs and Interspecies Interactions

There is increasing evidence that microbial or endophytic VOCs play cognate roles in mediating antagonism, mutualism, intra- and interspecies regulation of cellular and developmental processes, and modification of their surrounding environments. However, compared with plant VOCs, our knowledge about the biological and ecological roles of microbial VOCs is limited. Microbial volatile organic compounds (mVOCs) serve as chemical windows through which the fundamental information about the molecular basis of microbial activities is released [1, 24–26] (Fig. 1). There appears to be a multipartite basis for organisms' responses to mVOCs, and complex trophic interactions can result from the production of mVOCs. Moreover, species-specific mVOCs may also serve as marker compounds for the selective detection of fungal and bacterial species in the environment [27].

2.1 Microbial VOCs in Bacterial-Plant Interactions

Bacterial volatiles play an important role in bacterial-plant interactions, and plants respond strongly to mVOCs. Several studies have revealed that microorganisms are able to drastically alter biomass production by increasing mineral and nitrogen availability in the soil, hormonal pathways, and physiology of plants without direct physical contact [28–32] (Fig. 1). With the discovery of the plant growth-promoting properties of mVOCs, a search for possible mechanisms has been initiated. Ryu et al. (2003) reported the growth promotion of *Arabidopsis thaliana* which is attributable to two typical VOCs named 2,3-butanediol and acetoin emitted exclusively by two *Bacilli* [33]. Following up this observation, they discovered that volatiles emitted by *B. subtilis* strain GB03 regulate the homeostasis of auxin and cell expansion and increase photosynthetic capacity by enhancing photosynthetic efficiency and chlorophyll content [34]. Respective studies also indicated increased sugar accumulation. Kai et al. [59] have suggested that the growth promotion of *A. thaliana* in a sealed petri dish experiment was partially due to the CO_2 accumulation in the microenvironment [35]. *Bacilli* usually release CO_2 via the TCA cycle; however, many bacilli carry out incomplete oxidations when growing on carbohydrates and

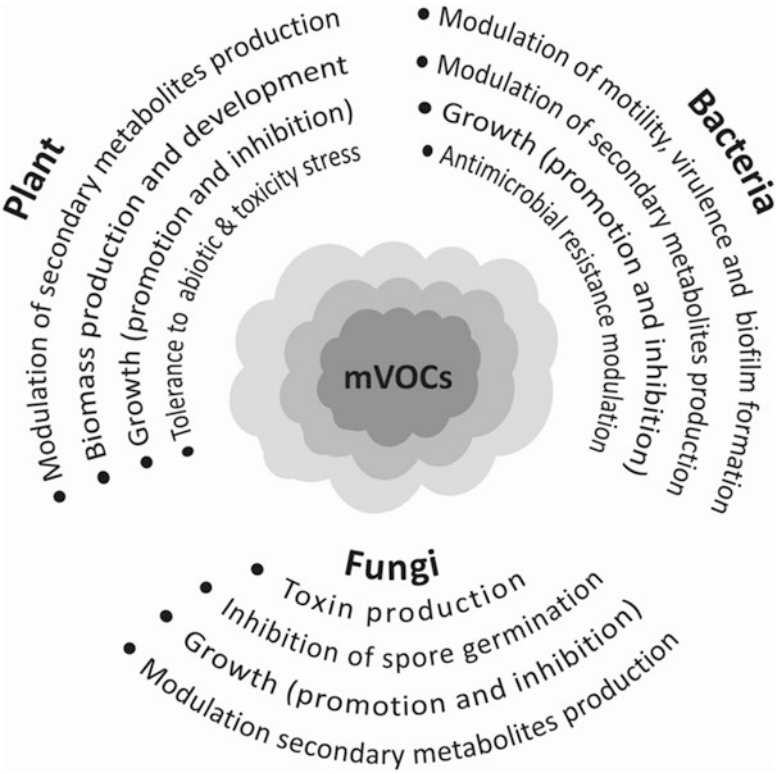

Fig. 1 Microbial volatile organic compounds (mVOCs)-mediated responses in plant, fungi, and bacteria. Both fungal and bacterial VOCs have positive impact on plant growth promotion. Secreted volatile compounds by microorganisms also contribute to their interspecies/inter-kingdom communication

under anaerobic conditions. Under these conditions the synthesis of the TCA cycle enzymes is repressed, and metabolites are partially converted to acetate, pyruvate, acetoin, and 2,3-butanediol. During this conversion process, two pyruvate units are condensed to form acetoacetate and release CO_2. Subsequently, acetoacetate decarboxylates to acetoin, which is then reduced to 2,3-butanediol and 2 moles of CO_2 are produced to yield 1 mole acetoin (or 2,3-butanediol as its reduced product). Therefore, CO_2 is simultaneously emitted during acetoin and 2,3-butanediol biosynthesis [28, 35].

Besides plant growth promotion, some recent studies have demonstrated that certain plant growth-promoting rhizobacteria (PGPR) strains produce mVOCs, which trigger plant tolerance to abiotic stresses, like drought and salt stresses, and nutrient deficiency [36]. Study by Zhang et al. (2009) has revealed that VOCs from *B. subtilis* strain GB03 increase iron uptake by *Arabidopsis* [37]. Under normal growth conditions, GB03 VOCs increase mRNA levels of the Fe-deficiency-induced transcription factor 1 (FIT1) and two of its target genes, ferric reductase FRO2 and

the iron transporter gene IRT1 which play an important role in mediating VOC-induced iron uptake [38]. Plants grown in calcareous soils have more chances of iron deficiency, and GB3 VOCs lead to rhizosphere acidification via two mechanisms, i.e., elevated proton exudation from roots and direct acidification by unknown VOC components [38]. Bacterial VOCs may also modulate the plant susceptibility to salt stress. In *Arabidopsis*, the sodium transporter AtHKT1 may function as a pivotal component in mediating VOC-induced salt tolerance. AtHKT1 functions in the removal of Na^+ from xylem sap; therefore, the presence of AtHKT1 in roots restricts the uploading of Na^+ to shoots of the plant, whereas in shoots this protein mediates Na^+ exclusion from the leaves [39]. Moreover, increasing studies have shown that AtHKT1 confers shoot-to-root Na^+ recirculation, possibly by loading Na^+ into phloem vessels, which lead to a higher proportion of Na^+ in the roots, with less Na^+ in the shoots [40]. The root-to-shoot ratio of Na^+ levels in VOC-treated plants is greater than that detected in control plants, which is consistent with the canonical role of root AtHKT1 in restricting Na^+ in the roots. It was shown that *B. subtilis* GB3 VOCs concurrently downregulated HKT1 expression in roots but upregulated it in shoot tissues, thereby orchestrating lower Na^+ levels in the whole plant [41, 42].

Drought stress limits the growth and productivity of crops, particularly in arid and semiarid areas. Some studies suggested that bacterial VOCs may lead to increased biosynthesis of two compatible solutes: choline and glycine betaine that protect cells from osmotic stress under dehydrating conditions, and this increased osmoprotection is not caused by alterations in ABA production, as osmotic-stressed plants exhibit VOC-independent accumulation of ABA in both shoots and roots [43]. The active component in VOCs shown to be necessary and sufficient for eliciting plant drought tolerance was found to be 2,3-butanediol [44].

2.2 Microbial VOCs in Fungi-Plant Interactions

The ability of soil fungi to produce plant growth-enhancing VOCs has been documented recently [45, 46]. Many *Trichoderma* strains commonly found in soil and root ecosystems have been extensively studied for their beneficial effects on plant growth. They are found to emit VOC mixtures that probably mimic plant metabolites and significantly enhance plant growth in *Arabidopsis* and tomato as measured by biomass, plant size, chlorophyll concentration, and root sizes depending on the duration of exposure [47]. Some studies demonstrated that increased CO_2 levels associated with microbial growth in a petri dish system can lead to plant growth promotion [35]; however, other studies found no significant differences in the CO_2 level in *Trichoderma* containing microhabitats and ambient air; also the sequestration of *Trichoderma*-produced CO_2 by absorption in the petri dish system did not reduce the growth promotion [47]. The volatile profile of fungi changes with their growth and maturation. Some VOCs like 1-hexanol, a truffle volatile, exhibit plant growth promotion effect at low concentrations [48] but inhibit growth at high concentrations [49]. Besides growth promotion fungal VOCs benefit plants by providing defense against pathogens of their hosts [16]. For example, an

endophyte, *Muscodor albus*, produces VOCs which include esters, alcohols, acids, lipids, and ketones that inhibit and kill plant pathogenic fungi and Gram-negative and Gram-positive bacteria [50]. The VOCs of *M. albus* operate in an additive or synergistic way. It has been shown that when mixtures of commercially available compounds, such as bulnesene, valencene, and synthesized compounds, like propanoic acid, 2-methyl, 3-methylbutyl ester, and 1-butanol, 3-methyl-, acetate, are tested against the pathogens, the results closely mimic the inhibitory and lethal activities of the *M. albus*, but compounds when used either individually or broken into several classes did not lead to the same inhibitory effects [50]. Additionally, VOCs of certain endophytic fungi may aid the survival of plants in some habitats. A *Phoma* sp. isolated from creosote bush emits VOCs that may contribute to the ability of this shrub to survive harsh desert habitats [51]. However, a range of plant pathogens, including *Verticillium*, *Ceratocystis*, *Cercospora*, and *Sclerotinia*, were inhibited or killed by the mixture of VOCs produced from this *Phoma* sp. [51].

Macias-Rubalcava et al. (2010) have demonstrated that the VOCs produced by *M. yucatanensis* are toxic to the roots and inhibit seed germination of amaranth, tomato, and barnyard grass. Besides this, the VOCs were also toxic to other endophytic fungi, potentially minimizing nutrient availability to endophytes by its host plants, thereby enhancing the growth of their hosts [16]. Mycofumigation, which is the use of volatile antimicrobial organic compounds produced by fungi to inhibit microbial growth, has become a promising alternative for controlling phytopathogenic fungi associated with post-harvest diseases in fruits and vegetables. Mercier and Jiménez (2004) have shown the toxic effect of VOCs from *M. albus* on peach pathogens, *Penicillium expansum, B. cinerea*, and *Monilinia fructicola*, and preventive effect of the volatiles on fungal contamination of post-harvest peaches over 1 week of storage [52]. Campos et al. (2015) revealed that VOCs of *M. albus* aid mycofumigation through DNA damage of the targets [53].

2.3 Microbial VOCs in Fungi-Bacteria Interaction

VOCs from fungi can play an important role in fungal-bacterial interactions and can lead to different phenotypical responses among partners. For example, according to Lutz et al. (2004), VOCs emitted by *Trichoderma atroviride* increased the expression of a biocontrol gene (*phlA*) in *P. fluorescens* that encodes the biosynthesis of 2,4-diacetylphloroglucinol [54]. Some other studies have demonstrated growth suppression of bacterial species by fungal VOCs, e.g., the oyster mushroom *Pleurotus ostreatus* have been shown to produce VOCs that exhibit inhibitory effects on *B. cereus* and *B. subtilis* [55, 56]. The phenotypic responses like growth alteration, antimicrobial activity, biofilm formation, or motility of soil bacterial strains to volatiles emitted by fungal and oomycetal can be affected either positively or negatively, and this reflects a potential strategy employed by the fungus to attract mutualistic bacteria toward itself and to repel

competitors by manipulating their motility through the use of VOCs [32, 57] (Fig. 1). It was shown that volatiles emitted by *Chryseobacterium* sp. AD48 and the mixture of *Chryseobacterium* sp. AD48 and *Tsukamurella* sp. AD106 inhibited the growth of *E. coli* WA321 significantly [58]. Changes in colony morphology of *S. marcescens* P87 were also observed when exposed to volatiles emitted by *Chryseobacterium* sp. AD48 and to volatiles emitted by the mixtures of *Dyella* sp. AD56 with *Janthinobacterium* sp. AD80.

Presently, the biological functions of many bacterial volatiles on fungi are not understood in detail. Studies have revealed that hundreds of soil bacteria produce bioactive volatiles, and volatiles from any one bacterial strain do not cause the same effect or the same degree of inhibition to all the fungi; rather, the responses depend on the specific fungi-bacteria combination. The following reasons might be responsible for these differences: (1) different fungi may respond to different component(s) of the volatile mixture, or (2) the sites of action may be different, or (3) the fungi might possess different abilities to detoxify the volatile metabolites [59]. Fungistasis is a phenomenon where fungal propagules are restricted in their ability to grow or germinate, and many soil bacteria can produce antifungal VOCs, thus contributing to fungistasis [60]. Fungistatic intensity varies with the physical and chemical properties of soil and microbial community composition [61, 62]. Although the production of volatiles is highly dependent on growth conditions and nutrient availability [63], the VOCs produced by *Streptomyces* spp. exhibit antifungal properties against *Rhizoctonia solani* and may contribute to plant disease suppression [64]. The VOCs produced from *Pseudomonas donghuensis* have strong antifungal and antioomycete activity [65], and other *Pseudomonas* strains have also been reported to have antioomycete activities [66, 67]. It was also shown that volatiles produced by monocultures and pairwise combinations of some selected bacteria are able to inhibit the growth of some phytopathogenic fungi and oomycetes [58].

3 Microbial VOCs Secreted by Plant Endophytes with Potential Aspects

The opulent diversity of volatile organic compounds produced by plant endophytes and progresses in chemical, biological, and genome analysis continue to prominently improve our understanding of these mysterious natural products. However, we are actually at the very beginning of exploring the nature and properties of this class of secondary metabolites and the vast potential they harbor. Till now several endophytes have been found to produce numerous classes of volatile secondary metabolites with plant growth stimulant, biofuel, biocontrol, and biopharmaceutical potentials. Here, we provide a general idea of the most important volatile organic compounds produced by endophytic organisms, their roles, and impact on our socioeconomic development (Table 1).

Table 1 List of microbial volatile organic compounds secreted from endophytic fungi and bacteria and their functions

Fungus name	Host species	Volatile compound	Functions
Muscodor albus	*Cinnamomum zeylanicum*	28 volatile compounds including isoamyl acetate	Produces biofuel, growth inhibition of Gram-positive and Gram-negative bacteria, phytopathogenic fungi, and oomycetes
Muscodor crispans	*Ananas ananassoides*	A mixture of antifungal and antibacterial volatile organic compounds	Wide range of activities against plant pathogens including fungus and bacteria, also have activity against some human pathogens
Daldinia concentrica	Olive tree	27 different compounds including alcohols, dienes, ketones, aldehydes, and sesquiterpenes	Antifungal, disinfecting activities
Oxyporus latemarginatus	*Capsicum annum*	5-pentyl-2-furaldehyde	Antifungal activity
Ascocoryne sarcoides	*Eucryphia cordifolia*	Hydrocarbons (preferentially produces several ketones and esters)	Capable of converting cellulose and glucose into short-chain hydrocarbons, have cellulase, lignolytic activities, produce biofuel
Phoma sp.	*Larrea tridentata*	15 volatile compounds including sesquiterpene with α-humulene or α-caryophyllene and several naphthalene derivatives	Antifungal activity, biofuel compounds
Phomopsis sp	Odontoglossum sp.	Sabinene (monoterpene), 1-propanol, etc.	Antifungal activity, aroma, and fragrance compounds
Myrothecium inundatum	*Acalypha indica*	Hydrocarbons and hydrocarbon derivatives (prevalently produce 3-octanone, 3- octanol, and 7-octen-4-ol and 1,4-cyclohexadiene, 1-methyl- and cyclohexane, etc.) produced in microaerophilic conditions	Antifungal activity
Gliocladium sp.	Ulmo trees	Hydrocarbon compounds similar to diesel fuel (hexene, benzene, 3,4-dimethylhexane, 1-octene, and m-xylene)	Produces biofuel

(*continued*)

Table 1 (continued)

Fungus name	Host species	Volatile compound	Functions
Muscodor yucatanensis	*Bursera simaruba*	Alcohols, esters, and ketones of saturated and unsaturated compounds, as well as benzene derivatives (octane, 2-methyl butyl, acetate, 2-pentyl furan, etc.)	Antifungal activity, inhibits seed germination of various grass weeds
Trichoderma atroviride	Soil borne, found in plant roots	Expresses biocontrol gene *phlA* that encodes 2,4-diacetylphloroglucinol	Controls the growth of some bacteria
Pleurotus pulmonarius (oyster mushroom)	Aspens and cottonwoods	3-octanone, 3-octanol, 1-octen-3-ol, benzaldehyde, and unidentified trace components	Antimicrobial activity against some bacterial strains
Bacteria name			
Pseudomonas donghuensis	Plant root	Dimethyl sulfide, S-methyl thioacetate, methyl thiocyanate, dimethyl trisulfide, 1-undecan, and HCN	Antifungal and antioomycete activities
Bacillus subtilis GB03 (PGPR)	Plant root	3-hydroxy-2-butanone (acetoin), 2,3-butanediol, choline, and glycine betaine	Promotes plant growth, increases photosynthetic capability, iron uptake, abiotic stress tolerance, drought tolerance
Streptomyces sp.	Plant root	Methyl 2-methylpentanoate, 1,3,5-trichloro-2-methoxy benzene	Antifungal properties against *Rhizoctonia solani*, acts in plant disease suppression

3.1 Endophytic VOCs as Plant Growth Stimulants

At the plant-microbe community level, substantial progress has been made in studying the multifaceted role in agroecosystems of microbial VOCs produced by endophytes, plant growth-promoting fungi (PGPFs), phytopathogens, and various strains of PGPR. For agriculture scientists, mVOCs are seen as biocontrol agents to control various phytopathogens and as biofertilizers for plant growth promotion. A growing body of evidence indicates that mVOCs are eco-friendly and can be exploited as a cost-effective sustainable strategy for use in agricultural practice as agents that enhance plant growth, productivity, and disease resistance. As naturally occurring chemicals, mVOCs have potential as possible alternatives to harmful pesticides, fungicides, and bactericides.

A new mechanism for growth promotion mediated by volatile organic compounds (VOCs) was reported by a group [33], who have shown that volatiles released by *Bacillus subtilis* GB03 induce growth of *Arabidopsis thaliana*. This

was the first evidence that volatile organic compounds can modulate growth, stress, nutrition, and health processes in plants (Table 1). To date, studies have achieved considerable progress in elucidating the mode of action of this type of compounds; however, it is still poorly understood.

Several endophytic *Bacillus* strains have been tested as growth inducers through the emission of volatiles [68] and have shown that volatiles emitted by *B. megaterium* XTBG-34 exhibited a 1.7-fold increase in fresh weight of *A. thaliana*. Moreover, the effect of VOCs on the root system was demonstrated by some researchers [69], who concluded that volatiles emitted by *Bacillus* species modified root architecture, eliciting the increase of total fresh weight, primary root length, lateral root number, and lateral root length on *A. thaliana*. Other endophytic fungi and bacteria that belong to the Gram-positive species have also been reported for their ability to release volatile organic compounds with growth-inducing activity.

Certain endophytes have been reported to emit VOCs that influence the defense response of their host plants [70]. Among the first bacterial volatiles that were found to confer plant resistance was 2,3-butanediol [28]. In tobacco, 2,3-butanediol (2,3-BD) produced by the rhizobacterium *Pseudomonas chlororaphis* induced resistance against *E. carotovora* [71]. Another study [72] showed that *Enterobacter aerogenes* (an endophyte)-derived emission of 2,3-BD influences resistance to pathogens and herbivorous insects and affects tritrophic interactions.

VOCs produced by fungi have received limited attention in terms of their relationship to plant pathogenesis or growth promotion. Fungi emit cocktails of dozens to hundreds of unique volatile compounds that fall into many chemical classes including alcohols, aldehydes, acids, ethers, esters, ketones, hydrocarbons, terpenes, and sulfur compounds [73]. The single most commonly reported volatile from fungi, 1-octen-3-ol also called “mushroom alcohol,” is used as an insect attractant [74, 75] (Table 1).

3.2 Volatile Biofuels from Endophytes

Growing environmental awareness, together with a gradual reduction of fossil fuel reserves, has led global research to recognize living organisms as an alternative sustainable source of fuel, and hence the phrase “biofuel” was introduced. By definition, biofuels are hydrocarbons that can be produced by or derived from living organisms as opposed to fossil fuels which are produced after years of decomposition of organic matter. Most of the existing modern biofuel production structures in deployment or under improvement are found on the bioconversion of plant materials into numerous hydrocarbon fuels, such as ethanol [76], isobutanol [77], and other fuel alternatives. These systems comprise of multiple processes like cultivation of oil-rich plants, collection and storage of plant biomass, disintegration of collected biomass, biochemical pretreatment, various chemical reactions (like scarification), fermentation with active microorganisms (mostly yeast), and finally collection of desired fuel product [78]. These multiple processes have many limitations such as high land usage for plant cultivation that causes demolition of natural territories by

being transformed into farmland and pressure on food materials that may pose a threat to some regions' food security. Moreover, pretreatment and scarification processes are expensive making the whole system economically less feasible, and the final product ethanol is not the most desired fuel for many of the present combustion engines as its energy density is quite low [79]. An unconventional approach, using active microorganisms that harbor metabolic pathways directed toward volatile hydrocarbon production, attempts to decrease cost allied with these multiple processes by directly producing fuel hydrocarbon volatiles using its own enzyme machineries [80]. In search of these active microorganisms, unique ecological niches such as comparatively untapped living tissues of higher plants are being explored for identification of novel symbiotic endophytes that may have the capacity to utilize its residing plant tissue by hydrolytic enzymes to produce volatiles with fuel potential. In recent times, numerous endophytes, especially fungi have been revealed to yield volatile organic metabolites which are analogous to fossil fuels in terms of their properties and potential. These are termed as mycodiesel [81]. As plant symbionts, endophytes may have special lingo-cellulolytic enzymes that give them the ability to both decompose plant material and transform it into mycodiesel. These two aptitudes designate that these endophytic organisms can be valuable sources for biofuel production. These new sources of fuel compounds are renewable, sustainable, as well as compatible with prevailing engine infrastructure [82].

3.2.1 Recent Research on Endophytes in Search for VOCs with Fuel Potential

Recent work has revealed the aptitude of endophytic microorganisms to produce diverse volatile hydrocarbons with fuel capacity. VOC production from a variety of endophytic fungi in the genera *Muscodor*, *Hypoxylon*, *Ascocoryne*, *Phomopsis* sp., and many more have been characterized for this purpose [81]. *Ascocoryne sarcoides*, an endophyte isolated from host plant *Eucryphia cordifolia*, emits a wide range of volatile hydrocarbons with fuel potential [83–86]. The volatile compounds formed by this fungus are different from those produced by other organisms. *Ascocoryne* emits a mixture of volatiles comprising of alkanes, alkenes, alcohols, acids, benzene derivatives, ester, ketones, and terpenes, which are analogous to biofuel molecules. Among them C8 compounds and C6–C9 alkanes as well as alcohols could also be a used as gasoline surrogates in fuel infrastructure [83, 86]. This endophyte has the distinctive competence of transforming cellulose or glucose into hydrocarbon chains of different lengths. These hydrocarbons are usually known to be used as diesel fuel.

Many of the endophytic fungi are known to have diverse enzymatic machineries like complex lingo-cellulolytic enzymes. They secrete these enzymes extracellularly, and these enzymes work directly on the lignocellulosic materials and convert them to glucose, and then this glucose is transformed into various high-energy hydrocarbons [87]. Their ability to utilize lignocellulosic substances as raw material makes them highly suitable as an economic agricultural resource for the production of volatile hydrocarbons. Hence, this discovery may lead to profitable biofuel production [86]. The general potential for endophytic fungi in biofuel development has been reviewed by many researchers. The specific role of endophytic fungi such as

Phoma sp., *Phomopsis* sp., *Hypoxylon* sp., *Myrothecium inundatum*, and *Gliocladium* spp. in this context has been explored, which lead us to the conclusion that these fungi can also be novel commercial hydrocarbon producers [51, 86–90]. Likewise, GC-MS analysis of many endophytic fungi, such as *Hypoxylon* sp. EC38, *Hypoxylon* sp. CI4A, *Hypoxylon* sp. CO27, and *Daldinia eschscholzii* EC12, has revealed the presence of hundreds of volatile terpenes (pinene, limonene, caryophyllene, chamigrene, gurjunene, selinene, and isoledene), which are used as jet or diesel fuels in aviation engines [91, 92].

3.2.2 Types of Biofuels Produced by Endophytes

Diesel Fuel

A variety of compounds recommended as diesel fuel have been identified as endophytic VOCs. This microbial diesel fuel consists mostly of four types of compounds, such as straight and branched alkanes, cyclic alkanes, benzene derivatives, and the poly-aromatic hydrocarbons [81]. Each of the compounds has been revealed to imitate diesel in numerous aspects comprising energy density and cetane number [93].

Biodiesel

Biodiesel, the mixture of fatty acid ethyl ester or fatty acid methyl ester, can serve the purpose of the most awaited sustainable fuel. Many endophytes are also known to be oleaginous in nature, and this property makes them rich in lipid or fatty acid content (Table 1). These lipids can be then transformed into fatty acid methyl esters or fatty acid ethyl esters through trans-esterification an efficient method for the production of biodiesel components [94]. As fuel alternative, biodiesel is analogous to petroleum-diesel in combustion properties. This allows it to function well in existing diesel-based engines [95–97]. Moreover, biodiesel is superior than petroleum-diesel in numerous features, such as environmental friendliness, renewability, reduced emission of CO_2, advanced combustion efficiency, upgraded lubricity, etc. [98]. Recent works have discovered many endophytic oleaginous fungi that are proficient in storing significant amounts of lipids and thus can serve as biodiesel feedstock [94, 99]. This indicates that significant quantities of biodiesel production from endophytic fungi are possible. Therefore, the pursuit for novel oleaginous endophytes with the applicable features is of prodigious importance.

Gasoline Substitutes

Mostly short-chain alcohols, like ethanol, are seen as gasoline surrogates. But, long-chain alcohols are also found to have similar fuel properties and thus can be recognized as a part of this group [93]. Some endophytic filamentous fungi have been detected to produce gasoline alcohols of C4–C10 chain lengths [50, 51, 83, 100]. These alcohols are produced through the Ehrlich pathway by degradation of amino acids, but the pathway by which long-chain alcohols are produced is yet to be determined [101].

Terpenes

One of the major class of constituents of endophytic volatiles are terpenes such as monoterpenes, diterpenes, and sesquiterpenes, like pinene, limonene, farnesene, bisabolene, caryophyllene, etc. [102, 103]. Endophyte *Phoma* sp. isolated from *Larrea tridentata* (creosote bush) produces an exclusive mixture of volatile organic compounds, comprising of a series of sesquiterpenoids reported to have fuel potential [51]. Terpenes are biosynthesized from the universal precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These molecules can be generated through either through the deoxyxylulose phosphate pathway or by the mevalonate pathway. Then enzymes named prenyltransferases assemble IPP and DMAPP into linear prenyl diphosphates, which are reorganized by terpene synthases into various diverse terpene molecules [82, 104]. Terpenes conventionally used as antimicrobials, fragrances, and flavors are now reported to have the potential to serve as eco-friendly fuel alternatives. For example, reduced farnesene has recently been determined as an advanced biosynthetic fuel [105]. Existing biofuels like ethanol has high oxygen content as well as many incompatibilities with prevailing petroleum-based fuel infrastructure, while the oxygen content in terpenes is almost zero and their energy density is also high [82]. These features make them attractive contestants as modern fuel alternatives as well as aviation fuel.

3.2.3 Endophytes' Genome Analyses as a New Frontier for Identification of Biofuel VOC Producing Pathways

The main obstacle in the commercialization of VOCs is the unavailability of information on the genetics, biochemical mechanisms, and metabolic pathways responsible for the production of the same because these information are prerequisite for the development of genetically engineered and modified strains for a scaled-up production of biofuel [86]. Determination of complete genome sequence and understanding of biosynthetic pathways and genes responsible for VOC production can resolve the withstanding difficulties [106]. Limited research has been conducted in this perspective. On the basis of such research, two biochemical pathways in microbes have been suggested to be responsible for the production of straight-chained hydrocarbons. These are the "elongation-decarboxylation" and the "head-to-head condensation" pathways [107]. Diesel fuel potential of these hydrocarbon elements marks the two mentioned pathways as the topmost important pathways [84]. Investigation on *A. sarcoides* indicates that the "head-to-head condensation" may have a major role in the production of biofuel like volatile hydrocarbons in this specific fungus [86]. This pathway would essentially include precursors like acetyl-CoA and malonyl-CoA condensing to elongate carbon chain length also going through other chemical reactions such as decarboxylation, decarbonylation, hydration, and oxidation-reduction, to produce hydrocarbon molecules. Other precursors, like L-methylmalonyl-CoA, might also have a role in the synthesis of hydrocarbons in *A. sarcoides*, as branched chain hydrocarbons are also formed. Other synthetic pathways, yet to be revealed, could possibly explain the presence of doubly substituted carbons in the branched alkanes that are generated by *A. sarcoides* [84]. Lastly, the alkane ester series found in GC-MS analysis of this fungus are the

result of an esterification reaction between acetic acid and the alkane alcohol. This fungus must also own additional lipid synthase enzymes as plentiful terpenoids have also been seen to be present [84]. Recent study [82] on four endophytes named *Daldinia eschscholzii* EC12, *Hypoxylon* sp. EC38, CI4A, and CO27 uncovered their capacity to produce a broad range of volatile biofuel hydrocarbons, the majority of which are monoterpenes and sesquiterpenes, including some other well-known biofuel components. Along with the metabolomics study, their genome investigation proved the presence of various carbohydrate-active enzymes (CAZymes) that act on both polysaccharides and lignin. Enzymes of terpene biosynthetic pathway, such as terpene synthases which can generate jet fuel-like compounds, have also been found. Moreover, the theory of horizontal gene transfer points at the probability of genetic material uptake by endophytes from their respective host plants, and as a result these endophytes produce an array of compounds that are analogues to their host plants' metabolites. Subsequently many aromatic plants have been found to harbor endophytes with high VOC production capacity [86, 108, 109].

3.2.4 Effect of Fermentation Method on Biofuel VOC Production by Endophytes

Moreover, ability of fungi to utilize cellulose for the production of hydrocarbons is a fascinating aspect because massive amount of food grains are presently exploited for fuel (alcohol) production. Although oatmeal fermentation medium has showed increased production of hydrocarbons than that of cellulose medium, development of fermentation techniques and genetic manipulations can support to make cellulose medium appropriate for increased production of hydrocarbon by endophytes [84]. So endophytic fungi can be explored for identification for such dual action performance for better production of biofuel.

Inappropriate fermentation method is another serious impediment that makes these endophytic fungi unsuitable for commercial production of biofuels. Limited oxygen supply in the fermentation system makes the fungi produce saturated or reduced VOCs which are highly desirable as fuel component, but in the case of aerobic fermentation system, these reduced VOCs become oxidized in the presence of oxygen. Oxidized VOCs are more likely to exhibit antibiotic activity rather than fuel potential which designates oxygen-limited condition most suitable for biofuel production [110]. Moreover varied elements or organisms added in the fermentation media can upsurge the production of the fuel components by fungi. For example, endophytic fungi *Gliocladium* spp. are perceived to generate double amount of VOCs when co-cultured with *Escherichia coli* in the fermentation media [87]. Therefore, critical modifications in current fermentation methodologies as well as imposing correct fermentation technique regarding the desired final metabolite are necessary for increased production of the desired product.

3.2.5 Future Challenges for Resourceful Biofuel Production from Endophytes

In order to make substantial impact by using endophytic fungi for biofuel production, efficient strategies are necessary to be undertaken. The strategies include improved

media condition for growth, upgraded nutrient circulation, and genetic engineering of the strains for increased production. There are also major challenges for researchers to design bioreactors that will be cheap enough for large-scale production of biofuel and to develop strains that will grow efficiently in low-nutrient supply condition as well as retain the capacity to utilize agriculture-derived lignocellulosic plant materials for the production of biofuel hydrocarbons. Fungi which have advanced and dynamic enzyme systems for lignocellulose degradation processes have advantages over other microorganisms in that they can consume biomass more easily and convert them to compounds with fuel potential. Thus highly efficient and cost-effective utilization of biomass as alternative energy supplement by endophytes promises to show huge impact on resolving fuel challenges. Therefore, substantial developments over prevailing technologies for the production of biofuel from endophytic fungi are necessary to be made. Synchronized strategies will also be a prerequisite to combine genetic modification practices with potential microbes.

3.3 Endophytic VOCs as Aroma and Flavor Compounds

Most of the aromatic plants are likely to have endophytes which can emit abundant VOCs with aroma and fragrance and are of great commercial value. Volatiles like terpenes and their derivative terpenoids, as well as ester molecules, are valuable flavor and aroma compounds used in various food and beverage preparations. Many fungal VOCs are found to be chemically similar as the desired plant molecules and are categorized as bioidentical natural flavoring and fragrance elements, therefore providing a huge range of prospects in the food, pharmaceutical, cosmetic, feed, and chemical industries [111]. These fungal endophytes are desirable for de novo fragrance production. Their volatiles contribute to the desirable flavor and aroma properties of certain cheese, nonalcoholic beverages, puddings, jellies, candies, baked goods, other food products, etc. One famous example is the production of methyl eugenol by fungal endophyte *Alternaria* sp. and *Aspergillus niger*, isolated from *Rosa damascene*. Methyl eugenol is also the major constituent of rose oil and is of huge demand in industries. Identification of rose oil aromatic compounds from rose plant endophytes confirmed the presence of a robust relationship between host plant and their associated endophytes [111]. An endophytic fungus identified as *Phialocephala fortinii* was isolated from *Pinus sylvestris*, and an interesting terpene molecule β-caryophyllene with spicy flavor was found in its volatiles [112]. Similarly, another uncommon endophytic strain *Phomopsis* (EC-4) obtained from *Odontoglossum* sp. was found to emit a peppery fragrance. GC-MS analysis of this fungus revealed that the major constituent of its volatile profile was a monoterpene named sabinene [90], an element normally found in orange peel oil [113] and assumed to be responsible for the peppery aroma [111]. In a recent study on endophytes of *Dipteryx alata* Vog. (baru fruit), three fungal endophytes were reported for their ability to produce verbenol, a potent terpenoid with mint-like flavor. It was obtained by utilizing α-pinene as a substrate and then bioconversion of this α-pinene to verbenol [114]. In another study, volatiles of an endophyte named *Urnula* sp., obtained from

Dicksonia antarctica, a tree fern, endemic to Australia, were investigated, and a plethora of fragrant molecules were reported that point at the commercial prospective of certain *Urnula* sp. volatiles as well as the significance of these individual volatiles to many industries such as food, beverage, flavoring, fragrance, etc. [115]. These growing amounts of research on volatile organic compounds secreted by endophytes reveal a rising attention to these microorganisms as a source of aroma compounds with various flavors.

3.4 Endophytic VOCs as Biopharmaceuticals and Mycofumigation Agents

Endophytic compounds being explored for the last few decades have been found to have antibacterial, immunosuppressant, anticancer, and antioxidant activities. Of them, the volatile organic compounds are being analyzed, and these molecules have turned out to contain great bioactive potential [116]. At the time of rampant antibiotic resistance, need for new potent antimicrobial therapeutics is on the rise. VOCs may have great potential as new age antibiotics that can be used to combat the microorganisms already resistant to novel antibiotics. Intensive exploration of endophytic VOCs may lead to the discovery of new antimicrobials for medical treatment and also for enhancing plant defense. Recent studies on plant endophytes support such possibilities as fungal endophytes play a key role in plant protection against biotic stress by producing a variety of novel secondary metabolites including volatile organic compounds which are involved in controlling pests and pathogens [117].

The endophytic fungus *Muscodor albus*, isolated from small branches of *Cinnamomum zeylanicum* (cinnamon tree), produces more than 25 volatile compounds, and these volatiles synergistically cause effective inhibition and death of a broad range of pathogens. For example, human pathogenic fungi, *Aspergillus fumigatus* and *Candida albicans* were strongly inhibited by the volatiles of *M. albus*. Among all the volatiles emitted by *Muscodor albus*, isoamyl acetate is assumed to be the most biologically active compound, responsible for the growth inhibition of pathogenic microorganisms [50]. *M. albus* was the first endophytic fungus proven to emit volatiles with strong antimicrobial activity against both plant and human pathogens. *M. albus* has been also demonstrated to harbor a high capacity for controlling numerous post-harvest diseases. The volatiles discharged by this organism have been efficiently used to regulate *Fusarium sambucinum*, *Helminthosporium solani*, and *Pectobacterium atrosepticum* infections named, respectively, as dry rot, silver scurf, and bacterial soft rot disease in stored potatoes (*Solanum tuberosum* L.) [118]. Its volatiles also strongly inhibit infection of three pathogenic fungi named *Tilletia horrida*, *T. indica*, and *T. tritici* that cause various diseases in rice and wheat plants [119].

Muscodor crispans, an endophytic fungus that was isolated from *Ananas ananassoides* (wild pineapple), produces a blend of antifungal and antibacterial volatiles that are effective against a wide range of plant and human pathogens. *Mycosphaerella fijiensis* and *Xanthomonas axonopodis* pv. citri which are well

known for causing serious plantation disease in bananas and citrus fruits, respectively, are strongly inhibited by the volatiles of *M. crispans*. Its volatiles are also effective against a wide range of human pathogens, including *Yersinia pestis*, *Salmonella choleraesuis*, and *Staphylococcus aureus* and *Mycobacterium tuberculosis*. An artificial mixture of these fungal VOCs was also found to kill three drug-resistant strains of *Mycobacterium tuberculosis* that upholds its potential as a new antimycobacterium drug [100].

Daldinia concentrica is a recently described novel endophytic fungus of *Olea europaea* L., a plant that grows in Israel. The fungus produces a mixture of volatile organic compounds; some of the major components of this mixture, as determined by GC/MS, are 3-methyl-1-butanol, (±)-2-methyl-1-butanol, 4-heptanone, isoamyl acetate, 4-heptanone, and trans-2-octenal. These volatiles were found to prevent mold formation on dried fruits and wheat grains and were capable to eradicate *Aspergillus niger* infection of peanuts in post-harvest assays indicating its possible applications in the food storage industries and agriculture [6].

Tangerine fruit faces huge post-harvest losses due to a green mold disease, caused by *Penicillium digitatum*, causing worldwide yield loss, and the use of synthetic agrochemicals has led to proliferation of resistant strains of the pathogen [120]. Volatiles emitted by *Muscodor suthepensis* strain CMU-Cib462 have been found to provide complete control of tangerine fruit rot caused by *P. digitatum* and proved its major volatiles, 2-methylpropanoic acid and 3-methylbutan-1-ol, to be alternatives to chemical fungicides [121].

Over the years, several other fungi have been identified to produce volatile antifungal metabolites. For example, in vitro and in vivo assays of 5-pentyl-2-furaldehyde, a volatile compound emitted by endophytic fungus *Oxyporus latemarginatus* isolated from *Capsicum annum* L., showed inhibition of the mycelial growth of pathogenic fungi named *Alternaria alternata*, *Colletotrichum gloeosporioides*, and *Fusarium oxysporum f.* sp. *lycopersici*. Moreover, *O. latemarginatus* is capable of inhibiting the growth of *Botrytis cinerea* and *Rhizoctonia solani*, which can cause post-harvest apple decay and root rot of moth orchids, respectively [122]. Moreover, *Aspergillus niger*, an endophytic fungi of *Rosa damascena* (rose), was found to produce a special volatile named 2-phenylethanol that has broad-spectrum antibacterial activity and great potential to be used as a preservative in pharmaceuticals [123]. Besides, endophytic fungus *Pichia guilliermondii* of medicinal plant *Paris polyphylla* var. *yunnanensis* produces three steroids and one triterpenoid which are ergosta-5,7,22-trienol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol, ergosta-7,22-dien-3β,5α,6β-triol, and helvolic acid, respectively. They have shown strong antibacterial activity and have also promisingly inhibited spore germination of rice blast fungus, *Magnaporthe oryzae.* Of these four VOCs from *Pichia guilliermondii*, helvolic acid holds prodigious prospect to be established as an antimicrobial agent in the coming years [124].

Recent researches also point at the impending prospect of using endophytic fungi as biocontrol agents against various parasitic nematodes. *Daldinia* cf. *concentrica* that produces volatiles capable of inhibiting growth of a wide range of plant pathogenic bacteria and fungi also displays nematistatic and nematicidal activities

against many plant parasitic nematodes, like *Meloidogyne javanica*. 3-methyl-1-butanol, (±)-2-methyl-1-butanol, 4-heptanone, and isoamyl acetate are the volatiles responsible for this bionematicidal activity. Although these four compounds harbor individual nematicidal activities, 4-heptanone has shown the most promising nematicidal activity among them in chemical assays. Application of these volatiles protects *M. javanica*-susceptible tomato plants from the harmful effects of this nematode [125]. Similarly, another endophytic fungi *Muscodor albus* with potent antimicrobial activity is also found to have powerful bionematicidal activity [126]. Likewise, *Fusarium oxysporum* isolated from coffee plants exhibits nematicidal properties against *M. incognita* nematodes [127].

Artificial mixtures of selected volatiles have great promise for application in food industry, agriculture, and pharmaceuticals. Moreover, the volatile nature of these antimicrobial compounds produced by endophytic fungi and their broad range of inhibition makes mycofumigation a low-cost and easily maintained method for preventing plant diseases.

4 Commercial Importance of mVOC and Their Future Perspectives

As methods for the analysis of gas-phase molecules are improving, it is becoming more and more apparent that endophytic VOCs are chemically more varied and biologically more active than it had been generally realized. In the identification of the volatile components, GC-MS remains the most common and valid method. However, the detection by this method is limited by the columns used in this spectrometry. These columns used are selective for detecting some chemical groups of VOCs but not the total VOCs [128]. Recently, a very sensitive detection technique, named proton-transfer reaction mass spectrometry (PTR-MS), has been developed to quantitate VOCs [51, 128]. PTR-MS uses gas-phase hydronium ions as ion source reagent. This analytical technique is used for online monitoring of volatile organic compounds in ambient air. A combination of GC-MS with PTR-MS has proven to be an effective tool for an over-all detection and identification of VOCs [128]. Even with such progresses made in the analysis, the compounds cannot be accurately identified due to poor matches in mass spectral library databases. New columns have recently been made available which can effectively collect large amounts of hydrocarbons and their derivatives. Such developments are expected to pave the way for isolation and identification of unknown chemical groups. It is apparent that the volatile compounds isolated from endophytes till date are only a tiny fraction of the total repertoire. But whatever the fraction of the actual number, the identified compounds have given us a wealth of chemicals some with already proven functions in plant growth promotion and biocontrol of pathogens as described earlier in this chapter. This hints at strong commercial value of such compounds.

The microbial VOCs are usually obtained as complex mixtures, and the production of volatiles is influenced by environmental conditions, which includes nutrient

content, composition of the microbial community, temperature, humidity, and pH. This large number of influences on the production of volatiles by the endophytes makes it difficult to identify either the effects of individual volatile molecules or how they work. Therefore in spite of the economic implications of these volatile compounds, their commercialization is very limited. Also it is now known that the effects of volatile compounds can vary from the lab to field conditions [129, 130], and this has led to contrasting results. Also there are reports suggesting that some of the microbial volatiles may modify growth or defense in a species-dependent manner. When used in the field, 2,3-BD was found to exert its effect only as a modulator of defense, and its effect as growth modulator could not be demonstrated under field conditions. Therefore it will be hard to assume growth or protection ability of volatile compounds without evaluating single or mixtures of volatiles on different species of crops under both laboratory and field conditions. Once the nature of either a single or groups of volatiles has been found to have growth promotion- or defense-related functions, the next challenge will be to decide on how to apply the same in open-field conditions since the volatile compounds evaporate fast.

Nonetheless the effectiveness of volatiles in plant growth promotion and as biocontrol agents against a wide range of plant and human pathogenic organisms [131, 132] has led to the commercialization of the endophytes themselves for such purposes. Capability conferred by mutualistic endophytes allows plant adaptation not only to biotic stresses but also to abiotic stress. Since the endophytes increase tolerance to drought and water stress, as well as tolerance to high temperature and high salinity, it is apparent that challenges faced in agricultural adaptation to climate change can be overcome by strategies which employ endophytes. Their use is proving to be excellent in mitigating the impacts of adversities on agricultural plant communities. The ability of mutualistic endophytes to make grasses disease, salt, and temperature resistant and the possibility of extrapolating the same abilities to plants of agricultural importance have led to the commercialization of quite a few endophytic formulations.

According to a company marketing endophytic preparations, formulations known as BioEnsure®-Corn and BioEnsure®-Rice can cause yield improvements for corn and rice under salt and drought stresses. BioEnsure®-Corn used in corn growth can lead up to a 25–80% yield increase under heavy drought stress and a 7% yield increase under low drought stress. These plants have been found to use 25–50% less water under normal conditions as well as under low drought stress. According to the company's claim, BioEnsure®-Rice can cause an increment in rice yield under drought and salt stresses and uses 25–40% less water. These products are sold as liquid formulations used for spraying onto seeds by personnel of commercial seed treatment companies. The endophytes which are fungal in nature remain dormant on the seed until the seeds germinate. The endophytes then establish a symbiotic association with seedlings (http://www.adaptivesymbiotictechnologies.com/press–publications.html).

One such endophyte belongs to the *Muscodor* species, whose volatiles have earlier been described to inhibit or kill a wide range of plant pathogenic fungi, bacteria, nematodes, insects, and even human pathogens. In November 2016,

Marrone Bio Innovations, Inc. (MBI), a global provider of bio-based pest management and plant health products for the agriculture and water treatment markets, received registration for use of *Muscodor vitigenus* as a biofumigant, from the United States Environmental Protection Agency (EPA) Biopesticides and Pollution Prevention Division (BPPD). *Muscodor vitigenus* is an endophytic fungus from the rain forest of the Peruvian Amazon. Its single volatile compound naphthalene, an active ingredient of common mothballs, acts as an insect repellent. It repels the adult stage of the wheat stem sawfly *Cephus cintus*.

It can be assumed that the future is going to see more uses of endophytes' volatiles in plant growth promotion and in controlling pathogens using either the whole organism or the volatile(s) once we know how to stabilize the volatile compounds under field conditions.

5 Concluding Remarks

Endophytic VOCs are a potential gold mine that calls for extensive exploration. Several plant growth-promoting volatile compounds have been studied at the laboratory levels, but field studies using the same remain in their infancy. More studies should be conducted to provide further scientific evidence that can be used to assess the cost-effective, eco-friendly, and sustainable use of naturally produced microbial VOCs for crop welfare. Advanced technologies with respect to profiling and analyzing VOCs, genome sequencing and functional genomics tools, and tools for studying the molecular, physiological, and cellular changes in plant and microbial systems no doubt will accelerate studies on the biosynthesis and modes of action of endophyte VOCs. In order to commercialize important compounds, efforts must focus on easy ways for the isolation, stabilization of the volatiles in field conditions, large-scale production and/formulation to ensure greatest efficacy and cost benefit.

Some volatile compounds may have toxic effects on humans. This needs to be addressed before certification for commercialization. Since some volatile metabolites found in endophytes may be structurally novel, they raise the possibility that these compounds could be potential health hazard if exposed to the same for a long time.

Because of the immense potentials, research on endophytic volatiles will keep drawing attention of both the academia and the industries. Safe and stable VOCs are expected to find worldwide use as growth promoters, biocontrol agents, biofuel, and aromatic compounds in the foreseeable future.

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Antidiabetic and Antioxidant Activities of Bioactive Compounds from Endophytes

13

Rosa Martha Perez Gutierrez and Adriana Neira González

Contents

Abstract

The aim of the present chapter is to appraise the phytochemical and pharmacological potential of the endophytes. This chapter will further highlight the future research prospects of the study of endophytes with antioxidant and antidiabetic activities. Informations on endophytes were obtained from related publications using electronic scientific databases. Based on previous reports, it could be said that the endophytes have emerged as excellent source of compounds which could be used for the treatment of skin diseases and microbial infections and as anticancer and anti-inflammatory agents. The studies provide new knowledge

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on the isolation and characterization of novel bioactives especially in the discovery of novel therapeutic drugs with antioxidant and antidiabetic properties. however, current research on the pharmacological properties of all the endophyte species including bioassay-guided isolation of phytoconstituents and their mechanism of action, pharmacokinetics, bioavailability, efficacy, and safety should be carried out in the future to add more value to this study.

Keywords

Endophytes · Medicinal plants · Marine plants · Antioxidants · Antidiabetic

Abbreviations

ABTS	2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
AGEs	Advanced glycation end products
AgNPs	Silver nanoparticles
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMPK	AMP-activated protein kinase
AST	Aspartate aminotransferase
CAT	Catalase
CE6	Not identified
CE9	Not identified
CEC12	*Cochliobolus* sp.
CED3	*Diaporthe* sp.
CED4	*Diaporthe* sp.
CED7	*Diaporthe* sp.
CEDp11	*Diaporthe phaseolorum*
CEDp2	*Diaporthe phaseolorum*
CEP1	*Phomopsis* sp.
CEP10	*Phomopsis* sp.
CEP4	*Phomopsis* sp.
CES13	*Sordariomycetes* sp.
CES8	*Sordariomycetes* sp.
CVD	Cardiovascular diseases
DAPG	2,4-Diacetylphloroglucinol
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EtOAc	Ethyl acetate
FRAP	Ferric reducing ability of plasma
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas chromatography mass spectrometry
GPx	Glutathione peroxidase
ITS	Internal transcribed spacer
MDA	Malondialdehyde
NCB	Gene sequencing
PMS-NADH	Phenazine methosulfate-nicotinamide adenine dinucleotide
ROS	Reactive oxygen species

SOD	Superoxide dismutase
T2D	Type 2 diabetes mellitus
TEM	Transmission electron microscopy
UV-Vis	Ultraviolet-visible spectroscopy
VOLF4	*Aspergillus* sp.
VOLF5	*Peniophora* sp.
VOR5	*Fusarium nematophilum*
XRD	X-ray diffraction

1 Introduction

Non-insulin-dependent diabetes also called type 2 diabetes is characterized by insulin resistance in tissues including the skeletal muscle and liver and fat tissues and impaired insulin secretion in the pancreas. Diabetes has been associated with a high incidence of complications which are initiated by glycation of proteins which commonly occur in chronic hyperglycemia. A series of subsequent molecular rearrangements and oxidations generate complex compounds of which the most reactive and unstable compounds are known as advanced glycation end products (AGEs) [1]. These modifications can alter the structure and function of proteins and promote cross-linking between them leading to pathological conditions [2]. With the increase of obesity in the population owing to poor lifestyle, consumption of high-calorie diets, and lack of exercise, the incidence of type 2 diabetes has increased considerably over the last decades. It has been estimated that currently around 385 million people are living with type 2 diabetes (T2D), and it is predicted to rise to 595 million by 2035 [3]. In the present scenario, medical treatment does not work for 50% of diabetic patients generating complications which reduce the overall life quality and produce mortality [4]. Chronic hyperglycemia is considered as the main cause of dysregulation of the metabolic signal transduction pathway generating reactive oxygen species (ROS). Excess ROS overfreights the antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) causing an imbalance between antioxidant defense and free radical production generating oxidative stress [5]. Oxidative stress plays an important role in β-cell dysfunction and in pathogenesis of insulin resistance [6] as disruption of cellular homeostasis leads to dysregulation of cell metabolism.

Oxidative stress causes functional and structural alterations in the cellular proteins, nucleic acid, and lipids inviting several complications in patients with diabetes [7]. Antioxidant enzymes such as GPx, SOD, and CAT act as free radical scavengers and form innocuous products donating electrons to ROS inactivating free radicals, thereby protecting cells against oxidative damage [8]. However, in diabetes these antioxidant enzymes are degraded [9]. Chronic hyperglycemia affects antioxidant defense system followed by injury of cellular organelles, development of insulin resistance, and increased level of lipid peroxidation [10]. Malondialdehyde (MDA) is the product of lipid peroxidation used as an indicator of cellular damage [11]. The liver is also damaged severely in diabetes, and therefore, levels of alanine

aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are widely used as indicators of liver functions [12].

Hundreds of plants have been used to treat diabetes mainly due to their hypoglycemic effect. Furthermore, numerous studies have reported the isolation and characterization of more than 200 compounds from medicinal plants [13, 14]. The benefits of such plants for treating diabetes are widely known, and they are regarded as alternatives to pharmaceuticals [15], e.g., *Magnolia grandiflora* contains honokiol as an active constituent which activates AMP-activated protein kinase (AMPK) [16]. AMPK is considered as a cellular energy sensor which helps regulate the energy balance and caloric intake and participates in the regulation of glycolysis, in the entry of glucose, in the oxidation of lipids, in the synthesis of fatty acids and cholesterol, and in gluconeogenesis. It has been considered as a white enzyme in the possible treatment of some diseases such as obesity, diabetes, and hepatic steatosis [17]. Metformin is an important oral antidiabetic drug which lowers blood glucose level and suppresses hepatic gluconeogenesis by activating AMPK in the skeletal muscle [18] and the liver [19].

The German scientist Heinrich Anton de Bary in 1866 used the term endophytes for all microorganisms that inhabit the tissues of healthy plants without showing symptoms of an identifiable disease in the host. Endophytes are bacterial, fungal, or actinomycete microorganisms which colonize healthy plant tissues. The relationship between host plant and the endophyte can be considered as symbiotic to near pathogenic [20] which is however poorly understood. In drug discovery, endophytes are major contributors in the production of compounds with diverse biological activities and novel chemical structures [21].

It has been estimated that there are approximately a million fungal endophytes living inside plant tissues without causing damage to hosts. In the last decades, they have been considered as important microbial resources [22] producing a large number of bioactive compounds. Starting from 2002, endophytic strains have generated nearly half of the newly discovered metabolites derived from fungi. Such metabolites show anti-inflammatory, antioxidant, antihypertensive, antidiabetic, anticancer, antifungal, immunomodulatory, and antibacterial activities [23].

Particularly tropical and subtropical plants are rich in diversity of endophytic microorganisms [24]. The biodiversity of endophytes is influenced by several factors such as the sampling site, the age of the tissue, and the associated vegetation [25]. The plant/microorganism association in many cases is influenced by the bioactive compounds produced by the microorganisms [26]. The compounds benefit the host plant in many cases by providing protection against infections and in others being crucial for their survival [27, 28]. Medicinal plants are producers of important bioactive compounds being a target for isolation of endophytic fungi [29–31]. Since endophytes are an important resource of bioactive compounds, it could be expected that they might have a solution for the treatment of diabetes. Thus, there is a need to study endophytes for the development of effective yet safe drugs.

In the last decades, marine organisms have attracted attention for their immense potential in producing widely diverse bioactives or secondary metabolites [32]. Among these, the study of fungi has become a foreground in the search for new

marine compounds specially after the discovery of penicillin [33]. Around 70,000 fungal species and 1500 species of marine-derived fungi from coastal ecosystems have been described worldwide [34]. Gareth Jones (1998) [35] conclude that as 70% of the earth comprises water bodies, there would be at least 72,000 species of marine fungi, indicating that the discovery of new bioactives is still underway. In addition, filamentous marine fungi is getting more attention of the pharmaceutical community for production of a wide variety of compounds that are pharmacologically active and structurally unique [36]. In this chapter, we have organized the research findings in this field with our prime focus on antioxidant and antidiabetic properties of the endophytes. By bringing the possible perspectives and trends for further studies of the endophytes in the limelight, this review could help in carrying out future research in this field.

2 Research Methodology

Relevant information on pharmacology of endophytes and isolation of their phytoconstituents were compiled based on scientific literature available from online databases such as Scopus, PubMed, Google Scholar, Scirus, ScienceDirect, SciELO, Web of Science, MEDLINE, SpringerLink, BioMed Central (BMC), and SciFinder. Informations derived from these databases were obtained using the keyword "endophytes." Furthermore, relevant scientific publications from different categories were also taken into consideration.

3 Antioxidative Potential of Endophytes

Oxidative stress is produced by an imbalance between the overproduction of reactive oxygen species and cellular antioxidant defenses resulting in the injury of macromolecules as proteins and lipids. Oxidative stress is mainly responsible for the pathogenesis of chronic diseases such as diabetes, cancer, and CVD [37] which leads to a global health problem causing disability and death of millions of people [38]. Numerous investigations indicate that a high consumption of vegetables and fruits rich in phenolic compounds significantly decrease the risk and/or incidence of cancer, diabetes, and CVD [39]. Diabetics have high concentrations of AGEs which have prooxidant effects and participate in the production of chronic complications in the diabetic patients [40]. Thus the treatment for increasing the effect of antioxidants and inhibiting the generation of AGES prevents complications in diabetes.

3.1 Endophytes from Medicinal Plants with Antioxidant Activities

3.1.1 *Achyranthes aspera*

Seventy-three isolates were obtained from the leaves of *Achyranthes aspera* as endophytic bacteria. Among them, AL2-14B showed higher DPPH radical

scavenging activity with IC_{50} value of 6.41 ± 0.11 mg/mL compared to the control plant with IC_{50} value of 8.11 ± 0.24 mg/mL. In β-carotene-linoleic acid assay, AL2-14B inoculated plants showed a range of 15.77–78.85, and β-carotene-linoleic acid assay of extract obtained from inoculated plant was found to be slightly higher than the control plant. In *A. aspera* leaves inoculated with AL2-14B, the reducing antioxidant power assay showed higher value than that of the control plant. The values ranged in the inoculated plant from 0.452 to 1.122 [41].

3.1.2 *Aegle marmelos*

One hundred sixty-nine strains of endophytes were obtained from 5 trees of *Aegle marmelos* of which 67 were pigmented endophytic fungi. The isolates were classified into Deuteromycota, Basidiomycota, and Ascomycota. In DPPH assay, the endophytes FC39BY, FC8ABr, FC2AP, FC75ABr, and FC30AGr showed 50% inhibition at a concentration of 174 μg/μL, 62 μg/μL, 43 μg/μL, 200 μg/μL, and 161 μg/μL, respectively. Among the extracts, FC8ABr and FC2AP showed a significantly higher antioxidant activity. In addition, FC2AP was found to have a higher reductive power than other endophytes [42].

3.1.3 *Caralluma acutangula*, *Moringa peregrina*, and *Rhazya stricta*

Twenty-one fungal endophytes, viz., *Cladosporium* sp. (one strain), *Bipolaris* sp. (one strain), *Alternaria* sp. (two strains), and *Phoma* sp. (six strains), were identified from various organs of medicinal plants like *Moringa peregrina*, *Rhazya stricta*, and *Caralluma acutangula* based on 18S rDNA sequencing and phylogenetic analysis. *Bipolaris* sp. exhibited significantly higher radical scavenging activity in DPPH, ABTS, and NADH/PMS assays and exerted a greater anti-lipid peroxidation effect than the other isolates. *Bipolaris* sp. even displays higher phenolic and flavonoid content [43].

3.1.4 *Centella asiatica* Used in the Biosynthesis of AgNPs

An endophytic fungus isolated from the medicinal plant *Centella asiatica* was used in the biosynthesis of silver nanoparticles (AgNPs). These nanoparticles were characterized using UV-Vis and FTIR spectrum, TEM analysis, particle size analysis, and zeta potential. The endophytic fungus was identified as *Aspergillus versicolor* ENT7 based on 18S rRNA gene sequencing (NCBI). Antioxidant activity of the AgNPs was evaluated by DPPH radical scavenging assay. AgNPs at a concentration of 100 μg/mL produce a radical scavenging activity of 60.04% compared to ascorbic acid (68.52%) used as standard at the same concentration [44].

3.1.5 *Costus spiralis*

Costus spiralis is a Brazilian Amazon plant known for its medicinal properties. Thirteen strains of fungal endophytes were obtained from *C. spiralis* and identified as *Phomopsis* sp. (CEP1), *Diaporthe phaseolorum* (CEDp2), *Diaporthe* sp. (CED3), *Diaporthe* sp. (CED4), *Phomopsis* sp. (CEP4), not identified (CE6), *Diaporthe* sp. (CED7), *Sordariomycetes* sp. (CES8), not identified (CE9), *Phomopsis* sp. (CEP10), *Diaporthe phaseolorum* (CEDp11), *Cochliobolus* sp. (CEC12), and

Sordariomycetes sp. (CES13). The antioxidant activities were measured using DPPH and FRAP assays. CEP1, CEDp11, CES13, CE6, and CEC12 showed highest antioxidant activities and were hence subjected to liquid-liquid fractionation with dichloromethane. The result suggested that coumarins were responsible for the antioxidant effect [45].

3.1.6 *Emblica officinalis*

Eleven endophytes were obtained from *Emblica officinalis*. These endophytes have been identified as homologues of *Diaporthe* sp., *Xylaria* sp., *Epacris* sp., and *Phomopsis* sp. Ethanolic extract of endophytic fungi showed significant activity in reducing power assay in the following order – *Phomopsis* sp. > *Xylaria* sp. > *Diaporthe* sp. > *Epacris* sp. – but they were less active than ascorbic acid used as standard. However, in DPPH assay, the scavenging activity was in the order *Phomopsis* sp. > *Diaporthe* sp. > *Xylaria* sp. > *Epacris* sp. where *Phomopsis* sp. showed the highest radical scavenging activity, *Diaporthe* sp. and *Xylaria* sp. showed moderate antioxidant effects, and *Epacris* sp. showed the lowest activity [46].

3.1.7 *Eugenia jambolana*

Ethyl acetate extracts of 21 different endophytic fungi associated with *Eugenia jambolana* Lam. contain terpenes and phenols as the main constituents responsible for producing antioxidant activity. The antioxidant activity of these extracts was evaluated using DPPH radical scavenging, reducing power and hydrogen peroxide scavenging assays. Among the isolated endophytes, the strains *Aspergillus niger*, *Aspergillus peyronelii*, *Aspergillus* sp., and *Chaetomium* sp. showed the highest antioxidant activity ranging from 50% to 80% compared to ascorbic acid used as a standard [47].

3.1.8 *Fritillaria unibracteata*

Fifty-nine strains of fungal endophytes were isolated from *Fritillaria unibracteata* var. *wabuensis*. The isolates were identified as 17 different taxa with abundant biodiversity. The most important taxa were *Fusarium redolens* (11 isolated) and *Fusarium tricinctum* (10 isolated), followed by *Clonostachys rosea* (8 isolated) as teleomorph, ochroleuca, and *Bionectria* and *Plectosphaerella cucumerina* (5 isolated). All the filtrates of fungal endophytes showed antioxidant effect in both FRAP and DPPH assays, and the values ranged from 84.60 ± 1.56 to 1104.44 ± 25.17 and from $6.88 \pm 0.14\%$ to $107.32 \pm 8.91\%$, respectively. Findings indicated that 62.0% of 30 isolates showed a value of more than 550 μM in FRAP activity. However, in two isolates (6WBY2 and 6WBK3) of the *Fusarium* genus and an unidentified endophyte WBS026, FRAP activities were greater than 1000 μM. 39.2% of 20 isolates exerted more than 50% DPPH radical scavenging. WBS027 isolated from the genus *Bionectria*, 7WBY2 from *Fusarium*, and an unidentified isolate WBS013 showed DPPH radical scavenging inhibition close to 100% inhibition [48].

3.1.9 *Guazuma tomentosa*

An endophytic *Phyllosticta* sp. of the fungi mycelium was isolated from *Guazuma tomentosa* H.B and K (Sterculiaceae) endophytic, and its filtrate was extracted in ethanol. The antioxidant activity was measured from ethanolic extract of the fungus in vitro using scavenging ABTS and DPPH radicals. The ethanolic extract of *Phyllosticta* sp. showed significant antioxidant activity against both ABTS and DPPH radicals with the EC_{50} value of 580.02 ± 0.57 μg/mL and 2030.25 ± 0.81 μg/mL, respectively [49].

3.1.10 *Gymnema sylvestre* Used in the Biosynthesis of AgNPs

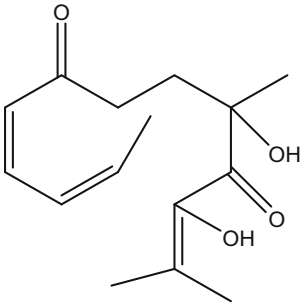
The endophytic fungi *Pestalotiopsis microspora* of phylum Ascomycetes was isolated from the leaves of *Gymnema sylvestre* and identified on the basis of the phenotypic characters. Biosynthesis of AgNPs was carried out with the fungal isolate of *P. microspora*, and then these nanoparticles were characterized using UV-Vis spectrum, FTIR spectrum, TEM analysis, XRD analysis, particle size analysis, and zeta potential analysis. Antioxidant activity of the biosynthesized AgNPs was measured by DPPH free radical scavenging assay using ascorbic acid as standard. Biosynthesis of AgNPs and fungal culture aqueous filtrate were found to be 76.95 ± 2.96 μg/mL and 182.89 ± 3.43 μg/mL, respectively. The biosynthesis AgNPs also showed a significantly high scavenging activity against H_2O_2 radicals at a concentration of 100 μg/mL (51.14% ± 1.78%), while the fungal filtrate showed scavenging activity of 31.28% ± 1.63% [50]. Further, *P. microspora* yields several bioactive compounds of biomedical and pharmaceutical importance [50, 51]. Bioactives like hydroxyl pestalopyrone, pestalopyrone, and ambuic acid are effective against human pathogens, and others like hydroxyl jesterone and jesterone are effective against plant pathogens [52].

3.1.11 *Kandis gajah*

The endophytic fungi *Acremonium* sp., *Chrysonilia sitophila*, and *Penicillium* sp. were isolated from *Kandis gajah*. The mycelia was extracted with ethyl acetate and was evaluated for radical scavenging activity using DPPH. The extract showed an IC_{50} value of 10.3 μg/mL compared to 9.8 μg/mL in case of ascorbic acid. The extract was isolated and identified as a sesquiterpene 3,5-dihydroxy-2,5-dimethyltrideca-2,9,11-triene-4,8-dione with antioxidant activity [53] (Fig. 1).

3.1.12 *Passiflora incarnata L.*

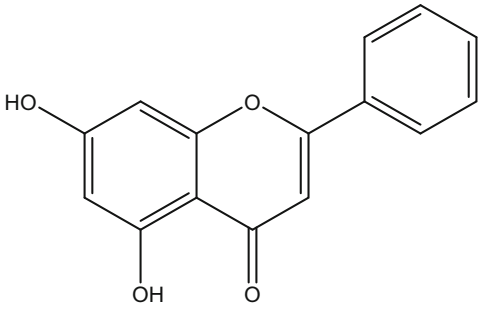
Three endophytes fungi are isolated from methanolic extract of *Passiflora incarnata L.* and identified as *A. alternata* (KT380662), *C. capsici* (KT373967), and *C. taiwanense* (PI-3 KX580307). *A. alternata* (KT380662) produce a high level of 5,7-dihydroxy-flavone (chrysin). The antioxidant activity was evaluated by the method of DPPH scavenging activity at different concentrations ranging from 20 to 100 μg/mL. Chrysin showed inhibition in the range of 9–27% compared to the standard BHT at 42–83% [54] (Fig. 2).



3,5-dihydroxy-2,5-dimethyltrideca 2,9,11-triene- 4,8-dione

Fig. 1 Isolate with antioxidant activity from *Kandis gajah*

Fig. 2 Isolate with antioxidant activity from *A. alternata*



Chrysin

3.1.13 *Polygonum cuspidatum*

The roots of *Polygonum cuspidatum* have been used for centuries for medicinal purposes. Endophytic actinomycete fungi *Streptomyces* sp. A0916 was isolated from *Polygonum cuspidatum*. The antioxidant activities were significantly inhibited by both extracts when subjected to DPPH radical assay. The results were not significantly different among both extracts, *P. cuspidatum* (92.7% inhibition) and *Streptomyces* sp. A0916 (93.2% inhibition), whereas ascorbic acid used as positive control showed an inhibition of 93.8%. It was inferred that both *P. cuspidatum* and *Streptomyces* sp. A0916 extracts showed strong antioxidant activities [55]. The chemical composition of *Streptomyces* sp. A0916 extract was 3-methyl-1-butanol, 4-methyl-1-pentanol, 1-nonanal, 6-methyl-2-oxiranyl-hept-5-en-2-ol, 2,6,11,15-tetramethylhexadecane, 2,6-dimethylocta-2, 7-dien-6-ol, 2,4-di-tert-butylphenol, glacial acetic acid, linoleic acid, 4-methylvaleric acid, 4-hexenoic acid, dehydroacetic acid, heptanedioic acid, 2-methyl butyric acid, and 1-p-menthen-8-ol identified by GC/MS.

3.1.14 *Rhodiola* Plants

Three hundred forty-seven endophytic fungi were isolated from rhizomes of three *Rhodiola* plants classified into 180 representative morphotypes (71, 57, and 52 isolates from Rc, Ra, and Rs, respectively) based on the characteristics of their cultures. In addition, these were also identified based on their related taxa or rRNA-ITS sequences. Isolated Rac88 from host *Rawas* is affiliated to phylum Glomeromycota and is placed in the genus *Entrophospora*. However, Rct60 was closely associated to *Mucor hiemalis* (99%), and Rac18 was assigned to *Umbelopsis* sp. (78%) of order Mucorales in Zygomycota. Isolates Rac69, Rac81, and Rac85 within Basidiomycota were closely matched to the sequences of *Ceratobasidium* sp. Rsc51 and Rsc45 were associated to *Rhizoctonia solani* (100%) and *Coprinellus xanthothrix* (99%), respectively. Other endophytic fungi belonging to classes Leotiomycetes, Dothideomycetes, and Sordariomycetes and phylum Ascomycota were also isolated [56].

Endophytic fungi in the rhizomes of *Rhodiola* spp. are diverse and abundant with 180 representative isolates distributed in 57 genera belonging to 4 fungal phyla. Isolates such as Rsc57, Rct45, Rac76, Rct64, and Rct63 exhibit strong antioxidant activity. Numerous investigations indicate that flavonoid and phenolic compounds could be considered as the main antioxidants in plants [57]. *Rhodiola* spices contain rosavins, *p*-tyrosol, and salidrosides [58]. Nevertheless, no comparative study has been carried out on their endophytes. The fermentation broth of Rac12 was seen to produce salidroside and p-tyrosol when subjected to HPLC. Data indicate that endophytes may produce the same bioactive chemicals as those of their hosts (Fig. 3).

3.1.15 *Salvia miltiorrhiza* Bge.f. alba

Endophytic fungi from *Salvia miltiorrhiza* Bge.f. alba have been considered a promising source of antioxidants. Fourteen fungal endophytes were identified by molecular and morphological methods as *Fusarium* and *Alternaría* species. However, six fungi were identified using internal transcribed spacer (ITS) rRNA gene sequence analysis as non-sporulating fungi. The results of phytochemical analysis carried out using ethanolic extracts of endophytic fungi from *S. miltiorrhiza* showed the presence of alkaloids, phenols, saponins, tannins,

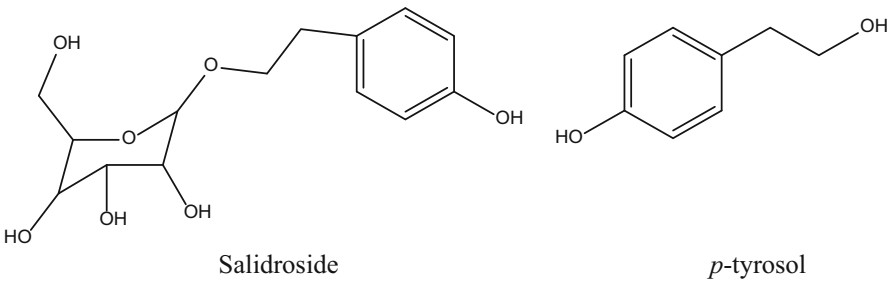


Fig. 3 Isolates with antioxidant effect from rhizomes of *Rhodiola* spp.

terpenoids, flavonoids, and steroids like those in the extracts of the roots of host plants [59]. The antioxidant activities of the extracts of endophytic fungi *F. proliferatum* SaR-2 and *A. alternata* SaF-2 and plant root were measured based on their ability to scavenge the DPPH free radicals compared to the standards, BHT and ascorbic acid. Findings indicated that both endophytic fungi at a concentration of 0.1 mg/mL showed higher radical scavenging activities than that of the plant root projecting 90.14%, 83.25%, and 80.23% values, respectively. Data showed that *F. proliferatum* SaR-2 could be a promising source of antioxidant compounds [60].

3.1.16 *Scapania verrucosa*

Forty-nine endophytic fungi were isolated from ethyl acetate extract of *Scapania verrucosa*. Based on their molecular and morphological characteristics, the isolated endophytic fungi were found to belong to the family Xylariaceae and seven genera *Creosphaeria*, *Nemania*, *Xylaria*, *Tolypocladium*, *Chaetomium*, *Penicillium*, and *Hypocrea*. However, the majority of these isolated endophytic fungi belonged to *Xylaria*, *Creosphaeria*, and *Chaetomium*. Forty-nine strains were evaluated for their in vitro antioxidant activities by different methods such as DPPH radical scavenging, hydroxyl radical scavenging, and reducing power and ferrous ion chelating assays. Of the isolated endophytic fungi strains, T24 (*Chaetomium globosum*) and T38 (*Creosphaeria* sp.) exhibited the highest antioxidant capacity [61].

3.1.17 Sudanese Medicinal Plants

Twenty-one endophytic fungi were isolated from Sudanese medicinal plants *Trigonella foenum-graecum*, *Vernonia amygdalina*, *Euphorbia prostrata*, *Catharanthus roseus*, and *Calotropis procera*. The isolated endophyte strains were assigned to 12 different taxa. Of them, ten strains were identified to belong to Ascomycetes, seven strains were found to be fungal, and four strains of Deuteromycetes belong to *Mycelia sterilia* [62]. *Chaetomium* and *Mycelia sterilia* were the most important fungal taxa isolated. The *Aspergillus* sp. endophyte isolated from *T. foenum-graecum* and *Curvularia* sp. from *V. amygdalina* exerted significant antioxidant activities in DPPH radical scavenging assay [63].

3.1.18 *Terminalia morobensis*

Pestalotiopsis microspora was isolated as an endophyte from *Terminalia morobensis* which grows in Papua New Guinea. *P. microspora* was of interest because of its antioxidant properties, and it produced two isobenzofuranones which have been isolated previously with substitutions at positions 5 and 7 with –OCH3, –CH3, or –OH functional groups. Isopestacin, having the basic structural features of an isobenzofuranone, possesses a 3-benzo substituent. Both pestacin and isopestacin showed similarities to flavonoids suggesting that it might possess antioxidant activity [64]. This was confirmed with their ability of scavenging the hydroxyl free radical ($OH^{\cdot}$) (Fig. 4).

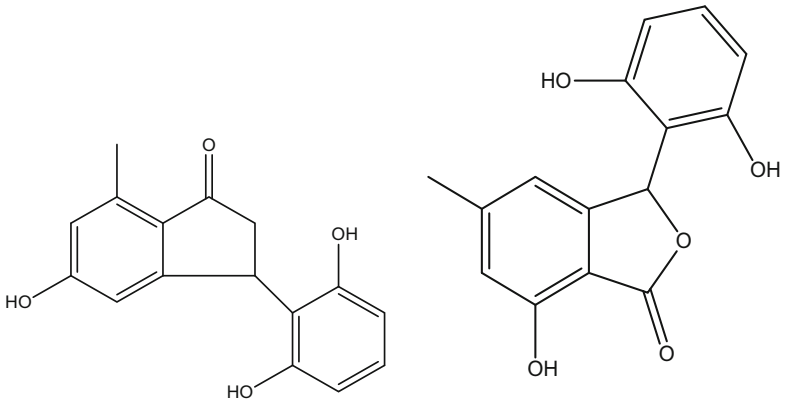


Fig. 4 Pestacin and isopestacin with antioxidant effect isolated from *Pestalotiopsis microspora*

3.1.19 *Trachelospermum jasminoides*

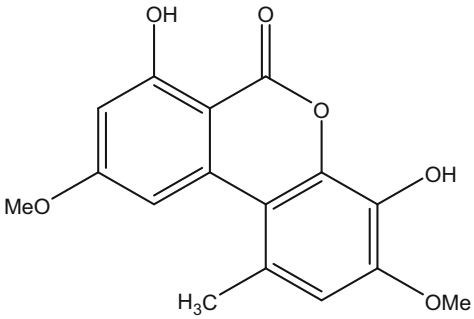
A total of 1626 endophytic strains were isolated from *Trachelospermum jasminoides* LINDL. Among them, endophytic fungus *Cephalosporium* sp. IFB-E001 which inhabits in the roots of *T. jasminoides* was extracted with $CHCl_3$:MeOH (1:1) containing graphislactone A as the most bioactive secondary metabolite with high antioxidant and free radical scavenging activities greater than those of ascorbic acid and butylated hydroxytoluene (BHT) used as standards [65] (Fig. 5).

3.1.20 *Taxus sumatrana*

Taxus sumatrana (Miq.) de Laub, found in Indonesia, is a plant known for its medicinal properties. Fourteen endophytic fungi were isolated from the plant, and their methanolic and ethyl acetate extracts were prepared [66]. The extracts were evaluated in vitro for their antidiabetic and antioxidative effects using α-glucosidase, DPPH free radical scavenging activity, and β-carotene bleaching assays [67]. Isolated endophytic fungi *Colletotrichum* sp. (TSC13) showed higher α-glucosidase inhibitor activity suggesting a promising antidiabetic activity, whereas TSC 24 showed higher antioxidant activity [68].

3.1.21 *Tinospora cordifolia*

Tinospora cordifolia, an Indian plant known as amrita (guduchi) in Sanskrit belonging to the family Menispermaceae, is used as a traditional medicinal plant. An endophytic fungus *Cladosporium velox* TN-9S was isolated from *T. cordifolia* and extracted using ethyl acetate, and total phenolic content was evaluated by Folin-Ciocalteu assay, and the antioxidant activity was measured by DPPH and FRAP methods. High phenolic content was recorded from the fungal extract which was found equivalent to 730 μg/mL of gallic acid. Significantly reduced radical scavenging activity was observed in DPPH assay with an IC_{50} value of 22.5 μg/mL [69].



Graphislactone A

Fig. 5 Compound with antioxidant effect from *Cephalosporium sp.*

3.2 Endophytes from Marine Plants with Antioxidant Activity

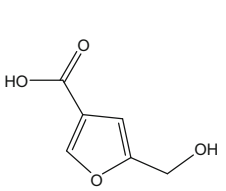
3.2.1 Mangroves

The endophytic fungi isolates from the leaves of mangroves *Rhizophora stylosa* and *R. mucronata* collected from the South China Sea were identified using a combination of phylogenetic analysis and morphology study of the internal transcribed spacer (ITS) sequences. Among them, 17 genera belonging to 8 taxonomic orders of Ascomycota were identified of which orders Xylariales (35.49%) and Diaporthales (27.61%) were the most common. Orders like Pleosporales, Hypocreales, Glomerellales, Eurotiales, Capnodiales, and Botryosphaeriales were also characterized. The radical scavenging ability was evaluated using DPPH and ABTS assays. Of the 46 mangrove isolates, fungal endophytes HHL38 and HHL55 showed the most potent antioxidant effect. Of the isolates, HQD-6 showed significant levels of flufuran in ABTS and DPPH radical scavenging assays [70].

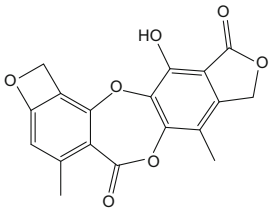
In other studies, a marine-derived endophytic fungi *Phomopsis* sp. A123 was isolated from the leaves of mangrove *Kandelia candel* (L) which contains a novel depsidone and phomopsidone A together with excelsione and four known isobenzofuranones, 7-methoxy-6-methyl-3-oxo-1,3-dihydroisobenzofuran-4-carboxylic acid, diaporthelactone, 7-hydroxy-4,6-dimethyl-3H-isobenzofuran-1-one, and 7-methoxy-4,6-dimethyl-3H-isobenzofuran-1-one [71]. These compounds showed weak antioxidant effect against DPPH radicals (Fig. 6).

3.2.2 Resveratrol Derivatives Isolated from the Endophytic Fungus *Alternaria* from Mangrove

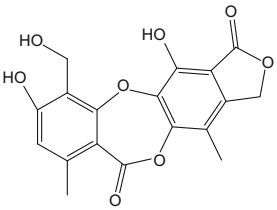
Three new stilbene derivatives, resveratrodehydes A, B, and C, were isolated from the mangrove endophytic fungus *Alternaria* which showed lower radical scavenging activity in DPPH assay with IC_{50} values of 447.62–572.68 μM compared to resveratrol (IC_{50} value of 70.22 ± 0.35 μM) [72]. However, resveratrodehyde B showed only few activities. Findings indicated that electron-withdrawing substituents such as COOH and COOR in *ortho*- or *para*-positions stabilize the phenol form of antioxidants and destabilize the phenoxy radical form to increase the O–H bond strength and decrease the antioxidant effect [73–75] (Fig. 7).



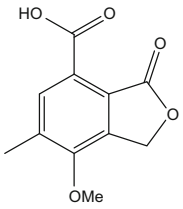
Flufuran



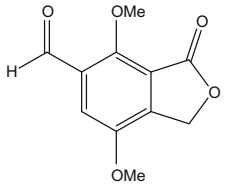
Phomopsidone A



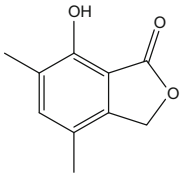
Excelsione



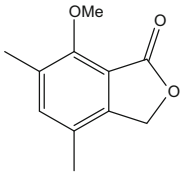
7-methoxy-6-methyl - 3-oxo-1,3 -dihydroisobenzofuran-4-carboxylic acid



Diaporthelactone



7-hydroxy-4,6-dimethy- -3H-isobenzofuran-1-one



7-methoxy-4,6-dimethy-3H-isobenzofuran-1-one

Fig. 6 Structure of antioxidants from *Phomopsis* sp. A123

3.2.3 Anthraquinone Derivatives from Endophytic Fungus *Eurotium rubrum* Associated with Mangrove *Hibiscus tiliaceus*

Seven compounds were isolated and identified from *Eurotium rubrum*, an endophytic fungus associated with mangrove *Hibiscus tiliaceus*. One new bisdihydroanthracenone derivative eurorubrin (**1**), 2-*O*-methyl-9-dehydroxyeurotinone (**2**), 4,2-Omethyl-4-*O*-(α-D-ribofuranosyl)-9-dehydroxyeurotinone (**3**), one new anthraquinone glycoside [6,3-*O*-(α-D-ribofuranosyl]questin] (**4**), and three known compounds, asperflavin (**5**), 2-*O*-methyleurotinone (6), and questin (**7**) were isolated. All of these compounds were evaluated using DPPH radical scavenging assay. Results suggested that compounds **1** and **6** showed strong activities which were stronger than that of the antioxidant butylated hydroxytoluene. Nevertheless, the other compounds showed moderate or weak activities [76] (Fig. 8).

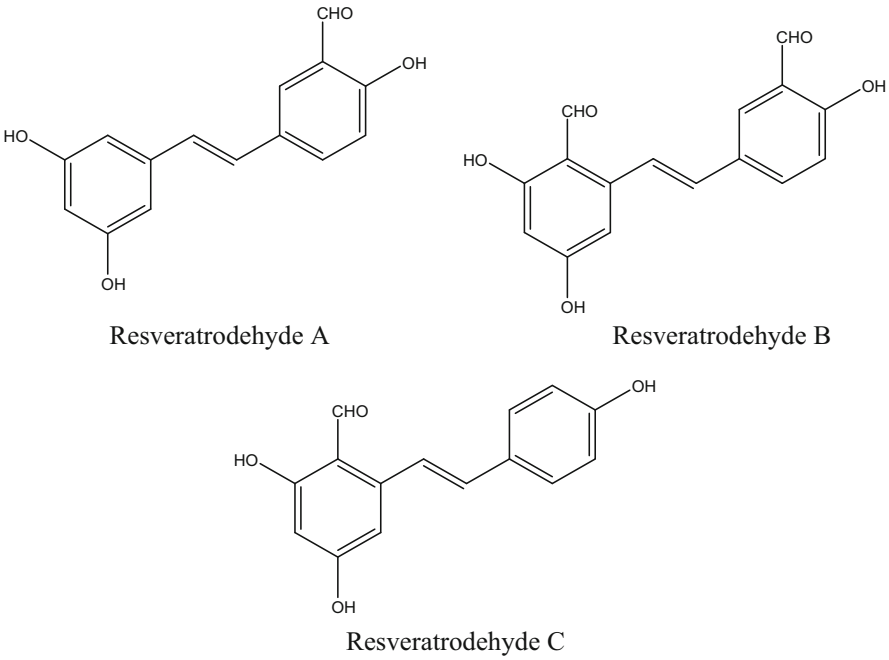


Fig. 7 Resveratrol derivatives with antioxidant activities

4 Endophytes with Antidiabetic Activity

4.1 Endophytes from Medicinal Plants with Antidiabetic Activity

4.1.1 *Acacia nilotica*

Thirty-six endophytic fungi were isolated and identified from methanolic extract of *Acacia nilotica*. An endophyte *Aspergillus awamori* produces the peptide lectin (*N*-acetylgalactosamine, 64 kDa) containing amino acids valine, tyrosine, threonine, and serine. The peptide showed inhibitory alpha-glucosidase activity (80%) and alpha-amylase activity (81%) with IC_{50} values of 5.625 and 3.75 μl/mL, respectively. The peptide is highly stable at optimum pH and temperature [77].

4.1.2 *Adhatoda beddomei*

An endophyte *Syncephalastrum* sp. was isolated from the plant *Adhatoda beddomei.* The mycelial endophyte was extracted with ethyl acetate, and the crude extract demonstrated an inhibitory activity of 75.2% on α-amylase with IC_{50} value 0.25 μg/mL compared to the IC_{50} value 0.75 μg/mL in case of acarbose. α-Amylase inhibitor blocks digestion and absorption of carbohydrate [78].

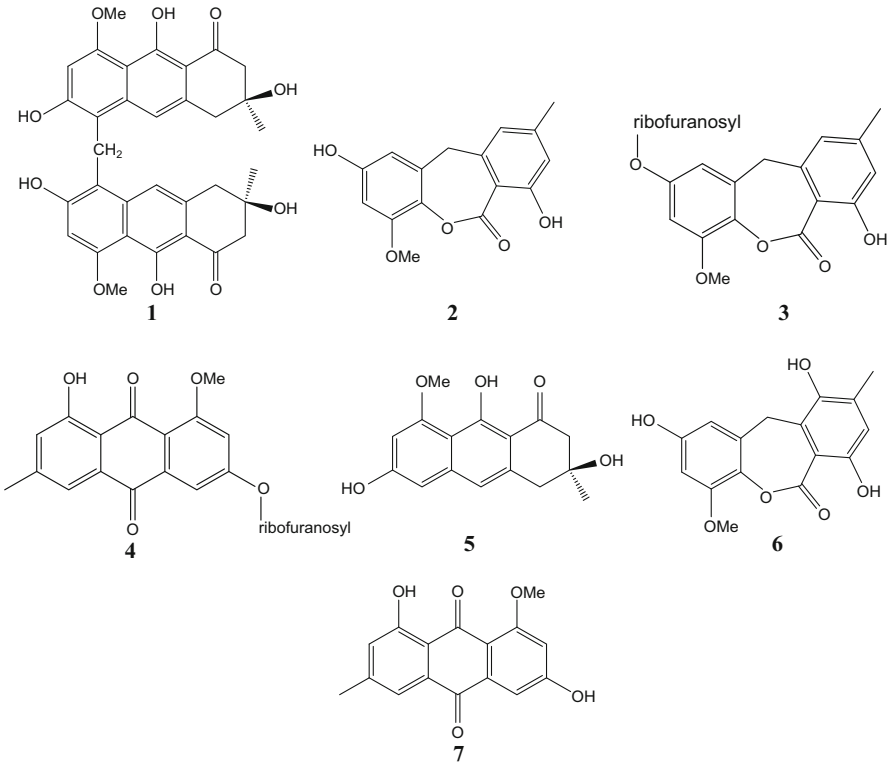


Fig. 8 Anthraquinone derivatives with antioxidant activities

4.1.3 *Ficus religiosa*

The leaves of *Ficus religiosa* carry the endophytic fungus *Dendryphion nanum.* From the EtOAc extract of *Dendryphion nanum* naphthoquinones, herbarin and herbaridine were obtained. Herbarin induced glucose uptake in rat skeletal muscles in the presence of insulin when rosiglitazone, a known glucose uptake activator, was used as standard in the assay. However, herbaridine did not show any such activity [79] (Fig. 9).

4.1.4 *Paeonia delavayi*

A chemical study conducted on fermentation product of *Phomopsis* sp. YE3250 derived from *Paeonia delavayi* led to isolation of seven new polyoxygenated cyclohexenoids named as phomopoxides A−G. All compounds showed significant α-glycosidase inhibition using acarbose as a positive control. In relation to the structural activity, the compounds D–G forming an epoxy moiety produce a weak α-glycosidase inhibition than those of A−C, indicating that tetrahydroxyl substitution in cyclohexene ring is crucial for α-glycosidase inhibition [80] (Fig. 10).

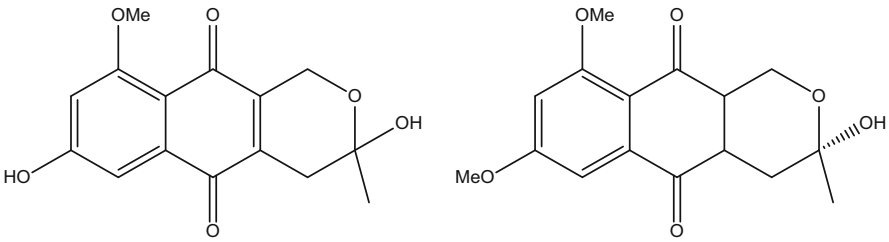


Fig. 9 Isolates with antidiabetic activity from *Dendryphion nanum*

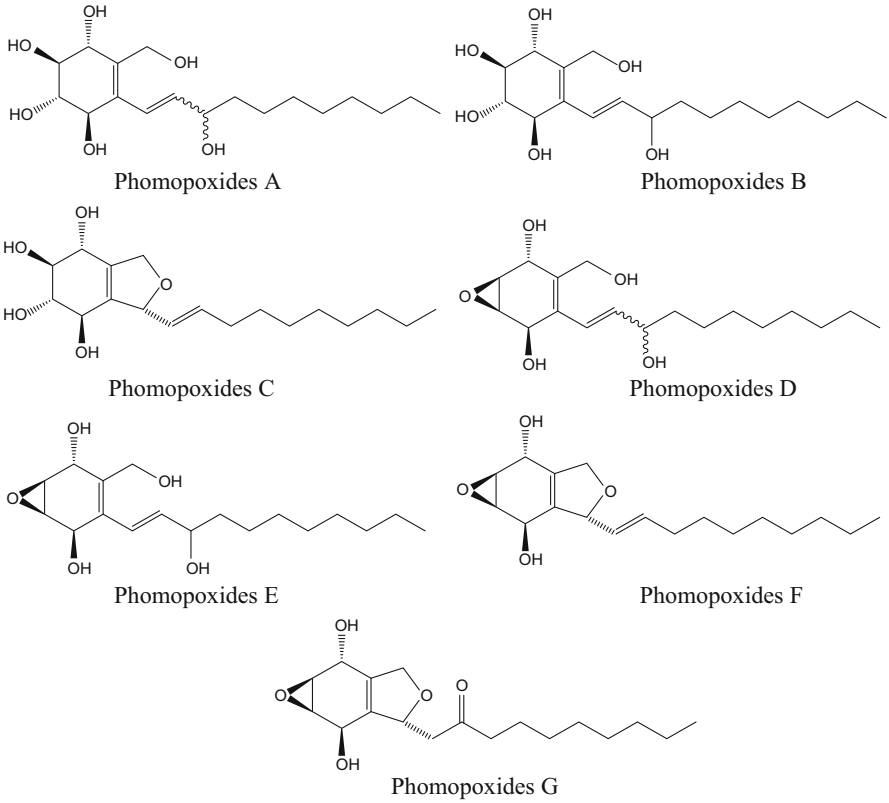
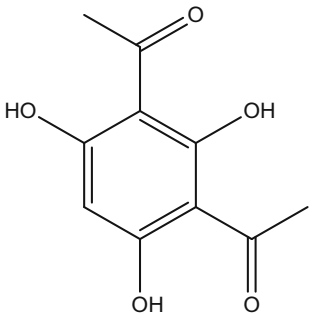


Fig. 10 Chemical structures of compounds phomopoxides A–G

4.1.5 *Piper auritum*

Pseudomonas protegens strain 8-1 was isolated from the leaves of *Piper auritum.* The ethyl acetate extract from the culture showed glycation inhibitory activity in vitro, and the isolated active compound was identified as the polyketide metabolite 2,4-diacetylphloroglucinol (DAPG). This compound inhibited protein



2,4-diacetylphloroglucinol

Fig. 11 2,4-Diacetylphloroglucinol inhibited protein glycation

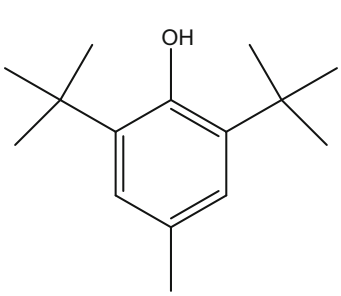
glycation much more than aminoguanidine used as standard in BSA-glucose model. DAPG also inhibited AGE formation as assessed by the three other assay models, BSA-MGO, fructosamine, and benzoate hydroxylation [81] (Fig. 11).

4.1.6 *Salvadora oleoides* Decne.

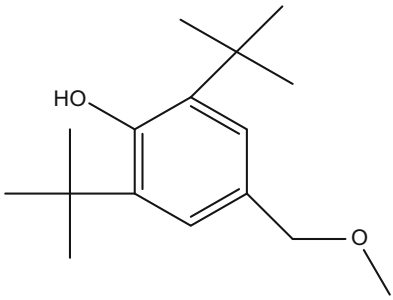
Seventeen endophytic fungi were isolated from *Salvadora oleoides* Decne (Salvadoraceae) which were classified as *Aspergillus* sp. and *Phoma* sp. The fungi mycelium were extracted with methanol (*Aspergillus* sp. JPY2 and *Aspergillus* sp. JPY1) and acetone (*Phoma* sp.). The antidiabetic activity of the extracts were evaluated using the model alloxan-induced diabetic rat. The extracts significantly reduced blood glucose levels in a range of 11.3%–28.04%, whereas the tolbutamide used as the standard drug reduced the blood glucose level up to 40%. The methanolic extract of *Aspergillus* sp. JPY1 produces 2,6-di-tert-butyl-p-cresol and phenol, 2,6-bis[1,1-dimethylethyl]-4-methyl as the main constituents [82] (Fig. 12).

4.1.7 *Tabebuia argentea*

Ten endophytes fungi were obtained from the *Tabebuia argentea* and identified as *A. niger*, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Alternaria* sp., and *Trichoderma* sp. which were used to obtain the methanolic extract and analyze phytochemical constituents by gas chromatography mass spectrometry (GC-MS). The methanolic extract was evaluated for its in vitro effect on α-glucosidase and α-amylase activity. Eighteen secondary metabolites were obtained by GC-MS, and their antidiabetic activities were evaluated against 21 different diabetic proteins/enzymes by in silico assay. Data indicated that octadecanoic acid methyl ester and 3 phthalates interacted more with all the 21 diabetic proteins/enzymes tested [83]. In addition, antioxidant activity was evaluated by various methods involving scavenging of free radical DPPH, FRAP, and TBA and superoxide radical FTC and iron. Results indicated that the methanolic extracts of *Aspergillus niger*, *Penicillium* sp., and *Trichoderma* sp. were found to be the most effective in showing in vitro antioxidant activity [84].



2, 6-di-tert-butyl-p-cresol



Phenol, 2, 6-bis [1,1-dimethyl ethyl] -4-methyl

Fig. 12 Isolate with antidiabetic activity from *Aspergillus* sp. JPY1

4.1.8 *Viola odorata*

Twenty-seven endophytes were isolated from *Viola odorata* Linn and were classified on the basis of microscopic and morphocultural characteristics. Anti-obesity potential of endophytic fungi associated with *Viola odorata* was evaluated using porcine pancreatic lipase (type II) employing 4-nitrophenyl butyrate as substrate. *Aspergillus* sp. (VOLF4) showed the most potent PL inhibitory effect followed by *Peniophora* sp. (VOLF5) and *Fusarium nematophilum* (VOR5). Previous data indicated that *Aspergillus* spp., *Penicillium*, and *Colletotrichum* showed good pancreatic lipase inhibitory activity [85].

4.1.9 *Viscum album*

A strain of the endophytic fungi *Alternaria* was isolated from *Viscum album*. The soluble proteins in crude extract were fractionated with ammonium sulfate to produce the peptide *N*-acetylgalactosamine, a 64 kDa protein lectin. The antidiabetic activity of peptide was evaluated in vitro by α-glucosidase, α-amylase, and sucrase assays and in vivo in alloxan-induced diabetes in rats. The *N*-acetylgalactosamine inhibited the enzymes α-amylase (85.26 ± 1.25), α-glucosidase (93.41 ± 1.27), and sucrase (81.61 ± 1.05). Also, diabetic rats showed significantly increased body weight (8.50%) compared to the standard drug (9.01%) after 14 days of treatment with the *N*-acetylgalactosamine. In addition, regeneration of pancreatic tissues and reducing the levels of urea (43.7 ± 5.8), creatinine (0.32 ± 0.01), serum cholesterol (103.54 ± 2.13), and triglycerides (124.68 ± 2.49) [86] were observed in the study.

4.2 Endophytes from Marine Plants with Antidiabetic Activity

4.2.1 Mangrove Endophytic Fungus *Xylaria* sp.

Endophytic fungus *Xylaria* sp. BL321 was isolated from the mangrove from which the four eremophilane sesquiterpenes were derived (**1**–**4**) and were then evaluated for their inhibitory effects on α-glucosidase employing an enzyme-based bioassay.

Compound **4** showed the most potent α-glucosidase inhibitory effect. However Compound **1** had a minimum effect on α-glucosidase [87] (Fig. 13).

4.2.2 Endophyte *Trichoderma* sp. 307 from Mangrove

A study of the simultaneous cultivation of aquatic pathogenic bacterium, *Acinetobacter johnsonii* B2 and endophyte *Trichoderma* sp. 307, from mangrove leads to the isolation of 2 new sesquiterpenes, microsphaeropsisin B (**1**), microsphaeropsisin C (**2**), 2 new de-*O*-methyllasiodiplodins, microsphaeropsisin (**3**), (3R, 7R)-7-hydroxy-de-O-methyllasiodiplodin (**4**), (3R)-5-oxo-de-*O*-methyllasiodiplodin (**5**), and 12 known compounds (3R)-7-oxo-de-*O*-methyllasiodiplodin (**6**), microsphaeropsisin (**3**), (3R)-5-oxolasiodiplodin (**7**), (3S)-6-oxo-de-*O*-methyllasiodiplodin (**8**), (3R)-de-*O*-methyllasiodiplodin (**9**), (3R,4R)-4-hydroxy-de-*O*-methyllasiodiplodin (**10**), (3R,5R)-5-hydroxy-de-*O*-methyllasiodiplodin (**11**), (3R,6R)-6-hydroxy-de-*O*-methyllasiodiplodin (**12**), (3R)-lasiodiplodin (**13**), (3S)-ozoroalide (**14**), (3S,5R)-5-hydroxylasiodiplodin (**15**), (E)-9-etheno-lasiodiplodin (**16**), and (3R)-nordinone (**17**).

The α-glucosidase inhibitory activities of all compounds isolated were evaluated. Findings indicated that compounds **4**, **5**, **8**, **9**, **10**, **16**, and **17** showed potent α-glucosidase inhibitory effect which was higher than that produced by the acarbose used as a positive control, whereas compounds **2**, **6**, **7**, and **14** showed moderate inhibitory activity. The other compounds **1**, **3**, **11**, **12**, **13**, and **15** were inactive. In relation to the structure activity, it was observed that the methoxy group at C-15 in the lasiodiplodin derivatives decreased the activity and the position of the hydroxyl and carbonyl groups also significantly altered the effect. However, the presence of C-9 to C-10 double bond was essential for the α-glucosidase inhibitory activity [88] (Fig. 14).

4.2.3 Endophytic Fungus *Nectria* sp. HN001 from Mangrove Plant *Sonneratia ovata*

Four new polyketides nectriacids A–C (**1–3**) and 12-epicitreoisocoumarinol (**4**) and three known compounds, citreoisocoumarinol (**5**), citreoisocoumarin (**6**), and macrocarpon C (**7**), were isolated from the endophytic fungus *Nectria* sp. HN001 associated with the mangrove *Sonneratia ovata* collected from the South China Sea. Compounds **2** and **3** exhibited stronger in vitro α-glucosidase inhibitory activity than acarbose used as positive control. Nevertheless, compounds **4**, **5**, and **6** showed moderate activity, while compound **7** showed no inhibitory activity compared to acarbose [89] (Fig. 15).

5 Conclusions

An extensive literature review revealed that very limited reports have focused on isolation of endophytes or extraction of their bioactives. Only few of the isolated compounds have been investigated so far. There is a need in the future to carry out research on endophytes through bioassay-guided isolation, chemical

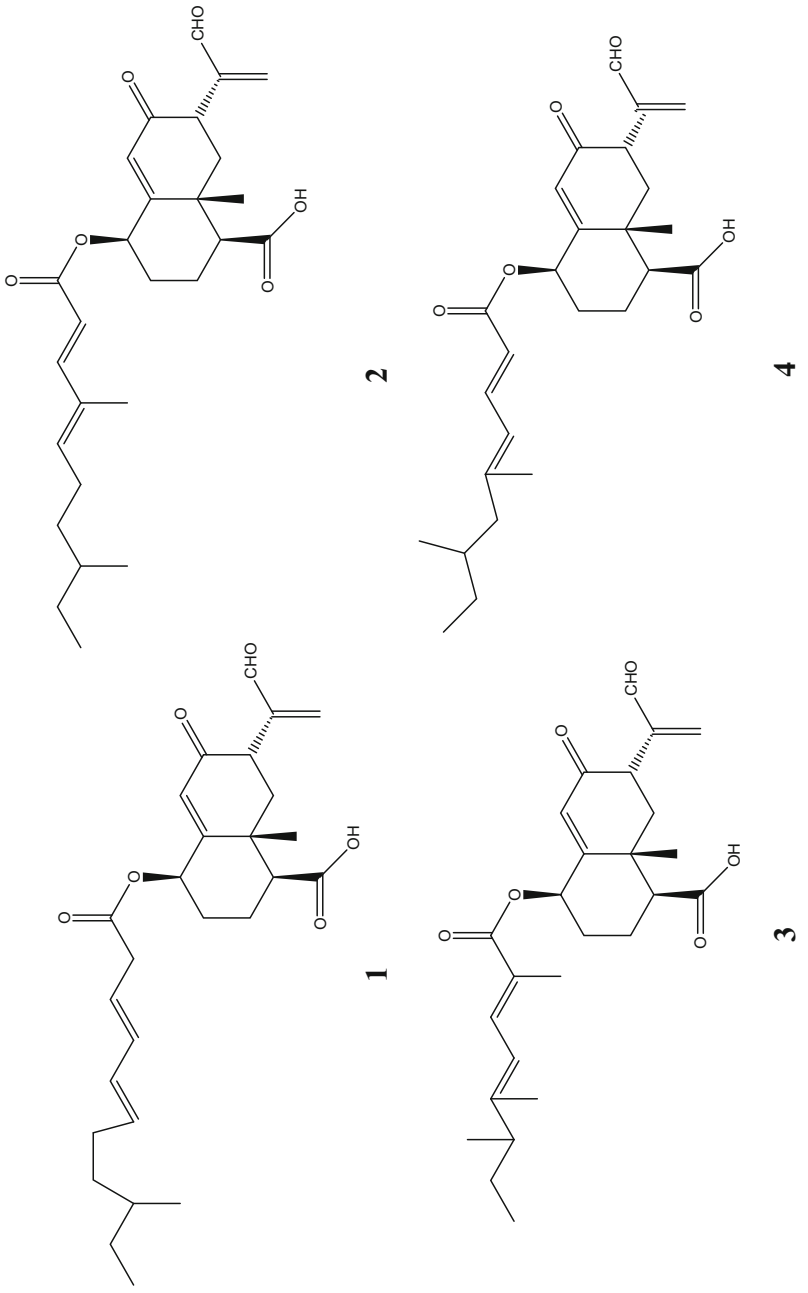


Fig. 13 Compounds with α-glucosidase inhibitory effect

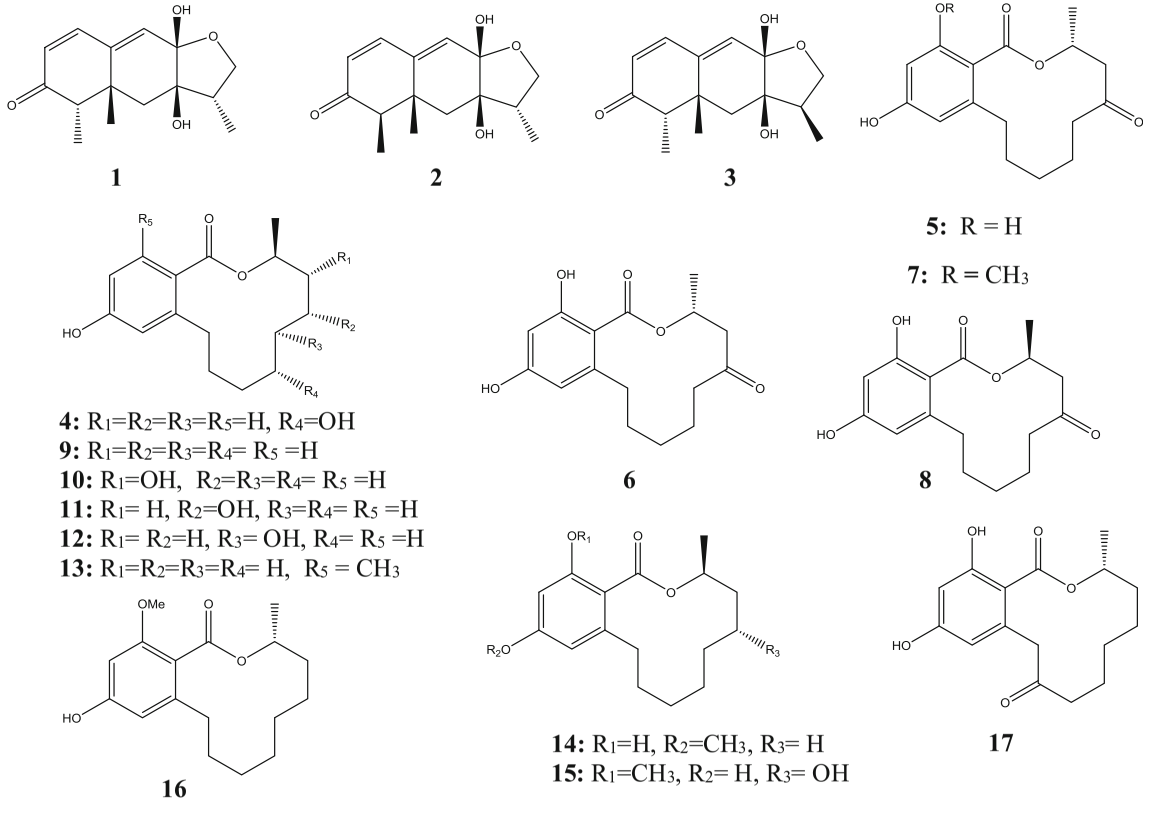


Fig. 14 Compounds with α-glucosidase inhibitory activities

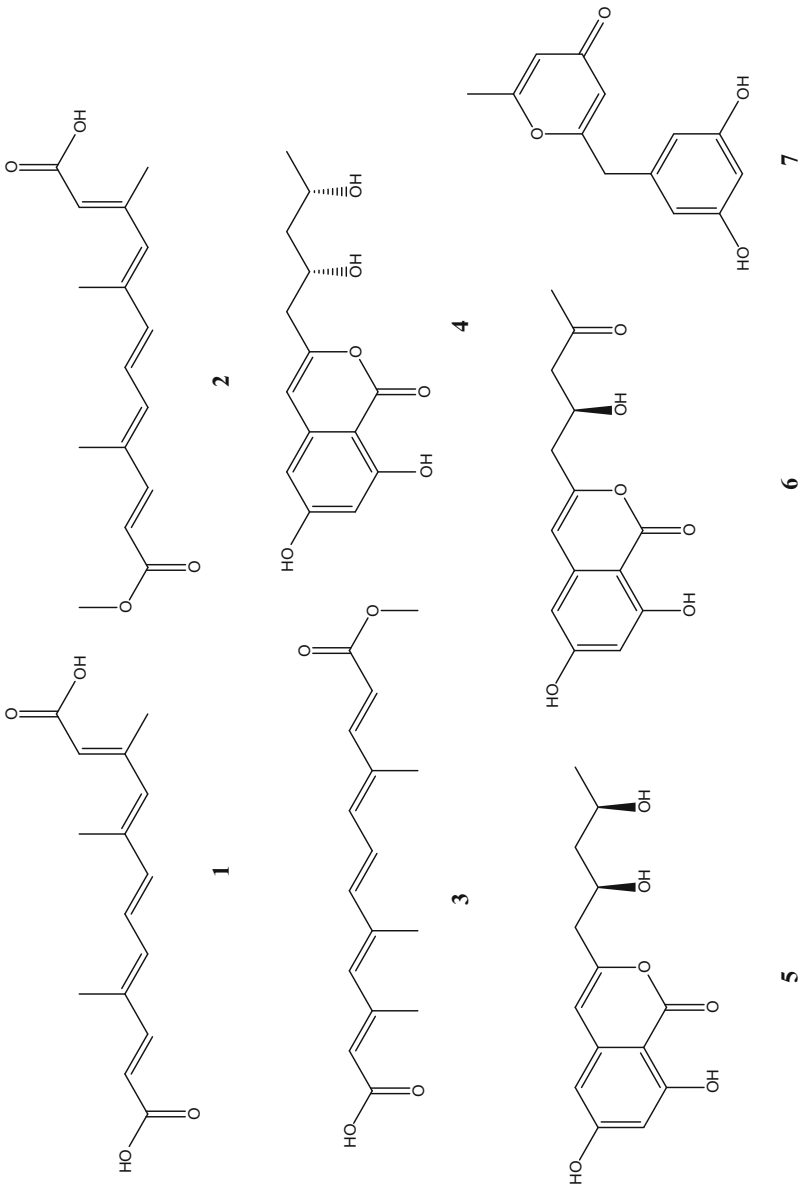


Fig. 15 Chemical constituents of *Nectria* sp. HN001

characterization, structure-activity relationship study, and mechanisms of action. Currently, all the studies found on antioxidant and antidiabetic activities from endophytes have been carried out in vitro, but using animal models for investigating their biological effects has not yet been carried out. This chapter might help the pharmacologists and chemists to investigate the pharmacological and phytochemical properties of endophytes.

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Fungal Endophytes: A Novel Source of Cytotoxic Compounds

14

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Contents

Abstract

Across the globe, cancer is the second most significant cause for mortality that was responsible for 8.8 million deaths in 2015, and the count is increasing at the alarming pace each year. The longer treatment protocols and the serious side effects of the existing anticancer drugs represent an urgent need to develop safe

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and effective anticancer drugs. Endophytic fungi offer the prolific source of novel metabolites that bears unique structural and functional capabilities with cytotoxic activity. In recent years, various bioactive metabolites possessing structural diversity have been identified from endophytic fungi and evaluated for their anticancer properties. Bioactive metabolites from endophytic fungi have potential to serve as a lead molecule for the pharmacological sector in the development of new drugs. The lower yield of metabolites is a major barrier for the utilization of these molecules for the treatment of cancer; therefore, alternate sources and production methods have been developed. The culture optimization to enhance yield and epigenetic means to activate silenced genes capable of producing novel metabolites were developed to obtain the fungal metabolites in higher quantities. The present review provides a comprehensive data of bioactive metabolites isolated from endophytic fungi harboring terrestrial plants during 2012–2018 (up to June 2018) with focus on their chemical structure, their cytotoxic capabilities, and their mechanism of action. The outlook of epigenetic modulation is discussed in perspectives of enhancing yield and identification of unidentified metabolites.

Keywords
Endophytic fungi · Anticancer compounds · Medicinal plants · Co-culture · Epigenetic modification

1 Introduction

The fungi are known to produce an array of biologically active metabolites with wide-ranging pharmacological activities. Twenty-seven years ago, 1.5 million was generally accepted as a conservative estimate of the number of species of fungi on Earth [1]. Since that time, molecular methods have enabled species concepts to be clarified in many genera, and the sequencing of environmental samples has led to the discovery of a substantial unexpected species diversity; as a result, the 1.5 million figure has been revised upward to between 2.2 and 3.8 million species [2]. Endophytes produce diverse metabolites, although these are relatively less explored microorganisms for identifying novel and structurally diverse molecules [3, 4]. Endophytes produce different classes of secondary metabolites such as steroids, xanthones, phenols, isocoumarins, perylene derivatives, quinines, furandiones, terpenoids, depsipeptides, and cytochalasins [5–9]. These metabolites include compounds of pharmacological importance such as anticancer, taxol [10], koningic acid [11]; antibacterial, sanguinarine [12] antimycobacterial, piperine [13]; antifungal, fusaripeptide A [14]; anti-inflammatory, ergoflavin [15]; antidiabetic, L-783,281 [16]; antiviral, alternariol and alternariol-(9)-methyl ether [17]; antioxidant, 3-epi-dihydroaltenuene A, altenuisol, 4-hydroxyalternariol-9-methyl ether [18]; enzyme inhibitors- fusaric acid derivatives [19]; α-glucosidase inhibitory activity, asperisocoumarins A and B [20] nectriacids B-C [21] and immunosuppressive agent, (−)-mycousnine [22]. Other metabolites include agriculturally important compounds such as cladosporin, [23], and 2-phenylethyl 1H-indol-3-yl-acetate [24], as

antifungals; azadirachtin A and B [25], as insecticidal etc. The present review highlights the recently reported metabolites having anticancer activity, discovered from endophytic fungi isolated from terrestrial plant, during 2012–2018 (up to June 2018). Among the 211 compounds discussed here, 81 compounds were found novel. A total of 96 endophytic fungi yielded 211 compounds, out of which 41 fungi belonging to 26 genera of Ascomycetes yielded 93 compounds, 31 fungi belonging to 5 genera of Hyphomycetes yielded 54 compounds, 21 fungi belonging to 7 genera of Coelomycetes yielded 56 compounds, 2 fungi belonging to 2 genera of Basidiomycetes yielded 6 compounds, and 1 unidentified fungus yielded 2 compounds (Fig. 1). A genera-wise pictorial representation of total compounds along with novel compounds identified from endophytic fungi during the mentioned period has been presented in Fig. 2. Novel anticancer compounds reported from endophytic

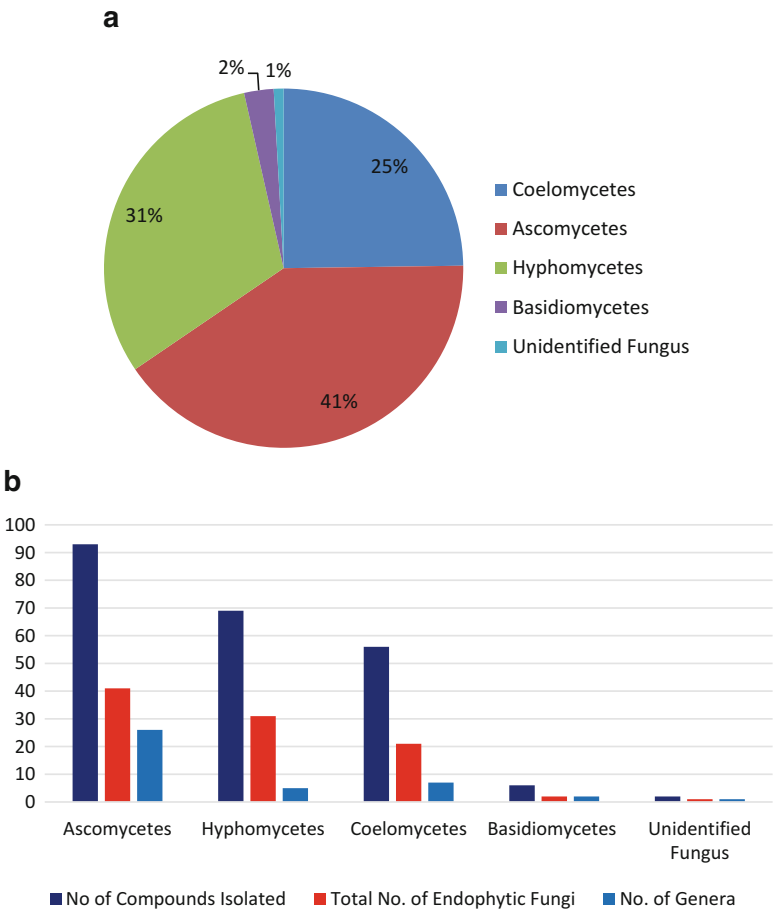

Fig. 1 (**a**) Percentage of anticancer compounds reported from different classes of endophytic fungi. (**b**) Comparative data of anticancer compounds reported from different class of endophytic fungi

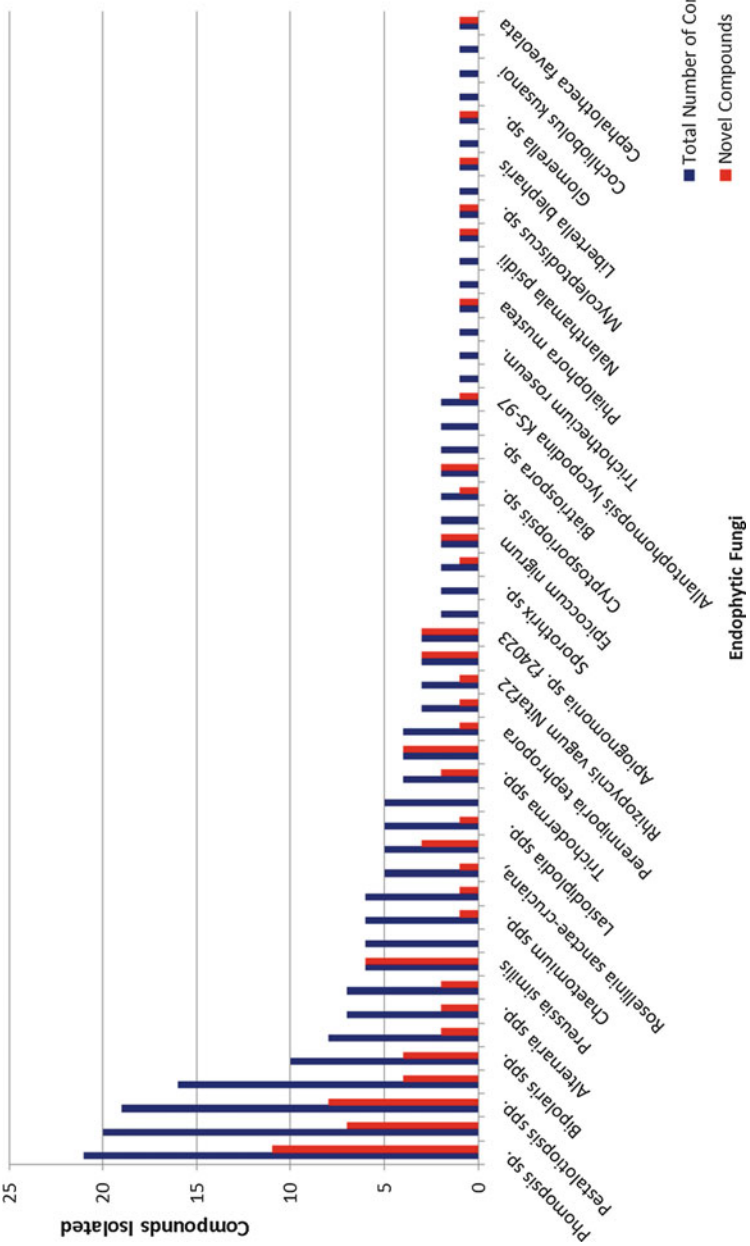

Fig. 2 Novel anticancer bioactive compounds reported from endophytic fungi

fungi are given in Table 1. The compounds that have been listed in this review are based on their source of origin. Mode of action of the isolated anticancer compounds has been described wherever possible.

2 Bioactive Metabolites from Endophytes

2.1 Compounds Produced by Coelomycetes

Four new diphenyl ether derivatives, sinopestalotiollides A–D **(1–4)** (Fig. 3), and a new natural α-pyrone **(5)** (Fig. 3) were isolated from an endophytic fungus *Pestalotiopsis palmarum* found in the leaves of medicinal plant *Sinomenium acutum* which was collected in Qinling Mountains, Shaanxi Province, China. Compound **(4)** exhibited potent cytotoxic activity against HeLa, HCT116, and A549 cell lines with IC_{50} values of 1.19, 2.66, and 2.14 μM, respectively, while the positive control doxorubicin displayed cytotoxic activity against HeLa, HCT116, and A549 cell lines with IC_{50} values of 8.96, 2.38, and 0.86 μM, respectively [26]. Compounds **(1–3, 5)** showed moderate cytotoxic activity toward HeLa, HCT116, and A549 cell lines with IC_{50} in the range of 12.8 to 47.82 μM.

Kaempferol **(6)**, quercetin **(7)**, rutin **(8)**, and genistein **(9)** (Fig. 3) were obtained from *Pestalotiopsis uvicola*, an endophytic fungus residing inside the traditional Chinese medicinal plant *Artemisia japonica* collected from Guizhou Province, China. Compounds **(6–9)** had different activities to reverse MDR in human breast adriamycin-resistant cell MCF-7/ADR and ovarian paclitaxel-resistant cell A2780/Taxol in vitro. Kaempferol **(6)** exhibited the strongest reversal activity at 40 μM concentration against A2780/Taxol (5.04-fold). Against the tumor cell line MCF-7/ADR, quercetin **(7)** showed the highest reversal (3.52-fold), followed by kaempferol **(6)** (2.71-fold) [27].

Pestallic acid E **(10)** and (+)-ambuic acid **(11)** (Fig. 3) were isolated from *Pestalotiopsis* sp. FT172 isolated from the leaves of *Myrsine sandwicensis* collected from Mokuleia Forest Reserve on the Oahu Island, Hawaii. Compounds **(10)** and **(11)** exhibited potent cytotoxicity against A2780 and cisplatin-resistant A2780 (A2780CisR) cell lines with IC_{50} values in the range of 3.3 to 17.0 μM [28].

A new compound (10S)-12,16-epoxy-17(15 → 16)-abeo-3,5,8,12,15-abietapentaen-2,7,11,14-tetraone **(12)** and a known compound uncinatone **(13)** (Fig. 3) were isolated from an endophytic fungus *Pestalotiopsis adusta*, residing inside *Clerodendrum canescens*, collected from Zhejiang Province, China. Compounds **(12)** and **(13)** demonstrated cytotoxicity against HL-60 tumor cell line with IC_{50} values of 12.54 and 15.66μM, respectively, comparable to with those observed for positive control cisplatin with IC_{50} value of 9.20 μM [29].

4-(3′,3′-Dimethylallyloxy)-5-methyl-6-methoxy-phthalide (DMMP) **(14)** (Fig. 3) was isolated from *Pestalotiopsis photiniae* associated with the branch of *Podocarpus macrophyllus* in Hainan, China [30]. DMMP **(14)** exhibited concentration-dependent antiproliferative activity with IC_{50} values of 36, 51, 81, and 147 μg/mL for HeLa, MDA-MB-231, MCF-7, and MRC5 cells, respectively. DMMP induced

Table 1 Novel anticancer bioactive compounds reported from endophytic fungi

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
Compounds produced by Coelomycetes							
1.	*Pestalotiopsis palmarum*	Leaves of *Sinomenium acutum*	Qinling Mountains, Shaanxi Province, China	Sinopestalotiollides A–C **(1–3)**, α-pyrone product **(5)**	HeLa, HCT116, and A549	IC_{50} in the range of 12.8 to 47.82 μM	[26]
				Compound **(4)**	HeLa, HCT116, and A549	IC_{50} of 1.19, 2.66, and 2.14 μM	
				Doxorubicin	HeLa, HCT116, and A549	IC_{50} of 8.96, 2.38, and 0.86 μM	
2.	*Pestalotiopsis uvicola*	*Artemisia japonica*	Guizhou Province, China	Kaempferol **(6)**, quercetin **(7)**, rutin **(8)**, genistein **(9)**	MDR in human breast adriamycin-resistant cell MCF-7/ADR and ovarian paclitaxel-resistant cell A2780/Taxol in vitro	Reversal activity	[27]
				Kaempferol **(6)**	2780/Taxol	Showed the reversal of 5.04-fold at 40 μM concentration	
				Quercetin **(7)**	MCF-7/ADR	Showed the reversal (3.52-fold)	
				Kaempferol **(6)**	MCF-7/ADR	Showed the reversal (2.71-fold)	
3.	*Pestalotiopsis* sp. FT172	Leaf of *Myrsine sandwicensis*	Mokuleia Forest reserve on the Oahu Island	Pestallic acid E **(10)** and (+)-ambuic acid **(11)**	A2780 and cisplatin-resistant A2780(A2780CisR) cell lines	IC_{50} values from 3.3 to 17.0 μM	[28]

4.	*Pestalotiopsis adusta*	*Clerodendrum canescens*	South Yandang, Zhejiang Province, China	(10S)-12,16-Epoxy-17(15 → 16)-abeo-3,5,8,12,15-abietapentaen-2,7,11,14-tetraone **(12)** and uncinatone **(13)**	HL-60 tumor cell line	IC 50 values of 12.54 and 15.66 μM Cisplatin IC_{50} of 9.20 μM	[29]
5.	*Pestalotiopsis photiniae*	Branch of *Podocarpus macrophyllus*	Hainan, China	4-(3′,3′-Dimethylallyloxy)-5-methyl-6-methoxy-phthalide (DMMP) **(14)**	HeLa, MDA-MB-231, MCF-7, and MRC5 cells	IC_{50} value of 36, 51, 81, and 147 μg/mL	[30, 31]
6.	*Pestalotiopsis fici*	Branches of *Camellia sinensis*	Hangzhou, China	Siccayne **(15)**	HeLa and HT29	IC_{50} values of 48.2 and 33.9 μM	[32]
				5-fluorouracil	HeLa and HT29	IC_{50} of 8.0 and 12.0 μM,	
7.	*Pestalotiopsis photiniae* (L461)	*Roystonea regia*	Hainan Province, China	Photipyrone B **(16)**	MDA-MB-231	Inhibitory rate at 25.0% and 23.0%, at 10 μg/mL	[33]
8.	*Pestalotiopsis karstenii*	Stems of *Camellia sasanqua*		Pestalrone B **(17)**	HeLa, HepG2 and U-251	IC_{50} values of 12.6, 31.7, and 5.4 μg/mL	[34]
				Pestalotin **(18)** and hydroxypestalotin **(19)**	U-251	IC_{50} values of 2.5 and 12.0 μg/mL	
9.	*Phoma* sp. YN02-P-3	Leaf of *Sumbaviopsis*	Yunnan Province, China	Phomones D–E **(20, 21)** and Rosellisin diacetate **(22)**	HL-60, PC-3, and HCT-116 cell lines	IC_{50} values of 0.52–9.85 μM	[35]
				Phomeketale C **(23)**	HL-60, Molm 13, and PC-3 cell line	IC_{50} value of 12.39, 37.81, and 48.40 μM	[36]
10.	*Phoma* species ZJWCF006	*Arisaema erubescens*	Zhejiang Province, China	Cercosporamide **(24)**	HT-29, SMMC-772, MCF-7, HL-60, MGC80–3, and P388 cell lines	IC_{50} values of 9.3 to 48.79 μM	[37]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
11.	*Phomopsis* sp.	Rhizome of *Paris daliensis*	Yunnan Province, China	Dalienxanthone C **(27)**, 3,8-dihydroxy-4-(2,3-dihydroxy-1-hydroxymethylpropyl)-1-methOxyxanthone **(28)**	SHSY5Y cells	IC_{50} values of 3.8 and 3.5 μM	[38]
				Dalienxanthones A–B **(25, 26)**, oliganthin E **(29)**, and cratoxylumxanthone D **(30)**	NB4, A549, SHSY5Y, PC3, and MCF-7 cell lines	IC_{50} values between 4.6 and 9.2 μM	
12.	*Phomopsis* sp. shj2	*Isodon eriocalyx* var. *laxiflora*	Kunming, China	Phomopchalasin C **(32)**	HL-60, SMMC-7721, and A-549 cell lines	IC_{50} values of 14.9, 22.7, and 21.1 μM	[39]
				Cisplatin	HL-60, SMMC-7721, and A-549 cell lines	IC_{50} values of 1.1, 4.6, and 4.7 μM	
				Phomopchalasin B **(31)** and phomopchalasin C **(32)**	Anti-migratory effect against MDA-MB-231	IC_{50} values of 19.1 and 12.7 μM	
				Cytochalasin D	Anti-migratory effect against MDA-MB-231	IC_{50} values of 0.2 μM	
13.	*Phomopsis* sp. BCC 45011	Leaf, *Xylocarpus granatum*	Nakhon Si Thammarat Province, Thailand	Mycoepoxydiene (**33**), deacetylmycoepoxydiene (**34**), phomoxydiene A (**36**) and C (**37**), and cytosporone E (**38**)	KB, MCF-7, NCI-H187, and Vero cells	IC_{50} values in the range of 1.49–40.17 μg/mL	[40]
				(−)-1893A **(35)**	NCI-H187 and Vero cells	IC_{50} of 45.5 and 16.93 μg/mL	[40]
				Mycoepoxydiene **(33)**	HepG2, A549, HCC-S102, HuCCA-1, KB,	IC_{50} value ranging from 0.27 to 2.80 μm/mL	[41]

					HeLa, MDA-MB-231, T47D, HL-60, and P388 cell lines		
				Deacetylmycoepoxydiene **(34)**	HepG2, A549, and HCC-S102 cell lines	IC_{50} value ranging from 1.05 to 1.95 μm/mL	[41]
14.	*Phomopsis glabrae*	Leaves of *Pongamia pinnata*	Karnala, Raigarh, India	Depsipeptide (**PM181110**) **(39)**	40 human cancer cell lines	IC_{50} value of 0.089 μM	[42]
					Ex vivo efficacy toward 24 human tumor xenografts	Mean $IC_{50} = 0.245$ μM	
15.	*Phomopsis amygdali*	Rhizome of *Paris axialis*	Shizhong, Yunnan, China	3-Methoxy-1,4,8-trihydroxy-5-(1′,3′,4′-trihydroxybutan-2′-yl)-xanthone **(40)**	A549	IC_{50} of 3.6 μM	[43]
				8-Methoxy-1,3,4-trihydroxy-5-(1′,3′,4′-trihydroxybutan-2′-yl)-xanthone **(41)**	SHSY5Y cells	IC_{50} of 4.2 μM	
				Dihydrosterigmatocystin **(42)**, secosterigmatocystin **(43)**, and vieillardixanthone **(44)**	NB4, A549, SHSY5Y, PC3, and MCF-7	IC_{50} values between 5.4 and 8.8 μM	
16.	*Phomopsis chimonanthi*	*Tamarix chinensis*	Yellow River Delta, Dongying, China	3S,22R,26-Trihydroxy-8,24E-euphadien-11-one **(45)**	A549, MDA-MB-231, and PANC-1 cell	IC_{50} values of 20.32, 19.87, and 30.45 μM (5-FU, IC_{50} of 0.47, 0.12, and 0.67 μM)	[44]
17.	*Allantophomopsis lycopodina* KS-97	Tree branch	Gassan stock farm in Yamagata, Japan	Allantopyrone A **(46)** Islandic acid-II methyl ester **(47)**	HL-60 cells	IC_{50} values of 0.32 and 6.55 μM	[45]
				Allantopyrone A **(46)**		Inhibits the NF-κB signaling pathway at	[46]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
						a step upstream of IκBα phosphorylation	
				Allantopyrone A **(46)**		Activates Keap1-Nrf2 pathway and protects PC12 cells from oxidative stress-induced cell death	[47]
18.	*Rhizopycnis vagum* Nitaf22	*Nicotiana tabacum*	China agricultural university	Rhizopycnin C **(48)**	A549 and HCT116 cell lines	IC_{50} values of 25.5 and 37.3 μM	[48]
				TMC-264 **(49)**	HCT-116, HepG2, BGC-823, NCI-H1650, and A2780	IC_{50} values of 4.2, 5.9, 7.8, 3.2, and 3.6 μM	
				Alternariol 9-methyl ether **(50)**	A549 cells	$IC_{50} = 70.4$ μM	
19.	*Diaporthe pseudomangiferae*	Leaves of *Sabicea cinerea*	Roura, French Guiana	Mycoepoxydiene **(51)**	KB, DA-MB-435, and MRC5 cell lines	IC_{50} values of 7.5, 17.7, and 15.8 μM	[49]
				Eremofortin F **(52)**	KB and MRC5 cell lines	IC_{50} value of 13.9 and 12.2 μM	
20.	*Diaporthe* sp.	*Taxus baccata*	Bhaderwah, Doda district, India	Trichalasin E **(53)**, F **(54)**, and H **(55)**	MCF-7 and HeLa cancer cell lines	IC_{50} values of 1058 and 1257 μg/mL	[50]
21.	*Libertella blepharis*	Leaf of *Olyra latifolia*	Province of colon, Republic of Panama	3-epi-Waol A **(56)**	MCF-7, HCT116, and H460 cell lines	IC_{50} values of 22.46, 6.20, and 1.0 μM	[51]

Compounds produced by ascomycetes							
22.	*Xylaria psidii*	Leaf of *Aegle marmelos*		Xylarione A **(57)** and (−) 5-methylmellein **(58)**	MCF-7, MIA-pa-ca-2, NCI-H226, HepG2, DU145 cell line	IC_{50} in the range of 16–37 μM	[52]
				Compounds **(57)** and **(58)**	fR2 (normal cell)	IC_{50} values of 79 and 76 μM	
				Compounds **(57)** and **(58)**	MIA-pa-ca-2 cells	IC_{50} values of 16.0 and 19.0 μM	
				Compounds **(57)** and **(58)**	MIA-pa-ca-2 cells	Cell cycle arrest at the sub-G1 phase	
23.	*Xylaria* sp. ZJWCF255	Leaf of *Ficus carica*		Cytochalasin Q **(59)**	SMMC-772, MCF-7, MGC80–3 cell lines	IC_{50} values of 17.24, 7.75, and 10.30 μg/mL	[53]
24.	*Chaetomium* sp. M336	*Huperzia serrata*	China	6-Formamide-chetomin **(60)**	HeLa, SGC-7901, A549	IC_{50} of 21.6, 23.0, and 27.1 nM	[54]
25.	*Cochliobolus kusanoi*	*Nerium oleander*		Oosporein **(61)**	A549 cells	IC_{50} of 21 mM	[55]
					A549 cells	IC_{50} 28.66 mM	[56]
26.	*Chaetomium* sp.	Leaves of *Sapium ellipticum*	Cameroon	SB238569 **(62)**	L5178Y cell line	IC_{50} value of 1 μM	[57]
27.	*Chaetomium globosum*	*Ginkgo biloba*	Linyi, Shandong Province, China	Chaetoglobosin A **(63)**, chaetoglobosin Fex **(64)**, 20-dihydrochaetoglobosin A **(65)**, chaetoglobosin fa **(66)**	HCT116 cells	IC_{50} values of 3.15,4.43, 8.44, and 5.85 μM (etoposide, IC_{50} = 2.13 μM)	[58]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
28.	*Talaromyces* sp.	Twigs of *Cedrus deodara*	Lolab Valley in the Western Himalayas of Kashmir, India	(−)-Ramulosin **(67)**	A-549, HEP-1, THP-1, PC-3, and HCT-116 cells	15, 23, 54, 23, and 44% cytotoxicity at 50 μM	[59]
				(3S,4aR,7S)-7,8-Dihydroxy-3-methyl-3,4,10,5,6,7–hexahydro -1H -isochromen-1-one **(68)**	A-549, HEP-1, THP-1, PC-3, and HCT-116 cells	35, 3, 40, 34, and 35% cytotoxicity at 50 μM	
				(−)-Epoformin **(69)**	A-549, HEP-1, THP-1, PC-3, and HCT-116 cells	98, 100, 50, 22, and 56% cytotoxicity at 50 μM	
				(1S*,3R*,5R*)-3-methyl −2-oxabicyclo[3.3.1]nonan-7-one **(70)**	A-549, HEP-1, PC-3, and HCT-116 cells	71, 26, 23, and 59% cytotoxicity at 50 μM	
				Paclitaxel	A-549, THP-1, HCT-116 cells	82, 71, and 72% cytotoxicity at 1 μM concentration	
				Fluorouracil	A-549, THP-1, and HCT-116 cells	22, 84, and 55% cytotoxicity at 20 μM concentration	
				Compounds **(65–68)**	HL-60 cells	Induce apoptosis and microtubule inhibition	
29.	*Talaromyces* sp. LGT-2,	*Tripterygium wilfordii*		3-Dehydroxymethylbisdethio-3, 10a-bis(methylthio) gliotoxin **(71)**, bisdethiobis(methylthio)gliotoxin **(72)**, didehydrobisdethiobis (methylthio)gliotoxin **(73)**	B16 cancer cell line	86, 82, and 78%, cytotoxicity at 500 μg/mL concentration	[60]

30.	*Dothiora* sp.	*Launaea arborescens*	Tabernas Desert (Almeria, Spain)	Hormonemate A **(74)**	HepG2 and MCF-7 cell line	IC_{50} values of 20.9 and 11.3 μg/mL	[61]
				Hormonemate B **(75)**	HepG2, MCF-7 cell line	IC_{50} 19.9 and 7.7 μg/mL	
				Hormonemate C **(76)**	MCF-7 cell lines	IC_{50} value of 20.9 μg/mL	
				Hormonemate D **(77)**	HepG2 and MCF-7 cell lines	IC_{50} value of 27.3 and 13.8 μg/mL	
				Hormonemate **(78)**	MCF-7 cell lines	IC_{50} value of 18.2 μg/mL	
				Hormonemate E **(79)**	HepG2, MCF-7, and MiaPaca_2	IC_{50} values of 18.7, 13.9, and 27.4 μg/mL	
				Doxorubicin	HepG2, MCF-7, and MiaPaca_2 cell line	IC_{50} values of 0.1, 0.8, and 1.0 μg/mL	
31.	*Cephalotheca faveolata*	Petiole of *Eugenia jambolana*	Mumbai, India	Sclerotiorin **(80)**	HCT-116, H460, ACHN, Panc-1, and Calu-1 cell lines	IC_{50} value of 0.63, 1.6 1.2, 1.6, and 2.1 μM	[62]
					MCF10A	$IC_{50} > 10$ μM	
					HCT116 cells	Induced apoptosis via the triggering of BAX and downregulation of Bcl-2 that results in stimulation of cleaved caspase-3 thereby causing the death of cancerous cells	

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
32.	*Cryptosporiopsis* sp.	*Clidemia hirta*		(R)-5-Hydroxy-2-methylchroman-4-one **(81)**	HL-60	IC_{50} of 4 μg/mL, induces G2 arrest	[63]
33.					K-562	IC_{50} value of 8 μg/mL	[64]
					HL-60 cells	Induces caspase-dependent apoptosis in cells and targets STAT-3 signaling cascade. HMC-mediated p-STAT-3 inhibition involves ubiquitin-dependent pathway	
34.	*Chaetoconis* sp. FT087.	Leaf of *Osmoxylon novoguineensis*	Waimea Valley on the Oahu Island, Hawaii	Dendryphiellin A1 **(82)**	A2780 cisplatin-resistant A2780CisR	IC_{50} values of 6.6 and 9.1 μg/mL	[65]
35.	*Bipolaris setariae*	*Parthenium hysterophorus*	Mumbai India	Ophiobolin A **(83)**	A2780, PC3, MDA-MB-231, MCF-7, MM1R, RPMI8226, U266B1 68, and Jurkat cells	IC_{50} of 0.4–4.3 μM	[66]
					hPBMC (normal cells)	IC_{50} of 20.9 μM	
						Inhibits multiple oncogenic signaling pathways, namely,	[66]

						PI3K/mTOR, Ras/Raf/ERK, and CDK/RB	
36.	*Rosellinia sanctae-cruciana*	Leaves of *Albizia lebbeck*		Jammosporin A **(84)**, 19,20-epoxycytochalasin D **(85)**, cytochalasin D **(86)**, 19,20-epoxycytochalasin C **(87)**, cytochalasin C **(88)**	MOLT-4	IC_{50} of 20·0, 10·0, 25, 8·0, and 6·0 μM	[67]
37.	*Nectria pseudotrichia* 120-1NP	*Gliricidia sepium*		Nectrianolins A **(89)** and B **(90)**, nectrianolin C **(91)**	HL-60 cell line	IC_{50} values of 1.7, 1.5, and 10.1 μM	[68]
					HeLa	IC_{50} values of 34.7, 16.6, and 52.1 μM	
38.	*Cryptosporiopsis* sp. H2–1 (NFCCI 2856)	*Clidemia hirta*	Hawaii	4-epi-ethisolide **(92)**	HL-60	IC_{50} values of 11 μM	[69]
39.	*Preussia similis*	*Globularia alypum*	Batna, Algeria	Preussilides A–F **(93–98)**	L929, KB3.1, A431, A549, SKOV-3 PC-3, MCF-7, and U2OS cell lines	IC_{50} values ranging from 2.5 to 80.0 μM	[70]
				Preussilides A **(93)** and C **(95)**	L929 and HeLa KB.3.1 and U2OS cells	IC_{50} values below 10 μM	
				Preussilide C **(95)**	MCF-7	IC_{50} values below 10 μM	
40.	*Periconia* sp. F-31	*Annona muricata*	Hainan Province, China	Periconiasin A **(99)**	HCT-8 and BGC-823 cell lines	IC_{50} values of 0.9 and 2.1 μM	[71]
				Periconiasin B **(100)**	HCT-8, Bel-7402, and BGC-823 cell lines	IC_{50} values of 0.8, 5.1, and 9.4 μM	

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Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
41.	*Periconia* sp. F-31	*Annona muricata*	Hainan Province, China	Periconiasin I **(101)**	MCF-7 tumor cell line	IC_{50} value of 4.8 μM	[72]
				Paclitaxel	MCF-7 tumor cell line	IC_{50} value of 0.2 nM	
42.	*Periconia* sp. F-31			Periconone E **(102)**	MCF-7 tumor cell line	IC_{50} value of 4.2 μM	[73]
43.	*Berkleasmium* sp.,	Rhizomes of *Dioscorea zingiberensis*	Hubei Province, China	Diepoxin δ **(103)** and palmarumycin C8 **(104)**	HCT-8, Bel-7402, BGC-823, A549, A2780	IC_{50} values of 1.28–5.83 μM	[74]
44.	*Lasiodiplodia pseudotheobromae* XSZ-3	*Camptotheca acuminata*	Panzhihua, Sichuan Province, China	Palmarumycin LP1 **(105)**, cladospirone B **(106)**, and scheme 50676 **(107)**	HL-60 cells	IC_{50} values of 2.39, 10.91, and 1.41 μM	[75]
				5-fluorouracil	HL-60 cells	IC_{50} of 1.87 μM	
45.	*Acremonium camptosporum*	Leaves of *Bursera simaruba*	Mexico	Acremoxanthone E **(108)**, acremoxanthone C **(109)**, acremonidin A **(110)** and B **(111)**, acremoxanthone A **(112)** and B **(113)**	U251 PC-3 K562 HCT-15 MCF-7 SKLU-1 cell line	IC_{50} in the range of 3 to 16 μM	[76]
46.	*Epicoccum nigrum*	Leaves of *Mentha suaveolens*	Morocco	Epicocconigrone A **(114)** and epicoccolide B **(115)**	Inhibition of at least 15 protein kinases with IC_{50} values ranging from 0.07 to 9.00 μM exerts mainly cytostatic effects in human	Inhibited HDAC activities with IC_{50} values of 9.8 and 14.2 μM	[77]

					lymphoma RAJI and U-937 cell lines		
47.	*Phialophora mustea*	*Crocus sativus*		Phialomustin B **(116)**	T47D,	IC_{50} of 1 μM	[78]
48.	*Nigrospora oryzae*	*Combretum dolichopetalum*	Nsukka region of eastern Nigeria	3,3′,4-tri-O-methylellagic acid **(117)**, 4-dehydroxyaltersolanol A **(118)**	L5178Y	IC_{50} values of 9.4 and 29.0 μM	[79]
49.	*Glomerella* sp. F00244	*Pinus massoniana*	Xiamen botanical garden, Fujian Province, China	Glometenoid A **(119)**	HeLa cell	21% growth inhibition at a concentration of 10 μM	[80]
50.	*Paraconiothynium brasiliense*	Branches of *Acer truncatum*	Bunge on Dongling Mountain, Beijing, China	Brasilamide E **(120)**	MCF-7, MGC	IC_{50} values of 8.4 and 14.7 μM	[81]
51.	*Biatriospora* sp. CCF 4378	*Ulmus laevis*	Libicky Luh Forest near Velky Osek, Czech Republic	6-Deoxyfusarubin **(121)** Ascomycone B **(122)**	HeLa cells	Dramatic changes of the cellular content and cell death	[82]
52.	*Trichothecium roseum*			Rosoloactone **(123)**	Reduced the survival rate of HeLa cells	IC_{50} value of ~ 8 μg/mL	[83]
53.	*Trichothecium* sp.	*Phyllanthus amarus*	Pune India	Trichothecinol A **(124)**	HeLa and B16F10 cells	50% cell death at 500 nM concentration	[84]
					B16F10	Induces apoptosis	
					MDA-MB-231 cells	Inhibits wound migration by 50% at 500 nM	

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
54.	*Stemphylium globuliferum*	*Mentha pulegium*	Morocco	Altersolanol A **(125)**	Induces cell death by apoptosis through the cleavage of caspase-3 and caspase-9 and through the decrease of anti-apoptotic protein expression		[85]
55.	*Mycoleptodiscus* sp.	*Desmotes incomparabilis*	Panama	Mycoleptodiscin B **(126)**	H460, A2058, H522-T1, PC-3, and IMR-90 cell line	IC_{50} in the range of 0.60–0.78 μM	[86]
56.	*Microsphaeropsis arundinis*	Stems of *Ulmus macrocarpa*	Dongling Mountain, Beijing, China	Arundinone B **(127)**	T24 and A549 cells	IC_{50} values of 35.4 and 81.6 μM	[87]
				Cisplatin	T24 and A549 cells	IC_{50} value of 3.72 and 8.45 μM	
57.	*Microsphaeropsis arundinis*	Stems of *Ulmus macrocarpa*	Dongling Mountain, Beijing, China	Arundinone B **(127)**	T24 and A549 cells	IC_{50} values of 35.4 and 81.6 μM, respectively (cisplatin, IC_{50} values of 3.72 and 8.45 μM)	[88]
58.	*Bipolaris sorokiniana* A606	*Pogostemon cablin*	Gaoyao, Guangdong Province, China	Cochlioquinone H **(131)**, cochlioquinone C **(133)**, cochlioquinone D **(134)**,	SF-268, MCF-7, NCI-H460, HepG2	IC_{50} in the range of 1.2 to 42.8 μM	[89]

				cochlioquinone E **(135)**, cochlioquinone B **(136)**			
				Cochlioquinone D **(132)**	SF-268, MCF-7 and HepG2 cell lines	IC_{50} values of 1.5, 2.4, and 1.2 μM	
				Isocochlioquinone D **(128)** Isocochlioquinone E **(129)** Cochlioquinone G **(130)** Isocochlioquinone C **(132)**	SF-268, MCF-7, NCI-H460, and HepG2	IC_{50} values of 11.3 to 50.6 μM	
				Cisplatin	SF-268, MCF-7, NCI-H460, and HepG2	IC_{50} value of 4.1, 2.9, 2.9, and 2.5 μM	
59.	*Dothideomycete* sp. CRI7	*Tiliacora triandra*	Nakhon Sawan Province, Thailand	Calbistrin F **(137)**	MOLT-3 cell line	IC_{50} values of 37.3 μg/mL	[90]
				Dothideomynone C **(138)**	HuCCA-1, A549, and MOLT-3 cell lines	IC_{50} values of 48.1, 46.5, and 17.4 μg/mL	
60.	*Myrothecium roridum*	*Ajuga decumbens*		Myrotheciumone A**(139)**	HepG2, SMMC-7721, A549, and MCF-7 cells and QSG-7701 and HL-7702 cell lines	IC_{50} value of 5.36, 6.56, 5.88, 7.56, 16.30, and 20.69 μM	[91]
						Promotes cytochrome c release from mitochondria and had cytotoxicity by inducing apoptosis in cancer cell lines	
61.	*Hypocrea lixii*	*Cajanus cajan*		Cajanol **(140)**	A549 and MC3T3-E1 cells and RAW264.7	IC_{50} value of 20.5, 48.7, and 40.2 μg/mL	[92]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
62.	*Gibberella moniliformis*	Leaves of *Coix lacryma-jobi* L. var. *ma-yuen*	Zhejiang Province in China	Triolein (trioleoylglycerol) **(141)**, ethyl acetate extract of *G. moniliformis* AH13	A549, HCT116, MDA-MB-231, and SW1990 cell lines	IC_{50} value of 42.28, 5.47, 7.86, and 12.19 μg/mL	[93]
	Nalanthamala psidii			Trichodermin **(142)**	MIA PaCa-2, BxPC-3, HPAC, KYSE −170, KYSE-510, MCF-7, MDA-MB-453, FTC-133, FTC-236, CL1–0, CL1–5, PC-3, and 22Rv1	IC_{50} value of 0.8, 1.2, 1.4, 2.3, 2.7, 3.8, 3.6, 3.6, 3.5, 2.8, 3.1, 3.3, and 2.5 μM	[94]
63.	*Apiognomonia* sp. f24023		Iwata, Shizuoka prefecture, Japan	MBJ-0011 **(143)**, MBJ-0012 **(144)**, MBJ-0013 **(145)**	SKOV-3 cells	IC_{50} value of 3.4, 63, and 54 μM	[96]
64.	*Sporothrix* spp. 4335 99KK29FL1	*Costus speciosus*	Kuala Keniam, National Park, Pahang, Malaysia	Trichodermol **(146)** and 7-epi-brefeldin A **(147)**	MCF-7	IC_{50} values of 0.83 and 0.35 μM Tamoxifen (IC_{50} = 0.11 μM)	
					WRL68	IC_{50} of 2.93 and 0.05 μM	
65.	*Lasiodiplodia theobromae* strain xsd08007	*Dendropanax laurifolius*	Kuala Keniam, National Park, Pahang, Malaysia	(3R,4S)-4-Hydroxymellein **(148)** and desmethyl-lasiodiplodin **(149)**	MCF-7 cells	IC_{50} value of 7.53 and 23.95 μM	[97]
					WRL68 cells	IC_{50} value of 175.61 and 159.67 μM	
				Compound **(149)**	MCF-7	Induces apoptosis	

	Compounds produced by Hyphomycetes						
66.	*Aspergillus terreus* PR-P-2	*Camellia sinensis* var. *assamica*	Yunnan, China	Butyrolactone I **(150)** and aspernolide A **(151)**	HL-60 cell line	IC_{50} values of 18.85 and 39.36 μM (5-FU IC_{50} of was 2.80 μM),	[98]
67.	*Aspergillus versicolor*	Rhizome of *Paris marmorata*	Dali, Yunnan, China	Versicoumarin D **(152)**	A549 and MCF-7 cell	IC_{50} of 5.8 and 8.0 μM	[99]
68.	*Aspergillus terreus* JAS-2	*Achyranthus aspera*	Varanasi, India	4,5-Dihydroxy-3-(1-propenyl)-2-cyclopenten-1-one **(153)**	A-549 cell lines	IC_{50} value of 121.9 μg/mL. At 150 μg/ml of compound maximum cells were found in sub G1 phase which represents apoptotic dead cells	[100]
69.	*Aspergillus fumigatus*,	Rhizomes of *Diphylleia sinensis*	Honghegu, Shanxi Province, China	Fumitremorgin D **(154)**, 4,8,10,14-tetramethyl-6-acetoxy-14-[16-acetoxy-19-(20,21-dimethyl)-18-ene]-phenanthrene-1-ene-3,7-dione **(155)**, fumitremorgin C **(156)**, 12,13-dihydroxyfumitremorgin C **(157)**, verruculogen **(158)**, 13-oxoverruculogen **(159)**	HepG2	IC_{50} values of 47.5, 139.9, 156.5, 4.5, 9.8, and 44.9 μM	[101]
70.	*Aspergillus glaucus*	Leaves of *Ipomoea batatas*		2,14-Dihydrox-7-drimen-12,11-olide **(160)**	MCF-7 cells, HepG2 cell	IC_{50} of 41.7, 61 μg/mL	[102]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
71.	*Aspergillus* sp. (strain no. YXf3)	*Ginkgo biloba*	Nanjing University, Nanjing, China	Sphaeropsidin A **(161)**	KB, SGC-7901, SW1116, and A549 cell lines	IC_{50} value of 9.03, 10.68, 7.02, and 6.74 μM	[103]
				4″-Dehydro-3-hydroxyterphenyllin **(162)**, 3-hydroxyterphenyllin **(163)**, 4″-deoxycandidusin A **(164)**	KB, SGC-7901, SW1116, and A549 cell lines	IC_{50} values ranging from 17.28 to 46.64 μM	
72.	*Aspergillus* sp.	Seeds of *Gloriosa superba*	Tirupati, India	6-Methyl-1,2,3-trihydroxy-7,8-cyclohepta-9,12-diene11-one-5,6,7,8-tetralene-7-acetamide **(165)**	A-549, HEP-2, MCF-7, CV-1, OVCAR-5 cell line	23, 70, 35, 43, and 80% growth inhibition at 100 μg/mL concentration	[104]
73.	*Penicillium decumbens* CP-4	Bark of *Cephalotaxus mannii*	Xishuangbanna in the Yunnan Province of China	Peniproline A **(166)**	Bel-7402 and HeLa cell lines	IC_{50} values of 8.1 and 15.5 μM	[105]
74.	*Penicillium brefeldianum*	Rhizome of *Pinellia ternata*	Nanjing, Jiangsu Province, China	Spirotryprostatin F **(167)**	HepG2 and MDA-MB-231 cells	IC_{50} values of 14.1 μM and 35.5 μM	[106]
				N-Demethylmelearoride A **(168)**	HepG2 cells	IC_{50} values of 36.6 μM	
				Cisplatin	MDA-MB-231 and HepG24 cells	IC_{50} values of 11.3 and 14.4 μM	
				Doxorubicin	MDA-MB-231 and HepG24 cells	IC_{50} values of 1.0 and 3.0 μM	
75.	*Penicillium pinophilum* MRCJ-326	*Allium schoenoprasum*	Kashmir, India	Dicatenarin **(169)** Skyrin **(170)**	MIA PaCa-2 cell line	IC_{50} values of 12 and 27 μg/mL	[107]

76.						Induce reactive oxygen species-mediated mitochondrial permeability transition and resulted in an increased induction of caspase-3 apoptotic proteins	
77.	*Penicillium* sp. HSZ-43	Leaves of *Tripterygium wilfordii*	Shanxi Province, China	Penifupyrone **(171)**	KB cells	IC_{50} value of 4.7 μM	[108]
78.	*Penicillium* sp.	Leaf of *Paris polyphylla*		Citrinin H1 (**172**), dehydroisopenicillide (**173**), penicillide (**174**), 5-hydroxy-2-pyridinemethanol (**175**)	HepG2 cell line	IC_{50} at 8.5, 12.5, 15.0, and 18.2 μg/mL	[109]
79.	*Penicillium* sp. SXH-65	Leave of *Tamarix chinensis*	Coast of Laizhou Bay in Dongying, China	Arisugacin B (**176**) Arisugacin F (**177**)	HeLa, HL-60, and K562 cell lines	IC_{50} values ranging from 24 to 60 μM	[110]
80.	*Penicillium melinii* Yuan-25	Roots of *Panax ginseng*	Changchun, Jilin Province, People's republic of China	Ginsenocin (**178**), penicillic acid (**179**)	MKN45, LOVO, A549, MDA-MB-435, HepG2, and HL-60 cells	IC_{50} values ranging from 0.49 to 7.46 μg/mL	[111]
	Penicillium janthinellum Yuan-27			Brefeldin A (**180**)		IC_{50} values <0.12 μg/mL	
81.	*Penicillium* sp.,	Leaves of *Garcinia nobilis*	Mount Etinde, southwest region, Cameroon	Penialidin A–C (**181, 182, 183**), citromycetin (**184**), p-hydroxyphenylglyoxalaldoxime (**185**), brefelfin A (**186**)	HeLa cells	IC_{50} values in the range of 0.88–9.21 μg/mL	[112]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
82.	*Trichoderma gamsii*	*Panax notoginseng*	China	Trichoderpyrone **(187)**	A549, HepG2, and HeLa cell lines	IC_{50} values of 16.9, 30.8, and 33.9 μM, etoposide (IC_{50} values 16.6, 16.1, and 15.0 μM)	[113]
83.	*Trichoderma gamsii*	*Panax notoginseng*		Aspochalasin D **(188)**	HeLa cells	IC_{50} value of 5.72 μM	[114]
84.	*Trichoderma* sp. 09	Root of *Myoporum bontioides*		Dichlorodiaportinol A **(189)**	MCF-7 and HepG2 cell lines	IC_{50} values of 17.8 and 39.6 μg/mL	[115]
85.	*Trichoderma gamsii*	*Panax notoginseng*		Aspochalasin J **(190)**	HeLa cells	IC_{50} value 27.8 μM	[116]
86.	*Alternaria alternata* KT380662	*Passiflora incarnata* L.	Tiruchirappalli, Tamil Nadu, India	Chrysin (5,7-dihydroxy flavone, ChR) **(191)**	HepG2 cells	Formation of condensed nuclei, membrane, blebbing, and apoptotic cell death against HepG2 cells	[117]
87.	*Alternaria tenuissima* CH1307	*Cephalotaxus hainanensis*	Hainan Province China and local National Parks, Thailand	Homoharringtonine **(192)**, the extract of the fermented broth of CH1307	K562, NB4, and HL-60 cancer cell	IC_{50} values of 67.25, 65.02, and 99.23 μg/mL	[118]

88.	*Alternaria* species G7	Leaves of *Broussonetia papyrifera*	Nanjing, Jiangsu Province, China	3,4′,5′-Trihydroxy-5-methoxy-6H-benzo[c]chromen-6-one **(193)**	A549, MG-63, and SMMC-7721 cell lines	IC_{50} values of 1.47, 2.11, and 7.34 μg/mL	[119]
				Altersolanol A **(125)**	MG-63 and SMMC-7721	IC_{50} values of 0.53 and 2.92 μg/mL	
89.	*Alternaria phragmospora*	*Vinca rosea*, leaves	Cairo, Egypt	5-Butyl-6-(hydroxymethyl)-4-methoxy-2H-pyran-2-one **(194)**	HL-60 and K562 cells	IC_{50} of 2.2 and 4.5 μM	[120]
				4-Methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one **(195)**	HL-60 and K562 cells	IC_{50} values and 0.9 and 1.5 μM	
90.	*Alternaria* sp.	*Erythrina variegata*	Samutsakorn Province, Thailand	Altersolanol A **(125)**		Antiangiogenic activity by suppressing all functions of endothelial cells, proliferation, tube formation, and migration At low concentration inhibits blood vessel formation in both ex vivo and in vivo assays	[121]
91.	*Fusarium chlamydosporium*	Leaves of *Anvillea garcinii* (Burm. f.) DC.	Al-Azhar university, Egypt	Fusarithioamide A **(196)**	BT-549 and SKOV-3 cell lines	IC_{50} values of 0.4 and 0.8 μM, doxorubicin (IC_{50}	[122]

(*continued*)

Table 1 (continued)

Sr. no.	Fungus	Plant parts/host plant(s)	Locality of host plants	Compounds isolated	Cell line	IC_{50}/EC_{50}/inhibition	References
						0.046 and 0.313 μM)	
				8-Acetylneosolaniol **(197)**	KB and SKOV-3 cell lines	IC_{50} 1.68 and 1.40 mM	
				Ergosta-7,22-diene-3β,5α,6β-triol **(198)**	KB, BT-549, SK-MEL, and SKOV-3 cell lines	IC_{50} values of 1.7, 1.9, 1.4, and 1.1 mM	
92.	*Fusarium* sp. PDB51F5			(R)-3,4-Dihydro-4,8-dihydroxy-6-methoxy-4,5-dimethyl-3-methyleneisochromen-1-one **(199)**	KB and NCI-H137 cell lines	IC_{50} values of 160 and 162 μM	[123]
				4 8-O-Methyljavanicin **(200)**	MCF-7 cell lines	IC_{50} value of 148 μM	
				Doxorubicine	KB, MCF-7, and NCI-H137 cell lines	IC_{50} of 0.35, 2.33, and 0.14 μM	
93.	*Fusarium equiseti* (Salicorn 8)	*Salicornia bigelovii* Torr.	Salt lake in Xinjiang, China	Diglucotol **(201)**	MCF-7, MDA-MB-231, and Caco-2 cancer cells	EC_{50} values of 97.56, 92.35, and 99.39 μM	[124]
				Cerevisterol **(202)**		EC_{50} values of 32.4, 41.5, and 37.56 μM	
				Ergosterol peroxide **(203)**		EC_{50} values of 64.5, 52.4, and 77.56 μM	

Compounds produced by basidiomycetes							
94.	*Perenniporia tephropora* Z41	*Taxus chinensis* var. *mairei*	Jingning, Zhejiang Province, China	Ergosterol **(204)**	HeLa, SMMC-7721, and PANC-1 cells	IC_{50} values of 1.16, 11.63, and 11.80 μg/mL	[125]
				Perenniporin A **(205)**, Rel-(+)-(2aR,5R,5aR,8S,8aS,8bR)-decahydro-2,2,5,8-tetramethyl-2H-naphtho[1,8-bc]genfuran-5-ol **(206)**, and albicanol **(207)**		IC_{50} values ranging from 6 to 58 μg/mL	
95.	*Ceriporia lacerate*	*Huperzia serrata*	Zhejiang Province, China	Ceriponols F **(208)** and K **(209)**	HeLa, HepG2, and SGC 7901 cell lines	IC_{50} values ranging from 32.3 to 173.2 μM	[126]
Compounds produced by unidentified fungus							
96.	Fungal strain, 2 L	*Ocimum basilicum*	Dhaka	Secalonic acid A **(210)**, secalonic acid D **(211)**	BxPC-3 cell line	IC_{50} values of 7.3 and 1.6 μM	[127]

apoptosis mediated by loss of membrane potential of mitochondria and caused cell cycle arrest in G1 phase in HeLa cells. In addition, upregulation of p53 and p73 protein levels were identified. Further the compound showed to enhance expression of mRNA specifically Bcl-2 family genes (PUMA, NOXA, Bax, Bad, and Bim). Reduction in mRNA levels of HPV E6–E7 was noticed [31].

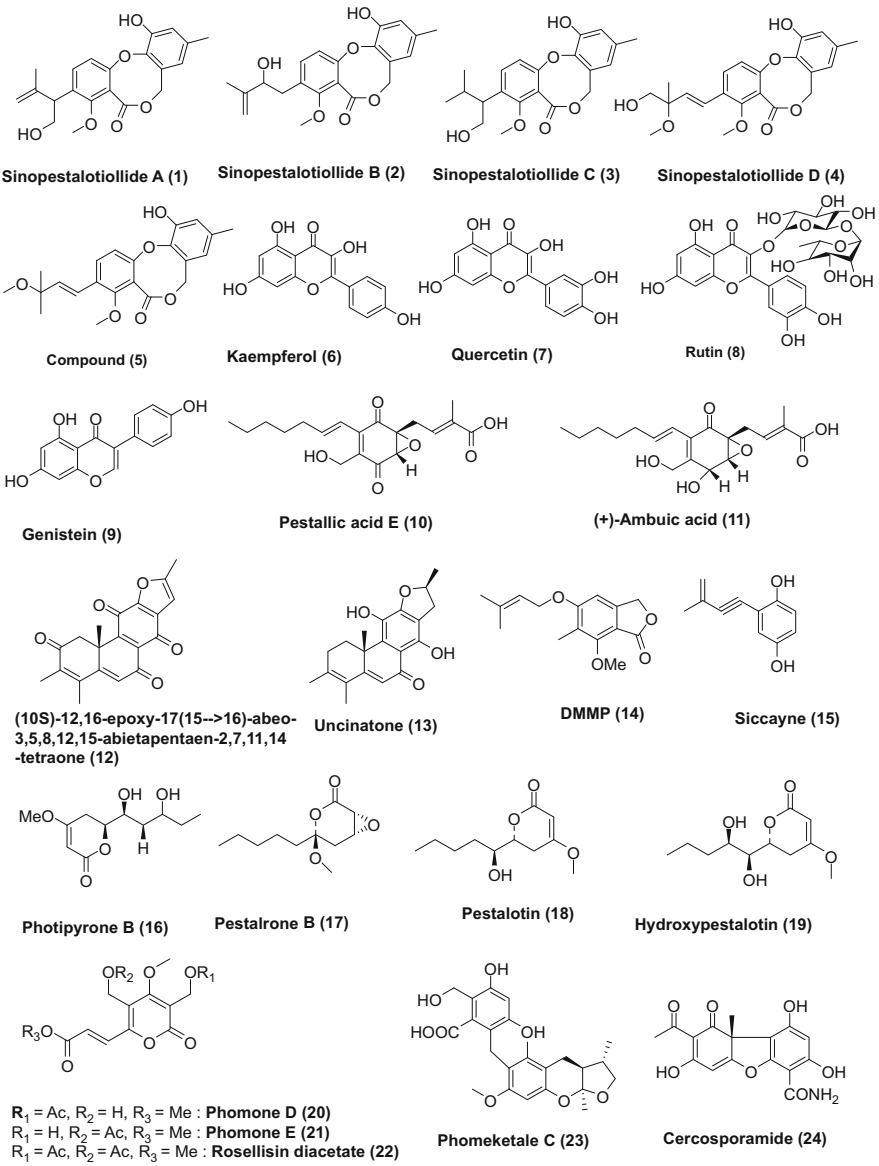


Fig. 3 Structures of metabolites isolated from Coelomycetes (**1–24**)

One known benzofuran derivative, siccayne **(15)** (Fig. 3), was extracted from an endophytic fungus *Pestalotiopsis fici* residing inside the *Camellia sinensis* collected from Hangzhou, China. Compound **(15)** exhibited cytotoxic activity with IC_{50} values of 48.2 and 33.9 μM, respectively, against HeLa and HT29 cell lines, while positive control 5-fluorouracil showed the cytotoxicity with IC_{50} values of 8.0 and 12.0 μM, respectively, against these cell lines [32].

Using one strain many compounds (OSMAC) approach, a new δ-lactone derivative named photipyrone B **(16)** (Fig. 3) was extracted from *Pestalotiopsis photiniae* (L461), an endophyte associated with *Roystonea regia* collected from Hainan Province, China. Compound **(16)** showed inhibitory rate at 25.0% and 23.0%, respectively, on the growth of MDA-MB-231 cell lines at 10 μg/ml [33].

Pestalotiopsis karstenii, an endophytic fungus residing inside the stems of *Camellia sasanqua*, was the source of new oxysporone derivative, pestalrone B **(17)**, and two known structurally related compounds pestalotin **(18)** and hydroxypestalotin **(19)** (Fig. 3). Compound **(17)** exhibited potent cytotoxic activity with IC_{50} values of 12.6, 31.7, and 5.4 μg/mL, respectively, against HeLa, HepG2, and U-251 cell lines. Compounds **(18)** and **(19)** exhibited potent cytotoxicity against U-251 cell lines with IC_{50} values of 2.5 and 12.0 μg/mL, respectively [34].

Two new α-pyrone derivatives, phomones D–E **(20, 21)** and rosellisin diacetate **(22)** (Fig. 3), were identified from *Phoma* sp. YN02-P-3, an endophytic fungus isolated from the leaf of *Sumbaviopsis* from Yunnan Province, China. Compounds **(20–22)** exhibited cytotoxicity with IC_{50} values in the range of 0.52–9.85 μM against HL-60, PC-3, and HCT-116 cell lines [35]. Phomeketale C **(23)** (Fig. 3), a new xyloketal, was also extracted from the same endophytic fungus. Compound **(23)** was found active with IC_{50} value of 12.39, 37.81, and 48.40 μM, respectively, against HL-60, Molm 13, and PC-3 cell line [36]. Compound cercosporamide **(24)** (Fig. 3) was isolated from *Phoma* species ZJWCF006, an endophytic fungus, associated with *Arisaema erubescens* collected from Zhejiang Province, China. Compound **(24)** exhibited cytotoxicity against HT-29, SMMC-772, MCF-7, HL-60, MGC80–3, and P388 cell lines with IC_{50} values of 9.3 to 48.79 μM [37].

Three new xanthones, dalienxanthones A–C **(25–27)** (Fig. 4), together with three known analogs, 3,8-dihydroxy-4-(2,3-dihydroxy-1-hydroxymethylpropyl)-1-methoxyxanthone **(28)**, oliganthin E **(29)**, and cratoxylumxanthone D **(30)** (Fig. 4), were obtained from *Phomopsis* sp., an endophytic fungus residing inside the rhizome of *Paris daliensis* collected from Yunnan Province, China. Compounds **(27)** and **(28)** exhibited cytotoxicity against SHSY5Y cell lines with IC_{50} values of 3.8 and 3.5 μM, respectively. Compounds **(25, 26, 29, 30)** also exhibited cytotoxic activity against NB4, A549, SHSY5Y, PC3, and MCF-7 cell lines with IC_{50} values between 4.6 and 9.2 μM [38].

Two novel compounds, phomopchalasin B **(31)** and phomopchalasin C **(32)** (Fig. 4), were obtained from *Phomopsis* sp. shj2, an endophytic fungus isolated from *Isodon eriocalyx* var. *laxiflora* collected from Kunming, China. Compound **(32)** exhibited moderate cytotoxic activity against HL-60, SMMC-7721, and A-549 cell lines with IC_{50} values of 14.9, 22.7, and 21.1 μM, respectively. The positive control cisplatin displayed cytotoxicity with IC_{50} values of 1.1, 4.6, and 4.7 μM,

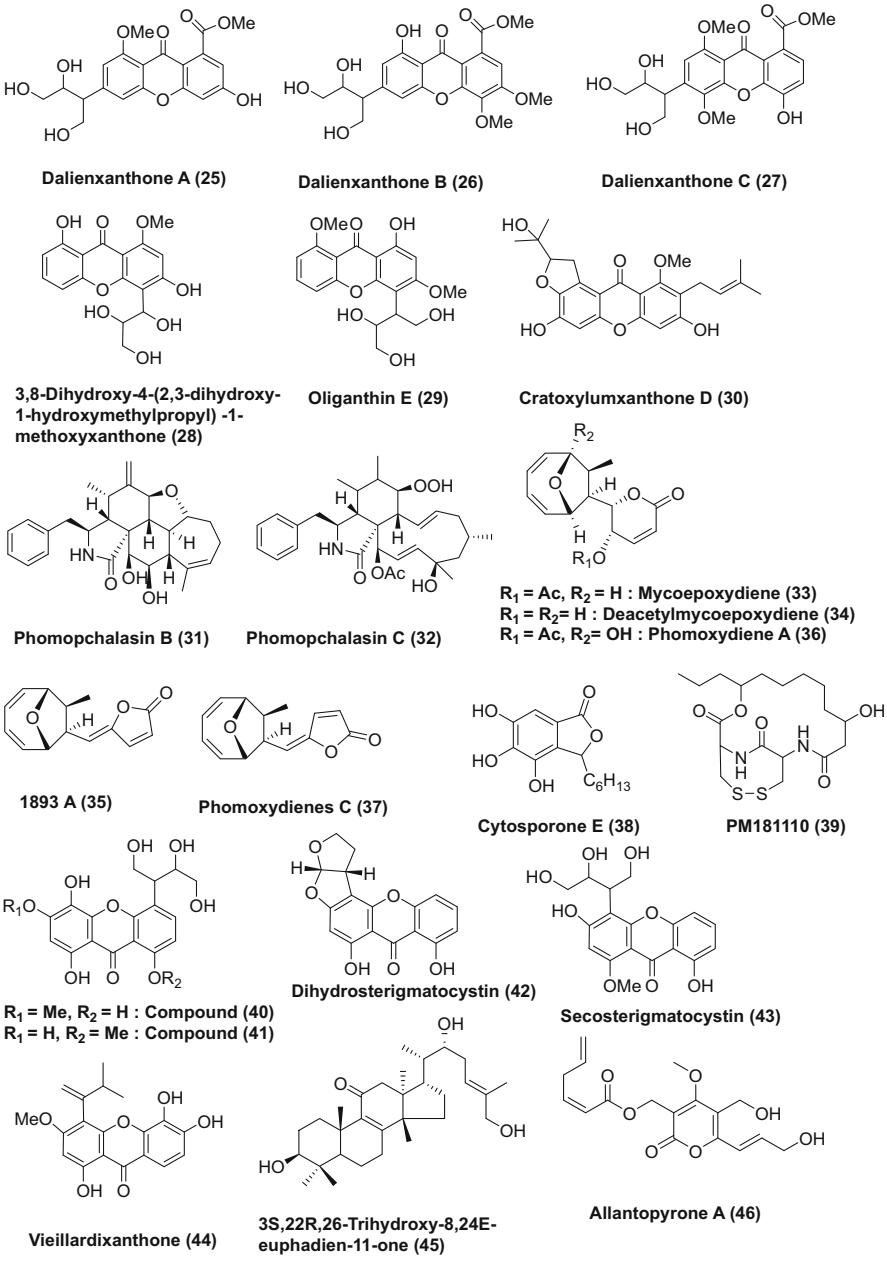


Fig. 4 Structures of metabolites isolated from Coelomycetes (**25**–**46**)

respectively, against HL-60, SMMC-7721, and A-549 cell lines. Compounds **(31)** and **(32)** exhibited an anti-migratory effect against MDA-MB-231 in vitro with IC_{50} values of 19.1 and 12.7 μM, respectively, while positive control cytochalasin D

exhibited an anti-migratory effect against MDA-MB-231 in vitro with IC_{50} value of 0.2 μM [39].

Mycoepoxydiene **(33)**, deacetylmycoepoxydiene **(34)**, 1893 A **(35)**, phomoxydiene A **(36)** and C **(37)**, and cytosporone E **(38)** (Fig. 4) were isolated from *Phomopsis* sp. BCC 45011, an endophytic fungus, associated with *Xylocarpus granatum* leaf, collected at Hat Khanom in Mu Ko Thale Tai National Park, Nakhon Si Thammarat Province, Thailand. Compounds (**33, 34, 36, 37, 38**) exhibited cytotoxicity against KB, MCF-7, NCI-H187, and Vero cells with IC_{50} in the range of 1.49–40.17 μg/mL. Compounds **(35)** and **(37)** were active against only NCI-H187 and Vero cells with IC_{50} of 45.5 and 16.93 μg/mL, respectively [40]. Compound **(33)** also showed cytotoxic activity with IC_{50} value ranging from 0.27 to 2.80 μM against HepG2, A549, HCC-S102, HuCCA-1, KB, HeLa, MDA-MB231, T47D, HL-60, and P388 cell lines. Compound **(34)** was also found active against HepG2, A549, and HCC-S102 cell lines with IC_{50} value ranging from 1.05 to 1.95 μM [41].

A new depsipeptide (PM181110) **(39)** (Fig. 4) was identified from an endophytic fungus *Phomopsis glabrae* isolated from the leaves of *Pongamia pinnata* collected from Karnala Birds Sanctuary, Raigarh, India. Compound **(39)** displayed activity against 40 different human cancer cell lines with mean IC_{50} value of 0.089 μM. When its ex vivo efficacy was evaluated against 24 human tumor xenografts, it exhibited mean IC_{50} value of 0.245 μM [42].

Two new xanthones, 3-methoxy-1,4,8-trihydroxy-5-(1′,3′,4′-trihydroxybutan-2′-yl)-xanthone **(40)** and 8-methoxy-1,3,4-trihydroxy-5-(1′,3′,4′-trihydroxybutan-2′-yl)-xanthone **(41)**, and three known xanthones, dihydrosterigmatocystin **(42)**, secosterigmatocystin **(43)**, and vieillardixanthone **(44)** (Fig. 4), were extracted from *Phomopsis amygdali* which was isolated from the rhizome of *Paris axialis* collected from Shizhong, Yunnan, China. Compounds **(40–44)** were tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF-7). Compound **(40)** showed cytotoxicity against A549 with IC_{50} values of 3.6 μM. Compound **(41)** showed cytotoxicity against SHSY5Y cells with IC_{50} value of 4.2 μM. Compound **(42)** showed cytotoxicity against NB4, SHSY5Y, and MCF-7 cells with IC_{50} value of 6.8, 7.6, and 8.5 μM, respectively. Compound **(43)** was only active against SHSY5Y cells with IC_{50} value of 8.2 μM, respectively. Compound **(44)** showed cytotoxicity against NB4, A549, SHSY5Y, PC3, and MCF-7 cells with IC_{50} values >10 μM. The positive control Taxol showed cytotoxic activity against NB4, A549, SHSY5Y, PC3, and MCF-7 cells with IC_{50} values of 0.03, 0.02, 0.02, 0.02, and 0.01 μM, respectively [43].

A novel euphane triterpenoid 3S,22R,26-trihydroxy-8,24E-euphadien-11-one **(45)** (Fig. 4) was extracted from *Phomopsis chimonanthi*, an endophytic fungus residing inside *Tamarix chinensis* collected from the Yellow River Delta, Dongying, China. Compound **(45)** exhibited cytotoxic activity against A549, MDA-MB-231, and PANC-1 cell lines with IC_{50} values of 20.32, 19.87, and 30.45 μM, respectively, whereas IC_{50} values for 5-fluorouracil were 0.47, 0.12, and 0.67 μM, respectively [44].

Allantopyrone A **(46)** (Fig. 4) and islandic acid-II methyl ester **(47)** (Fig. 5) were isolated from *Allantophomopsis lycopodina* KS-97, associated with a tree branch collected from Gassan stock farm in Yamagata, Japan, and exhibited cytotoxicity against HL-60 cells at IC_{50} values of 0.32 and 6.55 μM, respectively [45].

Allantopyrone A **(46)** inhibits the NF-κB signaling pathway at a step upstream of IκBα phosphorylation [46]. Proteomic analysis indicates that allantopyrone A **(46)** enhances the expression level of proteins mediated through transcription factor Nrf2. The compound showed to protect PC12 cell from deleterious effect of oxidative stress by activating the Keap1-Nrf2 pathway [47].

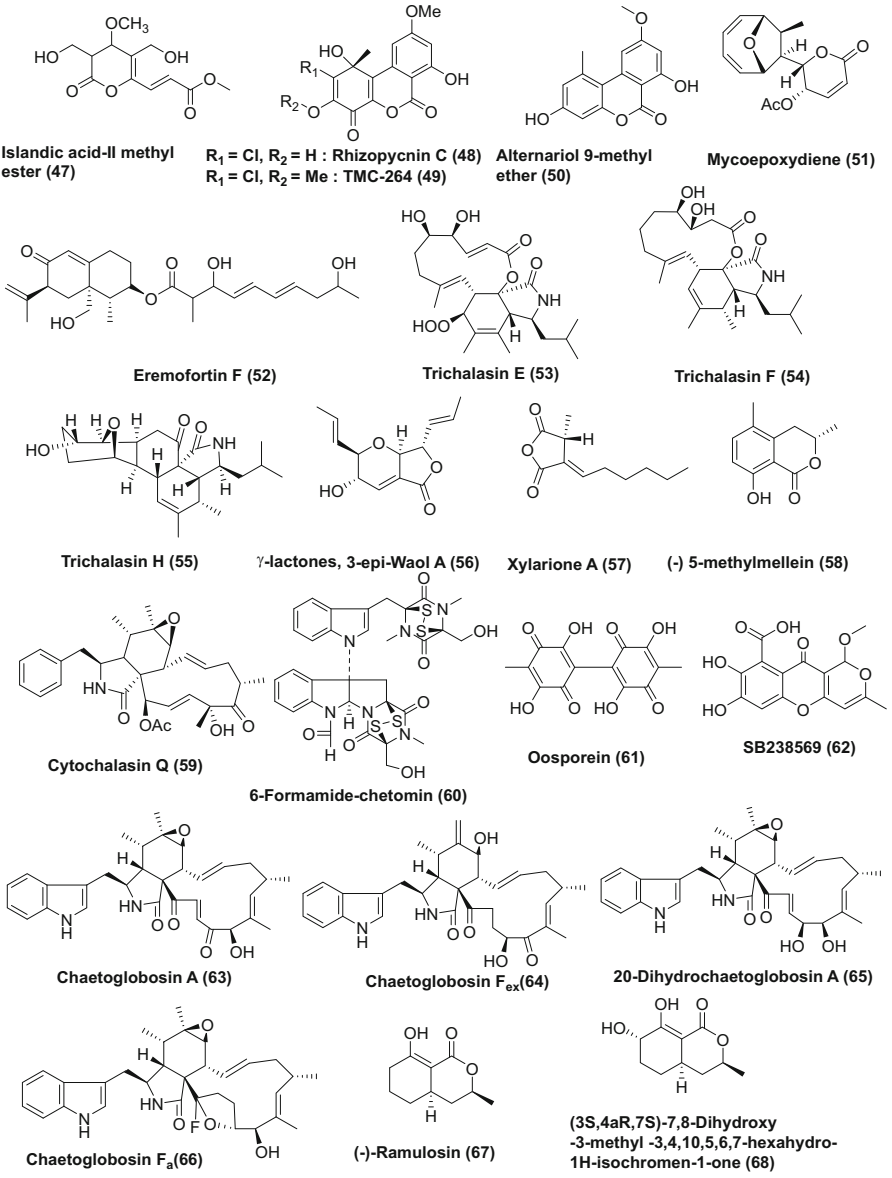


Fig. 5 Structures of metabolites isolated from Coelomycetes (**47**–**56**) and Ascomycetes (**57**–**68**)

A new dibenzo-α-pyrone, rhizopycnin C **(48)**, TMC-264 **(49),** and alternariol 9-methyl ether **(50)** (Fig. 5) were identified from *Rhizopycnis vagum* Nitaf22, an endophytic fungus obtained from *Nicotiana tabacum* grown in the greenhouse of the campus at China Agricultural University. TMC-264 **(49)** exhibited cytotoxic activity against selected cancer cell lines, namely, HCT-116, HepG2, BGC-823, NCI-H1650, and A2780, with IC_{50} values of 4.2, 5.9, 7.8, 3.2, and 3.6 μM, respectively. Rhizopycnin C **(48)** displayed mild activity toward A549 and HCT116 cell lines, with IC_{50} values of 25.5 and 37.3 μM, respectively. Alternariol 9-methyl ether **(50)** exhibited weak cytotoxic effect against A549 cells ($IC_{50} = 70.4$ μM) [48].

Diaporthe pseudomangiferae an endophyte isolated from the leaves of *Sabicea cinerea* was collected in Roura, French Guiana, and was the source of mycoepoxydiene **(51)** and eremofortin F **(52)** (Fig. 5). Mycoepoxydiene **(51)** exhibited cytotoxic activity against KB, MDA-MB-435, and MRC5 cell lines with IC_{50} values of 7.5, 17.7, and 15.8 μM, respectively. Compound **(52)** was active against KB and MRC5 cell lines with IC_{50} value of 13.9 and 12.2 μM, respectively. The positive control docetaxel exhibited cytotoxicity against KB and MDA-MB-435 cell lines, with IC_{50} values of 0.2 and 0.5 nM, respectively, while other positive control doxorubicin exhibited cytotoxicity against MRC5 cell line with IC_{50} values of 20 nM [49].

Vasundhara et al. [50] reported trichalasin E **(53)**, F **(54)**, and H **(55)** (Fig. 5), from an endophytic fungus *Diaporthe* sp. strain T1 isolated from *Taxus baccata* collected from Bhaderwah (Doda district, India). The crude extract of T1 showed cytotoxic activity against MCF-7 and HeLa cancer cell lines, with IC_{50} values of 1058 and 1257 μg/mL, respectively.

A new γ-lactone, 3-epi-Waol A **(56)** (Fig. 5), was extracted from *Libertella blepharis*, an endophytic fungus residing inside the leaf of *Olyra latifolia*, collected in the province of Colon, Republic of Panama. 3-epi-Waol A **(56)** display cytotoxicity with IC_{50} values of 22.46, 6.20, and 1.0 μM, respectively, against MCF-7, HCT116, and H460 cell lines [51].

2.2 Compounds Produced by Ascomycetes

A novel compound xylarione A **(57)** and a known compound (−) 5-methylmellein **(58)** (Fig. 5) were isolated from *Xylaria psidii*, an endophytic fungus residing inside the leaf sample of *Aegle marmelos*. Compounds **(57)** and **(58)** exhibited cytotoxic activity against MCF-7, MIA-Pa-Ca-2, NCI-H226, HepG2, and DU145 cell line with IC_{50} in the range of 16–37 μM, while against fR2 (normal) cell line, the IC_{50} value was 79 and 76 μM, respectively. Compounds **(57)** and **(58)** exhibited cytotoxicity against MIA-Pa-Ca-2 cells with IC_{50} values of 16.0 and 19.0 μM, respectively, and blocked cell cycle at sub-G1 stage. Compounds **(57)** and **(58)** induced apoptosis and displayed substantial decrease in membrane potential of mitochondria in concentration-dependent manner confirmed by flow cytometry analysis using rhodamine-123 [52].

Compound cytochalasin Q **(59)** (Fig. 5) was extracted from *Xylaria* sp. ZJWCF255, an endophytic fungus extracted from the leaf of *Ficus carica*. Compound **(59)** showed potent cytotoxicity against SMMC-772, MCF-7, and MGC80–3 cell lines with IC_{50} values of 17.24, 7.75, and 10.30 μg/mL, respectively [53].

A new compound 6-formamide-chetomin **(60)** (Fig. 5) was obtained from endophytic fungus *Chaetomium* sp. M336 that was isolated from *Huperzia serrata* which is the traditional Chinese medicine Qian Ceng Ta and grows at an altitude of 300–2700 m in damp forests and rock crevices in China. Compound **(60)** showed good cytotoxicity with IC_{50} values of 21.6 nM (HeLa), 23.0 nM (SGC-7901), and 27.1 nM (A549) [54].

Oosporein **(61)** (Fig. 5), which was characterized from an endophytic fungus, *Cochliobolus kusanoi* isolated from *Nerium oleander* L. Oosporein **(61),** showed activity against A549 cells with the IC_{50} of 21 μM [55]. Cytotoxic activity of oosporein against A549 cell lines showed IC_{50} of 28.66 μM [56].

From the leaves of *Sapium ellipticum* (Euphorbiaceae) obtained from west region of Cameroon, fungal endophyte *Chaetomium* sp. was isolated that led to identification of SB238569 **(62)** (Fig. 5). Compound **(62)** exhibited strong cytotoxicity against L5178Y cell line with an IC_{50} value of 1 μM [57].

An endophytic fungus *Chaetomium globosum*, associated with *Ginkgo biloba*, growing in Linyi, Shandong Province, China, was the source of cytochalasan, chaetoglobosin A **(63)**, chaetoglobosin Fex **(64)**, 20-dihydrochaetoglobosin A **(65)**, and chaetoglobosin Fa **(66)** (Fig. 5). Compounds **(63–66)** showed remarkable cytotoxicity against HCT116 cell lines with IC_{50} values of 3.15, 4.43, 8.44, and 5.85 μM, in comparison with the positive control etoposide with IC_{50} value of 2.13 μM [58].

Compounds (−)-ramulosin **(67)**, (3S,4aR,7S)-7,8-dihydroxy-3-methyl-3,4,10,5,6,7-hexahydro-1H -isochromen-1-one **(68)** (Fig. 5), (−)-epoformin **(69)**, and (1S*,3R*,5R*)-3-methyl-2-oxabicyclo [3.3.1]nonan-7-one **(70)** (Fig. 6) were isolated from *Talaromyces* sp. associated with twigs of *Cedrus deodara*, collected from the Western Himalayas of Kashmir, India. (−)-Epoformin **(69)** was found most active with 98, 100, 50, 22, and 56% cytotoxicity against A-549, HEP-1, THP-1, PC-3, and HCT-116 cells, respectively, at 50 μM concentration, followed by compound **(70)** with 71, 26, 23, and 59% cytotoxicity against A-549, HEP-1, PC-3, and HCT-116 cells, respectively, at the same concentration. Compound **(67)** also showed 15, 23, 54, 23, and 44% toxicity against A-549, HEP-1, THP-1, PC-3, and HCT-116 cells at 50 μM concentration. Compound **(68)** was least active with 35, 3, 40, 34, and 35% against A-549, HEP-1, THP-1, PC-3, and HCT-116 cells at the same concentration. The positive control paclitaxel exhibited cytotoxicity against A-549, THP-1, and HCT-116 cells with 82, 71, and 72% inhibition at 1 μM concentration, while another positive control fluorouracil showed 22, 84, and 55% cytotoxicity against A-549, THP-1, and HCT-116 cells at 20 μM concentration. All the isolated compounds were found to induce apoptosis in HL-60 cells using fluorescence and SEM studies. These compounds also caused significant microtubule inhibition in HL-60 cells [59].

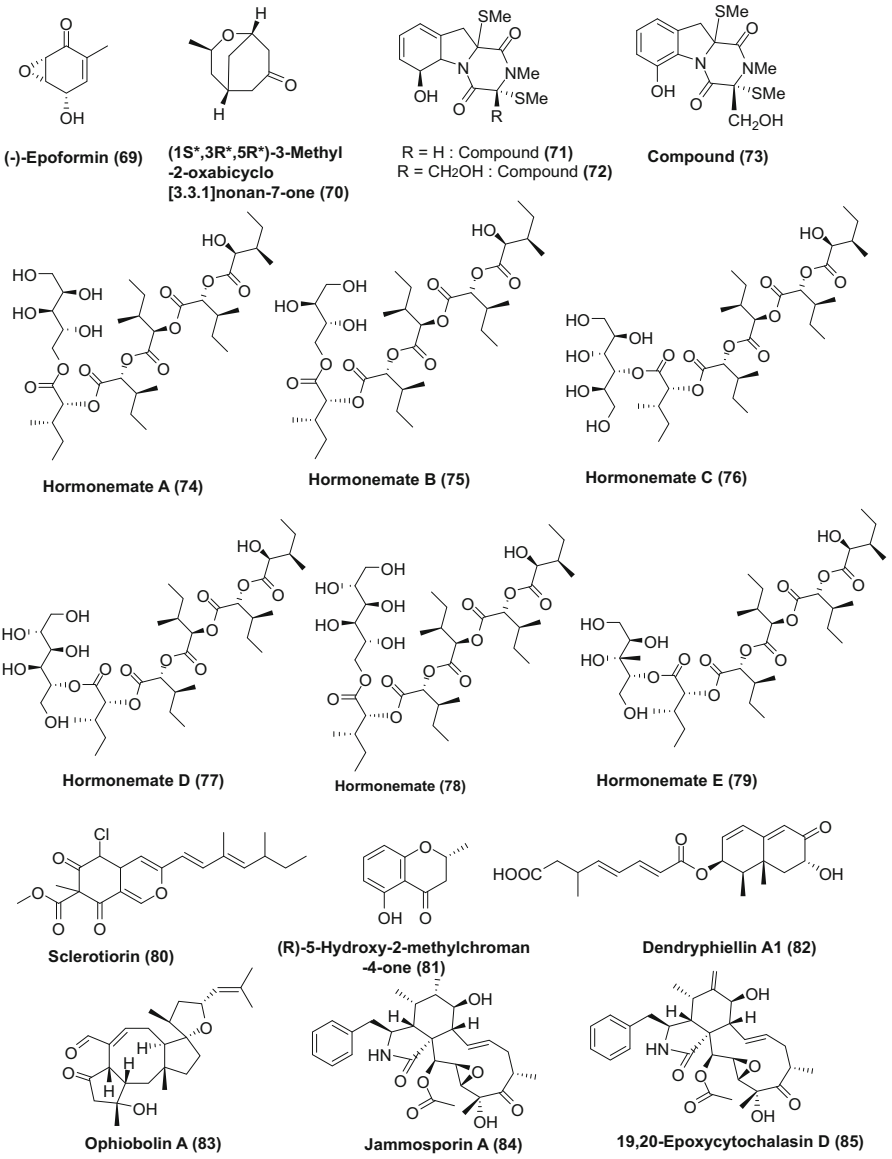


Fig. 6 Structures of metabolites isolated from Ascomycetes (**69–85**)

An endophytic fungus *Tripterygium wilfordii* was identified from *Talaromyces* sp. LGT-2 that led to isolation of three alkaloids, 3-dehydroxymethylbisdethio-3,10a-bis(methylthio)gliotoxin **(71)**, bisdethiobis(methylthio)gliotoxin **(72)**, and didehydrobisdethiobis(methylthio)gliotoxin **(73)** (Fig. 6). At a concentration of 500 μg/mL, compounds **(71–73)** exhibited cytotoxic activity against B16 cancer cell line with inhibitory rates of 86, 82, and 78%, respectively [60].

Hormonemates A–D **(74–77)**, hormonemate **(78)**, and hormonemate E **(79)** (Fig. 6) were isolated from *Dothiora* sp., an endophytic fungus associated with the endemic plant *Launaea arborescens* collected from Tabernas Desert (Almeria, Spain). Hormonemate E **(79)** was found active against HepG2, MCF-7, and MiaPaca_2 cell line with IC_{50} values of 18.7, 13.9, and 27.4 μg/mL, respectively. Hormonemate A **(74)** was found active against HepG2 and MCF-7 cell line with IC_{50} values of 20.9 and 11.3 μg/mL, respectively. Hormonemate B **(75)** was found active against HepG2 and MCF-7 cell line with IC_{50} values of 19.9 and 7.7 μg/mL, respectively. Hormonemate C **(76)** was active against MCF-7 cell lines with IC_{50} value of 20.9 μg/mL. Hormonemate D **(77)** was active against HepG2 and MCF-7 cell lines with IC_{50} values of 27.3 and 13.8 μg/mL. Hormonemate **(78)** was active against MCF-7 cell lines with an IC_{50} value of 18.2 μg/mL. Positive control doxorubicin was found active against HepG2, MCF-7, and MiaPaca_2 cell lines with IC_{50} values of 0.1, 0.8, and 1.0 μg/mL, respectively [61].

Sclerotiorin **(80)** (Fig. 6) was isolated from an endophytic fungus *Cephalotheca faveolata* residing inside the petiole of *Eugenia jambolana* collected from Mumbai, India. It showed cytotoxicity against HCT-116, H460, ACHN, Panc-1, and Calu-1 cell lines with the IC_{50} values of 0.63, 1.6 1.2, 1.6, and 2.1 μM, respectively, while in MCF10A, it showed an $IC_{50} > 10$ μM. Sclerotiorin (**80**) induced apoptosis in HCT116 cells via the triggering of BAX and downregulation of Bcl-2 that result in stimulation of cleaved caspase-3 thereby causing the death of cancerous cells [62].

An endophytic *Cryptosporiopsis* sp., isolated from *Clidemia hirta*, was the source of (R)-5-hydroxy-2-methylchroman-4-one (HMC) **(81)** (Fig. 6). Compound **(81)** exhibited cytotoxicity against HL-60 with an IC_{50} of 4 μg/mL and induced G2 arrest of the HL-60 cell cycle [63].

Compound **(81)** was also found active against leukemic cell line K-562 with IC_{50} value of 8 μg/mL. HMC exhibited cytotoxicity toward human leukemia cell lines blocking cell cycle at G2/M stage and hampered protein expression level indulged in regulating cell cycle. It targets STAT-3 signaling cascade and induced caspase-dependent apoptosis in HL-30 cells [64].

Dendryphiellin A1 **(82)** (Fig. 6) was isolated from *Chaetoconis* sp. FT087 which was isolated from *Osmoxylon novoguineensis* leaf collected in the Waimea Valley on the Oahu Island, Hawaii. Dendryphiellin A1 has a trinor-eremophilane skeleton. Dendryphiellin A1 **(82)** exhibited cytotoxic activity with IC_{50} values of 6.6 and 9.1 μg/mL, respectively, against A2780 and cisplatin-resistant A2780CisR cell lines [65].

The ophiobolin A **(83)** (Fig. 6) was derived from the endophytic fungus *Bipolaris setariae* of *Parthenium hysterophorus* collected from Mumbai, India. It inhibited solid (PC3, A2780, MDA-MB-231, MCF-7) and hematological (MM1R, RPMI8226, U266B1, and Jurkat) cancer cell proliferation with IC_{50} of 0.4–4.3 μM. In comparison, IC_{50} against normal cells (hPBMC) was 20.9 μM. Compound **(83)** was found to impart phosphorylation of S6 protein of PI3K/mTOR, Ras/Raf/ERK, and CDK/RB pathways. In cancer cell line MDA-MB-231, it led to cause apoptosis and blocked progression of cell cycle targeting signaling proteins. The anticancer property was as a result of simultaneous

blockage of different cancer regulatory pathways like PI3K/mTOR, Ras/Raf/ERK, and CDK/RB [66].

Jammosporin A **(84)**, a new cytochalasin, and four known analogs, 19,20-epoxycytochalasin D **(85)** (Fig. 6), cytochalasin D **(86)**, 19,20-epoxycytochalasin C **(87)**, and cytochalasin C **(88)** (Fig. 7), were extracted from *Rosellinia sanctae-cruciana*, an endophytic fungus isolated from the leaves of *Albizia lebbeck*. Compounds **(84, 85, 87, 88)** showed moderate cytotoxic activity against MOLT-4 cell line with IC_{50} values of 20·0, 10·0, 8·0, and 6·0 μM, respectively, while compound **(86)** showed an IC_{50} value of 25 μM [67].

Two sesquiterpene-epoxycyclohexenone conjugates, nectrianolin A **(89)** and B **(90)**, together with a sesquiterpene, nectrianolin C **(91)** (Fig. 7), were isolated from *Nectria pseudotrichia* 120-1NP, an endophytic fungus isolated from *Gliricidia sepium*. Compounds **(89–91)** exhibited cytotoxic activity against the HL-60 cell line with IC_{50} values of 1.7, 1.5, and 10.1 μM, respectively. Additionally, compounds **(89–91)** were found active against the HeLa cell line with IC_{50} values of 34.7, 16.6, and 52.1 μM, respectively [68].

Compound 4-epi-ethisolide **(92)** (Fig. 7) was obtained from *Cryptosporiopsis* sp. H2–1 (NFCCI 2856), an endophytic fungus associated with *Clidemia hirta* found in Hawaii. Compound **(92)** exhibited moderate activity with an IC_{50} value of 11 μM in HL-60 [69].

Six novel bioactive bicyclic polyketides, preussilides A–F **(93–98)** (Fig. 7), were extracted from *Preussia similis*, an endophytic fungus isolated from *Globularia alypum* collected in Batna, Algeria. Compounds **(93–98)** exhibited moderate to weak cytotoxic activity against L929, KB3.1, A431, A549, SKOV-3 PC-3, MCF-7, and U2OS cell lines with IC_{50} values ranging from 2.5 to 80.0 μM. Only two compounds **(93** and **95)** exhibited cytotoxicity against L929 and HeLa KB.3.1 and U2OS cells with IC_{50} values below 10 μM; in addition, only compound **(95)** was active against MCF-7 cells with an IC_{50} value of 7.3 μM. However, they were inactive against other cancer cell lines tested. All compounds caused nucleic fragmentation in the range of IC_{50} values in L929 cells [70].

New cytochalasans periconiasins A–B **(99–100)** (Fig. 7) were isolated from the endophytic fungus *Periconia* sp. F-31 isolated from *Annona muricata* collected from Hainan Province, China. Compound **(99)** showed cytotoxic activity against the HCT-8 and BGC-823 cell lines with IC_{50} values of 0.9 and 2.1 μM, respectively, while compound **(100)** showed cytotoxicity with IC_{50} values of 0.8, 5.1, and 9.4 μM, respectively, against the HCT-8, Bel-7402, and BGC-823 cell lines [71]. A new cytochalasan, periconiasin I **(101)** (Fig. 7), was extracted from the same fungus. Compound **(101)** showed in vitro cytotoxicity against human MCF-7 tumor cell line with an IC_{50} value of 4.8 μM, while positive control paclitaxel exhibited IC_{50} value of 0.2 nM [72]. Periconone E **(102)** (Fig. 7), a new polyketide-terpenoid hybrid molecule, was also obtained from *Periconia* sp. F-31. Compound **(102)** exhibited cytotoxic activity with an IC_{50} value of 4.2 μM against MCF-7 cell line [73].

Diepoxin δ **(103)** and palmarumycin C8 **(104)** (Fig. 7) were isolated from *Berkleasmium* sp., an endophyte isolated from the healthy rhizomes of medicinal plant *Dioscorea zingiberensis* collected in Hubei Province, China. Compounds **(103)**

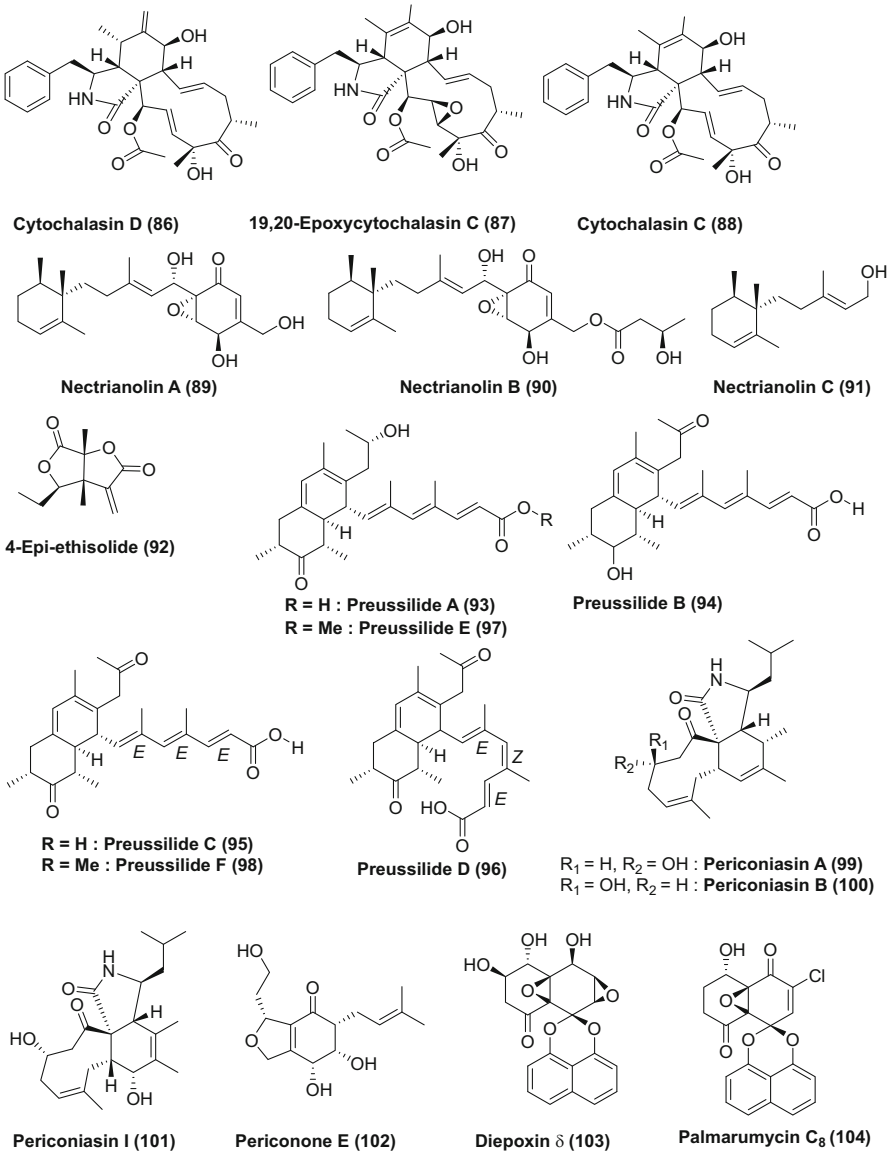
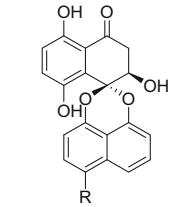


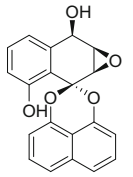
Fig. 7 Structures of metabolites isolated from Ascomycetes (**86–104**)

and **(104)** showed good cytotoxic activities against HCT-8, Bel-7402, BGC-823, A549, and A2780 cell lines with IC_{50} values in the range of 1.28–5.83 μM [74].

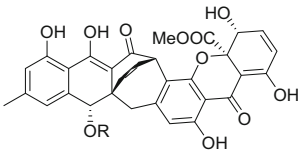
Palmarumycin LP1 **(105)**, cladospirone B **(106)**, and Scheme 50676 **(107)** (Fig. 8) were obtained from *Lasiodiplodia pseudotheobromae* XSZ-3, an endophytic fungus residing inside *Camptotheca acuminata* collected from Panzhihua, Sichuan



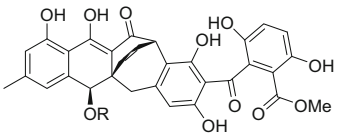
R= OH : **Palmarumycin LP1 (105)**
R= H : **Cladospirone B (106)**



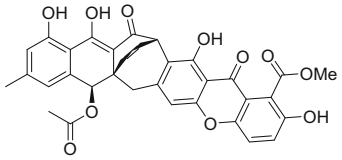
Sch 50676 (107)



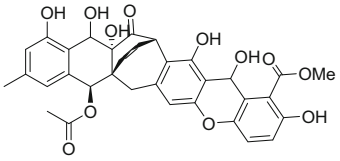
R = H : Acremoxanthone E (108)
R = Ac : Acremoxanthone C (109)



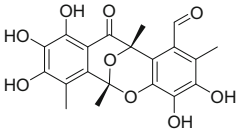
R= Ac : Acremonidin A (110)
R = H : Acremonidin B (111)



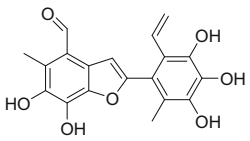
Acremoxanthone A (112)



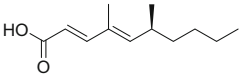
Acremoxanthone B (113)



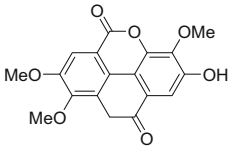
Epicocconigrone A (114)



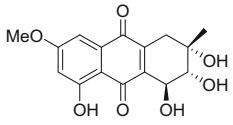
Epicoccolide B (115)



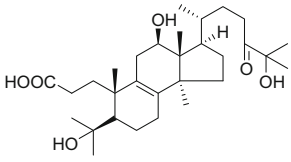
Phialomustin B (116)



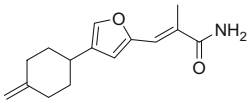
3, 3', 4-tri-O-Methylellagic acid (117)



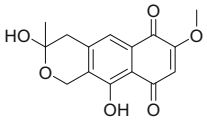
4-Dehydroxyaltersolanol A (118)



Glometenoid A (119)



Brasilamide E (120)



6-Deoxyfusarubin (121)

Fig. 8 Structures of metabolites isolated from Ascomycetes (**105–121**)

Province, China. Compounds **(105)** and **(107)** showed good cytotoxic activity against HL-60 cells with IC_{50} values of 2.39 and 1.41 μM, respectively. Compound **(106)** exhibited a moderate activity with an IC_{50} value of 10.91 μM, against HL-60 cells, and positive control 5-fluorouracil showed inhibitory effect against HL-60 cells with IC_{50} value of 1.87 μM [75].

An endophytic fungus *Acremonium camptosporum* isolated from the leaves of *Bursera simaruba* collected from the El Eden in the state of Quintana Roo, Mexico,

led to the isolation of six major heterodimeric polyketides, acremoxanthone E **(108)**, acremoxanthone C **(109)**, acremonidin A **(110)** and B **(111)**, and acremoxanthone A **(112)** and B **(113)** (Fig. 8). Compounds **(108–113)** exhibited cytotoxicity against U251, PC-3, K562, HCT-15, MCF-7, and SKLU-1 cell line with IC_{50} in the range of 3 to 16 μM, comparable to positive control cisplatin [76].

A new polyketide, epicocconigrone A **(114)**, and epicoccolide B **(115)** (Fig. 8) were isolated from *Epicoccum nigrum*, an endophytic fungus residing inside the leaves of *Mentha suaveolens* collected in Morocco. Compounds **(114, 115)** exhibited to block activity of 15 protein kinases with IC_{50} values lying between 0.07 and 9.00 μM. Compounds **(114, 115)** also inhibited histone deacetylase (HDAC) activities with IC_{50} values of 9.8 and 14.2 μM, respectively. Compounds **(114** and **115)** exert mainly cytostatic effects in human lymphoma RAJI and U-937 cell lines [77]. A new compound, phialomustin B **(116)** (Fig. 8), was obtained from *Phialophora mustea*, an endophytic fungus associated with corms of *Crocus sativus*. Compound (**116**) showed good cytotoxic activity against T47D cancer cell line, with an IC_{50} of 1 μM [78].

Compounds 3,3′,4-tri-O-methylellagic acid **(117)** and 4-dehydroxyaltersolanol A **(118)** (Fig. 8) were isolated from *Nigrospora oryzae*, an endophytic fungus residing inside the leaves of *Combretum dolichopetalum* which was collected from Nsukka region of Eastern Nigeria. Compounds **(118)** and **(117)** showed cytotoxicity with IC_{50} values of 9.4 and 29.0 μM, respectively, against L5178Y cell lines [79].

A new ring A-cleaved lanostane-type triterpenoid, glometenoid A **(119)** (Fig. 8), was obtained from *Glomerella* sp. F00244, an endophytic fungus associated with *Pinus massoniana* (mason pine) stem, which was collected from Fujian Province, China. Compound **(119)** exhibited weak antiproliferative activity against HeLa cell lines with 21% growth inhibition at a concentration of 10 μM [80].

Brasilamide E (**120)** (Fig. 8) was obtained from *Paraconiothynium brasiliense*, an endophytic fungus residing inside the branches of *Acer truncatum* collected from Dongling Mountain, Beijing, China. Compound **(120)** inhibited the proliferation, with IC_{50} values of 8.4 and 14.7 μM, respectively, of the MCF-7 and MGC cancer cell lines. Compound **(120)** inhibited the expression of hexokinase-II in MCF-7 cells, resulting in the dysfunction of glucose metabolism and ATP depletion which ultimately led to the inhibition of breast cancer cell proliferation [81].

A strain of *Biatriospora* sp. CCF 4378, an endophyte associated with *Ulmus laevis*, collected from Libicky Luh Forest near Velky Osek, Czech Republic, was the source of pyranonaphthoquinones, 6-deoxyfusarubin **(121)** (Fig. 8) and ascomycone B **(122)** (Fig. 8). Ascomycone B **(122)** and 6-deoxyfusarubin **(121)** showed dramatic changes of the cellular content and cell death in HeLa cells and primary human skin fibroblasts. The effect was rapid (in minutes) in both the cell types tested. During longer incubation times (24 h, 50 μg/mL), all cells died via necrotic cell death. The effect at microgram concentrations starts with a change in mitochondrial organization (from a filamentous network to individual perinuclear vesicles) and is followed by a reorganization of the actin cytoskeleton cellular filopodialisation, rounding, and blocked transferrin uptake and ends with a lack of detectable esterase and nucleic acid content [82].

A diterpenoid metabolite, rosoloactone **(123)** (Fig. 8), was extracted from the endophytic fungus *Trichothecium roseum*. Rosoloactone **(123)** reduced the survival rate of HeLa cells with IC_{50} value of approximately 8 μg/mL. Rosoloactone **(123)** inhibits the viability of HeLa cells and potentially led to ER-mediated apoptosis by inducing accumulation of misfolded proteins in the ER lumen as well as intrinsic apoptosis owing to mitochondrial dysfunction [83].

Trichothecinol A (**124**) (Fig. 8) was identified from an endophytic fungus *Trichothecium* sp. isolated from *Phyllanthus amarus* collected from Pune, India. Compound (**124**) at concentration of 500 nM led to 50% cell death in HeLa and B16F10 cells and caused apoptosis later. Trichothecinol A also checked migration of wound by 50% at 500 nM of MDA-MB-231 cells indicating its antimetastatic property [84].

The endophytic fungus *Stemphylium globuliferum* isolated from *Mentha pulegium* growing in Morocco was the source of altersolanol A **(125)** (Fig. 8) and, against myeloid K562 leukemia and A549 lung cancer, showed cytotoxic activity in a dose-dependent manner without affecting noncancerous cells' viability. Altersolanol A **(125)** leads to caspase-3 and caspase-9 cleavage thus imparting cell death and apoptosis [85]. Altersolanol A **(125)** exhibited cytotoxicity with mean IC_{50} values of 0.005 μg/mL (IC_{70} = 0.024 μg/mL) against 34 human cancer cell lines [86].

A novel reddish-orange alkaloid, mycoleptodiscin B **(126)** (Fig. 8), was obtained from the *Mycoleptodiscus* sp., an endophytic fungus associated with the plant *Desmotes incomparabilis* in Panama. Compound **(126)** was found active against H460, A2058, H522-T1, PC-3, and IMR-90 cell line with IC_{50} values in the range 0.60–0.78 μM [87].

A poly-oxygenated benzofuran-3(2H)-one dimer, arundinone B **(127)** (Fig. 8), was extracted from *Microsphaeropsis arundinis*, the endophytic fungus residing inside the stems of *Ulmus macrocarpa* collected from Dongling Mountain, Beijing, China. Compound **(127)** showed cytotoxicity against T24 and A549 cells with IC_{50} values of 35.4 and 81.6 μM, respectively, while the positive control cisplatin exhibited cytotoxicity against T24 and A549 cells with IC_{50} values of 3.72 and 8.45 μM, respectively [88].

The endophytic fungus *Bipolaris sorokiniana* A606, obtained from *Pogostemon cablin* which was collected at Gaoyao, Guangdong Province, China, isolated four new compounds named isocochlioquinones D–E **(128, 129)** and cochlioquinones G–H **(130, 131)** and five known cochlioquinone analogs, isocochlioquinone C **(132)**, cochlioquinone C **(133)**, cochlioquinone D **(134)**, cochlioquinone E **(135)**, and cochlioquinone B **(136)** (Fig. 8). Compounds (**131**) and (**133–136**) exhibited potent cytotoxicity in vitro against the four tumor cell lines, SF-268, MCF-7, NCI-H460, and HepG2, with IC_{50} in the range of 1.2 to 42.8 μM. Compound **(134)** showed excellent activity against SF-268, MCF-7, and HepG2 cell lines with IC_{50} values of 1.5, 2.4, and 1.2 μM, respectively. Other compounds **(128–130, 132)** exhibited moderate to poor activity against cell lines tested in the range of 11.3 to 50.6 μM. The positive control cisplatin exhibited cytotoxicity with IC_{50} value of 4.1, 2.9, 2.9, and 2.5 μM, respectively, against SF-268, MCF-7, NCI-H460, and HepG2 cell lines [89].

Using OSMAC approach, calbistrin F **(137)** and dothideomynone C **(138)** (Fig. 8) were extracted from *Dothideomycete* sp. CRI7, an endophytic fungus associated with the roots of a Thai medicinal plant, *Tiliacora triandra*, which was collected from Nakhon Sawan Province, Thailand. Calbistrin F **(137)** displayed weak cytotoxicity on the MOLT-3 cell line (IC_{50} = 37.3 lg/mL). Compound **(138)** exhibited cytotoxicity against HuCCA-1, A549, and MOLT-3 cell lines with IC_{50} values of 48.1, 46.5, and 17.4 lg/mL, respectively [90].

A new bicyclic lactone, myrotheciumones A **(139)** (Fig. 8), was extracted from an endophytic fungus *Myrothecium roridum*, associated with *Ajuga decumbens*. Compound **(139)** exhibited cytotoxicity with IC_{50} values of 5.36, 6.56, 5.88, 7.56, 16.30, and 20.69 μM, respectively, against HepG2, SMMC-7721, A549, MCF-7, QSG-7701, and HL-7702 cell lines. Myrotheciumone A **(139)** acts by promoting cytochrome c release from mitochondria and induces apoptosis in cancer cell lines [91].

Hypocrea lixii, an endophyte associated with *Cajanus cajan*, was the source of cajanol **(140)** (Fig. 8). R-18 produced the highest levels of cajanol (322·4 μg/l or 102·8 μg/g dry wt. of mycelium) after incubation for 7 days. Fungal cajanol **(140)** possessed strong cytotoxicity activity toward A549 cells with IC_{50} value of 20.5 μg/mL after 72 h treatment. The cajanol exhibited toxicity toward normal cells, MC3T3-E1 cells, and RAW264.7 with IC_{50} values of 48.7 and 40.2 μg/mL, respectively, after 72 h of treatment [92].

An endophytic fungus *Gibberella moniliformis* from the leaves of *Coix lacryma-jobi* var. *ma-yuen* was collected from hilly region of Taishun County in the Zhejiang Province in China and was the source of triolein (trioleoylglycerol) **(141)** (Fig. 8). The concentration of triolein produced by *G. moniliformis* AH13 reached 2.536 mg/g dry wt. of mycelium. Ethyl acetate extract of *G. moniliformis* AH13 showed strong antitumor activity against A549, HCT116, MDA-MB-231, and SW1990 cell lines with IC_{50} values of 42.28, 5.47, 7.86, and 12.19 μg/mL, respectively [93].

Trichodermin **(142)** (Fig. 9) was obtained from an endophytic fungus *Nalanthamala psidii* and exhibited cytotoxicity against MIA PaCa-2, BxPC-3, HPAC, KYSE-170, KYSE-510, MCF-7, MDA-MB-453, FTC-133, FTC-236, CL1–0, CL1–5, PC-3, and 22Rv1 with IC_{50} of 0.8, 1.2, 1.4, 2.3, 2.7, 3.8, 3.6, 3.6, 3.5, 2.8, 3.1, 3.3, and 2.5 μM, respectively. Trichodermin exhibited cytotoxicity against pancreatic cancer cells (MIA PaCa-2 and BxPC-3) via induction of caspase-dependent apoptosis along with intrinsic mitochondrial apoptosis. Trichodermin was found to induce DNA damage stress to activate p53 for causing apoptosis. When compared with gemcitabine, the compound activity was found similar which intensely reduced growth of tumor through induction of DNA damage [94]. It was reported that trichodermin **(142)** acts via mitochondrial dysfunction and ER stress [95].

MBJ-0011 **(143)**, MBJ-0012 **(144)**, and MBJ-0013 **(145)** (Fig. 10) were discovered as new cytotoxic compounds from an endophytic fungus *Apiognomonia* sp. f24023 which was isolated from a plant growing in Iwata, Shizuoka Prefecture, Japan. Compound **(143)** exhibited moderate cytotoxic activity against SKOV-3 cells with the IC_{50} of 3.4 μM. Compounds **(144)** and **(145)** showed weak cytotoxicity with IC_{50} value of 63 and 54 μM, respectively [96].

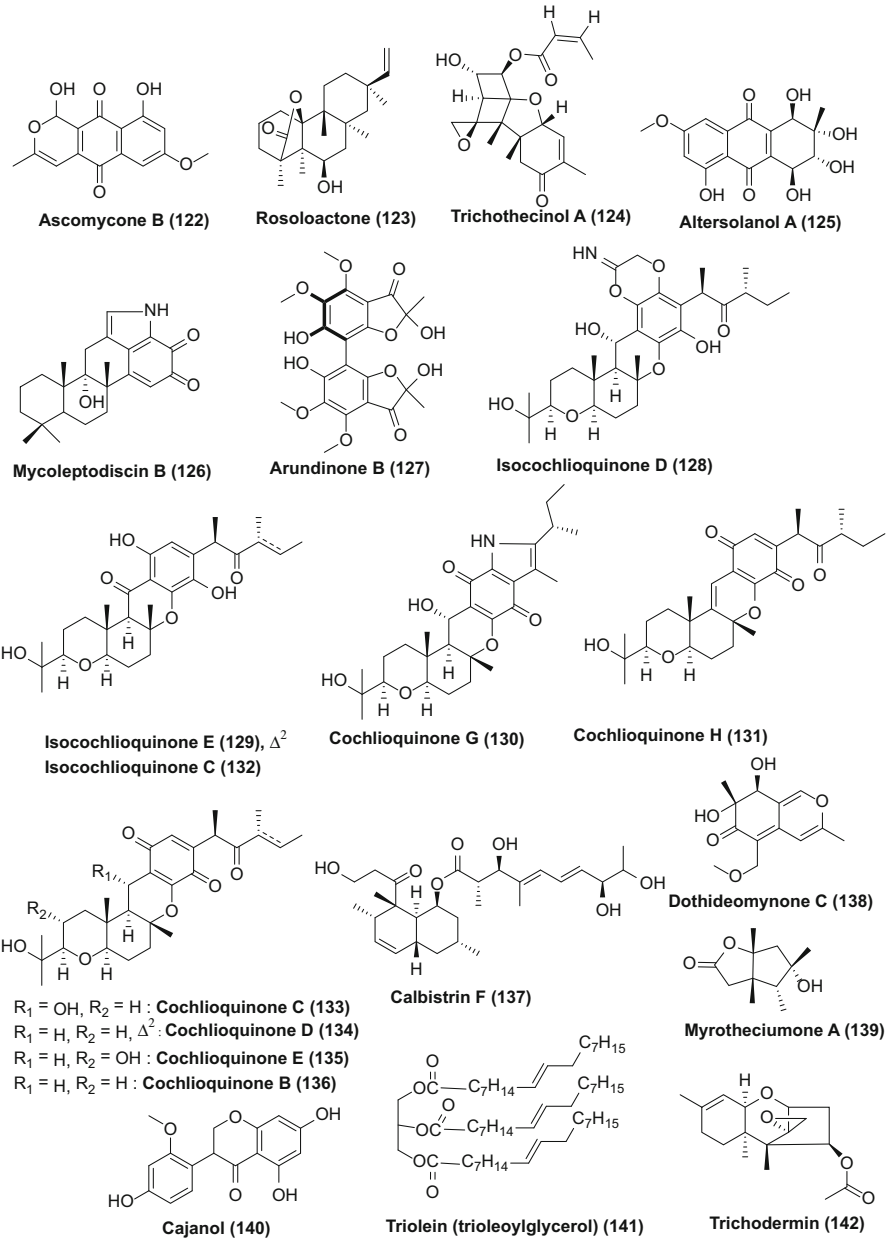


Fig. 9 Structures of metabolites isolated from Ascomycetes (**122–142**)

Trichodermol **(146)** and 7-epi-brefeldin A **(147)**, isolated from *Sporothrix* spp. 4335 99KK29FL1 associated with *Costus speciosus*, and (3R,4S)-4-hydroxymellein **(148)** and desmethyl-lasiodiplodin **(149)** (Fig. 10), isolated from *Lasiodiplodia*

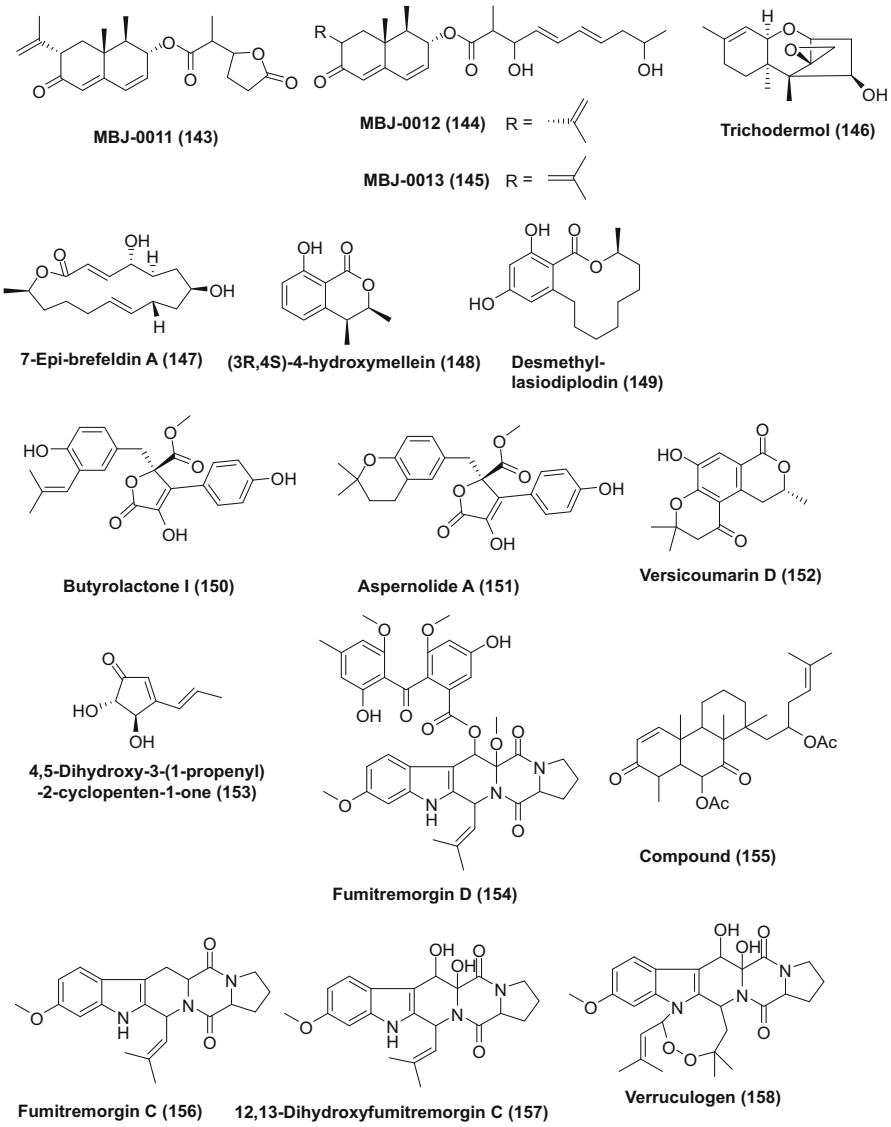


Fig. 10 Structures of metabolites isolated from Ascomycetes (**143–149**) and Hyphomycetes (**150–158**)

theobromae strain xsd08007 associated with *Dendropanax laurifolius*, were collected from Kuala Keniam, National Park, Pahang, Malaysia. Compounds **(146)** and **(147)** showed the greatest inhibitory activity against MCF-7, with IC_{50} values of 0.83 and 0.35 μM, respectively. In comparison with tamoxifen ($IC_{50} = 0.11$ μM), the activity of compounds was less effective. Compounds **(148)** and **(149)** were active against MCF-7 cells with IC_{50} value of 7.53 and 23.95 μM, respectively. Both the

compounds exhibited apparent differential cytotoxicity against WRL68 cells with IC_{50} value of 175.61 and 159.67 μM, respectively. Both the compounds were effective against MCF-7 but not against WRL68. Further compound **(149)** induces apoptosis in MCF-7, but compound **(148)** failed to induce apoptosis, significantly. Desmethyl-lasiodiplodin **(149)** inhibited growth of MCF-7 cells and resulted in IC_{50} seven times lower than normal cells. It induced the apoptosis and enhanced expression level of caspase 3, c-myc, and p53 [97].

2.3 Compounds Produced by Hyphomycetes

Butyrolactone I **(150)** and aspernolide A **(151)** (Fig. 10) were obtained from an endophytic fungus *Aspergillus terreus* PR-P-2 isolated from the plant *Camellia sinensis* var. *assamica* which was collected in Yunnan, China. Compounds **(150–151)** exhibited moderate cytotoxicity against HL-60 cell line with IC_{50} values of 18.85 and 39.36 μM (IC_{50} value of 5-FU was 2.80 μM), respectively [98].

A new isocoumarin, versicoumarin D **(152)** (Fig. 10), was isolated from the endophytic fungus *Aspergillus versicolor* associated with the rhizome of *Paris marmorata* collected from Dali, Yunnan, China. Compound **(152)** showed high cytotoxicity against A549 and MCF-7 cell with IC_{50} values of 5.8 and 8.0 μM, respectively [99].

An endophyte *Aspergillus terreus* JAS-2 isolated from *Achyranthus aspera* was the source of 4,5-dihydroxy-3-(1-propenyl)-2-cyclopenten-1-one **(153)** (Fig. 10). Compound **(153)** exhibited cytotoxicity against A-549 cell lines with IC_{50} value of 121.9 μg/mL. Its mechanism of action includes the apoptotic-induced cancer cell death [100].

Two new compounds, fumitremorgin D **(154)** and 4,8,10,14-tetramethyl-6-acetoxy-14-[16-acetoxy −19-(20,21-dimethyl)-18-ene]-phenanthrene-1-ene-3,7-dione **(155)**, and fumitremorgin C **(156)**, 12,13-dihydroxyfumitremorgin C **(157)**, verruculogen **(158)** (Fig. 10), and 13-oxoverruculogen **(159)** (Fig. 11) were extracted from *Aspergillus fumigatus*, an endophytic fungus associated with roots or rhizomes of *Diphylleia sinensis* which were collected from Honghegu, Shanxi Province, China. Compounds **(154)** and **(155)** showed poor cytotoxic activity with IC_{50} values of 47.5 and 139.9 μM, respectively, against the HepG2 cell line. Compounds **(157)** and **(158)** showed moderate cytotoxic activity against the HepG2 cell line with IC_{50} values of 4.5 μM and 9.8 μM, respectively. Meanwhile, compounds **(154, 156, 159)**, lacking C-12 and/or C-13 hydroxyls, showed weak activity with IC_{50} values of 47.5, 156.5, and 44.9 μM, respectively, against the HepG2 cell line [101].

Compound 2,14-dihydrox-7-drimen-12,11-olide **(160)** (Fig. 11) was extracted from *Aspergillus glaucus*, an endophytic fungus associated with the leaves of *Ipomoea batatas*. Compound **(160)** exhibits strong cytotoxicity against MCF-7 cells with IC_{50} of 41.7 μg/mL and moderate activity against HepG2 cell with IC_{50} value of 61 μg/mL, respectively [102].

Sphaeropsidin A **(161)**, 4″-dehydro-3-hydroxyterphenyllin **(162)**, 3-hydroxyterphenyllin **(163)**, and 4″-deoxycandidusin A **(164)** (Fig. 11) were isolated

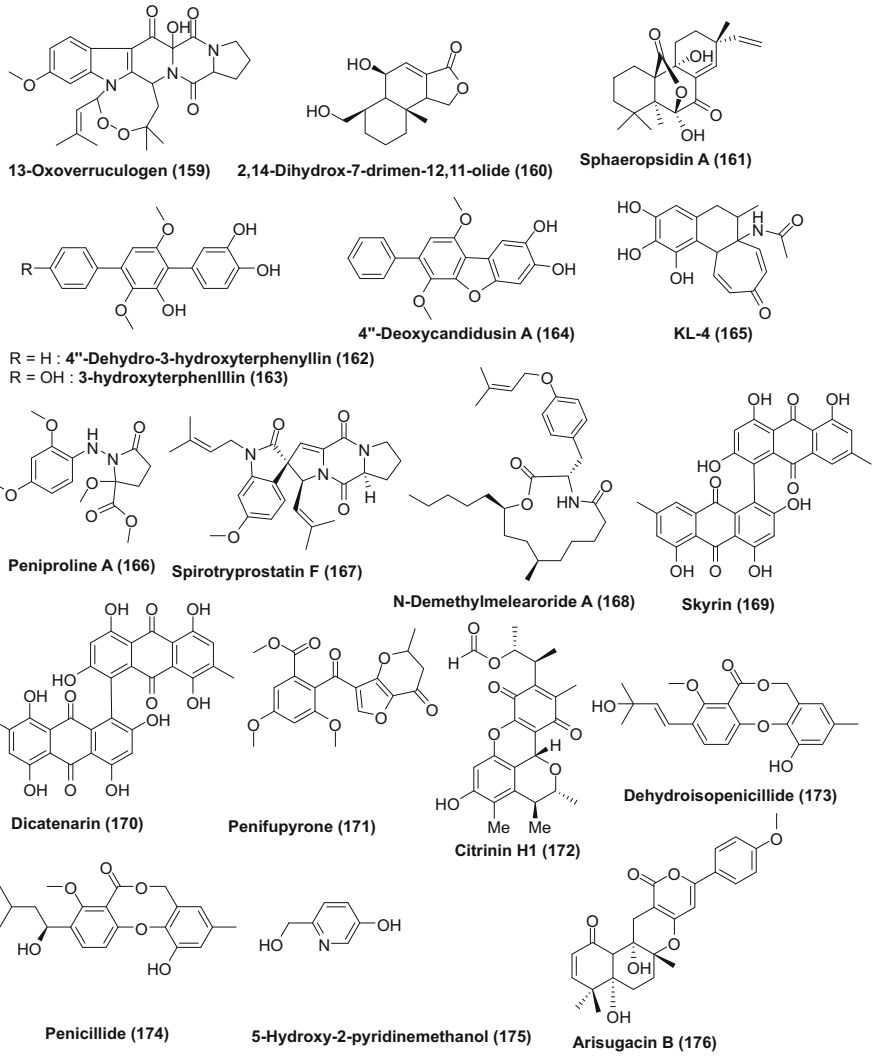


Fig. 11 Structures of metabolites isolated from Hyphomycetes (**159–176**)

from *Aspergillus* sp. (strain no. YXf3), an endophytic fungus from *Ginkgo biloba* collected on the campus of Nanjing University, Nanjing, China. Compound **(161)** was found active with IC_{50} value of 9.03, 10.68, 7.02, and 6.74 μM against KB, SGC-7901, SW1116, and A549 cell lines, respectively, while compounds **(162–164)** were found active against KB, SGC-7901, SW1116, and A549 cell lines with IC_{50} values ranging from 17.28 to 46.64 μM [103].

Compound 6-methyl-1,2,3-trihydroxy-7,8-cyclohepta-9,12-diene11-one-5,6,7,8-tetralene-7-acetamide (KL-4) **(165)** (Fig. 11) was extracted from *Aspergillus* sp., an endophytic fungus isolated from the seeds of *Gloriosa superba* collected from

Tirupati, India. Compound **(165)** showed 23, 70, 35, 43, and 80% growth inhibition in A-549, HEP-2, MCF-7, CV-1, and OVCAR-5 cell line at 100 μg/mL concentration [104].

One new compound, peniproline A **(166)** (Fig. 11), was isolated from endophytic fungus *Penicillium decumbens* CP-4 residing inside the bark of *Cephalotaxus mannii* which was collected from Xishuangbanna in the Yunnan Province, China. The peniproline A **(166)** exhibited cytotoxic activity with IC_{50} values of 8.1 and 15.5 μM, respectively, against Bel-7402 and HeLa cell lines [105].

One new indole-diketopiperazine, spirotryprostatin F **(167)**, and one new 13-membered macrolide, N-demethylmelearoride A **(168)** (Fig. 11), were isolated from *Penicillium brefeldianum*, an endophytic fungus, residing inside the rhizome of *Pinellia ternata* collected from suburb of Nanjing, Jiangsu Province, China. Compound **(167)** showed cytotoxic activity with IC_{50} values of 14.1 μM and 35.5 μM, respectively, against HepG2 and MDA-MB-231 cell lines. Compound **(168)** showed moderate activity against HepG2 cells with IC_{50} value of 36.6 μM. The positive control cisplatin showed cytotoxic activity against MDA-MB-231 and HepG24 cell lines with IC_{50} values of 11.3 and 14.4 μM, respectively. Another positive control doxorubicin showed cytotoxic activity against MDA-MB-231 and HepG24 cell lines with IC_{50} values of 1.0 and 3.0 μM, respectively [106].

Two anthraquinone compounds, skyrin **(169)** and dicatenarin **(170)** (Fig. 11), were obtained from *Penicillium pinophilum* MRCJ-326, an endophytic fungus strain residing inside *Allium schoenoprasum*, which was collected from Nathatop, J&K, India. Dicatenarin **(170)** and skyrin (**169**) showed good cytotoxic activity with IC_{50} values of 12 μg/mL and 27 μg/mL, respectively, against MIA PaCa-2 cell line. Compounds (**169**) and (**170**) induce apoptosis in cells via regulation of intracellular reactive oxygen species production, eventually disrupting mitochondrial transmembrane potential and enhanced caspase-3 apoptotic proteins in MIA PaCa-2 cells. Dicatenarin **(170)** showed higher efficacy than skyrin (**169**) due to additional phenolic hydroxyl group at C-4 which enhanced oxidative ROS generation [107].

A new funicone derivative, penifupyrone **(171)** (Fig. 11), was extracted from *Penicillium* sp. HSZ-43, an endophytic fungus associated with the leaves of *Tripterygium wilfordii*, collected from Shanxi Province, China. Compound (**171**) showed moderate cytotoxicity with IC_{50} value of 4.7 μM against KB cells [108].

Citrinin H1 (**172**), dehydroisopenicillide (**173**), penicillide (**174**), and 5-hydroxy-2-pyridinemethanol (**175**) (Fig. 11) were isolated from *Penicillium* sp., an endophytic fungus, associated with the leaf of *Paris polyphylla* collected from suburbs of Nanjing, Jiangsu Province, China. Compounds (**172–175**) showed inhibitory activity to HepG2 cell line with IC_{50} at 8.5, 12.5, 15.0, and 18.2 μg/mL [109].

Compounds arisugacin B (**176**) (Fig. 11) and arisugacin F (**177**) (Fig. 12) were isolated from the endophytic fungus *Penicillium* sp. SXH-65 associated with the leaves of *Tamarix chinensis* collected from the coast of Laizhou Bay in Dongying, China. Compounds (**176–177**) exhibited weak cytotoxic activity with IC_{50} values ranging from 24 to 60 μM against HeLa, HL-60, and K562 cell lines [110].

Two strains of endophytic fungi, *Penicillium melinii* Yuan-25 and *Penicillium janthinellum* Yuan-27, were isolated from the roots of *Panax ginseng* which was

collected in Changchun, Jilin Province, China. A new benzaldehyde derivative, ginsenocin (**178**), along with a known compound, penicillic acid (**179**) (Fig. 12), was isolated from Yuan-25 culture. The brefeldin A (**180**) was isolated from the Yuan-27 culture. Brefeldin A (**180**) exhibited cytotoxicity against MKN45, LOVO, A549, MDA-MB-435, HepG2, and HL-60 cells with IC_{50} values <0.12 μg/mL,

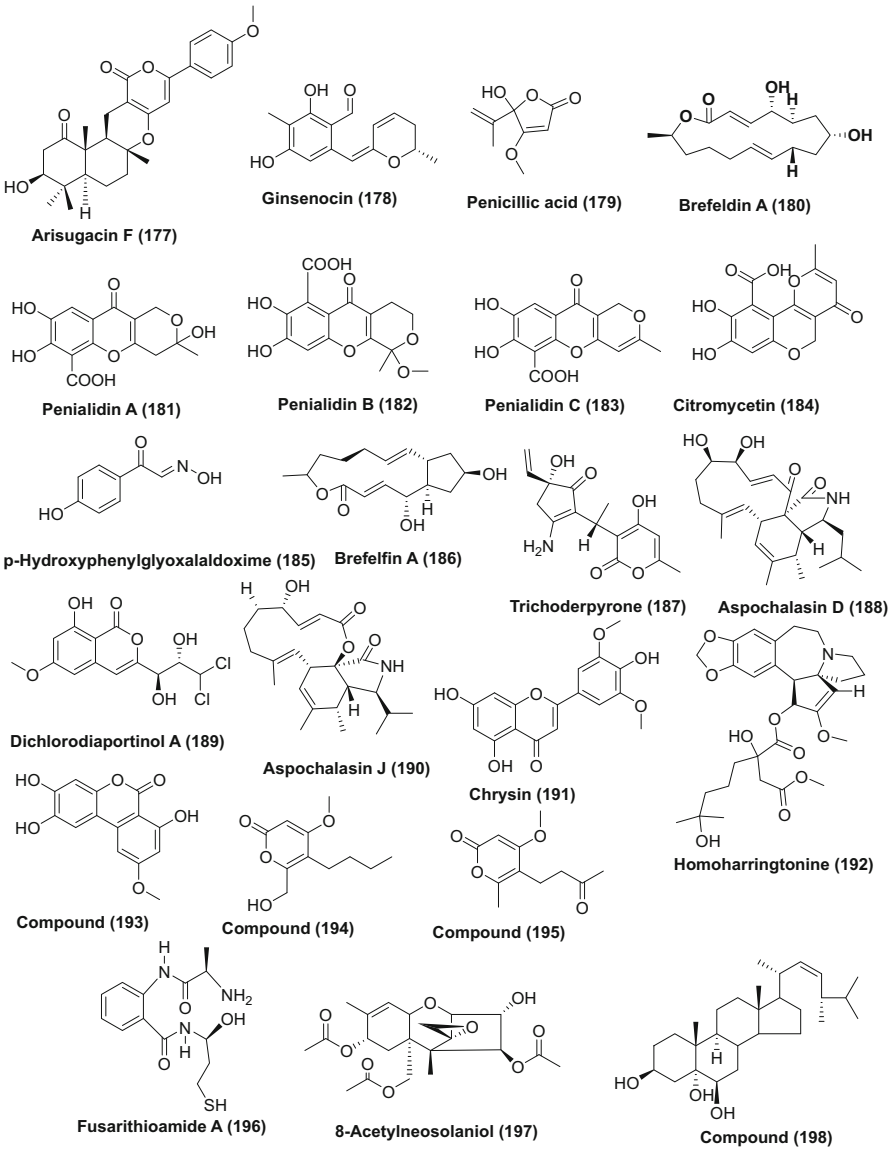


Fig. 12 Structures of metabolites isolated from Hyphomycetes (**177**–**198**)

while ginsenocin (**178**) and penicillic acid (**179**) exhibited potent cytotoxicity with IC_{50} values ranging from 0.49 to 7.46 μg/mL [111].

The endophytic fungus *Penicillium* sp., isolated from the leaves of *Garcinia nobilis* collected in Mount Etinde, Southwest Region, Cameroon, was the source of penialidins A–C (**181–183**), citromycetin (**184**), p-hydroxyphenylglyoxalaldoxime (**185**), and brefelfin A (**186**) (Fig. 11). Compounds (**181–186**) exhibited cytotoxicity against HeLa cells with LC50 values in the range of 0.88–9.21 μg/mL [112].

The compound trichoderpyrone **(187)** (Fig. 12), a unique polyketide with a cyclopentenone-pyrone hybrid skeleton, was extracted from *Trichoderma gamsii*, an endophytic fungus isolated from *Panax notoginseng*. Trichoderpyrone **(187)** exhibited cytotoxicity against A549, HepG2, and HeLa cell lines with IC_{50} values of 16.9, 30.8, and 33.9 μM, respectively, while positive control etoposide exhibited cytotoxic activity with IC_{50} values of 16.6, 16.1, and 15.0 μM, respectively [113].

Aspochalasin D **(188)** (Fig. 12) was isolated from the endophytic fungus *Trichoderma gamsii* which was isolated from the traditional Chinese medicinal plant *Panax notoginseng*. Compound **(188)** displayed moderate inhibitory activity against HeLa cells with an IC_{50} value of 5.72 μM [114].

Compound dichlorodiaportinol A **(189)** (Fig. 12), a new chlorine-containing isocoumarin, was extracted from *Trichoderma* sp. 09, an endophytic fungus associated with the root of *Myoporum bontioides* collected from Guangdong Province, China. Compound **(189)** exhibited cytotoxicity against MCF-7 and HepG2 cell lines, with IC_{50} values of 17.8 and 39.6 μg/mL, respectively [115].

The endophytic fungus *Trichoderma gamsii* inhabiting Chinese medicinal plant *Panax notoginseng* was the source of cytochalasan aspochalasin J **(190)** (Fig. 12). Compound **(190)** showed weak inhibitory activity with IC_{50} value 27.8 μM (Ding et al. 2012) [116].

Endophytic fungus *Alternaria alternata* KT380662, of *Passiflora incarnata* collected from Tiruchirappalli, Tamil Nadu, India, was the source of chrysin (5,7-dihydroxy flavone, ChR) (**191**) (Fig. 12). It is reported that chrysin **(191)**-treated HepG2 cells lose their viability in a time- and dose-dependent manner. Formation of condensed nuclei, membrane, blebbing, and apoptotic bodies clearly indicated that chrysin triggers immediate cellular responses and induces apoptotic cell death against HepG2 cells [117].

Endophytic fungus *Alternaria tenuissima* CH1307 associated with *Cephalotaxus hainanensis* collected from Hainan Province, China, and local national parks in Thailand was the source of homoharringtonine **(193)** (Fig. 12). The extract of the fermented broth of CH1307 showed antiproliferative activities against K562, NB4, and HL-60 cancer cell lines with IC_{50} values of 67.25, 65.02, and 99.23 μg/mL, respectively [118].

Compound 3,4′,5′-trihydroxy-5-methoxy-6H-benzo[c]chromen-6-one **(193)** (Fig. 12) and altersolanol A **(125)** (Fig. 8) were isolated from *Alternaria* species G7, an endophytic fungus residing inside the leaves of *Broussonetia papyrifera* collected from Nanjing, Jiangsu Province, China. Compound **(193)** showed potent cytotoxic activity with IC_{50} values of 1.47, 2.11, and 7.34 μg/mL, respectively, against A549, MG-63, and SMMC-7721 cell lines. Compound **(125)** exhibited good

cytotoxic activities against MG-63 and SMMC-7721 cell lines with IC_{50} values of 0.53 and 2.92 μg/mL [119].

Two new compounds, 5-butyl-6-(hydroxymethyl)-4-methoxy-2H-pyran-2-one **(194)** and 4-methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one **(195)** (Fig. 12), were isolated from *Alternaria phragmospora*, an endophytic fungus residing inside the leaves of *Vinca rosea*, collected in Cairo, Egypt. Compounds **(194** and **195)** were found active against HL-60 cells with IC_{50} values of 2.2 and 0.9 μM and against K562 cells with IC_{50} values of 4.5 and 1.5 μM, respectively [120].

Altersolanol A **(125)**, a hydroxylated tetrahydroanthraquinone extracted from *Alternaria* sp., an endophytic fungus associated with the leaves of *Erythrina variegata* collected in Samut Sakorn Province, Thailand**,** exhibited potent antiangiogenic activity by suppressing all functions of endothelial cells, proliferation, tube formation, and migration. It was found that altersolanol A inhibits blood vessel formation in both ex vivo and in vivo assays at low concentration [121].

A new benzamide derivative, fusarithioamide A **(196)**, and known compounds 8-acetylneosolaniol **(197)** and ergosta-7,22-diene-3β,5α,6β-triol **(198)** (Fig. 12), obtained from *Fusarium chlamydosporium* isolated from the leaves of *Anvillea garcinii* collected from the campus of Al-Azhar University, Egypt, possessed potent and selective activity toward BT-549, SKOV-3, SK-MEL, and KB cell lines with IC_{50} values of 0.4, 0.8, 9.3, and 7.7 μM, respectively, compared to doxorubicin (IC_{50} 0.046, 0.313, 0.171, and 0.027 μM, respectively). Compound **(198)** exhibited significant activity with IC_{50} values of 1.7, 1.9, 1.4, and 1.1 μM, respectively, toward SK-MEL, KB, BT-549, and SKOV-3 cell lines, respectively. However, **(197)** showed activity toward SK-MEL, KB, BT-549, and SKOV-3 cell lines with IC_{50} values of 14.0, 1.68, 9.6, and 1.40 μM, respectively [122].

Compounds (R)-3,4-dihydro-4,8-dihydroxy-6-methoxy-4,5-dimethyl-3-methyleneisochromen-1-one **(199)** and 8-O-methyljavanicin **(200)** (Fig. 13) were derived from the endophytic fungus *Fusarium* sp. PDB51F5. Compound **(199)** was weakly active against KB and NCI-H137 cell lines with IC_{50} values of 160 and 162 μM, respectively. Compound **(200)** exhibited weak cytotoxicity against MCF-7 cell lines with an IC_{50} value of 148 μM. Positive control doxorubicine exhibited cytotoxicity against KB, MCF-7, and NCI-H137 cell lines with IC_{50} values of 0.35, 2.33, and 0.14 μM, respectively [123].

A new glucitol, diglucotol **(201)**, together with known compounds cerevisterol **(202)** and ergosterol peroxide **(203)** (Fig. 13), was isolated from *Fusarium equiseti* (Salicorn 8), an endophytic associated with *Salicornia bigelovii* collected from a salt lake in Xinjiang, China. Compound **(201)** displayed mild activity against MCF-7, MDA-MB-231, and Caco-2 cancer cells with EC_{50} values of 97.56, 92.35, and 99.39 μM, respectively, whereas compound **(202)** exhibited high activity toward MCF-7, MDA-MB-231, and Caco-2 cancer cells with EC_{50} values of 32.4, 41.5, and 37.56 μM, respectively. Compound **(203)** exhibited less potent inhibitory activities than **(202)** against MCF-7, MDA-MB-231, and Caco-2 cancer cells with EC_{50} values of 64.5, 52.4, and 77.56 μM, respectively [124].

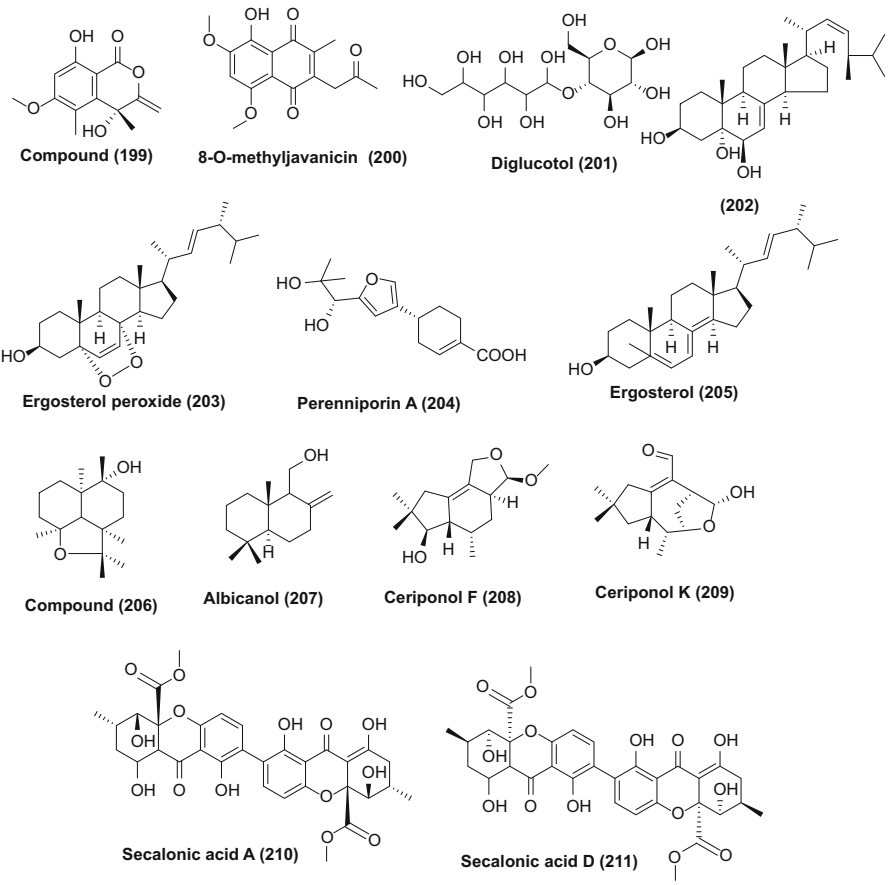


Fig. 13 Structures of metabolites isolated from Hyphomycetes (**199–203**), Basidiomycetes (**204–209**), and unidentified fungi (**210–211**)

2.4 Compounds Produced by Basidiomycetes

The endophytic fungus *Perenniporia tephropora* Z41, associated with *Taxus chinensis* var. *mairei*, collected in Jingning, Zhejiang Province, China, was the source of a new sesquiterpenoid, perenniporin A **(204)**, along with three known compounds, ergosterol **(205)**, rel-(+)-(2aR,5R,5aR,8S,8aS,8bR)-decahydro-2,2,5,8-tetramethyl-2H-naphtho [1,8-bc]genfuran-5-ol **(206)**, and albicanol **(207)** (Fig. 13). Compound **(205)** exhibited potent activity with IC_{50} values of 1.16, 11.63, and 11.80 μg/mL, against HeLa, SMMC-7721, and PANC-1 cells, respectively. Compounds **(204, 206, 207)** exhibited moderate cytotoxicity with IC_{50} values in the range of 6 to 58 μg/mL [125].

Two new tremulane sesquiterpenes ceriponols F **(208)** and K **(209)** (Fig. 13) were isolated from *Ceriporia lacerate*, a fungal endophyte residing in the stems of the

medicinal plant *Huperzia serrata* collected in Pan-An County, Zhejiang Province, China. Compounds **(208)** and **(209)** exhibited moderate cytotoxicity against HeLa, HepG2, and SGC 7901 cell lines with IC_{50} values ranging from 32.3 to 173.2 μM, respectively [126].

2.5 Compounds Produced by Unidentified Fungus

The fungal strain, 2 L, associated with *Ocimum basilicum* collected from Dhaka was the source of secalonic acid A **(210)** and secalonic acid D **(211)** (Fig. 13). Compounds **(210)** and **(211)** exhibited significant anti-pancreatic cancer activity with IC_{50} values of 7.3 and 1.6 μM, respectively [127].

3 Methods Used for the Activation of Silent Biosynthetic Genes

The production of different bioactive compounds by microorganisms occurs only under specific conditions, because of which, it often becomes difficult to detect them upon culturing them on standard laboratory media. This might be due to underexpression of corresponding biosynthetic genes for such "cryptic" or "orphan" pathways [128]. Efforts to manipulate nutritional or environmental factors enhanced the secondary metabolite biosynthesis leading to the identification of new natural products. Some of the approaches used are based on the modification of media composition, aeration, temperature, shape of culturing flask, application of stress conditions, and UV mutagenesis [129, 130]. It has been observed that interaction between organisms inhabiting the same or different species enhances the production of vast diversity of natural products. Hence, co-culture of two or more different microbes in a laboratory scale induces the cascade of genes responsible for biosynthesis that normally are masked under optimum culture parameters [131]. Also, epigenetic modifications by treating microbes with epigenetic modifiers improve the variation and diversity of produced metabolites [132].

4 The Co-culture Strategy

Optimization of media components and culture parameters of fungi have shown promising results for the isolation of various bioactive compounds. Co-cultivation has appeared as effective strategy where two or microbes are cultured together to induce production of unexplored bioactive microbial compounds [133, 134]. In co-cultivation strategy, the natural ecological niche is imitated, where different microbes coexist forming a complex network thus competing for the same resources within confined space. Microbes are challenged to compete under mimicked environment in the co-culture strategy in the laboratory, hoping the induction of those silent gene clusters which generally do not express under normal laboratory

conditions. It is expected that the under stress condition these silent genes get expressed leading to production of novel bioactive metabolites [135].

Co-cultivation of two mangrove-derived endophytic fungi led to production of the new alkaloids marinamide and marinamide methylether [136]. These compounds showed antiproliferative effect against HepG2, 95-D, MGC832, and HeLa cells [137]. In a similar way when *Libertella* sp., a marine-derived fungus, and *Thalassopia* sp., a bacterium, were cultured together, it produced novel diterpenoids, libertellenones A–D, which showed activity against HCT-116 cells displaying IC_{50} in range of 0.76 and 53 μM [138]. Glionitrin A, a novel diketopiperazine disulfide, was obtained from a mixed fermentation of a marine fungus *Aspergillus fumigatus* with bacterium *Sphingomonas* sp. of marine origin. Identitifed compound displayed cytotoxicity against HCT-116, A549, AGS, and DU145 cells with IC_{50} values of 0.82, 0.55, 0.45, and 0.24 μM, respectively [139]. An endophytic fungus *Paraconiothyrium* sp., obtained from the wood of *Taxus x media*, when subjected to co-cultivation with *Alternaria* sp. and *Phomopsis* sp. increase 2.7 and 3.8-fold production of paclitaxel with respect to axenically grown *Paraconiothyrium* sp. When all three fungi were cultured together, the production of paclitaxel was enhanced to 7.8-fold [140]. Li and Tao [141] reported that co-cultivation of cell suspension cultures of *Taxus cuspidate* with endophytic fungus *Fusarium mairei* produced paclitaxel of 12.8 mg/l (>two-fold higher) than endophytic fungal culture broth added to cell suspension culture of *T. cuspidata* which produced 6.11 mg/l. These results suggest that co-cultivation is an alternative strategy to increase the chemical diversity of metabolites together with enhancing the yield of previously identified metabolites produced by microbes during fermentation [142].

5 Epigenetic Modification

In recent times studies pertaining to genome mining have depicted the crucial role of various gene clusters responsible for secondary metabolism (dependent on non-ribosomal peptides (NRPS)-encoding genes and conserved polyketides) due to existence of cryptic gene clusters that persist silently under in vitro condition [143]. Studies have shown that activation of these silent gene clusters will open new avenues of research and might lead to production of novel unexplored biomolecules with potent biological activity. Overexpression of transcriptional factors regulating the expression of silent genes remains primary target for activating these silent gene clusters for generation of bioactive compounds. Activated transcriptional factor leads to enhanced expression of whole gene clusters driving the expression of even those genes which encode for products that hamper the production of few compounds. In *Aspergillus nidulans*, the overexpression of transcriptional factor gene *apdR* activated transcription of all genes in the cluster and led to discovery of aspyridones that have never been identified before from *A. nidulans*. This compound showed a moderate cytotoxicity [143].

In the heterochromatic region of filamentous fungi, many transcriptionally inactive silent gene clusters are located [144]. Histone modification is another method which

implies use of histone inhibitors, histone deacetylases (HDACs), or DNA methyltransferases (DMATs) to activate gene clusters which were previously silenced. HDACs target the functional group attached with histone, i.e., acetyl group from amino tails, and maintain the chromatin in an inaccessible state for the transcriptional machinery [145]. Suberoylanilide hydroxamic acid (SAHA), a HDAC inhibitor, stimulated the production of new cladochromes and calphostin B in *Cladosporium cladosporioides* [146]. When *Aspergillus niger* culture was supplemented with SAHA, the production of nygerone A was enhanced [147]. In another study when the fungus *C. cladosporioides* was grown in the presence of DMAT inhibitor 5-azacytidine, it induced the silent gene clusters which led to identification of various oxylipins and of two new polyketides, lunalides A and B by a *Diatrype* sp. [146].

6 Conclusions

Fungal endophytes are the novel source of compounds with a bland of anticancer properties. These endophytes provide structurally diverse heterocyclic scaffolds such as benzofuran (**15**), xanthone (**25**–**27**), chroman (**81**), glycoside (**8**), and steroids (**197, 201, 202**) along with stereospecific compounds such as cytochalasin Q (**59**), trichalasins (**53**–**55**), chaetoglobosins (**63**–**66**), and isocochlioquinones (**128, 129**). Chemical diversity is the prerequisite for bioactive compounds displaying similar activity via different modes and mechanisms of action. The identified fungal metabolites have shown anticancer activity through different mechanisms of action such as apoptotic cell death (sclerotiorin (**80**), rosoloactone **(123)**), inhibition of kinase proteins involved in signal transduction pathways (allantopyrone A **(46)**), and inhibition of histone deacetylase (**114, 115**). Although many reported fungal metabolites exhibited moderate cytotoxic activities, the rational derivatization of these metabolites and their high-throughput anticancer screening may lead to the molecules with better anticancer activity against a broad range of cancer cell lines. In addition, the metabolites with promising anticancer activity should be investigated systematically to establish their mechanism of action. This will help researchers to select the most appropriate metabolite for anticancer drug development.

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Endophytes as a Source of High-Value, Bioactive Metabolites

15

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Contents

Abstract

Endophytes, microbes that reside within plants, are capable of producing high-value bioactive metabolites with diverse biological activities such as antimicrobial, insecticidal, antidiabetic, antioxidant, anticancer, etc. Endophytes thus represent a subset of microbes that reside in unique niches and, if explored

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properly, may prove to be a reservoir of bioactive principles. Despite this, less than 5% of total plant diversity has been screened for its endophyte content. Moreover, detailed examination of natural products and their bioactivities have been carried out for even lesser number of endophytes. Further, genome sequencing of several microbes has revealed that the potential of microbes to produce secondary metabolites has been substantially underestimated because many of the secondary metabolite biosynthetic gene clusters are silent under standard laboratory growth conditions. This chapter provides an overview of microbial natural products that have been isolated from endophytes and discusses the above issues and possible mitigation strategies.

Keywords
Anticancer · Antidiabetic · Antimicrobial · Antioxidant · BGC · Bioactivity · Biosynthetic gene cluster · Cryptic gene cluster · Silent gene cluster · Secondary metabolite

1 Introduction

Natural products have been used for treatment of human diseases since time immemorial [1]. A large number of plants, microbes, marine or freshwater organisms, etc. have been explored for medicinal natural products [2]. Microbes' contribution to medicine has been prodigious. The serendipitous discovery of penicillin promoted the screening of microorganisms for new-generation novel drugs with antimicrobial and anticancer properties. Recently, antibiotic resistance in microbes has created a menacing state, and the necessity for new antibiotics is understandable [3]. It is now well established that discovery of bioactive metabolites with a novel chemical skeleton is much more efficient from natural resources as compared to combinatorial chemistry-based screens. Lately, pharmaceutical companies have been reducing the resources for combinatorial chemistry-based screens and are opting for natural product-based skeletons in their discovery programs [4]. This demands that new natural sources and niches should be continuously explored for novel bioactive metabolites.

De Bary (1866) introduced the term endophyte, as "an organism that lives inside the plant tissues" [5]. Endophytes, both bacteria and fungi, have been reported from several plant organs such as seeds, leaves, fruits, stem, and roots, as well as from the dead and hollow tissues of plants [6–8]. Approximately 300,000 plant species have been catalogued, and each may likely harbor one or more endophytes. However, endophytic biology of few plants has been completely studied [9]. Detailed natural product chemistry investigation and bioactivity characterization have been carried out on even lesser number of endophytic microbes. Thus, endophytes represent a microbial resource which is yet to be completely tapped for new bioactives.

Endophytes are cosmopolitan and show interactions like mutualism, and at times antagonism, with their host plants [10]. Mutualistic benefits bestowed to the plants include promotion of host growth, strengthening the resistance of plants to insect

pests [11] and pathogenic microbes, as well as providing protection from abiotic stresses (drought, high temperature, salinity, heavy metals, etc.) [12–15]. Extensive studies indicate that endophytes comprise a large variety of microorganisms including fungi, bacteria, and viruses [7, 16]. Most bacterial endophytes belong to the phyla *Proteobacteria*, *Firmicutes*, and *Actinobacteria* [17] and may be either gram-negative or gram-positive [18]. Previously, endophytic fungi were divided into clavicipitaceous (limited to grasses) and non-clavicipitaceous endophytes (associated with all land plants including ferns, conifers, and angiosperms) based on phylogeny and life history traits. Rodriguez et al. (2009) referred clavicipitaceous fungi as class 1 endophytes and further categorized non-clavicipitaceous endophytes in three different classes on the basis of host range, transmission, tissue colonized, the degree of colonization (*in planta*), *in planta* biodiversity, and types of fitness benefits imparted to the plant [19]. Class 2 endophytes may colonize both plant roots and shoots, whereas class 3 endophytes grow within the stem, and class 4 endophytes are restricted to the roots. Mostert et al. (2000) gave the concept of "true endophytes" or systemic endophytes, which coevolved with their host and generally do not exhibit much diversity [20]. They mostly reside within the host plants and do not show any symptoms of disease. In 1991, Petrini defined non-systemic endophytes as "the organisms that reside within the host for atleast a part of their lifecycle" [21]. Under adverse environmental conditions, they may become pathogenic. Non-systemic endophytes are transmitted only by horizontal means, while systemic endophytes mostly show vertical transmission via seeds or vegetative propagules but may rarely also exhibit horizontal transmission [22, 23].

Endophytes have been shown to be capable of producing various bioactive compounds of biotechnological application [11, 24, 25]. Several studies have also reported the production of host plant secondary metabolites by endophytes [26]. However, on repeated subculturing in an artificial medium, endophytes tend to lose the ability to produce host plant metabolites. This process is called "attenuation." For instance, *Fusarium proliferatum* isolated from *Dysoxylum binectariferum* was shown to produce rohitukine only for 2–3 generations after which it underwent attenuation [27]. Similarly, attenuation was observed in camptothecin-producing endophyte isolated from *Camptotheca acuminata* [28]. The molecular machinery used for the production of phytochemicals may be acquired from the host and may be lost outside the host when the endophyte is cultured in artificial media [29]. However, the secondary metabolites that are not phytochemicals, but compounds produced by the intrinsic biosynthetic pathways encoded in the genomes of endophytic microbes, continue to be produced by them for any number of generations (Fig. 1). Such metabolites are a unique reservoir for a natural product-driven modern drug discovery.

In this chapter, we will (a) cite examples of bioactive or high-value natural products produced by endophytes, (b) briefly outline the methodology for bioactivity screening, (c) discuss strategies for discovery of metabolic gene clusters and possible methods for their induction, as well as (d) provide future perspectives for greater exploitation of this resource for new natural products.

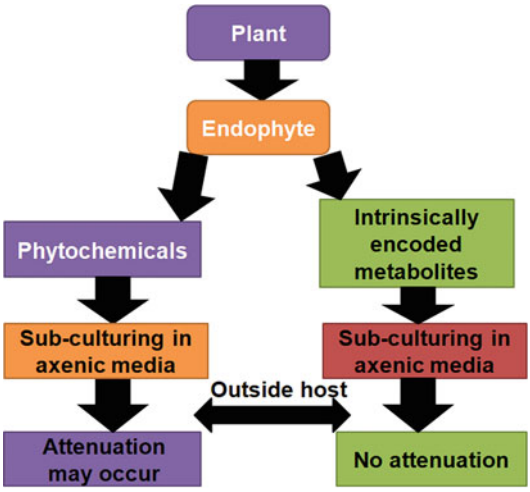


Fig. 1 Metabolic response of endophytic microbes on culturing in axenic media

2 Bioactive Metabolites Produced by Endophytes

Endophytes have now been acknowledged as synthesizers of diverse natural metabolites endowed with varied biological activities [30]. In terms of chemical properties, these metabolites may be alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, isocoumarins, chromones, etc. Several of them exhibit antifungal, antibacterial, anticancer, antidiabetic, or immunomodulatory activities [9, 11, 31–34].

2.1 Natural Products with Antimicrobial Activity

Xiamycin produced by *Streptomyces* sp. possesses anti-HIV activity [35]. Phomopsichalasin produced by *Phomopsis* sp. possesses antifungal and antibacterial activity [36]. A phenolic compound, colletotric acid extracted from liquid culture of endophytic fungus *Colletotrichum gloeosporioides* isolated from the stem of *Artemisia mongolica*, exhibited antifungal and antibacterial activity [37]. Endophytic fungus *Cryptosporiopsis cf. quercina*, isolated from the inner bark of *Tripterygium wilfordii*, was shown to produce cryptocin that inhibits the growth of *Pyricularia oryzae* and other phytopathogens [38]. It also yields cryptocandin, a potent antimycotic compound which displayed activity against phytopathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea* [39]. Antifungal compounds, epoxycytochalasin H, cytochalasin N, and cytochalasin H, were isolated from endophytic fungus *Phomopsis* sp. which resided within *Gossypium*

hirsutum. These compounds were effective against plant pathogens such as *Sclerotinia sclerotiorum*, *Bipolaris maydis*, *Fusarium oxysporum*, *Botrytis cinerea*, *Bipolaris sorokiniana*, *Gaeumannomyces graminis var. tritici*, *and Rhizoctonia cerealis* [40]. Gliotoxin, a sulfur-containing metabolite that displays immunosuppressive and antimicrobial activities, was isolated from *Chaetomium globosum* an endophytic fungus of *Ginkgo biloba* [40]. Steroidal compounds extracted from the liquid culture of *Colletotrichum* sp., an endophytic fungus from *Artemisia annua*, were shown to have antifungal activity against *Phytophthora capsici*, *Rhizoctonia cerealis*, *Helminthosporium sativum*, and *Gaeumannomyces graminis var. tritici* [41]. Cerevisterol, a metabolite which shows antibacterial and antifungal activity, was isolated from *Alternaria brassicicola* an endophytic fungus of *Malus halliana* [42]. Two sesquiterpenes, phaseolinone and phomenone extracted from *Xylaria* sp., isolated from the leaves of *Piper aduncum* showed antifungal activity against *Cladosporium cladosporioides* and *C. sphaerospermum* [43]. Chokols A-G, isolated from an endophytic fungus *Epichloe typhina* associated with *Phleum pratense,* were shown to be fungitoxic to the leaf spot disease-causing pathogen *Cladosporium phlei* [44]. Volatile organic compounds (VOCs) having effective fumigant property isolated from *Muscodor albus* an endophytic fungus *Cinnamomum zeylanicum*, was found to be useful for preserving the fruits and vegetables during storage. The antifungal activity of the VOCs was found to be due to synergistic effect of the components that comprised of esters and other compounds like alcohols, ketones, lipids, and organic acids [45, 46].

2.2 Natural Products with Anticancer Activity

Maytansinoids, which are 19-membered macrocyclic lactams related to ansamycin antibiotics that are exceptionally potent antitumor agents, were isolated from an endophytic actinomycete [47–49]. Lupinacidins, 6-alkylsalicylic acids, and salaceyins A and B were reported from endophytic actinomycetes. Lupinacidins showed cytotoxicity against murine colon cancer cell line, whereas the other two compounds exhibited activity against human breast cancer cell line [50, 51]. Similarly, pterocidin reported from *Streptomyces hygroscopicus* isolated from *Pteridium aquilinum* showed cytotoxicity against human cancer cell lines NCI-H522, OVCAR-3, SF539, and LOX-IMVI [52]. Naphthomycin A, another compound extracted from *Streptomyces* sp. isolated from *Maytenus hookeri*, was found to be cytotoxic against P388 and A549 human tumor cells [53, 54]. Endophytic bacteria *Bacillus licheniformis*, *B. pseudomycoides*, and *Paenibacillus dendritiformis* produced L-asparaginase efficiently [55]. Introduction of L-asparaginase in multidrug chemotherapy regime helped in the improvement of health of the patients affected with acute lymphoblastic leukemia [56]. Non-sporulating fungus isolated from *Knightia excelsa*, native to New Zealand, produced spiro-mamakine A, an analogue of spirobisnaphthalene which had cytotoxic potential against murine leukemia cell line [57]. Recently, an

endophytic fungus *Pestalotiopsis microspora* isolated from fresh fruits of the mangrove plant *Drepanocarpus lunatus* was shown to produce seven new 14-membered macrolides: pestalotioprolides C–H and 7-*O*-methylnigrosporolide. Pestalotioprolide E exhibited cytotoxicity against human ovarian cancer cell line, whereas 7-*O*-methylnigrosporolide and pestalotioprolides D-F indicated activity against murine lymphoma cell line [58]. Four bioactive compounds, mycoepoxydiene, altiloxin A, enamidin, and eremofortin F, were extracted from endophytic fungus *Diaporthe* sp. associated with the medicinal plant *Sabicea cinerea*. Eremofortin F exhibited cytotoxic effect against KB and MRC5 cells, while mycoepoxydiene showed remarkable activity against KB, MDA-MB-435, and MRC5 cancer cell lines [59]. The endophytic fungus *Pestalotiopsis microspora*, which was obtained from the *Torreya taxifolia* collected from Northern Florida, produced torreyanic acid. Torreyanic acid caused cell death by apoptosis and was found to be more effective toward cell lines sensitive to protein kinase C agonists and 12-O-tetradecanoylphorbol-13-acetate [60].

2.3 Natural Products with Antioxidant Activity

Natural antioxidants also known as scavengers, which provide protection from harmful free radicals, are commonly found in medicinal plants, vegetables, and fruits. Endophytes are also a promising source of natural antioxidant molecules [61]. To exemplify, an endophyte *Xylaria* sp. from *Ginkgo biloba* displayed antioxidant activity [61]. Two compounds, namely, pestacin and isopestacin, with antioxidant activity were extracted from the culture fluid of endophytic fungus *Pestalotiopsis microspora* [62, 63]. Interestingly, pestacin manifests greater antioxidant activity as compared to trolox, a vitamin E derivative [62]. Antioxidant compounds, 2,6-dimethoxy terephthalic acid and yangjinhualine A, were extracted from endophytic *Streptomyces* sp. isolated from the plant *Alpinia oxyphylla* [64]. Another endophytic actinomycete isolated *from Catharanthus roseus* possesses antioxidant activity [65].

2.4 Natural Products with Antidiabetic Activity

Endophytes have also been explored for their antidiabetic activity. Non-peptidal fungal metabolite (L-783,281), extracted from an endophytic fungus *Pseudomassaria* sp., was tested on two mouse models. The compound was shown to lower blood glucose level and was orally active, unlike insulin [66]. *Microbispora* sp. produced β-carbolines and indoles, which inhibit α-glucosidase, a target for antidiabetes therapy [67, 68]. α-Glucosidase inhibitor was also reported from other endophytic actinomycetes species, which were extracted from the roots of *Caesalpinia sappan*. *Streptomyces olivochromogenes* and another endophytic *Streptomyces* sp. derived from *Datura stramonium* also displayed significant antidiabetic potential [69, 70].

2.5 Natural Products with Insecticidal Activity

Use of endophyte derived compounds with pesticidal potential is gaining attention as it would help in reducing the load of synthetic pesticides. In such instances endophytes provide an eco-friendly option for production of powerful and selective insecticides and pesticides. An endophyte *Nodulisporium* sp. produced nodulisporic acids which are novel indole diterpenes that display significant insecticidal properties against the larvae of bowl fly, by activating insect glutamate-gated chloride channel [71]. Endophytic fungus *Phomopsis oblonga* was reported to display potent insecticidal activity against the beetle *Physocnemum brevilinenu* found on elm tree [72]. The endophytic fungus, *Muscodor vitigenus*, collected from liana plant (*Paullina paullinioides*), produced naphthalene as a major product. Being an active ingredient against common mothballs, it also showed a promising preliminary result as an insect deterrent and revealed its potency as insect repellent against wheat stem sawfly (*Cephus cinctus*) [73].

3 Screening of Bioactive Metabolites

3.1 Brief Outline for Isolation

The endophyte may be grown in different media and under various culture conditions as such factors may alter its metabolome [74]. Pure endophytic cultures or their extracts are generally subjected to preliminary simple and quick assays for identification of potential bioactivities. The cultures that are found to be positive in preliminary screens may then be taken up for isolation of pure molecules. Identification of new bioactive compounds from endophyte usually starts with scale up of fermentation process for production of large biomass. This is followed by the preparation of extracts and isolation of pure natural product molecules using various chromatographic techniques. The extracts or pure natural products may be screened against a panel of pharmacological targets to identify the potential bioactivity (Figs. 2, 3). In the case of extracts, this is followed by activity-guided fractionation and isolation of pure natural products [75]. Chemical structures of these natural products are then elucidated using various analytical chemistry and spectroscopy techniques. Promising scaffolds are then taken up in a typical medicinal chemistry program for improvement of pharmacological parameters.

3.2 Screening for Antimicrobial Activity

Antimicrobial activity may be performed using different protocols like diffusion methods (agar disk diffusion, agar well, agar plug, cross-streak, and poisoned food method), dilution methods (agar dilution, broth dilution), flow cytofluorometric and ATP bioluminescence assays, etc. [76]. Diffusion method, being simple and swift, is

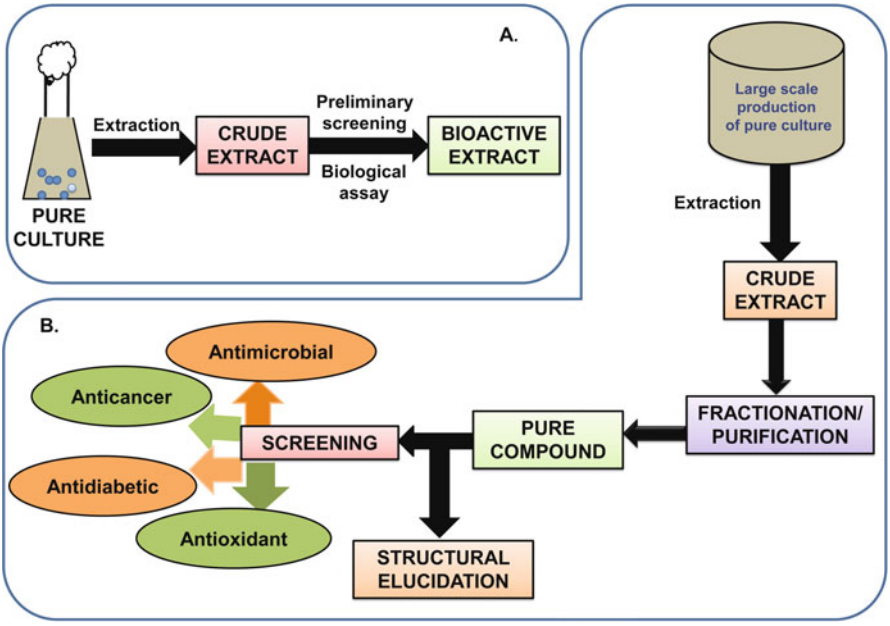
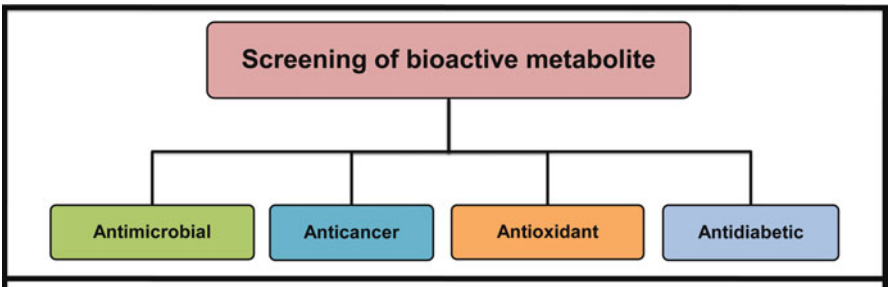


Fig. 2 Overview of isolation and screening of pure compounds



Screening	Methods/Assays
Antimicrobial	Dilution method, Diffusion method, Cross-streak, E-test, MIC, etc
Anticancer	Cytotoxicity assay, tetrazolium reduction assay, hollow fiber assay, enzyme assays, xenograft models, etc
Antioxidant	DPPH , ABTS assay, Serum catalase activity, etc
Antidiabetic	α-glucosidase inhibition, α-amylase inhibition, etc

Fig. 3 Methods for bioactivity screening of metabolites

routinely used to determine the antimicrobial potential of an endophyte, its extract, or pure compounds. In diffusion methods, antimicrobial activity of extract or pure compound is evaluated by measuring the zone of inhibition, formed due to the diffusion of antimicrobial agent in a solid medium, which inhibits the growth of test microbes. The endophyte may also be directly cross-streaked with the test microbe on a solid medium [77]. In this case, if metabolite(s) produced by the endophyte have antimicrobial activity, their diffusion in the medium inhibits the growth of cross-streaked test microbe. However, diffusion-based antimicrobial assays are not suitable for the calculation of MIC (minimum inhibitory concentration) [78]. Dilution methods (agar dilutions and broth medium) as well as E-test are most commonly used to calculate MIC [76, 79]. These methods have an edge over the diffusion-based methods as they permit a better quantitative estimation of antimicrobial activity. Another method to determine the antimicrobial efficacy of extract or pure compounds is the "time-kill" curve or "suspension tests/kill analysis" which reveals both time-dependent and concentration-dependent antimicrobial interactions to reveal either bactericidal or fungicidal activities [80]. ATP bioluminescence assay may also be used to determine the microbial population by quantifying the amount of adenosine triphosphate (ATP) produced by bacteria or a fungus [76]. Treatment with bactericidal antibiotics is known to non-specifically alter the membrane potential of test bacteria. Changes in membrane potential have been used in a flow cytometry-based assay to test the effectiveness of antibacterial compounds against wild-type and/or resistant strains of test microbes [81–83].

3.3 Screening for Antioxidant Activity

It is well known that reactive oxygen species *(*example O^{2-} and OH^{2-}) and free radical-meditated stress contribute to aging, cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, rheumatoid arthritis, etc. [84, 85]. Antioxidants are thought to be beneficial in the management of reactive oxygen species-mediated tissue injury [86]. Tirilazad [87] and NXY-059 [88, 89] are antioxidant molecules that exhibited neuroprotective activity in animal models of stroke [90]. Due to the believed general health promotion activity, antioxidants are also often found as key ingredients in food supplements. Different antioxidant assays are available to assess the radical scavenging activity of test compounds. DPPH (1,1-diphenyl-2- picrylhydrazyl)- and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))-based spectrophotometric assays are commonly used in vitro protocols for the assessment of antioxidant activity [91, 92]. Serum catalase and superoxide dismutase activities are often measured to test the in vivo effects of antioxidant molecules [93]. The lipid peroxidation status, which may be measured by the thiobarbituric acid reactive substance (TBARS) method, is also often used as an in vivo indicator of the antioxidant activity of test compounds administered in animal models [94].

3.4 Screening for Antidiabetic Activity

Antidiabetic potential of compounds or extracts may be tested by in vitro enzyme assays or through determination of in vivo antidiabetic activity. α – Glucosidase, which breaks down disaccharides into glucose resulting in increased blood glucose level, is one of the validated drug targets for management of diabetes. α-Glucosidase inhibition assay is a simple colorimetric method used for assessing the antidiabetic potential of compounds [95]. Similarly, α-amylase that catalyzes the hydrolysis of 1,4-glycosidic linkages of polysaccharides such as glycogen and starch into simpler sugars is another validated drug target for diabetes. Colorimetric methods for estimation of α-amylase activity have been used to assess the inhibition of this enzyme by test extracts or pure compounds [96]. Ex vivo antidiabetic activity assays that measure glucose uptake activity in cells have also been used to estimate the antidiabetic potential of extracts or pure compounds.

3.5 Screening for Anticancer Activity

Assessment of anticancer activity may be carried out by ex vivo (cell line based), target-based (in vitro), or animal model (in vivo)-based screening methods. A simple and quick preliminary assessment of potential anticancer activity of an extract or pure compound can be done by estimating their cytotoxicity against cancer cell lines. NCI-60, representing all major human cancer types, is the most commonly used panel of tumor cell lines for screening cytotoxicity of test extracts or compounds [97]. Here, the tumor cell lines are seeded in a medium containing test extracts or compounds, and then the decrease in the tumor cells vis-à-vis control untreated cells is monitored. Extracts of compounds possessing cytotoxicity may either decrease the cell number by directly killing the cells or by reducing the rate of their multiplication. Cell viability or cell death may be monitored in a cytotoxicity assay by using tetrazolium reduction methods that employ MTT, MTS, XTT, or WST-1 dye [98]. Cytotoxicity-based whole-cell screening method, though quick, does not give information immediately about the mode of action of the test compounds. On the other hand, large-scale screening of test compounds may be done by employing cell-free enzymatic assays of the validated drug targets for cancer therapy. PI3K/Akt/mTOR signaling pathway is commonly activated in cancers, and these three kinases are validated drug targets for anticancer therapy [99, 100]. CDKs (cyclin-dependent kinases), the master regulators of cell cycle, are also among the validated drug targets for cancer therapeutics [101]. Enzyme assays for these targets may be established in vitro, and the effectiveness of test compounds is measured by the degree of inhibition they cause, when added during the enzyme assay. Easy-to-use kits for carrying out these enzyme inhibition assays are also available in the market. Several in silico and high-throughput screens have been carried out against these kinases [102, 103], and US FDA-approved drugs targeting

these are available. Many compounds that target these kinases are presently in clinical trials [104]. In vivo humanized tumor models such as mouse xenografts and the hollow fiber are also available [105, 106]. Xenograft models may make use of either human tumor cell lines or patient derived tumor cells transplanted in mouse. Hollow fiber model makes use of a hollow fiber in which human tumor cells are seeded, and then the fiber is placed in the peritoneal cavity of the animal. These models are generally closer to the real-world scenarios and give information regarding pharmacokinetics of the test molecule as well as its efficacy when administered in vivo [107].

4 Genomics-Guided Identification of Biosynthetic Gene Clusters and Their Elicitation

Biosynthetic gene clusters (BGCs) are the set of closely linked genes that code for enzyme complexes like the nonribosomal peptide synthetase (NRPS) or polyketide synthases (PKS) responsible for metabolite production [108]. Besides, core biosynthetic enzymes many BGCs also harbor regulatory elements, transporters, and genes that mediate resistance to the host [109]. Genome mining of microbes revealed that they contain several BGCs which when activated may produce numerous secondary metabolites [110]. Bioinformatic tools such as anti-SMASH [antibiotics and Secondary Metabolite Analysis Shell] [111], SMURF [Secondary Metabolite Unknown Regions Finder] [112], CLUSEAN [CLUster SEquence ANalyzer] [113], CASSIS [Cluster Assignment by Islands of Sites], SMIPS [Secondary Metabolites by InterProScan] [114], and FungiFun [115] are available for identification of the BGCs responsible for PKS and NRPS enzymes, together with the function of the adjacent genes to aid in identification of secondary metabolite gene clusters in microbial genomes (Fig. 4). These tools have been successfully utilized to identify gene clusters such as nearly 50 clusters in *Aspergillus* sp. (genome size of 28–40 Mb) and 27 metabolite clusters in *Arthroderma benhamiae* (genome size of 22 Mb) [116, 117], etc.

4.1 Cryptic Biosynthetic Gene Clusters

There is an inconsistency found between the actual number of secondary metabolites produced by a microbe and the number of BGCs identified using bioinformatics tools [118]. Genome mining has shown that most microbes have the potential to produce many more metabolites compared to the natural products that are isolated when these microbes are grown in culture [119]. It has been observed that many of the BGCs are not expressed under laboratory conditions. In culture, such silent or "cryptic gene clusters" may express at a very low level and produce very minute quantities of metabolites which may not even be detectable. By sequencing the

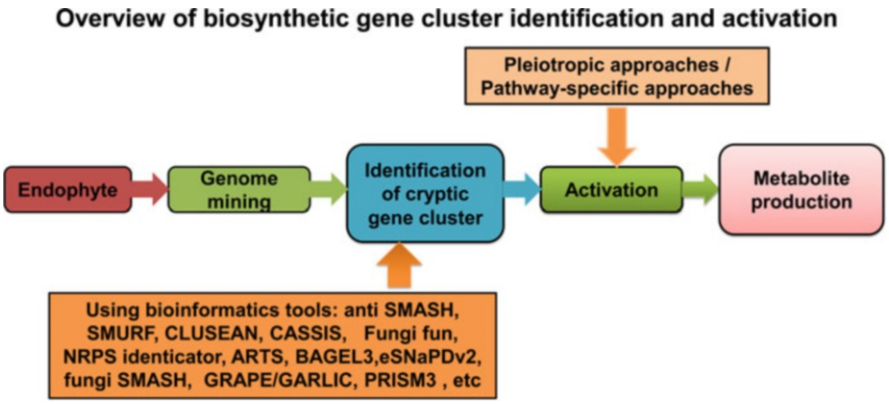


Fig. 4 Overview of biosynthetic gene cluster, their identification and activation

genome of *Streptomyces coelicolor* more than 20 BGCs were identified but until now less than six metabolites have been confirmed [120, 121]. Complete genome sequencing of many actinobacteria reveals the presence of many cryptic BGCs that may be responsible for novel metabolite production [122]. Furthermore, studies reveal that the filamentous fungi may contain higher number of BGCs as compared to actinobacteria [123]. It is possible that some of these "cryptic" BGCs are acquired horizontally from other microbes [124]. It is also feasible that signal molecules originating from other organisms, in specific ecological niches, are required for induction of such silent BGCs. Expression of BGCs may also be regulated by quorum sensing [125]. Moreover, production of natural products not directly involved in growth involves a high metabolic cost and hence is tightly regulated. In natural environments, these metabolites may help in better survival of the producer but in artificial pure cultures, in the absence of any competing organisms, their production may be suppressed [126, 127]. It has been postulated that if cryptic BGCs can be activated then the diversity of metabolites that microbes produce may be enhanced and newer natural products may be available for drug discovery screens. Consequently, pleiotropic and pathway-specific approaches have been attempted to activate BGCs (Fig. 5).

4.2 Induction of Cryptic Biosynthetic Gene Clusters: Pleiotropic Approaches

Pleiotropic approaches usually affect the expression of more than one BGC by impacting different regulatory pathways. Such methods result in the activation of multiple BGCs involved in the synthesis of several metabolites. Remarkably, such pleiotropic approaches can be used even when detailed genetic information about

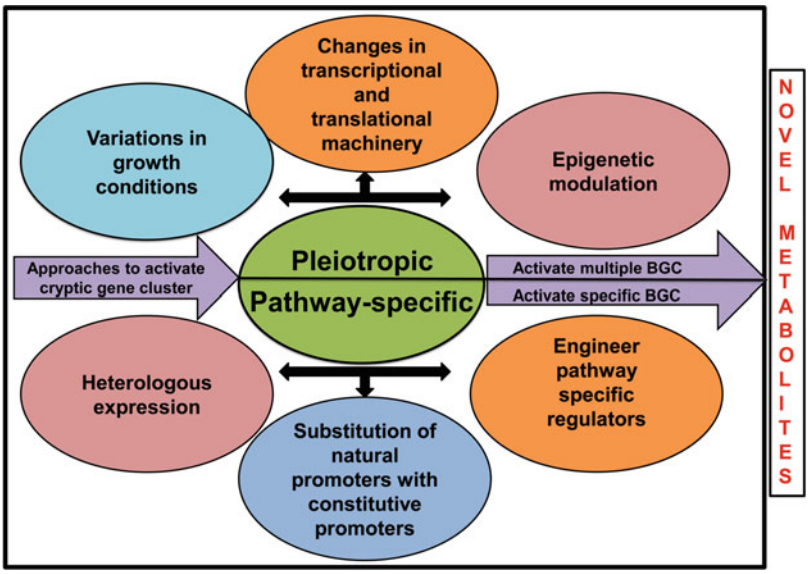


Fig. 5 Strategies for activation of cryptic gene clusters

the BGC is not available. Methods like changes in cultivation conditions, manipulation of growth factors, epigenetic modulation, and engineering the machinery involved in transcription and translation [128] may be helpful in pleiotropic activation of "cryptic" BGCs. A simple and rational approach, OSMAC (one strain-many compounds) that relies on changing fermentation conditions such as composition of the media, pH, salinity, aeration, shape of the culture vessel, etc., was found helpful in triggering cryptic BGCs [129]. Through OSMAC a novel antifungal agent polyene ECO-02301, from *Streptomyces aizunensis* [130], and three new compounds belonging to a class of 22-membered macrolides from *Streptomyces* sp. strain C34 [131] were identified. Novel in situ cultivation methods involving the culture of previously uncultivable microbes, in their natural niche, in the presence of signals emerging from other biotic and abiotic factors, were recently shown to result in production of novel antibiotic [132]. Such conditions may also be mimicked in the laboratory by employing co-cultivation or mixed cultivation methods which have been shown to induce "cryptic" BGCs [133]. It may be noted that the molecular cross-talk and signaling mechanisms in such co-cultivation environment still remain unexplored and may provide novel insights useful for artificial activation of cryptic BGCs. It was observed that the co-cultivation of *Aspergillus nidulans* with 58 actinobacteria resulted in the activation of cryptic BGC involved in the biosynthesis of orsellinic acid [134]. Similarly, co-cultivation of two microorganisms, the actinomycete *Salinispora arenicola* and fungus *Emericella* sp., triggered the expression of

BGC, encoding emericellamids, by 100-fold [135]. Recently, it was shown that sublethal concentrations of antibiotic trimethoprim caused activation of various gene clusters in *Burkholderia thailandensis* [136]. Compounds like piperidamycins [137] and coelimycins [138] were discovered by inducing alterations in transcriptional (inducing mutations in RNA polymerase) as well as translational machinery (mutations in ribosomal proteins) resulting in expression of silent gene clusters. Changing chromatin conformations through the use of chemicals led to the accumulation of new metabolites like cladochromes [139] and nygerone A [140], etc. Similarly, the use of histone deacetylase inhibitor valproic acid led to induction of fumiquinozoline C biosynthetic pathway genes and concomitantly an increased accumulation of the metabolite [141]. *Aspergillus nidulans* genome contains a single paralog of sumO gene which encodes for small ubiquitin-like protein SUMO. This gene is not considered to be important for its growth and expulsion of this gene resulted only in minor inhibition of growth [142]. Surprisingly, further investigation showed that deletion of the sumO gene resulted in enhancement of asperthecin production, while simultaneously the synthesis of sterigmatocystin and austinol /dehydroaustinol was decreased [143]. Novel elicitors such as animal, plant, fungal, or bacterial cell debris/extracts have also been used for stimulation of BGCs [144].

4.3 Induction of Cryptic Biosynthetic Gene Clusters: Pathway-Specific Approaches

Pleiotropic approaches may sometimes lead to activation of many BGCs at once and consequently production of several metabolites which can complicate their extraction and identification [128]. This difficulty may be overcome by applying target metabolite-specific strategies which regulate only specific gene clusters. Lately, new approaches like "knockout," overexpression of transcriptional factors, etc. were developed to activate silent BGCs [145]. Pathway-specific methods such as replacing natural promoters with constitutively expressing promoters in the BGC resulted in the production of burkholderic acid [146]. Whereas terferol analogues, avermitilol, epiisozizaene, and haloduracin biosynthesis could be achieved by expressing the entire BGC in heterologous host like *E. coli* [147–150]. However, employment of synthetic biology approach involving large-sized BGCs (sometimes >40 Kb) may pose an obstacle, which may be overcome by using vectors such as *E. coli-Streptomyces* artificial chromosomes (ESACs) [151].

Unraveling the genomes of endophytes may be helpful to fully understand their potential for novel bioactive metabolite synthesis. Recently, a novel endophytic actinobacteria *Paenibacillus dauci* isolated from carrot displayed numerous open reading frames (ORF) involved in the antibiotic metabolic process,

plant growth, antimicrobial peptide transport system, production of vitamins B12 and B6, etc. [152]. Similarly, genome sequencing of *Streptomyces wadayamensis* isolated from *Citrus reticulate* revealed the presence of 32 gene clusters, some of which encode the biosynthesis of terpenes, lasso peptide, bacteriocin-terpene, bacteriocin, siderophores, ectoine, lantipeptide, tiopeptide-lantipeptide, etc. [153]. In the same way, genome sequencing of the endophyte *Micromonospora lupini* revealed 15 BGCs with the potential to synthesize several secondary metabolites [154, 155]. Likewise, the complete sequencing of the genome of endophyte *Kibdelosporangium phytohabitans* extracted from *Jatropha curcas* revealed the presence of gene clusters involved in biosynthesis of nonribosomal peptides, polyketides, and compounds imparting plant growth promotion activity [156].

Recently, a novel phenazine compound 6-((2-hydroxy-4-metoxyphenoxy) carbonyl) phenazine-1-carboxylic acid (HCPCA) was extracted from *Streptomyces kebangsaanensis* an endophyte, associated with *Portulaca oleracea*. Nuclear magnetic resonance spectroscopy (NMR) was done to elucidate the molecular structure of the compound. Whole-genome sequencing of *S. kebangsaanensis* and its bioinformatic analysis was carried out to discover the biosynthetic pathways of this novel compound as well as other metabolites [157]. Here, antiSMASH software [111] was used for the analysis of the genome of *S. Kebangsaanensis*, which revealed the presence of 24 BGCs comprising of 170 known genetic modules involved in antibiotic and other secondary metabolite production. These gene clusters were also responsible for the biosynthesis of terpene, bacteriocins, butyrolactone, siderophores, nonribosomal peptide synthase enzymes, lantipeptide, and polyketide synthase type (PKS) II. It also contains four BGCs for bacteriocin biosynthesis and three gene clusters encoding biosynthesis of siderophore, nonribosomal peptide synthase, and polyketide synthase type II. *S. kebangsaanensis* genome also revealed the presence of the gene encoding enzymes, namely, isopentenyl-diphosphate and dimethylallyltransferase which play an important role in terpene biosynthesis [157]. Subsequent studies found another gene cluster responsible for the production of albaflavenone, a novel sesquiterpene, which was first isolated from *S. coelicolor* [158]. Genome analysis of *S. kebangsaanensis* had demonstrated its theoretical potential of producing secondary metabolites from 24 BGCs, some of which were confirmed with experimental results [157]. This investigation clearly shows that an in-depth understanding of secondary metabolite gene cluster is crucial for realizing the true metabolic potential of endophyte.

5 Conclusion and Future Prospects

Endophytes are a relatively less explored and little exploited group of microorganisms that may turn out to be a novel source of structurally diverse and bioactive metabolites with the potential to be utilized in medical,

agricultural, and industrial arenas [9]. Recently several plants have been studied with respect to the endophytes that they harbor. However, these may constitute less than 5% of total plant diversity, and a considerably enormous number of plants are yet to be studied [159]. Further it is well established that non-cultivable microorganisms inhabit the soil and other natural niches. It is likely that such non-cultivable microbes may also live inside plant tissues, and if this resource can also be tapped, it would further increase chances of isolation of novel bioactive natural products. One of the methods for this could be sequencing complete metagenomes from plant tissues and then digitally separating out the sequences that do not belong to the plant genome. The non-plant genome sequences may then be computationally assembled, analyzed, and characterized in detail to identify potential secondary metabolite biosynthesis genes. Through gene synthesis, they may then be cloned and expressed heterologously, and their biosynthetic potential may be assessed. Though with the presently available technology, this may be slightly far-fetched, but with the availability of single-cell genomics and development of specialized bioinformatics tools, it may become a viable possibility in future. Besides exploring endophytes for bioactive metabolites, other untapped sources of microbes that live inside animal and insect tissues [160, 161] may also be explored for new natural products.

Further, detailed natural product chemistry evaluation has been carried out on few endophytes. Moreover it is likely that the metabolites that are produced in traces would escape attention, due to technical issues. However, with the progress made in increasing the sensitivity, development of automated methods of chromatographic isolation, and advancements in spectroscopic techniques [2], it may be possible to identify and isolate natural products that are produced in trace quantities.

Even from the endophytes that have been studied in detail with respect to the metabolites that they produce, the full metabolic potential is yet to be realized. Recent advances in genome sequencing technologies and computational mining methods have shown the existence of a large number of cryptic BGCs in microbial genomes [162]. Developments of new culture methodologies, genetic manipulation protocols, synthetic biology procedures, etc. have made it possible to induce the functional expression of cryptic or silent BGCs [163, 164]. Transfer of silent or cryptic BGCs to heterologous hosts and/or their rational genetic manipulation has been shown to induce the expression of BGCs and concomitant production of encoded metabolites [149]. However these efforts have mostly been made on soil-isolated microorganisms [165, 166], and relatively sparse literature is available on endophytes with respect to such studies. It appears reasonable to assume that similar approaches could be exploited to unlock the metabolic potential of endophytes.

It is clear that this field is still in its infancy and increased efforts coupled with rapid strides in technological innovation may result in huge payoffs through the discovery of novel bioactive molecules from endophytic microbes (Table 1).

Table 1 Natural compounds produced by endophytes

Plant	Endophyte	Natural compound	References
Bruguiera gymnorrhiza.	*Streptomyces* sp.	Macrolide divergolides A–D	Ding et al. [167]
Capsicum frutescens	*Actinoallomurus fulvus*	Actinoallolides A–E	Inahasj et al. [168]
Carpobrotus edulis	*Blennoria* sp.	Blennolides A–G	Zhang et al. [169]
Mediterranean Alga	*Nodulisporium* sp.	Noduliprevenone	Pontius et al. [170]
Lycium intricatum	*Microsphaeropsis* sp.	Microsphaeropsones A–C	Krohn et al. [171]
Artemisia vulgaris	*Chalara* sp.	Isofusidienols A–D	Losgen et al. [172]
Lycopodiella cernua	*Paraphaeosphaeria neglecta*	Lycopodiellactone	Li et al. [173]
Knightia excels	non-sporulating endophytic fungus	Spiro-mamakone A	Van der Sar et al. [57]
Lysidice rhodostegia	*Penicillium dangeardii*	Penicillactones A-C	Liu et al. [174]
Torreya taxifolia	*Pestalotiopsis microspora*	Torreyanic acid	Lee et al. [60]
Clavaroids sp.	*Pestalotiopsis* sp.	torreyanic acid analogue	Ding et al. [175]
Imperata cylindrical	*Chaetomium globosum*	Chaetoglobins A and B	Ge et al. [176]
Carex aridula	strain of *Alternaria*	(−)-Alternarlactam 40	Zhang et al. [177]
Melia azedarach	*Fusarium* sp.	Fusarimine	Yang et al. [178]
Panax notoginseng	*Penicillium manginii*	Duclauxamide A1	Cao et al. [179]
Camellia sinensis	*Streptomyces* sp.	Rubrolone B	Yan et al. [180]
Trachelospermum jasminoides	*Cephalosporium acremonium*	Cephalosol	Zhang et al. [181]
Melia azedarach L	*Aspergillus* sp.	Aspertryptanthrins A–C	Lhamo et al. [182]
Annonsa muricata	*Periconia* sp.	Periconianone A	Zhang et al. [183]
Taxus brevifolia	*Pestalotiopsis* sp.	Pestalotiopsin Aand B	Pulici et al. [184]
Mangrove plant	*Aspergillus* sp.	Asperterpenoid A, asperterpenols A and B and aspterpenacids A and B	Huang et al. [185], Xiao et al. [186]
Panax notoginseng	*Trichoderma gamsii*	Trichoderones A and B, trichodermone	Ding et al. [187], [188]
Lycopodiella cernua	*Paraphaeosphaeria neglecta*	Paraphaeosphaeride A	Li et al. [189]

(*continued*)

Table 1 (continued)

Plant	Endophyte	Natural compound	References
Rhizophora stylosa	*Mucor irregularis*	Rhizovarins A-F	Gao et al. [190]
Codium fragile	*Aspergillus versicolor strain*	Asperverin	Ji et al. [191]
Marine red alga	*Paecilomyces variotii*	Varioxepine A	Zhang et al. [192]
Rhizophora mucronata	*Pestalotiopsis* sp.	Pestalotiopens A and B	Hemberger et al. [191]
Pritchardia lowreyana	*Peyronellaea coffeae-arabicae*	peyronellins A–C	Li et al. [193]
Paris polyphylla var. yunnanensis	*Aspergillus versicolor*	aspergillines A–E	Zhou et al. [194]
Brguiera sexangula var. rhynchopetala	*Daldinia eschscholtzii*	Cytochalasin metabolite ([11]-cytochalasa-5(6),13- diene-1,21-dione-7,18-dihydroxy-16,18-dimethyl-10-phenyl-(7S*,13E,16S*,18R)	Yang et al. [195]
Hibiscus tiliaceus	*Penicillium aurantiogriseum*	Peaurantiogriseols A–F	Ma et al. [196]
Sonneratia caseolaris	*Bionectria ochroleuca*	Pullularins E and F	Ebrahim et al. [197]
Tripterygium wilfordii	*Cryptosporiopsis* cf. quercine	Cryptocin	Li et al. [38]
Wheat	*Phomapsis* sp.	Phomapsichalasin	Horn et al. [36]
Gossypium hirsutum	*Phomapsis* sp.	Epoxycytochalasin H, Cytochalasin N, Cytochalasin H	Fu et al. [198]
Aspergillus fumigatus	*Cynodon dactylon*	Fumigaclavine C, fumitremorgin C	Cole et al. [199]
Zea maydis	*Acremonium zeae*	Pyrrocidine A and B	Donald et al. [200], He et al. [201]
Crocus sativus	*Penicillium vinaceum*	(−)-(1R,4R)-1,4-(2,3)-indolmethane-1-methyl-2,4-dihydro-1H-pyrazino-[2,1-b]-quinazoline-3,6-dione	Zheng et al. [202]
Ginkgo biloba	*Chaetomium globosum*	Epipolythiodioxopiperazine, Gliotoxin	Li et al. [40]
Lycium Intricatum	*Microdiplodia* sp.	1,4-oxazapan-7-one	Siddique et al. [203]
Prumnopytis andina	Penicillium janczewskii	Peniprequinolene , gliovictin, gliovictin acetate,mellein	Schmeda-Hirschmann et al. [204]
Cassia Spectabilis	*Phomopsis cassiae*	Cadinane sesquiterpenes	Silva et al. [205]

(*continued*)

Table 1 (continued)

Plant	Endophyte	Natural compound	References
Juniperus Communis	*Hormonema* sp.	Enfumafungin	Palaez et al. [206]
Piper aduncum	*Xylaria* sp.	Phomenone, Phaseolinone	Silva et al. [43]
Arisaema erubescens	*Phoma* sp.	Pestaphthalides A and B	Ding et al. [207]
Mallus halliana	Alternaria Brassicicola	Cerevisterol	Gu et al. [42]
Fagonia critica	*Microdochium bolleyi*	Isocoumarin derivative	Zhang et al. [208]
Piper aduncun	*Xylaria* sp.	Dihydroisocoumarins	Oliveira et al. [209]
Alibertia macrophylla	*Penicillium* sp.	Orcinol	Oliveira et al. [210]
Cynodon dactylon	*Aspergillus niger*	Fonsecinone A	Song et al. [211]
Artemisia mangolica	*Colletotrichum gloeosporoides*	Antifungal	Zou et al. [37]
Euconia ulmoides	*Sordariomycete* sp.	Chlorogenic acid	Chen et al. [212]
Terminalia morobensis	*Pestalotiopsis microspora*	Isopestacin	Harper et al. [62]
Bidens pilosa	*Botryosphaeria rhodina*	Botryorhodines A-B	Abdou et al. [213]
Macleaya cordata	*Chaetomium cupreum*	Oosporein	Mao et al. [214]
Conocarpus erecta	*Cytospora* sp.	Cytosporone B	Brady et al. [215]
Forsteronia spicata	*Diaporthe* sp.	Cytosporone B	Brady et al. [216]
Aegiceras corniculatum	*Dothiorella* sp.	Cytosporone B	Xu et al. [217]
Phleum pretense	*Epichloe Typhina*	Chokols A-G	Koshino et al. [44]
Fragraea bodenii	*Pestalotiopsis jesteri*	Jesterone Hydroxyjesterone	Li et al. [218]
Edenia gomezpompae	*Callicarpa acuminata*	Preussomerin EG1, 1b	Macias-Rubalcava et al. [219]
Unidentified tree	*Pestalotiopsis fici*	Pestalofones A-E	Liu et al. [220]
Erica Arboreal	*Nodulisporium* sp.	Nodulisporins D-F	Dai et al. [221]
Meliotus dentatus	*Unidentified Ascomycete*	Polyketides and steroids	Hussain et al. [222]
Mallus halliana	*Alternaria Brassicicola*	Herbarin A	Gu et al. [42]

(*continued*)

Table 1 (continued)

Plant	Endophyte	Natural compound	References
Cork Oak	*Trichoderma Citrinoviride*	Peptaibols	Maddau et al. [223]
Paris polyphylla var. yunnanensis Paris polyphylla var. yunnanensis Paris polyphylla var. yunnanensis Paris polyphylla var. yunnanensis	*Gliomastix murorum* and *Pichia guilliermondii*	Volatile oil	Zhao et al. [224]
Arisaema erubescens	*Phoma* sp.	β-sitosterol	Wang et al. [225]
Taxus mairei and *Torreya grandis*	*Paecilomyces* sp. and *Aspergillus clavatus*	Brefeldin A	Wang et al. [226]
Azadirachta indica	*Phomopsis* sp.	10-membered lactones	Wu et al. [227]
Garcinia atroviridis	*Penicillium Sclerotiorum*	Penicilazaphilones A and B and penicilisorin	Arunpanichlert et al. [228]

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Current Understanding and Future Perspectives of Endophytic Microbes vis-a-vis Production of Secondary Metabolites

16

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Contents

Abstract

Endophytes are the bacterial and fungal forms of organisms living within the plant system causing no ill effects to the hosts. They asymptomatically live in the cellular environment in the plants carrying out various complicated functions such as production of secondary metabolites and signaling molecules coupled to the responses of various external and internal stimuli for mutual

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survival. They are known to produce a range of metabolites of utility in treating various disorders in humans and also produce chemicals of utility in agriculture such as growth regulator and pesticides, in several economically important plants. Continued research findings on the range of metabolites produce by them and their promising utilities have raised hopes in finding biotechnological solutions ranging from prospecting to production of industrial relevance to find lasting sustainable solutions for economical exploitation. These aspects have been dealt in detail as evidenced through current scientific understanding coupled to the future perspectives.

Keywords
Endophytes · Plant adaptation · Agricultural application · Secondary metabolites · Biological activities · Signaling · Industrial potential

1 Introduction

Plant endophytic microorganisms have been topic of research interest in agricultural sciences in view of their diverse beneficial role in the improvement of plant growth and yield owing to their influence on various physiological functions. Historically the presence of the endophytes in plants came into attention as a result of suspicion when several herd of domesticated animals were harmed because of feeding with grass [1]. The microbial residents in plants were termed as endophyte by de Bary in 1866 [2] and were first described in *Lolium temulentum* [3]. The presence of bacteria within the tissues of healthy plants was first reported in 1926 by Freeman [3] who identified an endophytic organism in *Persian darnel* (annual grass). Moreover, these grasses with high endophyte content were found to be impervious to the assault by specific insects [4].

Endophytes constitute an extraordinarily large group of microorganism universal in plants and preserve a close relationship with their host for at least a part of their life cycle. Throughout this connotation, none of the interrelating partners are perceptibly harmed [5]. Roughly 300,000 plant species are host to at least excess of one endophyte [6], and the occurrence of biodiverse endophytes in enormous number plays an important role on the ecosystem in both the tropical and temperate rainforest [7–9]. Strobel et al. [6] reported the production of bioactive components by endophytes. They further opined that evolution of the endophytic microorganisms has occurred by incorporating the genetic information from the plant hosts, thereby conferring extraordinary adaptability by sharing some ecological functions, viz., defense from pathogen, grazing animals, and insect.

Endophytes are chemical synthesizers inside the plants [10] and are capable of producing wide range of bioactive secondary metabolites, viz., alkaloids, flavonoids, phenolic acids, terpenoids, tetralones, benzopyranones, chinones, quinones, steroids, xanthones, and others [6, 11, 12]. Such bioactive metabolites find wide-ranging application as agrochemicals, immune suppressants, antiparasitics, antitoxins, and anticancer agents [13–16]. Upon isolation and characterization of

bioactive principles, they may find potential use in agriculture, industry, and medicine. It was assessed that more than one million types of endophytes exist [17], thereby suggesting the enormous scope of the unexplored potential for varied applications.

The scope of this review encompasses the information contained in published literature on the type of endophytes and their ability to produce secondary metabolites with special reference to fundamental and applied research related to their sustainable production and utility for varied applications.

2 Definition, Distribution, and Origin of Endophytes

Endophytic microbes exist within plants for at least a part of their life cycle without causing any visible sign of disease [12, 18]. Sikora [19] expanded the definition of endophyte, as an organism colonized in the internal tissue of a plant throughout its life cycle no matter whether it was useful, harmful, or unbiased to its host. Endophyte originally was a concept of ecology but is an integral natural part of plant-microecology system [20]. According to Dreyfuss and Chapela [21], there are millions of endophytic fungi existing in special circumstance of 270,000 to 4,000,000 kinds in microtubule plant cells and intercellular space. McInroy and Kloepper [22] reported the incidence of a high density of endophytic bacteria in plant roots which were up to 10^4–10^6 CFU/g. A significant discovery of the endophytic fungus *Neotyphodium coenophialum* as the causative organism of "fescue toxicosis" a syndrome suffered by cattle fed in pastures of the grass *Festuca arundinacea* [23] provided direction to research on this topic. Schardl et al. [24] found that infected plants contained a number of poisonous alkaloids; however *Neotyphodium* species might be beneficial to their plant host, increasing their tolerance of biotic and abiotic stress factors.

3 Types of Endophytes

All the reported endophytes are fungi or bacteria (including actinomycetes). Endophytes are classified according to the microbe, mainly including endophytic fungi, endophytic bacteria, and endophytic actinomyces [6].

3.1 Endophytic Fungi

An endophytic fungus can multiply asymptomatically in the tissues of plants including stems, leaves, and roots. Bacon and White [12] reported that an endophytic fungus lives in mycelial form in biological organization within the living plant, at least for some time. Since the identification of a fungus from hyphal features alone is rarely possible, the identification techniques will require methods of immunofluorescence detection, DNA sequencing, and comparison of sequence to homologous

sequence registered in gene bank. Endophytic fungi are found to associate with aboveground tissue of liverworts, hornworts, mosses, lycophytes, equisetopsids, fern, and seed plants from the arctic to the tropics and from agriculture fields to the most diverse tropical forest [25]. Plants may benefit indirectly from endophytes by increased resistance to herbivores, by pathogen or stress, or by other unknown mechanisms [26]. Some studies have shown that endophytic fungi are able to protect their host plant from drought conditions [27]. Waller et al. [28] observed salt tolerance in infected plants with endophytes. Endophytic fungi also increase heat tolerance in their host. Redman et al. [29] reported that endophytes work as biological trigger to stimulate the stress response more rapidly and strongly than nonsymbiotic plants. Stoyke and Currah [30] initiated the form taxon "dark septate endophytes" (DSE) and used it for fungi that form moderately or completely melanized and having septate thalli within healthy root tissues. Strobel et al. [31] first isolated endophytic fungi (*Taxomyces andreanae*) from a medicinal plant (*Taxus brevifolia*). They also reported that a few species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal, and immunosuppressive compounds. Endophytic fungi may also produce metabolites with thermoprotective role.

Huang et al. [9] and Li [20] were able to identify a total 42 endophytic fungal strains from *Nerium oleander*. Endophytic fungi also exist in the marine plants [32]. Further the endophytic and obligate marine fungus *Ascochyta salicorniae* was reported in green algae [32]. Endophytic fungi also adopt widely by promoting plant growth and protecting the plant to reduce diseases and insect pests.

Fungal endophytes consist of two basic ecological groups: balansiaceous or "grass endophytes" and non-balansiaceous.

3.2 Balansiaceous Endophytes or Grass Endophytes

They are the best studied groups due to their ecological and economic importance. Balansiaceous endophytes form a distinctive group of closely related fungi with ecological requirements and adaptation discrete from those of other endophytes [33]. They grow systemically, epicuticularly, and intercellularly within all aboveground plant organs of grasses, resulting in vertical transmission of the endophytes through the seed. They belong to the clavicipitaceous genera *Epichloë* and *Balansia* and their anamorphs *Neotyphodium* and *Ephelis* [24]. The balansiaceous endophytes produce a diverse array of secondary metabolites. The toxic alkaloids consist of the anti-insect alkaloids peramine and lolines and the anti-vertebrate alkaloids loliterm B and ergovaline [34]. The primary benefits for the fungal partner are nutritional but also include fortification from abiotic stress, such as desiccation [12], and from competing epiphytic organisms [12]. The advantage of interaction for the plant is protection against herbivore by toxic alkaloids produced by fungal endophytes during symbiotic association, and they also mediate induced resistance through activation of

the host defense through constitutive and resistance [35].Shelby et al. [36] found ergopeptide variants that were actually modified by plant metabolism. Trace of loline alkaloid was reported even in uninfected plant *Festuca pratensis.* The lolines are insecticidal and insect-deterrent compounds that are produced in grasses infected by endophytic fungal symbionts of the genus *Epichloë* (anamorphic species: *Neotyphodium*). Lolines increase resistance of endophyte-infected grasses to insect herbivores and may also protect the infected plants from environmental stresses such as drought and spatial competition (Wikipedia).

3.3 Non-balansiaceous Endophytes

They are diverse, both phylogenetically and with respect to life history strategy. Non-balansiaceous endophytes belong to the *Ascomycota* and colonize either inter or intracellular, localized or systematic [26]. In the case of the non-balansiaceous types, the term "endophyte" usually refers to a fungus accomplished of cryptic activity of plant tissue and describe a momentary status [26]. The fungi are not obligate host specific; they have a certain level of adaptation to different hosts, while others are more specific and can only be found in specific organs of specific plant [26].

3.4 Endophytic Bacteria

Endophytic bacteria arise at inferior population densities than rhizospheric bacteria or bacterial pathogens [37]. Hallmann et al. [4] reported that endophytic bacteria might be better protected from environmental stresses than rhizospheric bacteria. Bacterial endophytes are able to repressing nematode proliferation thus would be of help to other crops in rotation with the host plants [38]. Endophytic bacteria usually exist in intercellular space and vascular tissue of the plant. More than 129 kinds of the endophytic bacteria were isolated from different crop plants, including both Gram-negative and Gram-positive species representing over 54 genera. The major bacterial taxa belong to the former *Pseudomonas* group and enterobacteriaceae [39, 40]. In soybean, 98 nonsymbiotic endophytic bacterial strains were isolated from 150 root nodules [41]. Gaiero et al. [42] have extensively studied bacterial root endophytes and plant growth promotion. Plant growth-promoting bacterial endophytes (PGPBs) were identified, but the predictive success at positively influencing plant growth in field condition has been limited. Rosenblueth and Martínez-Romero [43] studied molecular techniques on bacterial endophytes and their interaction with hosts. They have concluded that endophytes promote plant growth and yield, suppress pathogens, may help to remove contaminants, solubilize phosphate, or contribute assemble nitrogen to plants. Molecular analysis showed that defense responses limit bacterial population inside plants.

4 Economically Important Plants with Endophytes

Hyde and Soytong [44] reported that fungal endophytic community lives in different plant structures such as petioles, twigs, bark, leaves, and roots [44, 45]. Endophytic fungi and bacteria have been investigated in different parts of plant species of economic interest (Table 1).

Table 1 Isolation of endophytes from economic important plant with taxonomic group

Host plant	Isolation part	Taxonomic group	References
Citrus spp.	Leaves and seeds	*Colletotrichum gloeosporioides*, *Guignardia citricarpa*, and *Cladosporium* sp.	[46]
Glycine max (L.) Merr.	Root, leaves, and stem	*Ampelomyces* sp., *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides*, *Diaporthe helianthi*, *Guignardia mangiferae*, *Phoma* sp., *Fusarium oxysporum*, *Fusarium solani*, and *Fusarium* sp.	[47]
Lycopersicon esculentum	Leaves	*Streptomyces* sp.	[48]
Mangifera indica	Fruit	*A. bogorensis* M6	[49]
Moringa oleifera	Leaves	*Nigrospora* sp.,	[50]
	Leaves	*Gemmatimonas*	[51]
	Leaves	*Emericella* sp., *Aspergillus parasiticus*, *A. tamari*, *Bipolaris* spp.	[52, 53]
Musa acuminata Colla	Roots	*Agrobacterium*, *Bacillus*, *Aneurinibacillus*, *Enterobacter*, *Klebsiella*, *Lysinibacillus*, *Micrococcus*, *Paenibacillus*, *Rhizobium*, and *Sporolactobacillus*	[54]
Oryza sativa L.	Leaves and root	*Chaetomium globosum*, *Penicillium chrysogenum*, *Fusarium oxysporum*, and *Cladosporium cladosporioides*	[55]
Phaseolus vulgaris L.	Leaves	*Colletotrichum*, *Hannaella*, *Cochliobolus*, *and Phomopsis*	[56]
Saccharum spp.	Leaves	*Ascomycota phylum*	[57]
Sorghum bicolor	Leaves and stems	*Cellulomonas*, *Clavibacter*, *Curtobacterium*, and *Microbacterium*	[58]
Triticum aestivum L.	Leaves, stems, glumes, and grains	*Alternaria alternata*, *Cladosporium herbarum*, *Epicoccum nigrum*, *Cryptococcus* sp., *Rhodotorula rubra*, *Penicillium* sp., and *Fusarium graminearum*	[59]
Zea mays L.	Leaves and stems	*Alternaria alternata* and *Aureobasidium pullulans* var. melanigerum	[60]

5 Metabolic and Molecular Cooperation of Hosts and Endophytes

Many endophytes have the potential to synthesize various bioactive metabolites which may, directly or indirectly, be used as therapeutic agents against numerous diseases [61]. Their huge biological diversity coupled with their capability to biosynthesize bioactive secondary metabolites has provided the stimulus for a number of investigations on endophytes [62, 63]. Ekanayake et al. [64] reported that symbiotic associations between tall fescue grasses and asexual *Epichloë* fungal endophytes unveil biosynthesis of alkaloid compounds producing both detrimental and beneficial effects (Table 2).

6 Signaling Pathway of Secondary Metabolism in Endophytes

Collaborations among plants and helpful organisms are significant to the establishment and upkeep of stable biological communities, especially despite ecological anxieties. An ultimate model system for studying beneficial plant-fungal interactions

Table 2 Metabolites and molecular cooperation of host and endophytes

Host plant	Endophytic fungi	Mechanism	References
Anoectochilus formosanus	*Epulorhiza* sp.	Enhance enzyme activities of chitinase, β-1,3-glucase, phenylalanine ammonium lyase and polyphenol oxidase	[65]
Atracty lancea	*Sclerotium* sp.	Increase cell protection from desiccation and leaf metabolic capability of host	[66]
Cucumis sativus	*Penicillium* sp.	Secret phytohormones, viz., gibberellins and indoleacetic acid	[67]
Nicotiana attenuata	*Sebacina vermifera*	Enhance the absorption of nutrient and promote the growth and fitness of by inhibiting ethylene singling	[68]
Pecteilis susannae	*Epulorhiza* sp. *Fusarium* sp.	Enhance the absorption of N, P, and K element in plant promoting the seed germination of host	[69]
Pedicularis sp.	Dark septate endophytic fungi	Increase their nutrient utilization efficiency	[41]
Sesbania sesban	*Funneliformis mosseae*, *Rhizophagus intraradices*, and *Claroideoglomus etunicatum*	Secrete plant hormones	[70]

is the association between fungi and cool-season grasses [71]. Dupont et al. [72] studied the impact of endophyte infection and compared the expression profile base on RNA sequencing. Endophyte infection elicits reprogramming of host metabolism, favoring secondary metabolism at a cost to primary metabolism. Infection also induces changes in host development, mostly cell wall biogenesis and trichome formation. Carvalho et al. [73] reported nitrogen signaling in plant interaction with endophytic diazotrophic bacteria. Further, they found that diazotrophic bacteria have the ability to develop different type of root association with plant species and also biologically fix N_2 to plant-available ammonium. Ren et al. [74] studied the biosynthesis pathway of swainsonine, a new anticancer drug from endophytic fungi, *Undifilum oxytropis*, isolated from locoweeds. Swainsonine is being investigated for its significant roles in immune regulation and anticancer activity. Jie Yuan et al. [75] studied the mechanism of ethylene signaling induced by endophytic fungus, *Gilmaniella* sp. AL12, via induction of ethylene production in *Atractylodes lancea*. Pretreatment of plantlets with ethylene inhibitor aminooxyacetic acid (AOA) inhibited endophytic fungi-induced addition of ethylene and sesquiterpenoids. Studies on application of specific inhibitors such as Jasmonic acid inhibitors ibuprofen (IBU) and Salicylic acid (SA) inhibitor paclobutrazol and 2-aminoindan-2-phosphonic acid to host plants inoculated with fungi showed lack of expression of sesquiterpenoid in A. lancea [76]. This work extensively established the signaling pathways of sesquiterpenoid biosynthesis and provided a theoretical basis for the industrialization of active compounds in *A. lancea* and also will provide a reference for the biosynthesis of other active compounds such as ginseng saponin, menthol, paclitaxel, glycyrrhizic acid, and artemisinin and will help to further clarify plant-endophyte interactions. Jasmonic acid is involved in the signaling pathway for fungal endophyte-induced volatile oil accumulation of *Atractylodes lancea* plant. They observed that jasmonic acid acts as a downstream signaling molecule in hydrogen peroxidase and nitric oxide-mediated volatile oil accumulation induced by endophytic fungus and has a matching interaction with the SA signaling pathway.

7 Induction and Production of Various Secondary Metabolites by Endophytes and Industrial Potential

Endophytes produce natural products, mostly secondary metabolites, in response to external incentives such as foreign infection or nutritional changes [77]. Newman and Cragg [78] are of the opinion that endophytes contain almost 50% of the new drugs introduced to the market from 1981 to 2010. Most bioactive natural products have the capability to target specific proteins coded by essential genes [79]. However it is not easy to extrapolate the studies for targeting genetically linked diseases in humans due to complex human protein-protein interactions [80]; however they have been broadly investigated for the treatment of infectious diseases [79]. For example, beta-lactam antibiotics, such as the *penicillins* and the *cephalosporins*, are

largely used for their broad antibacterial spectrum and outstanding safety profile for human use [80] (Table 3).

Endophytes provide extensive types of bioactive secondary metabolites with selected structure including flavonoid, alkaloids, chinones, benzopyranones, phenolic acids, steroids, quinones, tetralones, terpenoids, xanthones, and others [88–91]. A quite good number of reports emphasized that endophytes promote bioactive metabolite accumulation in host plants [92–94] and also able to synthesize such compounds or similar metabolites in host plants [5]. In view of a good number of contributions in this area of research nowadays, both plant and endophytes are considered as equal partners in bioactive secondary metabolite production. *In vitro* biotransformation of plant secondary metabolites to novel bioactive for value addition by using endophytes was reviewed by Ludwig-Muller [95]. The bioactivity of endophyte-mediated metabolites produced by plants is not limited to antimicrobial alone but also reported to exhibit anti-inflammatory, antiproliferative, or cytotoxic activity toward human cancer cell lines [96]. Several secondary metabolites producing endophytic fungi have been isolated from medicinal plants *Garcinia mangostana* (fruits), *Costus speciosus* (leaves), and *Flacourtia inermis* (fruits) which yielded helminthosporal acid, helminthosporol, GKK1032B, citrinin, shikimic acid, and ergosterol, respectively. These are structurally different compounds with promising bioactivities. Bioactive secondary metabolites are also isolated from conifer-associated endophytic fungi which are having anti-inflammatory, antimicrobial, antiproliferative, or cytotoxic activity toward human cancer cell lines and activity against plant insect pests or plant pathogens [96]. Such bioactive metabolites find wide-ranging application as anticancer, antiparasitics, agrochemicals, antibiotic, immune suppressants, and antioxidant agents (Table 4). As of now efforts to translate the well-established endophyte-mediated bioactive metabolite production protocols into commercial scale processes are required.

Table 3 Endophytes and their potential biological activities

Name of endophytes	Chemical nature	Activities	References
Azotobacter	Tryptophan	Hormone production	[81]
Diazotrophic endophytes	Lipopeptide	Antioxidant, biofertilizer, biocontrol agent	[82]
Nodulisporium sp.	Volatile organic compound	Biological control	[83]
Penicillium canescens	Tetrapeptide	Antifungal	[84]
Pseudomonas syringe	Lipopeptide	Antifungal	[85]
Pseudomonas viridiflava	Lipopeptide	Antifungal	[86]
Streptomyces sp.	Pentacyclicindolosesquiterpine	Antibacterial and anti-HIV	[87]

Table 4 Secondary metabolite production by endophytes

Plants	Endophytes	Compound	Activity	References
C. spectabilis	*P. cassiae*	3,11,12-trihydroxycadalene	Antifungal	[97]
Dicerandra frutescence	*Phomopsis longicolla*	Dicerandrol A	Anticancer	[98]
Ephedra fasciculate	*Chaetomium chiversii*	Radicicol	Antifungal, antimalarial	[99]
Erythrina crista-galli	*Phomopsis* sp.	Mevinic acid	Anti-inflammatory	[100]
Eugenia jambolana	*Cephalotheca faveolata*	Sclerotiorin	Antimicrobial	[101]
Gloriosa superba	*Aspergillus* sp.	6-methyl-1,2,3-trihydroxy-7,8cyclohepta-9,12-diene-11-one-5,6,7,8-tetralene-7-acetamide	Anticancer	[102]
Mangrove	*Phomopsis* sp.	Phomopsin A, B, C; cytosporone B	Antifungal	[17]
Mangrove	*Halorosellinia* sp.	Anthracenedione	Antimalarial	[103]
Xylopia aromatica	*Periconia atropurpurea*	Periconicin B	Antibacterial	[104]

8 Challenges in Endophytic Research for the Production of Secondary Metabolites

Endophytes have emerged as a precious source of new metabolites, as industrially significant enzymes, and as stress relievers of host plant, but still many aspects of endophytic biology are yet to be addressed. Despite significant leads on various aspects of endophytes of both fungal and bacterial origin influence on plant growth, yield, and bioactive metabolite production, still certain areas have to be investigated in-depth to better understand the type of secondary metabolites produced in endophyte association zones of host and also in the host plant in response to triggering effect of chemicals released by endophytes. Large-scale cultivation of endophytes in bioreactors is yet to be explored although this method is used to produce some anticancer metabolites wherein an endophyte was used [105]. However the production of anticancer alkaloid camptothecin in bioreactor using endophytic fungus *Entrophospora infrequens* has been reported. Currently, there is a growing thrust on deep understanding of the host plant-endophyte niche with the help of "omics" tools [106] such as genome sequencing, comparative genomics, microarray, next-generation sequencing, metagenomics, and metatranscriptome. This will help in understanding genetic and metabolic diversity of similar or related microbes. Similarly, in studies pertaining to sharing of the pathway by hosts and endophytes, large-scale cultivation of endophytes in bioreactors is lacking and needs to be focused in the future.

9 Conclusion

The coevolution of plant with endophytic association has not been broadly studied to improve our understanding of their effect on plant physiology, biochemistry, and adaptation to changed territories. Expression of various biochemical molecules in plants is a subject of intense research. However, the potential of endophytes to produce metabolites of utility value is beginning to be understood. Biotechnological production of endophyte-derived compounds both under *in vitro* and *in vivo* conditions has been investigated. However they need to be studied for the sustainable production of the secondary metabolites up on cultivation of endophytes in the bioreactors. The aspects of signaling of the pathways from precursor production to the formation of the end product in a consistent manner needs clear demonstration in model systems. Synthetic biology approach to the formation of the endophytic secondary metabolite is to be pursued vigorously. Such an approach will certainly be of biotechnological importance to produce novel molecules.

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Secondary Metabolite Production by Endophytic Fungi: The Gene Clusters, Nature, and Expression

17

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Contents

Abstract

With the recent advancements in drug discovery, the bioprospecting of endophytic fungi for the search of secondary metabolites of pharmaceutical importance and novel medicinal properties has become one of the prime targets. The biosynthetic pathways that are responsible for secondary metabolites have genetic basis for their production. But the expression of the gene clusters responsible for

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secondary metabolites remains cryptic under laboratory conditions. The large-scale production of these metabolites is severely distressed by its attenuation in axenic cultures. Our insights into these clusters, their regulation, and expression may lead to the mining of more novel bioactive metabolites. This approach of genome mining for the production of novel metabolites is assuring. Major challenges lie in the understanding of the regulatory mechanisms which drive the expression of these cryptic genes. Gaining knowledge on various strategies for the identification as well as induction of these silent clusters is the need of the hour. With the help of multidisciplinary scientific approaches involving bioinformatics, molecular genetics, genome mining, metabolomics, etc., we can explore the hidden treasures of the endophytic fungal diversity.

Keywords
Bioactive compounds · Epigenetic modifications · Genome mining · Metabolomics

Abbreviations

BGCs	Biosynthetic gene clusters
SMs	Secondary metabolites

1 Introduction

With a dramatic increase in the multidrug resistance pathogens and a need for new antibiotics, our focal point has shifted to search for novel secondary metabolites (SMs) and their potential [1]. The diverse nature of SMs with properties ranging from chemical or pharmaceutical drugs, antibiotics, immunosuppressants to potent anticancer drugs, toxic nature for agricultural and industrial use, depicts the hallmark of SMs' importance [2]. Approximately about 170,000 natural products are known to be from marine and terrestrial organisms [3]. Out of 22,500 microbial bioactive compounds, including antibiotics, that have been reported so far, 38% are from fungi [4]. But the discovery of antibiotic channels is hurdled by the slower rate of antibiotic development than the developing antibiotic resistance [1]. Consequently, there is an urgent requirement for more as well as new compounds in the drug discovery pipeline. The empirical use of natural products by human is ancient, but understanding their nature, character, and structure has been a recent practice. The genomic era has provided much insight into the understanding of the secondary metabolites' nature, expression, regulation, and exploitation.

2 Fungal Endophytes as Producers of Secondary Metabolites

Microorganisms are known to synthesize SMs. As far as endophytic fungi are concerned, they are very well-known repertoire of bioactive secondary metabolites. Endophytic fungi, the term coined by de Bary (1986), colonize plant tissues [5]

and live asymptomatically inside the host tissues without causing apparent symptoms [6]. Endophytic association with plant hosts had been traced back to over 400 million years [7]. They are ubiquitous, thriving in various geographical and climatic zones, diverse ecosystem, and an extensive host range, viz., mosses, ferns, grasses, herbs, shrubs, trees, etc. [8]. Most of these fungi belong to *Ascomycota*, while a few belong to *Basidiomycota*, *Zygomycota*, and *Oomycota* [8]. The relationship between endophytes and their hosts is mostly mutualistic as they confer many benefits to the hosts such as production of various secondary metabolites [8], stress resistance and host growth promotion [8], resistance to diseases, and/or herbivores [9], act as decomposers [10], etc. In addition to these benefits, endophytes also collude with their partners in degrading the harmful contaminants of the rhizosphere [11].

3 Secondary Metabolites: Nature and Role

Fungal endophytes biosynthesize numerous SMs. Secondary metabolites are known to confer many benefits to the host. These chemical substances impact communications and act as inhibitors for competitors [12]. Various pharmaceutical applications have been found to be associated with the secondary metabolites. The potential application of secondary metabolites in the field of agriculture, food, cosmetics, etc. is a very well-acknowledged fact [13]. The SMs play a crucial role in various physiological functions of the host as well as the endophyte itself as they share an intricate relationship evolved during the time and happen to produce the same compound, very often [14]. Consequently, profile of the SMs and their chemical diversity confer them with the required niche security [15].

The idea of getting compounds from the endophytes, which were earlier known to be obtained from plants, has revolutionized the ongoing discovery of secondary metabolites [16]. For example, Paclitaxel, a multimillion dollar compound, was initially known to be extracted from Northwest Pacific yew tree *Taxus brevifolia*. But later on several endophytes were reported to produce this compound thus saving the sacrifice of thousands of yew trees [17]. Various categories assigned to theses metabolites are alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, saponins, tannins, terpenoids, tetralones, xanthones, and many others [18]. These molecules are an excellent source of antibiotics and anticancerous properties [19]. Secondary metabolites show a wide range of bioactivities including antibacterial, anticancerous, antifungal, anti-inflammatory, antioxidant, cytotoxic, and plant growth-promoting properties [19].

Future alternatives could be provided by exploring SMs of endophytic fungi against conventional drug therapies [20]. Even though the secondary metabolite production by endophytic fungi has attracted many investigators, the mystery behind the pathways involved in their production has remained a gray area. Metabolic diversity of SMs seems to be reflective of endophytic fungal diversity as per some of the contemporary metagenomic studies. Secondary metabolites are low-molecular-weight organic compounds that play an indirect role in the growth and

survival of producer organisms. Secondary metabolites find their usage in varied applications such as antibiotics, immunosuppressants, pesticides, plant growth promoters, pharmaceutics, etc. [19]. The symbolic attribute of the genes involved in SM biosynthetic pathway is their physical clustering on to the chromosome into biosynthetic gene clusters (BGCs) [21]. Secondary metabolite production has evolved hundreds of millions of years as endophytes utilize them as communicating signals, defense, inhibiting competitors, etc.

4 Gene Clusters for Secondary Metabolite Production and Their Characteristics

Contemporary genome mining shows that only a small portion of SMs is known out of the total potential metabolites that fungi can synthesize [22]. A multitude of silent gene clusters, under laboratory conditions, could be unmasked by different approaches to get them switched on [23–25]. Secondary metabolites are often biosynthesized by multienzyme pathways, and the proteins comprising one pathway are often encoded within a clustered set of genes, termed as biosynthetic gene cluster (BGC) [21, 26, 27] and are co-regulated. The arrangement of gene clusters responsible for modulating the chemical scaffold, transportation of products or substrates, regulatory and resistance functions, etc. are lined up contiguously in the genome and hence leading to the concept of gene clusters [25].

The first gene cluster to be identified in *Penicillium chrysogenum* and *Aspergillus nidulans* was penicillin cluster [28, 29]. The traditional view on secondary metabolite gene clusters has changed in the light of advancements in the fungal genome sequencing. For example, two distinct genomic positions were recorded for (i) the spore pigment production in *A. nidulans* and (ii) trichothecenes production in *Fusarium* spp. [30]. Thus the disparity between the numerous new gene clusters found in *Aspergillus* sp. and the number of gene clusters known for a species so far leads to the idea of the presence of silent gene clusters. These biosynthetic gene clusters (BGCs) are signatures for fungal genomes comprising co-localized contiguous genomes, and they participate in the same metabolic pathways. Their link with specialized metabolism, evolving into fungal lineages, sustaining distinct ecological requirements, utilizing uncommon nutrient sources (e.g., galactose and allantoin), and synthesizing bioactive pharmacological metabolites or virulent factors (e.g., aflatoxin and melanin), is most often observed [31].

Gene clusters instigating the biosynthesis of SMs spans around 10,000 bases or more [32]. The main biochemical pathways that guide the biosynthesis of SMs are polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS), hybrids (PKS-NRPS), terpene synthases (TPS), terpene cyclases (TCs), and prenyltransferases (PTs) or combinations thereof. Some SMs are known to be synthesized by ribosomes as well as post-translationally modified peptides (RiPPs) that help in regulation, carriage, and metabolite tailoring [32]. For example, generic scaffolds of most SMs are framed by PKS and NRPS enzymes that use malonyl CoA and amino acids, respectively, as building blocks, to manufacture the core

architecture of SMs [32]. These multimodular enzymes, being organized into modules, exhibit a great architectural similarity and product assemblage mechanism, despite considerable substrate difference [32].

Few precursor metabolites such as short-chain carboxylic acids (e.g., acetyl-CoA) or amino acids, which are linked together by backbone enzymes such as PKSs, NRPSs, TCs, or dimethylallyl tryptophan synthetases (DMATSs), result into the biosynthesis of SMs. Resultant oligomers thereupon are modulated by tailoring enzymes governed by common regulation [2]. Those SM clusters appearing to be species specific or confined to taxonomic distribution are found in less number of species, whereas those reported in wide distribution are highly divergent among species. Thus, amidst dozens of SM gene clusters, their identity, and number varies among closely correlated genomes of species, displaying synteny and high sequence conservation [2]. The significance of gene clustering lies in the coordinated transcriptional regulation through sharing far-distance regulatory elements or by administering changes in chromatin alignment [33]. The physical proximity of these clustered genes might be important for their co-inheritance, co-regulation of the combination of functionally important genes, and formation of end product in a pathway, favoring genomic assortment for conserving them together [34].

5 Genetic Basis of Biosynthesis and Identification of BGCs

The knowledge of genetic basis of biosynthesis allows to employ metabolic strategies for the optimization of the product manufacture by endophytes and making it cost-effective. Low yield of secondary metabolite production, requirement of optimal growth conditions, and several orphan compounds, i.e., compounds with unknown biosynthetic mechanism, are few problems to be circumvented via application of metabolic engineering. Therefore, understanding the substructure of genetics involved in SM biosynthesis paves a path for rearrangement of genes to obtain new products [2, 35]. Nielsen and Nielsen [36] have mentioned three ways to achieve the genetic basis of biosynthesis:

(a) Targeted approach that involves the comparison of similar BGCs, reducing them to probable BGCs, conjointly with retro-biosynthetic analysis that aims to presume enzymes and precursors instigating the production of targeted compounds. It leads to discovery of the genomic loci responsible for production of various SMs in *Penicillium aethiopicum* [37, 38]. A resistance-gene-guided genome mining and homology search for a high similarity to identify orphan compounds are few direct approaches to identify BGCs [39, 40].
(b) Untargeted approach: Untargeted approach involves exploiting the databases such as ClusterMine 360, IMG-ABC, and MIBiG to assess information such as the genome for biosynthetic potential, affinities among BGCs and their linkage, mapping new entries, correlating the BGCs with conserved motifs, grouping them into families, etc. These are some of the indirect approaches toward understanding the SMs and their BGCs [36].

(c) Metabolomics approach: When blended with mass spectrometry (MS), metabolomics helps in compound detection with respective BGCs in a sequenced genome. Peptidogenomics along with computational tools such as antiSMASH, NP.searcher, Pep2Path, MS-guided discovery, etc. made the detection of BGCs more plausible [36].

6 Silent and Orphan Gene Clusters

Genome mining has rapidly progressed the identification of putative genes responsible for SM biosynthesis. Identifying a central PKS- and/or NRPS-encoding gene, assumed to have regions containing gene clusters concerning particular metabolite formation, conjointly with anticipated function of adjoining genes, forms the basis of identifying those genes [41]. The term "orphan" and "silent" gene clusters, introduced by "Gross," refers to the clusters that are yet to be discovered for corresponding metabolites and genes that are not or less expressed, respectively [42]. In the absence of environmental cues, multifaceted communities, and host interaction, BGCs often remain silent in laboratory conditions.

7 Approaches to Access and Express the Silent Ones

One of the drawbacks of the routine bioactive potential screenings of endophytic fungal cultures is that it omits the biosynthetic pathways of SM production. Envisaging the plausible physiological role of BGCs in axenic cultures is a daunting task.

7.1 Co-culturing

Culturing two microbes together in an exclusive condition is referred as interspecies cross talk [43]. This technique exploits the fact that fungi are evolved to reside in specialized environmental conditions that involve interactions with other microbiota. Thus, there is a possibility that such interactions may cause the biosynthesis of diverse SMs involved in symbiotic associations [44]. Liquid co-cultures, viz., *Emericella* sp., a marine fungus with *Salinispora arenicola*, (a marine actinomycetes), and solid co-cultures of *Eutypa lata* and *Botryosphaeria obtusa*, both wood-decay fungi, grown in petri dishes, have shown a high potential of inducing chemical diversity of SMs displaying antimicrobial, anticancer, and phytotoxic potentials [45, 46]. Fischer et al. [47] summarized the mutual induction of SM production in fungi and bacterial co-culture cross talks with the ability to trigger diverse SMs. For example, fermenting *Streptomyces bullii* with *A. fumigatus* together culminated into formulation of SMs belonging to diketopiperazine alkaloids and

ergosterol [48]. Li et al. [49] found a novel cyclic tetrapeptide, viz., cyclo-(L-leucyl-trans-4-hydroxy-L-prolyl-D-leucyl-trans-4-hydroxy-L-proline), when two mangrove fungi, viz., *Phomopsis* sp. K38 and *Alternaria* sp. E33, are co-cultured [49].

7.2 OSMAC Approach

"One strain producing many compounds" is one of the classical approaches followed so as to culture the endophytes in different media to target silent BGCs [50]. The term OSMAC, coined by Zeeck and co-workers, refers to the capability of a strain to produce several unique but different SMs when introduced into varying growing conditions [51]. Various growth parameters such as aeration, flask shapes, pH, temperature, light source, etc. also trigger the cryptic biosynthetic pathways, when altered, as is shown in case of *Aspergillus ochraceus* [51]. *Spicaria elegans*, a marine fungus when introduced into ten different conditions, brought out a striking shift in the SM profiles, which included two known aspochalasins, a novel spicochalasin A and five new aspochalasins M–Q [52]. These reports provide an evidence to show that carbon sources and growth media have a great impact on SM profiles of endophytes.

7.3 Genetic Mutations

Profiling followed by comparing the metabolites of the mutant (deletion) as well as wild type is a typical strategy to observe the modulation in SM biosynthesis. For example, about 25 SM synthases/synthetases were characterized in chemical profiling of single-gene deletion mutants in *Aspergillus nidulans* [53]. These deletions also helped in establishing the role of core components of nuclear complex, i.e., VeA and LaeA (light-regulated developmental factor), in SM biosynthesis. Similarly, in *Aspergillus nidulans*, when a conserved subunit of the COP9 signalosome, csnE/CSN5, was deleted, a silenced cluster containing polyketide synthase gene got activated and resulted in the production of an antibiotic 2,4-dihydroxy-3-methyl-6-(2-oxopropyl)benzaldehyde (DHMBA) [54]. Various plausible ways to intervene into the regulatory circuits involved in SM biosynthesis are adopted. Genome mining has bestowed us with knowledge of putative gene encoding biosynthesis. Brakhage (2013) reported that strong inducible promoters, causing endogenous promoters, exchange in gene clusters [2]. The *acvA* gene that encodes the tripeptide synthetase, its activity is rate-limiting for the penicillin biosynthesis. Therefore, when exchanged against *alcA,* which is a strong inducible alcohol dehydrogenase promoter, the transformants showed drastic increase in penicillin production in *Aspergillus nidulans* [55].

7.4 Epigenetic Modification to Instigate Silent Clusters

Many silenced gene clusters are located within the heterochromatic regions and subsequently transcriptionally repressed. Heterochromatic regions comprise several silenced gene clusters, i.e., transcriptionally repressed [56, 57]. Epigenetic modifications, such as acetylation, ADP ribosylation, glycosylation, methylation, phosphorylation, and ubiquitination, play key roles in regulation of expression in genes. DNA methylation and histone modification causing agents modulate and induce cryptic fungal gene clusters. For example, 5-aza-20-deoxycytidine, 5-azacytidine, hydralazine, procainamide, procainamide, etc. are known DNA methyltransferase (DNMT) inhibitors, whereas suberoylanilide hydroxamic acid (SAHA), sodium butyrate, valproic acid, etc. are known inhibitors of DNA methyltransferase (DNMT). These inhibitors are capable of expediting the awakening of dormant cryptic clusters. HDAC results into deacetylation of histones at amino groups to maintain the inaccessible state of chromatin [58]. Negative regulation of penicillin and sterigmatocystin in *Aspergillus nidulans* and attenuation of NRPS gene cluster transcription in *Aspergillus fumigatus* depict the role of HDAC in SM biosynthesis [59, 60]. De novo synthesis of different lunalides and oxylipins by *Diatrype* sp. when 5-azacytidine was added and synthesis of new calphostin B and cladochromes by *Cladosporium cladosporioides* once SAHA was introduced depict the epigenetic role in breaking the silence of cryptic fungal gene clusters [61]. *Fusarium tricinctum*, an endophyte of *Aristolochia paucinervis*, when co-cultivated with *Bacillus subtilis*, resulted into 78-fold increase in constitutive SMs. This resulted in the production of three new natural products, viz., macrocarpon C, (−)-citreoisocoumarinol, and 2-(carboxymethylamino) benzoic acid [62]. When histone H3 deacetylase HdaA was inactivated in *Chaetomium* sp., a foliar endophyte of *Sapium ellipticum*, it leads to pleiotropic activation and overexpression of over and above two thirds of the biosynthetic genes. Furthermore, new structural compounds, viz., arbumelin, arbumycin, a meroterpenoid arbuscullic acid B, and the diterpenoid arbuscullic acid A, were obtained in its metabolic profile [63].

Furthermore, not only chemical epigenetic modifiers but also dietary items, such as grapes, green tea, soybean, turmeric, and cruciferous vegetables known to cause epigenetic change, affect the dormant gene clusters of endophytes. Sharma et al. [64] reported induction of cryptic bioactive SMs in *Colletotrichum gloeosporioides*, an endophyte of *Syzygium cumini*, by the addition of grape skin and turmeric extracts having resveratrol and curcumin, respectively, as their major components.

8 Computational Tools to Explore BGCs

Identification of homology, chemical product, enzymes encoded may give insights into BGC prediction [65]. A whole new dimension has been introduced by the computational aspects of genome mining with increase in genomic data toward the prediction of bioactive gene clusters, engineering and manipulation of genes of biosynthetic pathways, alternative analogues of active biomolecules, identifying

novel molecules, etc. [35]. Identifying the unfamiliar biosynthetic gene clusters (BGCs), encoding the enzymatic pathways for metabolite synthesis of putative gene clusters may unleash a large repertoire of compounds. Thus we may gain insights into unexplored BGCs; those are inactive in standard laboratory conditions depicting habitat intricacy and may hold promising pharmaceutical potential [32].

8.1 Databases

Databases focusing on clusters NRPS and PKS genes such as ClustScan Database (CSDB) and Recombinant ClustScan Database (rCSDB) are analyzed by ClustScan software [66]. "Database of biosynthesis clusters curated and integrated" (DoBISCUIT) targets PKS and NRPS biosynthetic gene clusters with information on genes clusters involved in tailoring the enzymes [67]. ClusterMine360 database [67] has 200 PKS and NRPS gene clusters which could be further subjected to analysis with antiSMASH. Ichikawa et al. [67] reviewed softwares such as SEARCHPKS, MAPSI, and Natural Product Domain Seeker (NaPDos) that address specific classes of enzymes.

8.2 Motif-Independent BGC Identification Approach (MIDDAS-M Algorithm)

Two or more transcriptomic datasets form the basis of identifying co-expressed genes in "chromosomally adjacent clusters" with an adjustable window, is how the algorithm works for a correlation between the co-expression to target gene that might encode a pathway of biosynthesis [68]. Secondary Metabolite Unique Regions Finder (SMURF) is a motif-based tool used to predict the endophytic fungal gene clusters such as PKS, NRPS, DMAT, and SMURF, and it is utmost specific. Both annotated and non-annotated genomes could be used here to predict SM clusters [69]. Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) is another database designed to predict the repertoire of compounds by detailed analysis of architecture of PKS/NRPS functional domains and orthologous gene clusters and predicts their core structure [26]. In the proteomic approach, through the usage of Proteomic Investigation of Secondary Metabolism (PriSM), BGCs and their respective metabolites can be investigated. The limitation in this is that it is restricted to PKS- and NRPS-based SM clusters whose enzyme products are >100 kDa in size [70].

8.3 HGT Transfer of BGCs Genes

Horizontal gene transfer or HGT can contribute to accelerated procurement of genes linked to ecologically essential attributes [71]. Apart from prokaryotes, HGT occurrence in eukaryotes is thoroughly evidenced, and fungi are no exceptions to it, which

include various fungal BGCs that encountered HGT encrypting diverse specialized metabolic pathways (horizontal gene transfer in eukaryotes: the weak-link model), and the examples include fungal SMs such as bikaverin [72], gliotoxin [72], and sterigmatocystin [72]. The propensity of clustered genes instigating SM biosynthesis, undergoing HGT, is 1.66-fold higher than the non-clustered genes.

9 Regulation of BGCs

Environmental cues, biotic and abiotic stresses, and interaction with all communities are the challenges met by endophytes in natural habitat. In response to these factors, endophytes produce SMs mediated via transcription factors. Regulation of SM gene clusters happens at different levels. Apart from environmental cues, the regulators are global or SM gene cluster-specific ones. Global regulatory proteins control these gene clusters in conjunction with other genes not associated with secondary metabolism, whereas SM gene cluster-specific ones target a particular transcription factor responsible to regulate a specific enzyme in the gene cluster [56].

9.1 Global or Indirect Regulation

LaeA, a protein with nuclear methyltransferase-domain, was first characterized in *Aspergillus nidulans* and marked as a hallmark of global regulators. It depicted both the positive and negative regulation in SAM biosynthesis of several species such as *Aspergillus*, *Fusarium*, and *Penicillium* spp. [73]. AreA, a transcription factor belonging to GATA family, is one of the highly conserved global transcription factors with characteristic Cys_2Hys_2 zinc finger DNA-binding domain. It is reported to repress nitrogen metabolism when ammonium and glutamine are present [74]. Besides the primary metabolism, it affects the SM regulatory genes *aflR* and *aflJ*, regulating aflatoxin and sterigmatocystin, respectively, in species-specific mycotoxin responses in *Aspergillus parasiticus* [75]. Similarly PacC, belonging to family of Cis_2His_2 zinc finger transcription factor, globally regulates number of physiological processes along with SM biosynthesis in response to surrounding pH [76]. β-Lactam, cephalosporin expression in *A. chrysogenum*, was found to be under the regulation of PacC as it is bound to structural genes of promoter region [77]. CreA, Cys_2His_2 zinc finger transcription factor, largely concerned with carbon catabolite repression [78], also mediated SM regulation. Deregulation of mutant CreA was observed with respect to wild type in *A. chrysogenum*, which resulted in overproduction of cephalosporin [79]. The heterotrimeric Velvet Complex, i.e., the velvet proteins VeA and VelB and LaeA, is a developmental regulator sensing light, linking SM biosynthesis with sexual development in response to light [80]. Duran et al. [81] reported that VeA modulates the regulatory aflR gene in *Aspergillus flavus* and consequently affected aflatoxin production. In *A. chrysogenum*, *A. nidulans*, and *P. chrysogenum*, β-lactam biosynthesis is also governed by light-dependent regulator VeA [33]. CBC, i.e., CCAAT-binding complex, is responsible for regulation in redox

status and iron deprivation. SidC, a core enzyme encoded by *sidC* in *A. nidulans*, is required for an SM siderophore, viz., ferricrocin which is needed for iron homeostasis and development. In iron-depriving conditions, *sidC* interacts with the bZIP protein HapX and gets upregulated by CBC [82].

9.2 Pathway-Specific or Direct Regulation

Influencing the gene clusters, in which they are found in, the pathway-specific transcription factors positively regulate the expression. $Zn(II)_2Cys_6$ family of transcription factors, reported only in fungi, are most commonly committed to SM regulation. The regulator AflR, which regulates aflatoxin/sterigmatocystin, is a well characterized and established one for regulation studies. In *Aspergillus nidulans*, *aflR* deletion caused sterigmatocystin repression even under stimulating conditions, whereas its overexpression in unfavorable aflatoxin conditions has resulted in the expression of biosynthetic genes for aflatoxin [83, 84]. The *aflR* is very well known to be present in sterigmatocystin cluster of *A. nidulans* and aflatoxin clusters of *A. parasiticus* and *A. flavus* [84–86]. Cys_2His_2 family of transcription factors, reported in fungi and eukaryotes, bind DNA as monomers, are classical regulators. Proteins, such as Cmr1p in *Colletotrichum lagenarium*, Pig1p in *Magnaporthe grisea*, Cmr1 in *Cochliobolus heterostrophus*, and BMR1in *Bipolaris oryzae*, positively regulate melanin pigment synthesis that in turn results in withstanding stresses [74].

Basic leucine zipper (bZIP) transcription factors that guide DNA binding and dimerization of proteins are regarded as factors responding to environmental stresses and seem to have a link with SM biosynthesis [87]. Sterigmatocystin and asperthecin that are regulated by RsmA in *Aspergillus nidulans*, aflatoxin regulated by AtfB in *Aspergillus parasiticus*, and ochratoxin regulated by Aoyap1 in *Aspergillus ochraceus* are stress and SM regulators associated with bZIPs [88–90]. Winged Helix transcription factor family includes a group of proteins with helix-turn-helix structure. *Acremonium chrysogenum*, which is a well-known producer of antibiotic cephalosporin C, was first time reported to be under cluster-specific regulation factor CPCR1 belonging to subfamily of RFX proteins [66].

10 Conclusion

Endophytic fungal secondary metabolism is a complex process as it does not follow a strict categorized command but overlaps, interconnects, and is regulated by multilevel regulators. Challenges and pitfalls in SM investigation are lacking of a complete knowledge on (i) detection, identification, and characterization of all the metabolites, (ii) influence of silent gene clusters under standard laboratory conditions, (iii) true function and nature of SM in their native state, and (iv) effect of combined and critical role of SMs in various biosynthetic pathways. The state-of-the-art technologies used currently in natural product discovery show an exciting

phase. In the genomic era, we have information flow through efficient sequencing technologies and computational approaches to predict and identify the targets. However, the important task is to understand the global networks of interaction between the key regulators, which may provide more insights to bridge the gaps in our understanding between the primary and secondary metabolism. This approach may open up an alternative source to phytochemicals. To explore and appreciate the true potential of fungal endophytic BGCs, we need to bring in multidisciplinary approaches including bioinformatics, molecular biology, chemical characterization of SMs, understanding the physical environmental effects, metabolomics, proteomics, etc.

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Secondary Metabolites Produced by Endophytic Fungi from Marine Environments

18

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Contents

Abstract

Endophytes are symptomless organisms thriving within the living host tissues. Some endophytic fungi have been shown to be producing the same compounds produced by their hosts, e.g., taxol produced by *Pestalotiopsis microspora*

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isolated from *Taxus wallichiana*. Hence, there has been lot of interest to screen the secondary metabolites of endophytic fungi. In recent times the focus on endophytic fungi and the secondary metabolites they produce has shifted to marine environments. Unlike terrestrial environments where more research has been conducted on secondary metabolites from living plant substrata, the secondary metabolites produced by endophytic fungi isolated from marine environments are equally from macroalgae (seaweeds) and sponges in addition to mangrove and different shoreline plant substrata. Many promising secondary metabolites that have therapeutic potential including in antimicrobial, antiviral, antimalarial, and anticancer applications have been reported from endophytic fungi isolated from seaweeds, sponges, and plants from maritime environments. For example, the compound 3-*O*-methylfunicone isolated from *Talaromyces* sp., in mangrove habitat, has shown antifungal, antitumor, and lipid-lowering properties and required beyond academic research wherein pharmaceutical industry needs to take it further. A *Cladosporium* L037 species from the brown alga *Actinotrichia fragilis*, collected off Seragaki Beach at Okinawa Island, Japan, produced two 12-membered macrolides, namely, sporiolides A and B. Both these metabolites exhibited potent cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 0.37 and 3.1 um, respectively. A cyclic tetrapeptide compound produced by *Petriella* sp., an endophyte of the sponge *Suberites domuncula*, showed cytotoxicity against murine L5178Y lymphoma cells at an ED_{50} value of <0.1 μg/ml. The present chapter updates and consolidates the information available on the secondary metabolites produced by endophytic fungi isolated from marine environments.

Keywords
Antimicrobial · Anticancer · Bioactive compounds · Macroalgae · Marine drugs · Natural products · Sponges

1 Introduction

Three quarters of surface of the earth comprises marine environment and reflects a fusion of all diverse living microbes [1]. Oceans are not only the habitats for diverse living beings but are also luxuriant resources of diverse natural products. Diversity is found not only in forms and structures of different marine organisms but also reflected in the resulting metabolites produced by them [2]. The fact that there has been a growing increase in the number of marine-derived natural products reported from endophytic fungi is encouraging to take further steps and extensive explorations for natural bioactive compounds from marine environment in drug discovery perspective. In a short period, the number of new metabolites reported has risen from 108 to 142 from 2014 to 2016, which illustrates the potential of marine endophytes to deliver the promising drugs to mankind and their need [3].

Several drugs of clinical importance prove the worth of chemical skeleton these marine natural compounds possess and their importance to human welfare [4]. Nonetheless, new incidences of resistance and infection clearly indicate the need of more

reliable and robust drugs to combat the burning issues of resistance, infections, epidemics, etc. [5]. The intricate intercommunications and the various niches in the marine environment culminate into complex and diverse metabolite profile that are still not well characterized and poorly understood [6].

2 Endophytic Fungi Isolated from Macroalgae (Seaweeds)

Macroalgae (seaweeds) harbor many endophytic fungi. Natural products from macroalgae are wide ranging starting from therapeutic to cosmetics and have been widely exploited for various human uses. Specialized ecological niches have always been the factors for the diversity in the secondary metabolites secreted by microbe when in association with a host. Factors such as extended exposure of sunlight, higher and varying salt concentration, and interaction with various other microbiota add the complexity of the interactions that the corresponding algae have. Thus in turn, the endophytes associated with hosts also undergo the stress, and one may predict the secondary metabolites (SMs) to be highly diverse and potent.

2.1 Secondary Metabolites with Anticancerous Activities

Several fungi associated with algae produce SMs with anticancerous activity. *Paecilomyces variotii*, an endophyte of marine algae, is reported to produce varioloid A and varioloid B, which are indole derivatives with anticancerous activity [7]. *Microsporum* sp. associated with *Lomentaria catenata*, a red algae, produces physcion, which is capable of inducing apoptosis in HeLa cells [8]. *Leptosphaeria* sp. extracted from *Sargassum tortile*, a brown alga, was found to produce epipolysulfanyldioxopiperazines showing topoisomerase II inhibition and thus cytotoxic against P-388 leukemia cells [9]. Mycoendophytes are also known to produce bioactive compounds belonging to steroid groups. *Aspergillus ochraceus* is found to produce cinnamolide derivative and compound insulicolide with anticancerous properties [10]. *Aspergillus ochraceus* isolated from macroalga *Sargassum kjellmanianum* was also investigated to produce steroidal derivative, 3β, 11α-dihydroxy ergosta- 8,24(28)- dien-7-one, an uncommon 7-nor-ergosterolide, an anticancer nor-ergosteroid compound [11]. *Chaetomium* sp. QEN-14 associated with the marine green alga *Ulva pertusa* was characterized to produce even new cytochalasan derivatives, cytoglobosins A−G. Out of seven, cytoglobosins C and D showed anticancer activity against A-549 tumor cell line [12]. Isolation and characterization of chaetopyranin, a new benzaldehyde secondary metabolite, from *Chaetomium globosum*, an endophyte of red alga *Polysiphonia urceolata*, possess anticancerous activity against several tumor cell lines [13]. Also an isolate of *Xylaria* sp., from *Bostrychia tenella*, a Brazilian marine seaweed, was reported to produce cytochalasin D, which showed moderate activity against SF-295 and HCT-8 cancer cell lines [14]. These examples show that the endophytic fungi from marine macroalgae have good potential in producing secondary metabolites that have anticancerous activity and hence are in need of further research.

2.2 Secondary Metabolites with Antimicrobial Activities

Sun et al. [15] isolated *Aspergillus wentii*, an endophyte of *Gymnogongrus flabelliformis*, marine red alga. They characterized three new xanthone derivatives: yicathin A, yicathin B, and yicathin C. All three were screened against *Colletotrichum lagenarium* and *Fusarium oxysporum*, phytopathogens, as well as against bacterial pathogens *Escherichia coli* and *Staphylococcus aureus*. Yicathin B was potent against *E. coli* and yicathin C against *E. coli*, *S. aureus*, and *C. lagenarium* [15]. Furthermore, the first report on cyclopiane diterpenes, showing antibacterial activity, was from *Penicillium chrysogenum* QEN-24S, an algicolous fungus derived from marine red alga. Conidiogenone B, tetracyclic diterpenes, displayed distinct activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *P. aeruginosa*, *Pseudomonas fluorescens*, and *Staphylococcus epidermidis* [16].

2.3 Secondary Metabolites with Antioxidant Activities

Epicoccum sp., isolated from marine brown alga *Fucus vesiculosus*, produced a novel compound epicoccone, viz., 4,5,6-trihydroxy-7-methylphthalide, which has potent antioxidant activity with 95% radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 25 μg/mL and 62% inhibition of Thiobarbituric acid reactive substances (TBARS) at 37 μg/mL [17]. Two compounds, i.e., 2,3,6,8-tetrahydroxy-1-methylxanthone, 2,3,4,6,8-pentahydroxy-1-methylxanthone (a xanthone derivatives), and 5-(hydroxymethyl)-2-furanocarboxylic acid from *Wardomyces anomalus*, isolated from marine macroalgae, displayed a significant antioxidant activity as they inhibit $p56^{lck}$ tyrosine kinase [18]. Similarly, two novel compounds 7-isopropenylbicyclo[4.2.0]octa-1,3,5-triene-2,5-diol and 7-isopropenylbicyclo- [4.2.0]octa-1,3,5-triene-2,5-diol-5-â-D-glucopyranoside, hydroquinone derivatives, were isolated from *Acremonium* sp.; inhabitant of tissues of the *Cladostephus spongiosus*, a brown alga, displayed significant antioxidant activity with up to 90% scavenging activity of DPPH radical and a moderate lipid peroxidation activity [19]. 2-Hydroxycircumdatin C, a benzodiazepine analogue, derived from *Aspergillus ochraceus*, isolated from *Sargassum kjellmanianum*, was first time reported to have been isolated from a natural source. This compound showed excellent DPPH radical scavenging activity and found to be 8.9 times more potent than butylated hydroxyl toluene, a well-known standard used in antioxidant assays [20].

A new fungus-derived benzodiazepine analogue, 2-hydroxycircumdatin C (1), and a compound which has been isolated from a natural resource for the first time but has been previously synthesized, namely, (11aS)-2,3-dihydro-7-methoxy-1H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H,11aH)-dione (2), along with five structurally related known alkaloids (3–7), were isolated from *Aspergillus ochraceus*, an endophytic fungus isolated from the marine brown alga *Sargassum kjellmanianum*. Compound 1 displayed significant DPPH radical-scavenging activity with an

IC_{50} value of 9.9 mm, which is 8.9-fold more potent than that of butylated hydroxytoluene (BHT), a well-known synthetic positive control.

2.4 Secondary Metabolites with Other Activities

Tyrosine-kinase inhibitor: Chaetominedione and 5-(hydroxymethyl)-2-furancarboxylic acid, isolated from *Chaetomium* sp., an endophytic fungus isolated from *Valonia utricularis*, from Atlantic Ocean, were reported to have tyrosine-kinase inhibitor activity by 100% at 200 μg/ml [21]. Inhibition of acetylcholinesterase was shown by 3β,4α-dihydroxy-26-methoxyergosta-7,24(28)-dien-6-one (2), a new steroid, and (8E,12Z)-10,11-dihydroxyoctadeca-8,12-dienoic acid (1), a new oxylipin, isolated from an endophytic fungus, *Aspergillus flavus*, associated with *Corallina officinalis*, a red alga.

3 Endophytic Fungi Isolated from Mangrove Plants

Mangroves are halotolerant plants in coastal wetlands and mangrove habitat acknowledged as one of the richest as well as productive habitats with pronounced economic, ecological, and social significances [22]. The species diversity of both flora and fauna of mangroves is well documented and hence often termed as "hotspots" for marine fungi [23]. The fungi associated with mangroves are also called as manglicolous fungi that include higher fungi belonging to ascomycetes and basidiomycetes and lower fungi belonging to oomycetes and thraustochytrids. The vegetation in mangroves is specialized to thrive in adverse conditions such as extreme temperature, high salinity, high wind velocity, tidal extremes, and anaerobic clayey soils concomitantly; fungi associated with them are also equipped with secondary metabolites which can withstand against these odds [22]. The manglicolous fungi are the second largest ecological group of marine fungi [24]. Undeniably, SMs from mangrove fungi are biotechnologically interesting as these bioactive metabolites play a decisive role to cope up extreme environmental conditions. Among various groups of mangrove microbiota, endophytes are one of the producers of novel metabolites with pharmaceutical and nutraceutical importance such as antidiabetic, antibiotic, anti-inflammatory, antiviral, antioxidant, anticancer, and therapeutic agents and immunosuppressive drugs [25]. Mangrove endophytes are not restricted to aforementioned role, but rather endophytes are well-known for remediation process [26] including biosorption of heavy metals also [27].

3.1 Secondary Metabolites with Anticancer Activities

Penicillium brocae MA-231, isolated from mangroves, produced spirobrocazines C and brocazine G with cytotoxic activity against cancer cell lines such as A2780

and A2780 CisR cell [28]. *Lasiodiplodia* sp. 318, isolated from mangrove plant *Excoecaria agallocha*, was found to secrete 2,4-dihydroxy-6-nonylbenzoate having cytotoxic activity [29]. *Pestalotiopsis microspora*, associated with *Drepanocarpus lunatus*, produced 7-*O*-methylnigrosporolide (78), pestalotioprolides D–F [30]. Similarly, *Stemphylium globuliferum*, isolated from *Avicennia marina* in Egypt, secreted dihydroaltersolanol C, altersolanols A, B, and N, and alterporriol E [31]. Pestalpolyol I, a new polyketide derivative, was produced by *Pestalotiopsis clavispora*, isolated from *Rhizophora harrisonii*, and has anticancer activity. Pullularin A, pullularin C, verticillin D, and pullularins E and F had been characterized from *Bionectria ochroleuca*, from inner tissue of *Sonneratia caseolaris*, showing anticancerous activity [31]. *Annulohypoxylon* sp., an endophyte of mangrove plant *Rhizophora racemosa*, was reported to produce daldinone derivatives having apoptotic activity against cancer cells [31].

3.2 Secondary Metabolites with Antimicrobial Activities

Penicillium aculeatum (No. 9EB), a foliar endophyte of the mangrove *Kandelia candel*, resident from the South China Sea, was found to secrete (2′S*)-2-(2′-hydroxypropyl)-5-methyl-7, 8-dihydroxy-chromone, a new chromone derivative, which displayed antibacterial activity against *Salmonella*, a gram-negative bacteria, with an MIC value of 2.00 ± 0.02 μM. Two more compounds, bacillisporin A and bacillisporin B, displayed antibacterial activity against *Bacillus subtilis*, a gram-positive bacteria with MIC values of 0.13 ± 0.02 μM [32]. *Stemphylium* sp. 33231, inhabiting *Bruguiera sexangula* var. *rhynchopetala* in South China Sea, secreted four new anthraquinone derivatives as well as four new alterporriol-type anthranoid dimers, when cultivated on rice medium. Anthraquinone derivatives happened to display more antibacterial activity when compared to anthranoid dimers [33]. Similarly, antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, and *Micrococcus tetragenus* was exhibited by 1-(2,6-dihydroxyphenyl) butan-1-one, a compound isolated from *Penicillium citrinum*, a foliar endophyte isolated from *Bruguiera sexangula* var. *rhynchopetala.* Two dihydroisocoumarin penicimarins also isolated from it displayed antibacterial activity against the five pathogenic bacteria: *S. epidermidis*, *Escherichia coli*, *S. aureus*, *Bacillus cereus*, and *Vibrio alginolyticus* [34]. *Neosartorya udagawae*, a root endophyte of mangrove plant *Avicennia marina*, was found to yield a unique 6/6/6/5 quinazoline ring system connected directly to a 6/5/5 imidazoindolone ring, neosartoryadins A and B, as active metabolite displaying antiviral property against virus A (H1N1) [34]. Two new cyclic tetrapeptides were derived from the co-culture of two mangrove fungi, *Phomopsis* sp. and *Alternaria* sp., which displayed moderate to high antifungal activity against *C. albicans*, *G. graminis*, *H. sativum*, *R. cerealis*, and *F. graminearum* [35].

3.3 Secondary Metabolites with Antioxidant Properties

Mangrove vegetation experiences a constant stress due to the marine environment where the plants thrive. Very few studies have focused on how endophytes contribute to counter the abiotic stresses such as temperature, salinity, drought, and other factors [36]. Endophytes secrete anti-stress biochemical compounds to act as stress busters. An ascomycetous fungal strain SK2YWS-L, derived from mangroves, reported to produce three novel 2,3-diaryl indone derivatives, ascomindones A−C, exhibiting excellent antioxidant properties with one of the compound displaying potency more than ascorbic acid [31].

3.4 Secondary Metabolites with Antituberculosis Activity

An endophytic fungus *Aspergillus* sp. 16-5C was reported to produce asperlones A and B, dinaphthalenone derivatives that displayed excellent activity against *Mycobacterium tuberculosis* protein tyrosine phosphatase B (MptpB) [31]. Similarly, another *Aspergillus* sp. 16-5c, a mangrove endophyte, was found to produce a novel sesterterpenoid, asperterpenoid A (1), with a new carbon skeleton, characterized with the help of extensive spectroscopic methods, which displayed an activity against MptpB with an $IC_{(50)}$ value of 2.2 μM [37].

Furthermore, characterization of alterporriol-type dimers from a mangrove fungus *Alternaria* sp. (SK11) displayed potent antagonistic activity against MptpB, in particular against atropisomer 2 with an IC_{50} value of 8.70 μM [38]. Furthermore, *Penicillium dipodomyicola*, HN4-3A, a resident of mangrove plant *Acanthus ilicifolius*, also displayed MptpB inhibition due to peniphenones A–D [38].

3.5 Secondary Metabolites with Anti-inflammatory Activities

Aspergillus terreus, from mangroves of South China Sea, residing in *Kandelia obovata*, was reported to produce meroterpenoid, an anti-inflammatory compound [39].

3.6 Secondary Metabolites with α-Glucosidase Inhibitory Activity

Secondary metabolite of an endophytic fungus *Meyerozyma guilliermondii*, from mangrove plant *Kandelia obovata* of South China Sea, grown on solid substrate, was reported to have three new depsidones, botryorhodines E–G, and two new isoindolinones, meyeroguillines A and B, with α-glucosidase inhibitory activity [32].

Talaromyones A and B, two new depsidones, out of seven compounds, are produced by *Talaromyces stipitatus* SK-4, an endophyte isolated from *Acanthus ilicifolius*. Talaromyones B and two other depsidone analogues showed inhibition of α-glucosidase [19].

4 Endophytic Fungi Isolated from Sponges

Marine sponges are also treasure for bioactive SMs and have yielded diverse pharmaceutical products during various investigation carried so far and covered a wide range from novel anticancer, anti-inflammatory, and antibiotics agents [40]. Reports suggest that more than 5000 metabolites with diverse chemical skeleton and properties belong to various classes such as alkaloids, macrolides, terpenoids, polyethers, peptides, and nucleoside derivatives [40]. Endophytic fungi harboring marine sponges are also a prime focus of research, and they are known to produce the highest number of SMs giving more insights into the chemistry of natural products [40]. Fungi associated with sponges such as *Aspergillus similanensis*, *Trichoderma harzianum*, *Hypocrea koningii*, and *Emericella variecolor* produced new chemical compounds with various biological activities such as antifungal, antibacterial, antioxidants, antitumorous, and antihyperlipidemic effects [41]. Cytochalasin K and 10-phenyl-[12]-cytochalasin Z16 were isolated from endophytic fungus *Arthrinium arundinis* ZSDS1, isolated from a marine sponge, *Phakellia fusca*, found in Xisha islands of China. Both the compounds displayed cytotoxic effect on various cell lines [42]. Similarly, from the endophyte *Penicillium raistrickii*, associated with *Axinella corrugata*, a sponge, compound 1,3,6-trihydroxy-8-methyl- 9H-xanthen-9-one, a norliquexanthone, was isolated, and it possesses antimicrobial and cytotoxic activities [43]. Two new cyclodepsipeptides, isolated from a sponge-derived fungus *Scopulariopsis brevicaulis*, have shown cytotoxic effect as well as antibacterial activity [44]. In another study, two disydonols, A and B, out of three noble phenolic bisabolane sesquiterpenoid dimers, produced by *Aspergillus* sp., isolated from a sponge *Xestospongia testudinaria* in the South China Sea, were found to be effective against HepG-2 and Caski human tumor cell lines and hence were cytotoxic [45]. Similarly, four new bisabolane-type sesquiterpenoids along with one known compound were produced by *Aspergillus* sp. isolated from the same sponge, i.e., *Xestospongia testudinaria*, and these have shown antibacterial activity. Marilines A–C, novel phthalimidine, produced by *Stachylidium* exhibited inhibitory activity against human leukocyte elastase (HLE) having an IC_{50} value of 0.86 μM [40].

In the above paragraphs, few examples on secondary metabolites produced by endophytic fungi isolated from macroalgae, mangroves, and sponges have been presented to show the trends available. More information is provided in Table 1 along with references, and the readers are advised to consult the individual publications for further information.

5 Discussion

Marine fungal natural products are diverse in nature as they are synthesized in response to the environmental cues to manage stress and support the growth. Despite the fact that marine endophytes are strong contenders for providing metabolite that can play a part in drug discovery pipeline, this area is still less explored. The lifestyle, survival strategies, defense mechanism, communication, etc. are some of

Table 1 Secondary metabolites produced by endophytic fungi isolated from algal, mangrove, and sponge hosts and their activity

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
1	*Aspergillus wentii*	Yicathin A–C	Antibacterial and antifungal activity	*Gymnogongrus flabelliformis*	Algae	[15]
2	*Acremonium* sp.	Acremonisol A and (3R)-7-hydroxy-5-methylmellein	Antimicrobial, cytotoxic activity	Red alga	Algae	[46]
3	*Acremonium* sp.	Phthalide derivative, acremonide, isocoumarin derivatives, acremonones A-H	Antifungal activity	*Rhizophora apiculata*	Mangrove plant	[47]
4	*Acremonium* sp.	7-isopropenylbicyclo[4.2.0]octa-1,3,5-triene-2,5-diol, 1,2-(1-hydroxy-1-methyl)-2,3-dihydrobenzofuran-5-ol, 2, 2-dimethylchroman-3, 6-diol,2-(3-dihydroxy-3-methylbutyl) benzene −1,4-diol	Antioxidant activity	*Cladostephus spongiosus*	Sponges	[48]
5	*Alternaria* sp.	Xanalteric acids I and II	Antimicrobial	*Sonneratia alba*	Mangrove plant	[50]
6	*Alternaria* sp.	Alternariol, perylene quinones	Cytotoxic activity	*Sonneratia alba*	Mangrove plant	[51]
7	*Alternaria* sp.	Alterporriol K–M	Cytotoxic activity	*Aegiceras corniculatum*	Mangrove plant	[47]
8	*Alternaria* sp.	10-oxo-10H-phenaleno[1,2,3-de] chromene-2-carboxylic acids, xanalteric acids I and II	Antifungal activity	*Sonneratia alba*	Mangrove tree	[47]
9	*Alternaria* sp.	Cyclohexenone and cyclopentenone derivatives	Antifungal activity	Mangrove plant	Mangrove plant	[52]
10	*Alternaria* sp. (SK11)	Atropisomer 2	MptpB inhibitor	Mangrove plant	Mangrove plant	[38]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
11	*Alternaria* sp. SK6YW3L	Altenusin	a-glucosidase inhibitory activity	*Sonneratia caseolaris*	Mangrove plant	[53]
12	*Alternaria tenuis*	Azepine alkaloid Sg17-1-4	Anticancer activity	Chinese alga	Algae	[53]
13	*Alternaria tenuis*	Isocoumarin	Cytotoxic activity	Marine alga	Algae	[54]
14	*Annulohypoxylon* sp.	Daldinone I	Cytotoxic activity	*Rhizophora racemosa*	Mangrove tree	[56]
15	*Apiospora montagnei*	Diterpene myrocin, polyketide apiosporic acid, 9-hydroxyhexylitaconic acid monomethyl ester, (+)-hexylitaconic acid,+)-epiepoxydon	Antibacterial, cytotoxic activity	Sponges, jelly fish, and algae	Sponges, jelly fish, and algae	[57]
16	*Arthrinium arundinis*	Cytochalasin K, 10-phenyl-[12]-cytochalasin Z16	Cytotoxic activity	*Phakellia fusca*	Sponge	[42]
17	*Ascochyta salicorniae*	Tetramic acids, polyketide ascosalipyrone	Enzymatic activity	*Ulva* sp.	Algae	[46]
18	Ascomycota sp. CYSK-4	Desmethyldichlorodiaportintone	Anti-inflammatory activity	*Pluchea indica*	Mangrove plant	[58]
19	*Ascochyta saliconiae*	2,3-dihydro-2-hydroxy-2,4-dimethyl-5-trans-propenylfuran-3-one	Tyrosine kinase inhibition	*Ulva* sp.	Algae	[48]
20	*Aspergillus aculeatus*	Aspergillusol A	Enzymatic activity, cytotoxic activity	*Xestospongia testudinaria*	Sponge	[59]
21	*Aspergillus carneus*	Drimane sesquiterpene lactone, 9α-hydroxy-5α-drim-7-ene-6-one-11,12-olide	Cytotoxic activity	*Laminaria sachalinensis*	Algae	[60]

22	*Aspergillus flavipes*	Cytochalasin derivatives Z16-Z20	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[47]
23	*Aspergillus flavus*	5-hydroxy-2-pyrones	Cytotoxic activity	*Enteromorpha tubulosa*	Algae	[46]
24	*Aspergillus flavus*	Alkaloids	Cytotoxic activity	*Enteromorpha tubulosa*	Algae	[46]
25	*Aspergillus fumigatus*	Indole alkaloid fumigaclavine C	Cytotoxic activity	Alga	Algae	[61]
26	*Aspergillus glaucus*	Aspergiolide A	Cytotoxic activity	Mangrove roots	Mangrove plant	[46]
27	*Aspergillus nidulans*	Quinazolinone alkaloids, aniquinazolines A–D	Antibacterial, cytotoxic activity	*Rhizophora stylosa*	Mangrove tree	[62]
28	*Aspergillus niger*	Ergosterimide	Enzymatic activity	*Colpomenia sinuosa*	Algae	[46]
29	*Aspergillus niger*	Pyranonigrin A	Enzymatic activity	Mangrove wood	Mangrove tree	[46]
30	*Aspergillus niger*	Asperamides A and B, sphingolipid	Antifungal activity	*Colpomenia sinuosa*	Algae	[46]
31	*Aspergillus niger*	Nigerapyrones A–E, asnipyrones B	Cytotoxic activity	*Avicennia marina*	Mangrove tree	[47]
32	*Aspergillus niger*	Phenethyl-α-pyrone derivative, isopyrophen	Antibacterial activity	*Colpomenia sinuosa*	Algae	[54]
33	*Aspergillus ochraceus*	7-nor-ergosteroid, steroidal derivatives	Cytotoxic activity	*Sargassum kjellmanianum*	Algae	[49]
34	*Aspergillus ochraceus*	Nitrobenzoyl sesquiterpene	Chemotaxonomic activity	*Coelarthrum* sp.	Algae	[60]
35	*Aspergillus ochraceus*	2-hydroxycircumdatin C	Antioxidant activity	*Sargassumk jellmanianum*	Algae	[48]
36	*Aspergillus oryzae*	Asporyzin A, asporyzin B, indoloditerpene, asporyzin C	Antibacterial activity	*Heterosiphonia japonica*	Algae	[49]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
37	*Aspergillus parasiticus*	Parasitenone, gabosine derivative	Antioxidant activity	*Carpopeltis cornea*	Algae	[49]
38	*Aspergillus pseudodeflectus*	Pseudodeflectusin	Anticancer activity	*Sargassum fusiform*	Algae	[49]
39	*Aspergillus* sp.	Halimide	Anticancer activity	Algae	Algae	[48]
40	*Aspergillus* sp.	Dehydroxychlorofusarielin B	Antibacterial activity	*Sargassum horneri*	Algae	[59]
41	*Aspergillus* sp.	Terpeptin analogues	Antioxidant activity	*Sargassum* sp.	Algae	[49]
42	*Aspergillus* sp.	Dehydroxychlorofusarielin B	Antibacterial activity	*Sargassum horneri*	Algae	[46]
43	*Aspergillus* sp.	Terpeptins A and B	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[46]
44	*Aspergillus* sp.	Chlorogentisyl alcohol	Antioxidant activity	*Hypnea saidana*	Algae	[55]
45	*Aspergillus* sp.	Terpeptins A and B	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[47]
46	*Aspergillus* sp.	Aspergillumarins A and B	Antibacterial activity	*Bruguiera gymnorrhiza*	Mangrove plant	[47]
47	*Aspergillus* sp.	Sesquiterpenes (+)-sydowic acid, (+)-sydonic acid	Antibacterial activity	*Dichotella gemmacea*	Corals	[60]
48	*Aspergillus* sp. 085242	Asperterpenols A and B	Inhibit acetylcholinesterase	Mangrove plant	Mangrove plant	[63]
49	*Aspergillus* sp. 085242	Asperisocoumarins B, furoisocoumarins, and asperisocoumarins E and F	α-glucosidase inhibitory activity	Mangrove plant	Mangrove plant	[64]

50	*Aspergillus* sp. 085243	Asperisocoumarins A, asperisocoumarins C	Antioxidant	Mangrove plant	Mangrove plant	[64]
51	*Aspergillus* sp.	Fumiquinazoline J	Cytotoxic activity	*M. angulosa*	Corals	[41]
52	*Aspergillus* sp.	Isydonols A and B	Cytotoxic activity	*Xestospongia testudinaria*	Sponge	[45]
53	*Aspergillus* sp.	Aspergiterpenoid A, (−)-sydonol, (−)-sydonic acid, (−)5-(hydroxymethyl)-2-(2′,6′,6′-trimethyltetrahydro-2H-pyran-2-yl)phenol, (Z)-5-(hydroxymethyl)-2-(6′-methylhept-2′-en-2′-yl)phenol	Antibacterial activity	*Xestospongia testudinaria*	Sponge	[65]
54	*Aspergillus sydowii*	Chlorinated 2,5-diarylcyclopentenones, sydowin A and B, aromatic butenolides, aspernolides A and B	Cytotoxic activity	*Acanthophora spicifera*	Corals	[46]
55	*Aspergillus terreus*	Butenolides, butyrolactones VI and VII	Anti-mycobacterial activity, antiplasmodial activity	Tree hole	ND	[49]
56	*Aspergillus terreus*	Butyrolactone	Enzymatic activity	*Laurencia ceylanica*	Algae	[54]
57	*Aspergillus tubingensis*	Dimeric naphtho-γ-pyrones, monomeric naphtho-γ-pyrones	Cytotoxic activity	*Pongamia pinnata*	Plant	[47]
58	*Aspergillus ustus*	Drimane sesquiterpenoids, threo-isomers	Cytotoxic activity	*Suberites domuncula*	Sponge	[51]
59	*Aspergillus ustus*	Ustusoranes A–F	Cytotoxic activity	*Bruguiera gymnorrhiza*	Mangrove plant	[46]
60	*Aspergillus ustus*	Drimane sesquiterpenes	Cytotoxic activity	*Acrostichum aureum*	Mangrove plant	[55]
61	*Aspergillus ustus*	Drimane sesquiterpenoids ustusols A–C	Cytotoxic activity	*Bruguiera gymnorrhiza*	Mangrove plant	[60]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
62	*Aspergillus ustus*	Isomeric strobilactone B esters of (E,E)-6,7-epoxy-2,4-octadienoic acid	Larvicidal activity	*Codium fragile*	Sponge	[60]
63	*Aspergillus versicolor*	6,8-di-*O*-methylaverantin	Antibacterial activity	*Sargassum thunbergii*	Algae	[54]
64	*Aspergillus wentii*	Asperolides A–C, tetranorditerpenoid derivative, wentilactones A and B, botryosphaerin B	Antibacterial, cytotoxic activity	*Sargassum* sp.	Algae	[60]
65	*Aureobasidium* sp.	Aureobasidin, hydroxylated decanoic acids	Antifungal, antilarval, antibacterial activity	*Posidonia oceanica*	Algae	[46]
66	*Beauveria feline*	Destruxins, pseudodestruxin C	Cytotoxic and antituberculosis activity	*Caulerpa* sp.	Algae	[46]
67	*Bionectria ochroleuca*	Pullularins E and F, verticillin D	Cytotoxic activity	*Sonneratia caseolaris*	Mangrove plant	[47]
68	*Botryosphaeria* sp. SCSIO	Botryosphaerin B.	Anti-inflammatory	*Kandelia candel*	Mangrove plant	[66]
69	*Botrytis* sp.	α-pyrone, (E)-6-(hept-1-enyl)-2H-pyran-2-one	Enzymatic activity	*Hyalosiphonia caespitosa*	Algae	[46]
70	*Botrytis* sp.	Cyclopentenone bromomyrothenone B, cyclopentenone botrytinone	Tyrosinase inhibitory, antioxidant, antimicrobial activity	*Enteromorpha compressa*	Algae	[46]
71	*Cadophora malorum*	Hydroxylated sclerosporin derivatives, 15-hydroxysclerosporin, 12-hydroxysclerosporin, 11-hydroxysclerosporin, 8-hydroxysclerosporin	Fat-accumulation inhibitory activity	*Enteromorpha* sp.	Algae	[54]

72	*Campylocarpon* sp. HDN13-307	Campyridones D, ilicicolin H	Cytotoxic activity	*Sonneratia caseolaris*	Mangrove plant	[67]
73	*Chaetomium globosum*	Alkaloids	Cytotoxic activity	*Artemisia annua*	Plant	[49]
74	*Chaetomium globosum*	Cytoglobosins A–G	Cytotoxic activity	*Ulva pertusa*	Sponge	[49]
75	*Chaetomium globosum*	Chaetoglocins A–D	Antibacterial activity	*Cynodon dactylon*	Sea grass	[55]
76	*Chaetomium globosum*	Chaetopyranin	Cytotoxic activity	*Polysiphonia urceolata*	Algae	[13]
77	*Chaetomium globosum*	Chaetopyranin, 2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl) benzaldehyde and isotetrahydroauroglaucin, erythroglaucin	Antioxidant activity	*Polysiphonia urceolata*	Algae	[13]
78	*Chaetomium globosum* QEN-14	Cytoglobosins C and D	Cytotoxic activity	*Ulva pertusa*	Sponge	[12]
79	*Chaetomium* sp.	Chaetocyclinones A–C	Cytotoxic activity, antifungal activity	Marine alga	Algae	[46]
80	*Chrysosporium synchronum*	1-*O*-(α-D-mannopyranosyl) chlorogentisyl alcohol	Antioxidant activity	*Sargassum ringgoldium*	Algae	[55]
81	*Cladosporium* sp.	Cinnamic acid and bis (2-ethylhexyl) phthalate	Antifouling activity	*Mangrove*	Mangrove plant	[59]
82	*Cladosporium* sp.	Sporiolides A and B, sporiolides	Cytotoxic activity	*Actinotrichia fragilis*	Algae	[54]
83	*Coniochaeta* sp.	Oxepinochromenones, conioxepinols A–D, one furochromenone, coniofurol A, one xanthone, and conioxanthone A	Cytotoxic activity	*Xanthoria mandschurica*	Lichen	[49]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
84	*Coniothyrium cereale*	Phenalenone derivatives, conioscleroderolide	Antibacterial activity	*Enteromorpha* sp.	Algae	[54]
85	*Cordyceps dipterigena*	Verticinols A and B	Antifungal activity	*Desmotes incomparabilis*	Plant	[55]
86	*Corynespora cassiicola*	Decalactones, xestodecalactones D–F, corynesidone C	Protein kinase activity	*Laguncularia racemosa*	Mangrove plant	[47]
87	*Cosmospora vilior*	Cosmochlorins B	Glycogen synthase kinase (GSK)-3b inhibition	*Sonneratia alba*	Mangrove plant	[68]
88	*Curvularia* sp.	Apralactone A, curvularin macrolides, dimeric curvularin	Cytotoxic activity	*Acanthophora spicifera*	Algae	[46]
89	*Curvularia* sp.	Curvulide A	Antibacterial, antifungal activity	*Acanthophora spicifera*	Algae	[46]
90	*Cytospora* sp.	Cytosporone C	Cytotoxic activity	Mangrove plant	Mangrove plant	[46]
91	*Daldinia eschscholzii*	Lactone helicascolide C	Antifungal activity	*Gracilaria* sp.	Algae	[54]
92	*Diaporthe phaseolorum* SKS019	5-deoxybostrycoidin and fusaristatin A	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[69]
93	*Diaporthe* sp.	Dicerandrol D	Antimalarial activity	*Mangrove trees*	Mangrove trees	[70]
94	*Diaporthe* sp.	Sesquiterpenoids, diaporols A– I	Cytotoxic activity	*Rhizophora stylosa*	Mangrove plant	[60]
95	*Dichotomomyces cejpii*	Dichotomocej, pityriacitrin	Cytotoxic activity	*Lobophytum*	Corals	[71]
96	*Dothiorella* sp.	Dothiorelone B	Cytotoxic activity	Mangrove plant	Mangrove plant	[46]

97	*Drechslera dematioidea*	Sesquiterpenoids, isosativenetriol, drechslerines A and B, 9-hydroxyhelminthosporol. Drechslerines C–G, sativene epoxide	Antiplasmodial activity	*Liagora viscida*	Algae	[54]
98	*Emericella nidulans*	Arugosins G and H	Antibacterial, antifungal, and antialgal activities	Green alga	Algae	[46]
99	*Emericella nidulans*	Prenylated polyketides arugosins G and H	Antifungal activity	Green alga	Algae	[54]
100	*Emericella* sp.	Emericellamides A and B	Antibacterial activity	*Halimeda* sp.	Algae	[46]
101	*Emericella* sp.	Isoindolones emerimidine A and B	Cytopathic activity	*Aegiceras corniculatum*	Mangrove plant	[47]
102	*Epicoccum nigrum*	Thiodiketopiperazines, epicoccins I–T, and ent-epicoccin G	Anti-inflammatory activity	*Lysidice rhodostegia*	Plant	[49]
103	*Epicoccum* sp.	4,5,6-trihydroxy-7-methylphthalide (1, epicoccone)	Antioxidant activity	*Fucus vesiculosus*	Seaweed	[48]
104	*Eurotium cristatum*	Anthraquinone glycoside, 3-*O*-(α-D-ribofuranosyl)-questinol	ND	*Sargassum thunbergii*	Algae	[54]
105	*Eurotium rubrum*	Prenylated diketopiperazine derivatives	Cytotoxic, antioxidant activity	*Hibiscus tiliaceus*	Plant	[46]
106	*Eurotium rubrum*	Alkaloid and anthraquinone derivatives	Antibacterial, antifungal, and cytotoxic activities	*Hibiscus tiliaceus*	Plant	[72]
107	*Eurotium rubrum*	12-demethyl-12-oxoeurotechinulin B and 9-dehydroxyeurotinone	Antibacterial activity	*Hibiscus tiliaceus*	Plant	[47]
108	*Exophiala* sp.	Chlorohydroaspyrones, A and B	Antibacterial activity	*Halichondria panicea*	Sponge	[59]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
109	*Fusarium chlamydosporum*	Sulfur-containing diketopiperazine derivatives, fusaperazines A and B	Antioxidant activity	*Carpopeltis affinis*	Algae	[54]
110	*Fusarium* sp.	Anthraquinone derivative	Anticancer activity	Mangrove plant	Mangrove plant	[73]
111	*Fusarium* sp.	Isoflavone, 5-*O*-methyl-2′-methoxy-3′-methylalpinumisoflavone	Cytotoxic activity	*Kandelia candel*	Mangrove plant	[49]
112	*Fusarium* sp.	Anthraquinone, 5-acetyl-2-methoxy-1,4,6-trihydroxy-anthraquinone	Antimicrobial activity	Mangrove plant	Mangrove plant	[46]
113	*Geniculosporium* sp.	Botryane sesquiterpenoids	Herbicidal, antifungal, and antibacterial activities	*Polysiphonia species*	Algae	[54]
114	*Gliocladium* sp.	4-keto-clonostachydiol	Antimicrobial and cytotoxic activity	*Durvillaea antarctica*	Seaweed	[46]
115	*Guignardia* sp.	Methoxyver mistatin and hydroxyvermistatin	Cytotoxic activity	*Kandelia candel*	Corals	[47]
116	*Guignardia* sp.	Meroterpenes, guignardones F–I	Antibacterial activity	*Scyphiphora hydrophyllacea*	Plant	[47]
117	*Guignardia* sp.	R-3-hydroxy undecanoic acid methylester-3-*O*-α-L-rhamnopyranoside	Antibacterial activity	Mangrove plant	Mangrove	[47]
118	*Halorosellinia* sp.	Anthraquinone SZ-685C	Anticancer activity	Mangrove plant	Mangrove	[74]
119	*Leptosphaeria* sp.	Leptosin A	Anticancer activity	*Sargassum tortile*	Algae	[48]

120	*Leptosphaerulina* sp. SKS032	Leptospyranonaphthazarin A, leptosnaphthoic acid A, diaportheins B	Antibacterial activity	*Acanthus ilicifolius*	Mangrove plant	[73]
121	*Massrison* sp.	Spiro-5, 6-lactone ring skeleton, including massarigenin D, spiromassaritone, and paecilospirone	Antifungal activity	*Rehmannia glutinosa*	Plant	[55]
122	*Microsporum* sp.	Anthracene glycoside, asperflavin ribofuranoside	Antioxidant, antibacterial activity	*Lomentaria catenata*	Algae	[46]
123	*Microsporum* sp.	Asperflavin ribofuranoside	Antibacterial activity	*Lomentaria catenata*	Algae	[54]
124	*Monodictys putredinis*	Monomeric xanthones, monodictysins A–C, and monodictyxanthone	Enzymatic activity	Green alga	Algae	[54]
125	*Myrothecium* sp.	Roridin R	Cytotoxic activity	*Sponge*	Sponge	[75]
126	*Neosartorya udagawae*	Neosartoryadins A and B	Antiviral activity	*Avicennia marina*	Mangrove tree	[34]
127	*Nigrospora* sp.	Nigrosporapyrones A–D	Antibacterial activity	Sea fan	Sea fan	[46]
128	*Nigrospora* sp.	Nigrosporanenes A and B	Cytotoxic activity, antioxidant activity	*Annella* sp.	Corals	[46]
129	*Nigrospora* sp.	Nigrospoxydons A–C	Antibacterial activity	*Annella* sp.	Corals	[46]
130	*Nigrospora* sp.	2,3-didehydro-19a-hydroxy-14-epicochlioquinone B	Antibacterial activity	*Pongamia pinnata*	Plant	[47]
131	*Nigrospora* sp.	Bostrycin	Anticancer activity	Mangrove plant	Mangrove plant	[61]
132	*Nodulisporium* sp.	Noduliprevenone	Anticancer activity	ND	ND	[48]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
133	*Paecilomyces* sp.	Paeciloxocins A and B	Cytotoxic activity, antifungal	Mangrove plant	Mangrove plants	[49]
134	*Paecilomyces* sp.	Prenylated xanthone, paeciloxanthone	Cytotoxic activity, antifungal	Mangrove tree	Mangrove tree	[46]
135	*Paecilomyces* sp.	Paeciloxocins A and B	Anticancer activity	Mangrove plant	Mangrove	[74]
136	*Paecilomyces variotii*	Butenolides, butyrolactone IX, aspulvinone O	Antioxidant activity	*Grateloupia turuturu*	Algae	[54]
137	*Penicillium aculeatum*	(2′S*)-2-(2′-hydroxypropyl)-5-methyl-7, 8-dihydroxy-chromone	Antibacterial activity	*Kandelia candel*	Mangrove plant	[61]
138	*Penicillium aculeatum*	Bacillisporin A and B	Antibacterial activity, α glucosidase inhibition	*Kandelia candel*	Mangrove plant	[61]
139	*Penicillium chermesinum*	Azaphilones, chermesinones A–C, p-terphenyls (6′-*O*-desmethylterphenyllin, 3-hydroxy-6′-*O*-desmethylterphenyllin, 3″-deoxy-6′-*O*-desmethylcandidusin B)	Inhibitory activities	*Kandelia candel*	Mangrove plant	[55]
140	*Penicillium chermesinum*	Azaphilone sesquiterpenoids, chermesinones A–C	Enzyamatic activity	*Mangrove plant*	Mangrove plant	[60]
141	*Penicillium chrysogenum*	Polyketide derivatives, glycerol derivatives, monoterpene derivative	Antifungal, cytotoxic activity	*Red algal Laurencia*	Algae	[76]
142	*Penicillium chrysogenum*	Penicisteroids A and B	Cytotoxic activity	*Laurencia*	Algae	[55]

143	*Penicillium chrysogenum*	Penicitides A and B one glycerol derivative 2-(2,4-dihydroxy-6-methylbenzoyl)-glycerol, one monoterpene derivative penicimonoterpene	Antifungal activity	*Red algal species*	Algae	[55]
144	*Penicillium chrysogenum*	Penicisteroid A	Antifungal activity	*Red algal species*	Algae	[77]
145	*Penicillium chrysogenum*	Sorbicillacton A	Cytotoxic activity	Mangrove plant	Mangrove	[78]
146	*Penicillium citrinum*	Pentacyclic alkaloids, citrinadins A and B	Cytotoxic activity	Marine algae	Algae	[54]
147	*Penicillium citrinum*	Dihydroisocoumarin penicimarins, meroterpenoids	Antibacterial	*Bruguiera sexangula*	Mangrove shrub	[79]
148	*Penicillium citrinum*	(Z)-7,40-dimethoxy-6-hydroxy-aurone-4-*O*-b-glucopyranoside	Neuroprotective activity	*Bruguiera gymnorrhiza*	Mangrove plant	[79]
149	*Penicillium dipodomyicola*	Peniphenone B and C	MptpB inhibitor	*Acanthus ilicifolius*	Mangrove plant	[46]
150	*Penicillium expansum*	Polyphenols	Cytotoxic activity	*Excoecaria agallocha*	Mangrove plant	[49]
151	*Penicillium expansum*	Expansols A	Cytotoxic activity	*Excoecaria agallocha*	Mangrove plant	[47]
152	*Penicillium expansum*	Bisabolane sesquiterpenoids	Cytotoxic activity	*Excoecaria agallocha*	Mangrove plant	[60]
153	*Penicillium griseofulvum*	4-hydroxyphenethyl methyl succinate and 4-hydroxyphenethyl 2-(4-hydroxyphenyl) acetate	Antioxidant, cytotoxic activity	*Lumnitzera racemosa*	Mangrove plant	[46]
154	*Penicillium oxalicum* EN-201	Penioxamide A, 18-hydroxydecaturin B	Brine shrimp lethality	*Rhizophora stylosa*	Mangrove plant	[80]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
155	*Penicillium pinophilum*	Azaphilone derivatives, pinophilins A and B	Cytotoxic activity	Marine alga	Algae	[54]
156	*Penicillium raistrickii*	1,3,6- trihydroxy-8-methyl-9H-xanthen-9-one	Cytotoxic activity	*Axinella corrugata*	Coral reefs	[43]
157	*Penicillium sacculum*	1-hydroxy-3-methoxy-6-sulfo-8-methylxanthone	Cytotoxic activity	Mangrove plant	Mangrove plant	[54]
158	*Penicillium* sp.	Ctrinal A	Cytotoxic activity	*Blidingia minima*	Seaweed	[48]
159	*Penicillium* sp.	Penicipyrone, penicilactone	Antimicrobial activity	Sea fan	Sea fan	[59]
160	*Penicillium* sp.	Chromanone A	Anticancer, antioxidant activity	*Ulva* sp.	Sponge	[59]
161	*Penicillium* sp.	Penicinoline	Insecticidal activity, cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[49]
162	*Penicillium* sp.	Penisporolides A and B	Enzymatic activity	*Kandelia candel*	Mangrove plant	[46]
163	*Penicillium* sp.	Ketal penicipyrone, ϒ-lactone, penicilactone	Antifungal activity	*Annella* sp.	Corals	[46]
164	*Penicillium* sp.	6,8-dihydroxy-3,4,7-trimethylisochroman-1-one	Cytotoxic activity	*Bruguiera sexangula*	Mangrove shrub	[46]
165	*Penicillium* sp.	Redoxcitrinin, phenol A, citrinin H2	Antioxidant activity	*Ulva pertusa*	Sponge	[46]
166	*Penicillium* sp.	Leptosphaerone C, penicillenone, and 9-demethyl FR-901235	Cytotoxic activity	*Aegiceras corniculatum*	Mangrove plant	[46]
167	*Penicillium* sp.	Arugosin I	ND	*Aegiceras corniculatum*	Mangrove plant	[46]
168	*Penicillium* sp.	Janthitrem-type indole triterpenes	Antioxidant activity	*Aegiceras corniculatum*	Mangrove plant	[46]

169	*Penicillium* sp.	Penilumamide	Antimicrobial and cytotoxic activity	*Laurencia* sp.	Algae	[46]
170	*Penicillium* sp.	Isomeric pyrrolyl 4-quinolinone alkaloid penicinoline	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[46]
171	*Penicillium* sp.	Tetramic acids, penicillenol A1, A2, B1, B2, C1, and C2	Cytotoxic activity	*Aegiceras corniculatum*	Mangrove plant	[46]
172	*Penicillium* sp.	*N*-deoxy analogues	Antimicrobial and cytotoxic activity	*Xiphophora gladiata*	Algae	[46]
173	*Penicillium* sp.	Penicinoline	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[47]
174	*Penicillium* sp.	Leptosphaerone C, penicillenone, arugosin I, and 9-demethyl FR-901235	Cytotoxic activity	*Aegiceras corniculatum*	Mangrove plant	[47]
175	*Penicillium* sp.	Indole triterpenes, shearinines D–K	Channels blocking activity	*Aegiceras corniculatum*	Mangrove plant	[47]
176	*Penicillium* sp.	Shearinine A	Cytotoxic activity	Mangrove plant	Mangrove plant	[46]
177	*Penicillium* sp.	Communesins A and B	Cytotoxic activity	Marine algae	Algae	[54]
178	*Penicillium* sp.	15-Hydroxy-6α,12-epoxy-, 7α,10αH,11βH-spiroax-4-ene-12-one	Cytotoxic activity	*Avicennia marina*	Mangrove plant	[81]
179	*Penicillium* sp. FJ-1	4-(20,30-dihydroxy-30-methyl-butanoxy)-phenethanol, and 15-, hydroxy-6a,12-epoxy-7b,10aH,11bH-spiroax-4-ene-12-one	Antiproliferative activities	*Avicennia marina*	Mangrove plant	[81]
180	*Penicillium* sp.	7-Epiaustdiol, stemphyperylenol, secalonic acid A	Antibacterial activity	*Kandelia candel*	Mangrove plant	[82]
181	*Penicillium steckii*	(S)-8-methoxy-3,5-dimethylisochroman-6-ol	Antibacterial, anticancer activity	*Sargassum*	Algae	[43]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
182	*Penicillium terrestre*	Trimeric terrestrol A	Cytotoxic, antioxidant activity	Mangrove plant	Mangrove	[54]
183	*Penicillium thomi*	Biphenyl derivative 4-(3-hydroxypropyl)-5,6-dimethoxybiphenyl-3,40-diol	Anticancer activity	*Bruguiera gymnorrhiza*	Mangrove plant	[46]
184	*Penicillium* sp.	Peniphenone, xanthones	Immunosuppressive activity	*Sonneratia apetala*	Mangrove tree	[83]
185	*Pestalotia* sp.	Chlorinated benzophenone pestalone	Antibacterial activity	*Rosenvingea* sp.	Algae	[84]
186	*Pestalotiopsis heterocornis*	Heterocornols A-C, F-H, methyl-(2-formyl-3-hydroxyphenyl) propanoate, agropyrenol, and vaccinol G	Cytotoxic, antibacterial	Sponge	Sponge	[85]
187	*Pestalotiopsis* sp.	Cytosporones, cytosporones J–N, coumarins, pestalasins A–E, Pestalotiopsoid A, pestalotiopsones A–F	Anticancer	*Rhizophora mucronata*	Mangrove plant	[59]
188	*Pestalotiopsis* sp.	Pestalotiopsones A–F	Cytotoxic activity	*Rhizophora mucronata*	Mangrove plant	[46]
189	*Pestalotiopsis* sp.	Pestalotiopsoid A, cytosporones, and coumarins	ND	*Rhizophora mucronata*	Mangrove plant	[46]
190	*Pestalotiopsis* sp.	Diphenyl ethers, pestalotethers A–D, pestalochromones A–C, xanthone, pestaloxanthone	Antifungal activity	*Rhizophora apiculata*	Mangrove plant	[55]
191	*Pestalotiopsis* sp.	Pestalotiopsones A–F	Cytotoxic activity	*Rhizophora mucronata*	Mangrove plant	[47]
192	*Pestalotiopsis* sp.	Sesquiterpenoids	Enzyme inhibitory activity	*Sargassum horneri*	Algae	[60]

193	*Pestalotiopsis vaccinii*	Vaccinal A	Anti-enterovirus 71 activity	*Kandelia candel*	Mangrove plant	[86]
194	*Petriella* sp.	Infectopyrone derivatives, cyclic tetrapeptide WF-3161	Cytotoxic activity	*Suberites domuncula*	Sponge	[59]
195	*Phaeosphaeria spartinae*	Spartinoxide, A82775C	Enzymatic activity	*Ceramium*	Algae	[49]
196	*Phaeosphaeria spartinae*	Spartinols A–D	Cytotoxic activity	*Ceramium* sp.	Algae	[46]
197	*Phaeosphaeria spartinae*	Spartinoxide	Enzymatic activity	Red alga	Algae	[46]
198	*Phoma herbarum*	Bromochlorogentisylquinones A and B	Antioxidant activity	*Gloiopeltis tenax*	Algae	[46]
199	*Phoma* sp.	Lactone, xanthones	Cytotoxic activity	*Avicennia marina*	Mangrove plant	[46]
200	*Phoma* sp.	Epoxyphomalins A and B	Cytotoxic activity	*Ectyplasia perox*	Sponge	[86]
201	*Phoma tropica*	5-Hydroxyramulosin	Antioxidant activity	*Fucus spiralis*	Algae	[54]
202	*Phomopsis* sp.	Aliphatic compounds	Antifungal activity	Mangrove plant	Mangroves	[87]
203	*Phomopsis* sp.	Hexaketide γ-lactones; oblongolides W1, W2, X, and Y; and 2-deoxy-4α-hydroxyoblongolide X	Antiviral, cytotoxic activity	*Musa acuminata*	Plant	[49]
204	*Phomopsis* sp.	Phomopsis-H76 A, B, and C	Antibacterial, cytotoxic activity	*Excoecaria agallocha*	Mangrove plant	[49]
205	*Phomopsis* sp.	Excelsione	Cytotoxic activity	*Mangrove plant*	Mangrove plant	[46]
206	*Phomopsis* sp.	Ethyl 2-(3-hydroxy-2-(7-hydroxyoctanoyl)-5-methoxyphenyl)acetate	Cytotoxic activity	*Excoecaria agallocha*	Mangrove plant	[46]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
207	*Phomopsis* sp.	Terpenoids	Enzymatic activity	*Hibiscus tiliaceus*	Plant	[46]
208	*Phomopsis* sp.	Isoquinoline alkaloid	Cytotoxic activity	Mangrove plant	Mangrove plant	[46]
209	*Phomopsis* sp.	Phomoindene A	Cytotoxic activity	Mangrove plant	Mangrove	[46]
210	*Phomopsis* sp.	Phomochromone A and B, phomotenone	Antifungal activity	*Cistus monspeliensis*	Plant	[55]
211	*Phomopsis* sp.	Naphtho-α-pyrone, 5-hydroxy-6,8-dimethoxy-2-benzyl-4H-naphtho[2,3-b]-pyran-4-one	Cytotoxic activity	*Excoecaria agallocha*	Mangrove plant	[47]
212	*Phomopsis* sp.	2-(7′-hydroxyoxooctyl)-3-hydroxy-5-methoxybenzeneacetic acid ethyl ester	Cytotoxic activity	Mangrove tree	Mangrove tree	[47]
213	*Phomopsis* sp.	1,7-dihydroxy-2-methoxy-3-(3-methylbut-2-enyl)-9H-xanthen-9-one, 1-hydroxy-4,7-dimethoxy-6-(3-oxobutyl)-9H-xanthen-9-one	Cytotoxic activity	*Avicennia marina*	Mangrove plant	[47]
214	*Phomopsis* sp.	Phomopsin A	Cytotoxic activity	Mangrove tree	Mangrove tree	[47]
215	*Phomopsis* sp.	Cyclotetrapeptides	Antimicrobial activity	Mangrove plant	Mangrove	[88]
216	*Phomopsis* sp. PSU-MA214	(2R,3S)-7-ethyl-1,2,3,4-tetrahydro-2,3,8-trihydroxy-6-methoxy-3-methyl-9,10-anthracenedione	Cytotoxic, antibacterial	*Rhizophora apiculata*	Mangrove plant	[89]
217	*Phomopsis* sp.	Phomopsichalasin G	Cytotoxic activity	*Xylocarpus granatum*	Mangrove plant	[90]

218	*Pseudallescheria* sp.	Dehydroxybisdethiobis (methylthio)gliotoxin	Antibacterial activity	*Agarum cribrosum*	Algae	[46]
219	*Pseudolagarobasidium acaciicola*	Terpene endoperoxides	Cytotoxic activity	*Bruguiera gymnorrhiza*	Mangrove plant	[91]
220	*Rhizopus stolonifer*	Cyclopentenone bromomyrothenone B	Antioxidant, antimicrobial activity	*Sargassum horneri*	Algae	[46]
221	*Scopulariopsis brevicaulis*	Scoparides A and B	Cytotoxic and antibacterial activity	Sponge	Mangrove plant	[44]
222	*Rhytidhysteron rufulum*	Rhytidchromones A, B, C, and E	Cytotoxic activity	*Bruguiera gymnorrhiza*	Sponge	[91]
223	*Scytalidium* sp.	Marilines A–C	Anti-leukamic activity	*Callyspongia* cf. *C. flammea*	Sponge	[40]
224	*Scytalidium* sp.	Halovirs A–E	Antifungal, antiviral	*Halodule wrightii*	Seaweed	[84]
225	*Spicellum roseum*	Spicellamide A, and spicellamide B	Cytotoxic activity	*Ectyplasia perox*	Sponge	[59]
226	*Stemphylium globuliferum*	Tetrahydroanthraquinone, tetrahydroanthraquinone heterodimers	Antimicrobial activity	*Mentha pulegium*	Plant	[55]
227	*Stemphylium* sp. 33231	Anthraquinone derivatives	Antibacterial activity	*Bruguiera sexangula*	Mangrove plant	[33]
228	*Talaromyces amestolkiae*	Sescandelin B, 3,4-dimethyl-6,8-dihydroxyisocoumarin, aspergillumarin A	α-glucosidase inhibitor activity	*Kandelia obovata*	Mangrove plant	[92]
229	*Talaromyces amestolkiae*	5-hydroxy-7-methoxy-2-methylbenzofuran-3-carboxylic acid, 5-hydroxy-7-methoxy-2-methylbenzofuran-3-yl)ethan-1-one	Antibacterial activity	*Kandelia obovata*	Mangrove plant	[92]
230	*Talaromyces flavus*	Norsesquiterpene peroxides, talaperoxides A–D	Anticancer activity	*Sonneratia apetala*	Mangrove tree	[60]

(*continued*)

Table 1 (continued)

Sr. No.	Fungi name	Secondary metabolites	Property	Host	Host category	References
231	*Talaromyces flavus*	Talaperoxides A–D	Brine shrimp toxicity, cytotoxic	*Kandelia candel*	Mangrove plant	[82]
232	*Talaromyces* sp.	7-Epiaustdiol, 8-*O*-methylepiaustdiol, stemphyperylenol, and secalonic acid A	Antibacterial activity	*Kandelia candel*	Mangrove plant	[49]
233	*Talaromyces* sp.	Isochromenones 7-epiaustdiol and 8-*O*-methylepiaustdiol	Antioxidant activity	*Kandelia candel*	Mangrove plant	[46]
234	*Talaromyces* sp.	3-*O*-methylfunicone	Antifungal, antitumor, and lipid-lowering activity	Mangrove plant	Mangrove	[93]
235	*Talaromyces* sp. cf-16	Z -roquefortine C, viridicatol, penitrem A, penijanthine A	Antibacterial activity	*Sargassum* sp.	Seaweed	[48]
236	*Talaromyces* sp. (HZ-YX1)	Talaramide A	Inhibition of mycobacterial PknG activity	*Kandelia obovata*	Mangrove plant	[94]
237	*Talaromyces* sp. ZH-154	Secalonic acid A	Cytotoxic activity	*Kandelia candel*	Mangrove plant	[82]
238	*Talaromyces stipitatus* SK-4,	Talaromyones B	Antibacterial activity	*Acanthus ilicifolius*	Mangrove plant	[19]
239	*Talaromyces stipitatus* SK-4,	Depsidone	Cytotoxic activity	*Acanthus ilicifolius*	Mangrove plant	[19]
240	*Trichoderma atroviride*	3-hydroxybutan-2-yl 4-(2-hydroxy-*N*-(3-oxobutan-2-yl)propanamido)butanoate		*Ceriops tagal*	Mangrove tree	[46]
241	*Trichoderma sp.* Xy24	(9R,10R)-dihydro-harzianone	Cytotoxic activity	*Xylocarpus granatum*	Mangrove plant	[95]
242	*Tryblidiopycnis* sp.	Monoterpenes		Mangrove plant	Mangrove plant	[60]

243	*Verticillium tenerum*	Verticinols A and B	Antibacterial, antifungal, antialgal, antiplasmodial, antiviral, cytotoxic, and enzymatic activity	Marine alga	Algae	[46]
244	*Wardomyces anomalus*	Anomalins A and B	Antioxidant activity	*Enteromorpha* sp.	Algae	[54]
245	*Wardomyces anomalus*	2,3,6,8-tetrahydroxy-1-methylxanthone, 5-(hydroxymethyl)-2-furanocarboxylic acid	Antioxidant, tyrosine kinase inhibition	*Enteromorpha* sp.	Algae	[18]
246	*Xylaria cubensis*	Succinic acid derivatives, xylacinic acids A and B	Antibacterial activity	*Bruguiera parviflora*	Mangrove	[47]
247	*Xylaria psidii*	2-carboxy-8-methoxy-naphthalene-1-ol	Antifungal, cytotoxic activity	*Kappaphycus alvarezii*	Algae	[96]
248	*Xylaria* sp.	Cytochalasin D	Cytotoxic, antibacterial activity	*Bostrychia tenella*	Coral	[14]
249	*Xylaria* sp.	Sesquiterpenoids, mairetolide F	Antibacterial, cytotoxic activity	*Licuala spinosa*	Mangrove tree	[49]
250	*Xylaria* sp.	Xyloketal H	ND	Mangrove tree	Mangrove tree	[46]
251	*Xylaria* sp.	Xylarisin	Antibacterial activity	*Annella* sp.	Corals	[46]
252	*Xylaria* sp.	Xylopyridine A	DNA-binding affinity	Mangrove plant	Mangrove plant	[46]
253	*Xylaria* sp.	Allenic ethers	ND	Mangrove tree	Mangrove tree	[46]
254	*Xylaria* sp.	Eremophilane sesquiterpenes	α-glucosidase inhibitor	*Avicennia marina*	Mangrove plant	[14]
255	*Xylaria* sp.	Xyloketal F	L-calcium channel blocking activity	*Avicennia marina*	Mangrove plant	[14]
256	*Xylaria* sp.	Cytochalasin D	Antimicrobial, cytotoxic activity	*Bostrychia tenella*	Algae	[14]

the various factors that lead to the complexity and diversity of the metabolic profile of marine endophytes [97]. The number of bioactive SMs reached to 700 compounds by 2010, which has shown a phenomenal sevenfold increase [46]. As evident from Table 1, endophytes belonging to the genera *Aspergillus* and *Penicillium* are the major contributors, i.e., nearly half of the active metabolites belong to them. The classes of compounds are diverse such as derivatives of cytochalasins, isocoumarins, phenolics, pyrones, steroids, xanthones, etc. Properties such as immunosuppressive activity [39], neuroprotective activity [98], *Mycobacterium tuberculosis* protein tyrosine phosphatase (MptpB) inhibitor activity [99], and antimicrobial, antioxidant, and cytotoxic activities [46] have been reported from metabolites secreted by *Penicillium* species. More than 40% of the metabolites showed anticancerous property, followed by antimicrobial nature of metabolites. Other endophytic fungal genera that produce a considerable number of secondary metabolites are *Talaromyces*, *Phomopsis*, *Xylaria*, *Alternaria*, and *Chaetomium*. *Xylaria* sp. possessed SMs belonging to different classes of secondary metabolites including alkaloids, phenolics, terpenes, despidone, etc., responsible for various activities such as α-glucosidase inhibition, tyrosine kinase inhibition, antifungal, calcium channel blocking activity, cytotoxicity, etc. [46, 47]. Atropisomer 2 MptpB inhibitor, altenusin having α-glucosidase inhibition, phthalide and isocoumarin derivatives having antifungal properties, etc. have been reported from *Alternaria* spp. As evident from Table 1, it is clear that most of the natural products are from mangrove habitat followed by algal sources and then from sponges.

6 Challenges

One of the major limitations in the mining of marine products is the cultivation of marine endophytes in laboratory conditions. Not only endophytes, this implies to every other marine microbe which leads to "oceans' dark matter" that still remains unexplored [100]. One of the universal facts as well as a flaw in the endophytic fungal research is that often the slow-growing endophytes are overlooked by the faster-growing endophytes and thus a gap is still left in the exploration of actual diversity, consequently SMs [48]. This gap can be filled in by adopting novel isolation techniques that would also allow rare fungi to prop up and separate. The inability of secondary metabolite gene clusters to express or silence gene clusters in standard laboratory conditions is one of the greatest demerits with the endophytes. Accordingly, gimmicks involving awakening of silent/orphan gene clusters such as co-culture method, epigenetic modifications, varying growth conditions, genome mining, mutation etc. should be considered to estimate the full potential of the endophytes.

Another important concern relates to destruction of mangrove habitats as that would not only affect the environment and ecology but also would deprive us from isolating fungal isolates of therapeutic use. Hence, the immediate task is to study, conserve, record, quantify, and explore the marine ecosystems for the benefit of

forthcoming generations without disturbing the habitats but in a "live and let live" manner [101].

To profile the metabolite patterns, an efficient expression system is needed that can express small molecule biosynthesis in a high yield, genetic tools that can assess the full potential of endophytic fungi, and activating the silent biosynthetic gene clusters.

7 Conclusions

Myriads of bioactive molecules are reported every year from the marine sources. The complexity and diversity of the secondary metabolites are highlighted from the diverse chemical skeletons characterized during their exploration. Various properties possessed by these SMs are defined, but their role in cellular mechanisms or pathways is still a gray area. More detailed analysis of the activities of various compounds have to be dealt with and take up clinical trials of potential drugs. Parallel to this, in-depth analysis on molecular characterization of SMs and cellular mechanisms, a repository (culture collection) to facilitate preservation, inventory, and screening activities, should also be undertaken. Seabeds, mangrove interior, seaweed collection, etc. are few of the hardships related to marine research. To make this more accessible, we need modern equipment and centers to counter the aforementioned problems.

Growing antimicrobial resistance resulting in multidrug-resistant pathogens requires intensive research to look for novel drugs from different other habitats, and marine environment is one which is a promising area. Since marine environment is an extreme environment, the physicochemical conditions prevailing in such natural environment should be mimicked to dig novel bioactive compounds that have various properties including antimicrobial, antioxidant, antituberculosis, anticancerous, antiviral, immunosuppressant, anti-inflammatory, and many more, for the next generation.

Though a momentum in endophytic fungal secondary metabolite profiling and drug discovery has been initiated, we are still way behind in the number of fungi explored from marine environment [102]. We need to gain better insights into biochemical and genetic level of biosynthetic pathways that would open new vistas toward harnessing and designing new molecules for drug discovery. Marine endophytes are highly qualifying candidates for drug development as they possess intricate chemically and structurally diverse metabolites with potent activities.

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Fungal Endophytes from Medicinal Plants as a Potential Source of Bioactive Secondary Metabolites and Volatile Organic Compounds: An Overview

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Humeera Nisa and Azra N. Kamili

Contents

Abstract

In this chapter, we provide a general overview of secondary metabolites, especially easily volatilized molecules, namely, VOCs, isolated and identified from endophytic fungal communities of different medicinal plants. A fungal endophyte spends the whole or part of its life cycle colonizing inter- and/or intracellularly inside the healthy tissues of the host plants, causing no apparent symptoms of disease. Endophytic fungi produce a wide array of secondary metabolites and volatile organic compounds with important biological functions, displaying a broad range of useful antibiotic and pharmaceutical activities as well as immunomodulatory and toxic activities. Some of their biological activities are still unknown to mankind. These microbial metabolites have drawn enormous attention as potential agents of medicinal properties. Fungi are well known for emitting a complex mixture of volatile organic compounds (VOCs). Fungal

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VOCs commonly form a bioactive interface between plants and numerous microorganisms. Fungi emit plethora of unique volatile compounds that belong to a number of chemical classes including alcohols, aldehydes, acids, ethers, ketones, hydrocarbons, terpenes, and sulfur compounds. VOCs are gases, carbon-based compounds having characteristic odors, and are produced during primary and secondary metabolism. The diverse functions of fungal VOCs can be used in biotechnological applications as biofuel, biocontrol, and mycofumigation.

Keywords
Endophytes · Fungi · Secondary metabolites · Volatile organic compounds

1 Introduction

Fungal endophytes are fungal microorganisms which spend the whole or part of its life cycle colonizing inter- and/or intracellularly inside the healthy tissues of the host plants, causing no apparent or overt symptoms of diseases [1]. These fungal microorganisms have been isolated from many species of woody plants and grasses [1, 2]. Endophytic fungi are polyphyletic group of highly diverse, primarily ascomycetous fungi that are defined by their occurrence within plant tissues without causing any immediate overt effects [2, 3]. These fungi are found in liverworts, hornworts, mosses, lycophytes, equisetopsids, ferns, and seed plants from the arctic tundra to the tropics [2, 4–9]. Endophytes live in the intercellular spaces of stems, petioles, roots, and leaves of plants causing no discernible manifestation of their presence and are unnoticed [10]. In plant-endophyte symbiotic relationship, the former protects and feeds the latter which produces "in return" bioactive (plant growth regulatory, antibacterial, antifungal, antiviral, insecticidal, etc.) substances to enhance the growth and competitiveness of the host in nature [11]. High species diversity is another characteristic of endophytic mycobiota surveys showing assemblages consisting of more than 30 fungal species per host plant species [12].

Recently, several studies have led to the discovery of important plant secondary metabolites from endophytic fungi and thus increased the prospect of using such organisms as an alternative source of these metabolites [13]. In addition, endophytes accumulate other structurally diverse and biologically active compounds that are unprecedented in nature [14–20]. Such compounds are known to have great importance for drug discovery, agriculture, etc. [4, 21, 22]. Endophytes are accepted as an important and largely untapped reservoir of unique chemical structures that have been modified through evolution and are believed to be involved in host plant protection and communication [19]. Fungal endophytes are known to mimic the structure and function of host compounds [23] and can produce plant growth hormones such as gibberellins [24]. Endophytes constitute a major portion of the unexplored fungal diversity. A large part of the work on fungal endophytes has been carried out on diversity studies, but reports on their practical biotechnological applications are few. The studies for searching taxonomic novelty in endophytes are comparatively less, partly due to unavailability of appropriate expertise in fundamental systematics.

Endophytic fungi represent an important component of fungal biodiversity and are known to have an effect on plant community diversity and structure [25–27]. A study reported a new species *Idriella licualae* from a tropical palm tree *Licuala ramsayi* (F. Muell.) Domin in rainforests of Queensland [28]. Subsequently after 2 years, they described three new species *I. euterpes*, *I. asaicola*, and *I. amazonica* from *Euterpe oleracea* in Brazilian Amazon forest [29]. Liu et al. isolated an endophytic species, *Colletotrichum yunnanense* from *Buxus* sp. in China [30]. *Penicillium coffeae* was isolated as a new endophyte from *Coffea arabica* L. in Hawaii by Peterson et al. [31]. Similarly, *Ceratopycnidium baccharidicola* [32] from Argentina and *Preussia mediterranea* (Sporormiaceae) from Mediterranean region [33] were also reported as new endophytes. Although most of the newly described species are largely mitosporic fungi, there are few reports on new ascomycetous species. Jacob and Bhat [34] described *Kumbhamaya indica* and *Gonatobotryum bimorphosporum* as two new endophytic conidial fungi of India. Later on, *Echinosphaeria pteridis* and its anamorph *Vermiculariopsiella pteridis* have been described as endophytes of a pteridophyte by Dhargalkar and Bhat [35]. Singh et al. studied endophytic assemblages of two medicinal plants collected in India and isolated two species, viz., *Gnomoniella pongamiae* from *Pongamia pinnata* and *Thielavia icacinacearum* from *Nothapodytes nimmoniana* [36, 37]. A study successfully isolated a total of 25 endophytic fungi from the inner bark of *Taxus baccata* grown in Iran by the aseptic technique [38]. Interestingly, this was the first report of the genus *Stemphylium* as a taxol-producing taxon. In a preliminary report, the species diversity and the frequency of colonization of endophytic fungi in the aerial parts of *Chamaecyparis thyoides* were presented by Bills and Polishook [39]. A total of 961 fungal isolates were cultured representing 88 species of filamentous fungi. In a study, a total of 130 endophytic fungi were isolated from 12 Chinese traditional medicinal plants collected in Yunnan province, Southwest China, and were further tested for antitumor and antifungal activities [40].

2 Biodiversity of Endophytic Fungi Isolated from Different Medicinal Plant Species

Fungi are one of the most diverse life forms on this planet, and the number of fungal species is considered important among mycologists [41]. Hawksworth [42] predicted that there are 1.5 million species of fungi; of these, about 74,000 are currently known [43]. Recent studies from tropical forests [44–46] suggest that fungal diversity is greater in the tropics than in the temperate regions, and many tropical mycologists view 1.5 million as a conservative figure [43]. Some researcher however, think that the figure of 1.5 million is too high [47–49]. Arnold et al. [50] concluded that fungal endophytes are hyperdiverse in the tropics and that the figure of 1.5 million may markedly underestimate fungal diversity. Recent studies in a forest in Guyana [51] and four forests in Mudumalai Wildlife Sanctuary, southern India [52], revealed that certain tropical forests are not hyperdiverse with reference to fungal endophytes. Of the myriad of ecosystems on earth, those having the

greatest biodiversity seem to have greatest number of endophytes. Tropical and temperate rainforests are the most biologically diverse terrestrial ecosystems on earth. The most threatened of these spots cover only 1.44% of the land's surface, yet they harbor more than 60% of the world's terrestrial biodiversity [53]. As such, one would expect that areas of high plant endemicity also possess specific endophytes that may have evolved with the endemic plant species [54].

Four hundred bark and leaf segments were studied, and a total of 732 isolates representing 28 taxa, including 3 morphotypes, were isolated. The genera *Glomerella* and *Gibberella* were first reported in *Taxus* by Xiong et al. [55]. Eighty-one endophytic fungi were isolated from *Taxus media* which were grouped into eight genera based on their morphological and molecular identification. *Guignardia* and *Colletotrichum* were seen to be the dominant genera, whereas the remaining genera were infrequent groups. Three representative species of the distinct genera gave positive results upon screening through molecular marker and were capable of producing taxol which were validated by HPLC-MS. An ecological investigation of foliar endophytic fungal communities on *Musa acuminate* (Banana) species complex was undertaken in Hong Kong and Queensland, Australia, by Brown et al. [56]. Twenty-four taxa were isolated out of which *Colletotrichum gloeosporioides*, *Pestalotiopsis palmarum*, and *Nigrospora oryzae* were the dominant endophytes. Isolates of the family Xylariaceae and a *Phoma* species were most frequently isolated from indigenous banana in the Wet Tropics of north Queensland. *Sarocladium* species are frequently associated with grasses as mutualistic endophytes. A species of *Sarocladium* (anamorphic Hypocreales) was isolated as endophytic fungus from the coastal grass *Spinifex littoreus* (Poaceae) by Yeh and Kirschner [57].

3 Endophytic Fungi as the Potential Source of Plant Bioactive Secondary Metabolites

Fungi are key resources of bioactive metabolites [44, 58]. Among fungi, endophytic fungi which inhabit within their host plant without causing any disease symptoms are important to screen biologically active secondary metabolites [59]. In endophyte-hos symbioses, secondary metabolites produced by endophytes contribute positively to the host [60]. Secondary metabolites, defined as low molecular weight compounds, are not required for growth in pure culture but are produced as an adaptation for specific functions in nature. They play vital roles in numerous metabolic interactions between fungi and their plant hosts, such as signaling, defense, and regulation of their symbiosis [59]. In endophyte-host symbiosis, endophytes are known to successfully prevent the host plant from attacking fungi and pests by producing secondary metabolites and in return demanding nutrition [54]. The array of metabolites and other chemicals synthesized by the endophytes endow the plants with more resistance to nematodes, insects, and livestock. Plants inhabited with specific endophytes are often able to grow faster due to the production of phytohormones and become so competitive that they dominate in a particular environment. Endophytes are the chemical synthesizers inside plants [62]. The secondary metabolites

produced by endophytes associated with medicinal plants can be exploited for curing many diseases [63]. Strobel isolated about 6500 endophytic fungi and concentrated over novel endophytic microbes [23]. As discussed earlier, a large number of bioactive metabolites extracted from endophytic fungi have been characterized for over 12 years, which belong to diverse structural groups, i.e., alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, xanthones, chinones, isocoumarines, benzopyranones, tetralones, cytochalasines, perylene derivatives, furandiones, depsipeptides, and enniatines [59, 61]. Some of them represent novel structural groups, e.g., the palmarumycins and a new benzopyroanone [16]. Such substances are synthesized through polyketide pathway from mevalonate-derived C5 units and (or) using non-ribosomal protein synthesis. A literature survey reveals that the number of novel chemical structures produced by endophytes (51%) is significantly higher than the soil fungus (38%), suggesting that these frequently overlooked endophytes are the novel sources of bioactive secondary metabolites [59, 61]. Plants and their endophytic microbes emit a wide range of volatile acids, alkanes, alkenes, carbonyls, alcohols, esters, terpenoids, and other small molecules into the biosphere.

Microbial metabolites seem to be distinctive of certain biotopes, both at environmental and organism level. Thus, it appears that the search for novel secondary metabolites should core on organisms that inhabit exceptional biotopes. Fungal secondary metabolites produced may vary with the biotope in which the fungus grows and to which it is adapted. The production of cyclosporine A, echinocandin B, papulacandins, and verrucarins varied with both habitat and substrate in a study [44]. Endophytic fungi are the sources for screening natural products and in optimizing new bioactive secondary metabolites. The secondary metabolites synthesized by endophytic fungi may correspond with its respective ecological niche and continual metabolic interactions between the fungus and plant resulting in an increase in the synthesis of these secondary metabolites [64]. Recently, several studies have led to the discovery of important plant secondary metabolites from endophytic fungi, thus raising the prospect of using such organisms as alternative sources of these metabolites [65]. In addition, endophytes accumulate other biologically active and structurally diverse natural products that are unprecedented in nature [15–20, 54] and are of importance for drug discovery or for agriculture [4, 21, 22]. Endophytes are an important and largely untapped reservoir of unique chemical structures that have been modified through evolution and are believed to be involved in host plant protection and communication [19]. Fungal endophytes are known to produce metabolites that mimic the structure and function of host compounds [23] and can produce plant growth hormones such as gibberellins [12, 24].

4 Fungal Volatile Organic Compounds

Volatile organic compounds (VOCs) are carbon-based solids and liquids that readily enter the gas phase by vaporizing at 0.01 kPa pressure at a temperature of approximately 20 °C [67]. VOCs have generally low to medium water solubility and often

have a distinctive odor [66]. Many of the best known VOCs are made by industrial activities which are used in paint thinners, air fresheners, automotive products, dry cleaning fluids, etc. They are generated by combustion and evaporation of petroleum-based products and used to manufacture products like plastics, pharmaceuticals, and other major goods of modern society. Some anthropogenic VOCs are associated with air pollution and the contamination of groundwater [68]. Volatile organic compounds (VOCs) produced by microorganisms are regarded as important infochemicals in the biosphere which influence the dynamics of the ecosystem and vice versa [81]. The emissions of microbes affect atmospheric chemistry [73–75]. Microbial species produce consistent and reproducible VOC profiles under standard culture conditions. A number of studies have been done on VOCs of bacterial origin and their role in signaling in terrestrial environments [76–78]. However, less attention has been paid on the ecological role of VOCs of fungal origin [79, 80].

Over 300 distinct VOCs have been identified from fungi [69–71], and among these, the volatile sesquiterpenoids have been the focus of particular attention [72]. With the discovery of the mycodiesel-producing organism, previously identified as *Gliocladium roseum* subsequently reclassified as *Ascocoryne* sp. [82], it is clear that fungi produce a wide array of VOCs with great potential in industrial and agricultural applications as alternative fuels, perfumery, biodegradation, and decontamination of human and animal wastes as well as post-harvest food processing [83]. Volatile organic compounds (VOCs) comprise part of an organism's "metabolome," providing a useful indication of chemical diversity as a representative class of natural products [84]. It is thought that VOCs help in mediating relationships between fungi and other organisms such as host plants in case of endophytes. About 250 VOCs have been demonstrated to be produced by fungal endophytes [85]. Endophytic VOCs are proposed to have potential uses as biocontrol agents, antibiotics, commodity chemicals, and biofuels [82, 85, 86]. Unlike other fungal products, they have the advantage of being easily identified and quantified without extraction. Therefore, the endophytes are considered as ideal candidates to survey the diverse range of natural products especially the VOCs, quickly and efficiently.

Fungi produce various mixtures of gas-phase, carbon-based compounds VOCs that are able to diffuse through the atmosphere and soils, due to their small size. Fungal volatile organic compounds (VOCs) are commonly formed at bioactive interface between plants and microorganisms, and fungal-plant interactions symbolize intriguingly biochemically complex and challenging scenarios that are discovered by metabolomic approaches. All fungi produce VOCs, but their composition varies with the species of fungus and the environmental situation in which the fungus is grown. These fungal VOCs are mixtures of alcohols, aldehydes, acids, ethers, esters, ketones, terpenes, thiols, and their derivatives having characteristic moldy odors associated with damp indoor spaces [87]. VOCs are proved to have significant effects on growth, development, and defense system of plants under controlled environments. Many fungal VOCs are also reported to support dynamic processes leading to countless interactions between plants, antagonists, and mutualistic symbionts [88].

Despite some methodological and technological constraints, researchers have detected and characterized approximately 250 fungal VOCs, many of which have characteristic odors and are produced during primary and secondary metabolism [86]. Fungal VOCs may contribute to a controversial medical diagnosis called "sick building syndrome" and may also be important in the success of some biocontrol species of *Trichoderma*. VOCs also play important signaling roles for fungi in their natural environments. Many ecological interactions are mediated by VOCs, including those between fungi and plants, arthropods, bacteria, and other fungi. The diverse functions of fungal VOCs made it possible to use them in biotechnological applications as for biofuel, biocontrol, and mycofumigation [86].

5 Conclusions

Bioprospecting of secondary metabolites and VOCs of potential pharmacological, industrial, or other commercial value, especially from endophytic fungi from different medicinal plants, hopes for finding novel biotechnological products. These fungal endophytes may produce a wealth of novel bioactive metabolites and VOCs that have yet to be discovered. These fungal-originated VOCs are produced in small quantities with potent biotechnological use as biocontrol agents. Most of the research on fungi and biofuel has focused on finding efficient enzymes for degrading biomass into fermentable substances. In this twenty-first-century fungal bioprospecting, a concerted search for new biotechnological fungal VOCs will require a paradigm shift in the scientific community. Thus, VOCs represent a new frontier in bioprospecting as these compounds promise the discovery of new compounds for human exploitation and will generate new hypotheses in fundamental biology.

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Part IV

Applications in Agriculture and Industry

Endophytic Fungi: A Cryptic Fountainhead for Biodiversity, Functional Metabolites, Host Stress Tolerance, and Myco-mediated Nanoparticles (Nps) Synthesis

20

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Abstract

The view on association of higher plants and fungi from past few decades confirms the belief that all plants foster their own endophytic fungal diversity as a host, and all plant species studied till date are found to harbor one or more endophytes. The diversity of endophytic fungi can have deep impressions on plant communities through adding fitness to their concerned host conferring tolerance against abiotic and biotic stresses. While endophytes have been outlined to biosynthesize a wide array of molecules, genome sequencing of such organisms has revealed that these have the potential to provide many more secondary metabolites than usual. Recently, various methods have been advanced to aid in the activation of cryptic biosynthetic pathways. Since the most important medicinal compound taxol (paclitaxel) has been isolated from the endophytic fungus therefore, more plant mimetic compounds may be expected from this hidden microbial source. Various enzymes (amylase, lipase, cellulase, protease, lactase, pectinases, peroxidase, catalase, and penicillinase) and toxins (aflatoxin, zearalenone, ochratoxin, citrinin, T-2 toxin, and fumonisins) may be isolated from this repertoire. Cell-free extract of many endophytic fungal isolates may also be utilized to synthesize the nanoparticles like copper (Cu), silver (Ag), platinum (Pt), and gold (Au) from respective metal salt solutions. This chapter also discusses different approaches such as co-culture of microbes, altering growth media and culture conditions, genetic as well as epigenetic strategies for obtaining the biochemical treasure hidden within these unique microbes.

Keywords

Cryptic metabolites · Nanoparticles · Epigenetics · Biodiversity · Stress resistance · Natural product

Abbreviations

ABA	Absiscic acid
AgNPs	Silver nanoparticles
AZA	5-Azacytidine
CPT	Camptothecin
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
GA	Gibberellic acid
HDAC	Histone deacetylases
IAA	Indole acetic acid
JA	Jasmonic acid
RPCs	Rat prolactinoma
SA	Salicylic acid
SAHA	Suberoylanilide hydroxamic acid
VOC	Volatile organic compound

1 Introduction

The term endophytes refer to the microorganisms residing within healthy plant tissues with symbiotic association and it was first introduced by De Bary [1]. Several decades of research indicate that almost living plants are symbiotic with fungal endophytes/mycorrhizal fungi [2]. The definition was further elucidated as "microbes that colonize living, internal tissues of plants without causing any immediate and overt negative symptoms" [3]. A number of fungal diversity studies have shown the recovery of different genera and species from a single plant [4].

The endophytes apparently stay in a mutualistic association with the host plants and can be a novel source of metabolites of agriculture, medical, and therapeutic interests. Endophytes have the potential to produce similar secondary metabolites as their host. Hunting for novel, efficacious, and safer antimicrobials is necessity of the time as the pathogens of humans and plants have developed resistance to the existing antimicrobials, thus posing a greater challenge. The fungal endophytes-derived bioactive compounds provide us with new choices of novel antimicrobials and therapeutics, which can effectively be used against diseases of plant and human (Fig. 1). Many endophytic fungal strains have been reported to produce novel broad-spectrum bioactive compounds belonging to alkaloids, macrolides, terpenoids, flavonoids, glycosides, xanthones, isocoumarins, quinones, phenylpropanoids, aliphatic metabolites, and lactones. However, the antimicrobial compounds isolated till date from those fungal endophytes are only a small part.

The importance of endophytic fungi may be understood as more than hundred anticancer compounds including the taxol (paclitaxel), a host mimetic compound from *Taxomyces andreanae* have been isolated from them [5]. A lot of enzymes like amylase, lipase, cellulase, protease, lactase, pectinase, peroxidase, catalase,

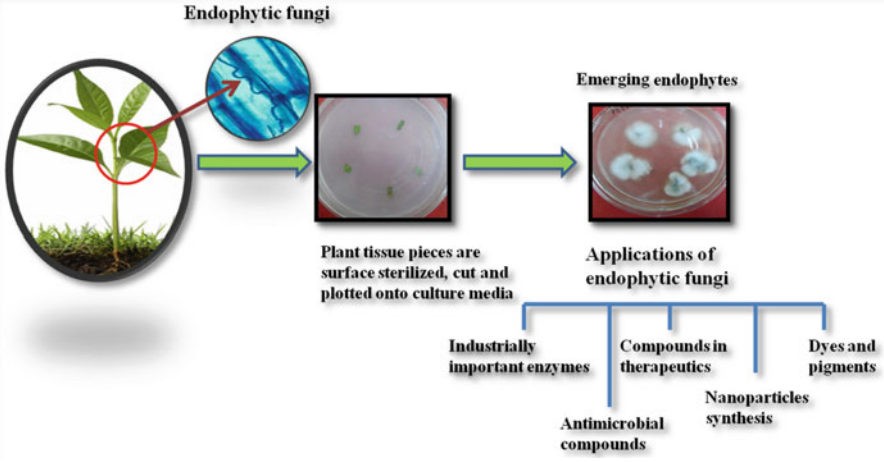


Fig. 1 Isolation of endophytes from plant tissues and areas of their application

penicillinase are reported from this group of microbes. Several mycotoxins like aflatoxin, zearalenone, ochratoxin, citrinin, T-2 toxin, fumonisins have also been isolated from endophytic fungi. Also, the fungal biomass and cell-free filtrate can be used to synthesize the metal nanoparticles like copper (Cu), silver (Ag), platinum (Pt), and gold (Au) [6–8]. Elucidation of symbiotic association of endophytic fungi with plants has been defined based on fitness benefits or impacts to macroscopic hosts and microscopic symbionts [9].

2 A Cryptic Fountainhead for Biodiversity of Fungal Endophytes

There is leading research and improving knowledge on the diversity of endophytic fungi, but statistics of dependence on hosts and their lifestyle information is limited to us which reduces interpretation of their ecological functions. There is some biogeographical system and compared diversity of cultivable and noncultivable endophytes and evaluation of their appearance is determined by distinct ecological factors [10]. Based on ratio of vascular plants to fungal species, more than one million species of endophytic fungi is estimated to exist as in mosses [11], grasses [12], shrubs [13], and deciduous and coniferous trees [14–16]. Nevertheless, the recognition of fungal endophyte diversity influenced by environmental variables is still limited at present. Traditional techniques such as cultivation-dependent methods have been routinely used in previous studies related to endophyte diversity (Fig. 1). Traditional methodology is not so successful due to the presence of some non-sporulating and non-culturable endophytic fungi in natural environments; however, next generation sequencing (NGS) and DNA fingerprinting techniques have successfully been employed in the detection and identification of even the non-culturable endophytic fungi which forms a major part of community composition.

2.1 *Synnemapestaloides ericacearum* Species

A fungus belonging to the subfamily *Xylariomycetidae* was isolated from leaves of mountain laurel (*Kalmia latifolia*) and Labrador tea (*Rhododendron groenlandicum*) which were collected from coastal southern New Brunswick. A novel Synnemapestaloides (Sporocadaceae) species reported here as synonym of *Ericacearum* was confirmed on the basis of phylogenetic and morphological studies. The filtered cell-free extract of this new species displayed a potent antifungal activity against *Microbotryum violaceum*, a biotrophic pathogen. Synnemadoxins, a postulated precursor, synnemadi acid A and 1, 3-benzodioxin-4-one scaffold, a rare natural product was characterized as new structures in its metabolites and assessed for antimicrobial activity. In-vitro analyses of isolated compounds showed inhibitory

activity effects on pathogenic fungi *M. violaceum* at 2.3 μg mL-1 besides expressing some impressive antibiotic activity [17].

The diversity of fungal order Xylariales is reviewed on the basis of secondary metabolites and correlations between structural and chemical diversity. The predominant fungal endophytes belonging to the order Xylariales are the producer organisms of pharmaceutically important lead molecules including the antiparasitic nodulisporic acids and antimycotic sordarins, with an addition of a commercial drug, emodepside. To support classical morphology and molecular phylogenetic approaches, recently the secondary metabolite profile is being considered as additional parameters to reconstruct evolutionary relationships among these fungi. Xylariales order is the example of such recent taxonomic rearrangement which depend on advanced approaches, as certain metabolite groups appear to have importance at the species, genus, or family level, respectively, while other group of metabolites are only limited to definite taxa and their production is mostly dependent on the culture conditions and manipulation. Diversity on the basis of metabolic profiles may be recognized in a single species or like *Hypoxylon rickii*, *Pestalotiopsis fici,* and *Daldinia eschscholtzii*. It indicates towards the future, because diversity of secondary metabolite will improve our knowledge by undertaking on certain genera that have so far been neglected [18].

2.2 *Nigrospora* Species

The members of genus *Nigrospora* show different characteristics changing with host such as endophytes, plant pathogens, or saprobes. Nowadays, the phylogenetic relationships, as well as familial placements among *Nigrospora* species remain arguable. *Nigrospora* belongs to Xylariales, whose confirmation was based on a phylogeny inferred from LSU sequence data. To avoid dilemma on *Nigrospora* species, a multilocus phylogeny was created based on TEF1-α, TUB2, and ITS in conjunction with host associations, morphological characters, and ecological data employed. Additionally, the identification of 165 isolates collected from China and 3 from Europe was ascertained based on multilocus phylogeny. Out of these, 13 novelties were suggested including 12 new species and 1 new union. The new species in mentioned above and described under genus *Nigrospora* were *N. bambusae*, *N. aurantiaca*, *N. chinensis*, *N. camelliae-sinensis*, *N. guilinensis*, *N. hainanensis*, *N. osmanthi*, *N. lacticolonia*, *N. vesicularis*, *N. pyriformis*, *N. rubi,* and *N. zimmermanii*. These findings suggest the importance of endophytic fungi adding to the diversity of *Nigrospora*, many of which were previously unknown. Taxa that clustered to *Nigrospora* have wide host range whereas others those diverged from cluster later favored to restricted host range. Therefore, the genus *Nigrospora* associates from a wide to a narrow host range according to recent data and general evolutionary guidance [19].

2.3 *Colletotrichum* Species

Diversity of *Colletotrichum* spp. associated with *Citrus* and related genera in European orchards, nurseries, and gardens are reviewed as plant pathogens, endophytes, and saporbes. The diseases caused by different species of *Colletotrichum* are registered as stem-end rot, fruit rot, tear stain, postbloom, fruit drop, and wither-tip of twigs. During 2015 and 2016 survey in Greece, Malta, Portugal, and Spain, a total of 174 *Colletotrichum* strains were isolated from symptomatic fruits, leaves, petals, and twigs. On the basis of seven genomic loci (GAPDH, ITS, CAL, ACT, CHS-1, HIS3, and TUB2), and the morphological characters of the isolates, a multilocus phylogeny was established. *Colletotrichum* strains were identified as members of *Colletotrichum gloeosporioides* s.str., and two novel species (*C. hystricis* and *C. helleniense*) were identified in the *C. gloeosporioides* species complex. *Colletotrichum novae-zelandiae*, *C. karstii*, *C. limonicola* and *C. catinaense* were kept in the *C. boninense* species complex, and *C. acutatum* s.str. as members of *C. acutatum* species complex. Out of all, *C. karstii* and *C. gloeosporioides* were evaluated as the chief species [20].

2.4 *Alternaria, Neofusicoccum*, and *Preussia* Species

Medicinal plant *Artemisia* has many medicinal applications but association of fungal endophytes with this plant has been rarely studied. Ten plants sampled from Tenerife and La Palma of *Artemisia thuscula* was examined to isolate the diversity of endophytic fungi. On the basis of multilocus phylogeny (LSU, ITS) and morphology, 37 fungal species were identified associated to 25 fungal genera where *Alternaria alternata* (CF = 18.71%), *Neofusicoccum* sp. (CF = 8.39%), and *Preussia* sp. (CF = 3.23) were the predominant species, respectively. The rate of colonization varied among plants (CR = 25–92.11%). Lack of host specificity and reduced host diversity were also observed. Sorensen-Dice index indicated that out of 45 cases, 27 were with zero similarity. Only one case was with 57% similarity (TF1 and TF7) and one with 50% while rests were ranging between 11% and 40% in similarity. Fisher's alpha and Simpson index of diversity indicated higher species richness in plants from La Palma than in plants from Tenerife. Three nutrient media (i.e., lignocellulose agar-LCA, potato dextrose agar-PDA, and tomato juice agar-V8) were used in a case study in terms of colonization rate, but when data was averaged, no differences were found. Colonization frequency indicated several species with partiality for nutrient medium (63% of the species were isolated from only one nutrient medium). By using the Bayesian method, phylogeny was constructed and 54 endophytic fungal ITS sequences and associated GenBank sequences were examined. Ten orders (Dothideales, Diaporthales, Hypocreales, Botryosphaeriales, Trichosphaeriales, Capnodiales, Xylariales, Amphisphaeriales, Pleosporales, and Eurotiales) were recognized [21].

3 Endophyte-Derived Natural Products

The role of secondary metabolites has always been a matter of argue, but these are usually known to play a chief part in interspecies protection and transmission. Secondary metabolites produced by microbes are the organic compounds which are not involved immediately in their development, reproduction, and growth. Secondary metabolites have been used as various purposes like pharmaceuticals, flavoring, and decorating agents. Alkaloids, glycosides, phenolics, and terpenoids are in nature of secondary metabolites. In recent past, endophytes have been a crucial and alternative source for the production of novel bioactive compounds in the area of pharmaceuticals, agricultural, and drug discovery. Discovery of penicillin and streptomycin as antibacterial agents isolated from *Penicillium* sp. and *Streptomyces* sp., respectively brought a great revolution in the exploration of microbes for the human welfare. Further, many antibiotics were isolated from different microorganisms as lovastatin (from *Aspergillus* sp.); amino glycosides, tetracycline, streptomycin, and other polypeptides (from *actinomycetes*); immunosuppressive agents, like cyclosporine (from *Trichoderm*a and *Tolypocladiyn* sp.) and rapamycin (from *Streptomyces* sp.); cholesterol-lowering agents like mevastatin (from *Penicillium* sp.); and antihelminthics and antiparasitic drugs, like ivermectins (from *Streptomyces* sp.) are just a few examples from a wide library of bioactives recovered from microorganisms [22].

3.1 Antibacterial Compounds from Fungal Endophytes

3.1.1 Phomopsichins A-D and Phomoxanthone A

Phomopsichins A-D **(1–4)** and phomoxanthone A **(5)** (Fig. 2) are compounds isolated from endophytic fungus *Phomopsis* spp. Structural properties of these compounds were explained by spectroscopic analysis coupled with single-crystal X-ray diffraction. It shows a tricyclic structure, with dihydropyran ring fused with

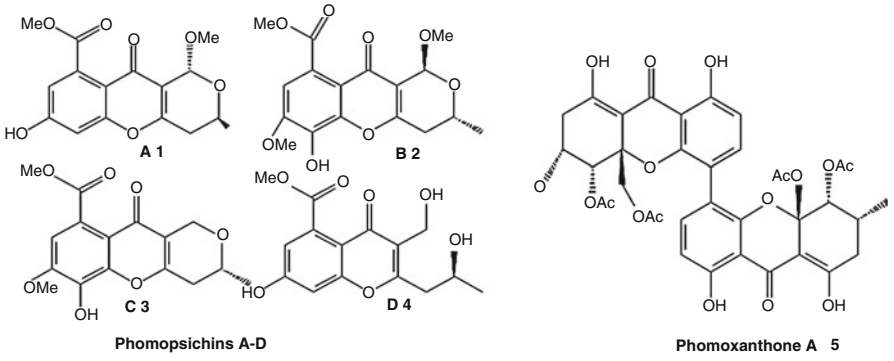


Fig. 2 Chemical structures of phomopsichins A-D **(1–4)** and phomoxanthone A **(5)**

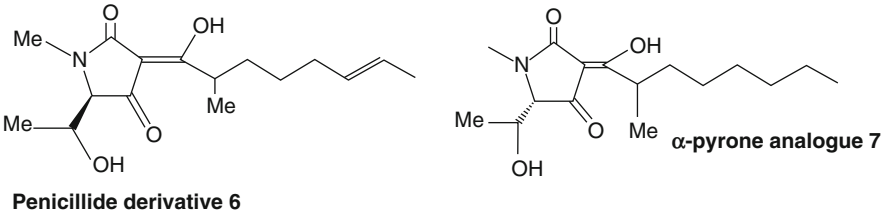


Fig. 3 Chemical structures of penicillide derivative and α-pyrone analogue

the chromone ring. Phomopsichins **1–4** showed inhibitory activities against acetyl cholinesterase and slight antimicrobial activities. It also scavenge free radical scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) as well as OH. Their other bioactivities are worthy of further study, considering their unique molecular structures [23].

3.1.2 Penicillide Derivatives

Endophytic fungi have attracted attention due to their functional diversity. *Pestalotiopsis sydowiana* isolated from a halophyte *Phragmites communis trinus* produced 11 compounds, including 4 penicillide derivatives **(6)** and 7 α-pyrone analogues **(7)** (Fig. 3). All these compounds were identified based on spectroscopic data. It was found to show inhibitory effects on 20S proteasome. Modest proteasome inhibition activity was shown by 1–3, penicillide derivatives and 5, 9–10 α-pyrone analogues and of these, pestalotiopyrone G showed the strong activity with an IC50 value 1.2 ± 0.3 μM. The endophytic fungus *P. sydowiana* might be a good resource for proteasome inhibitors [24].

3.1.3 Fusarubin, 3-O-methylfusarubin, and Javanicin

An endophytic isolate displaying the antibacterial and antituberculosis properties was identified as *Fusarium solani* through rDNA sequencing. This strain was cultured to large-scale fermentation for extraction of its bioactive metabolite using column chromatography. Biologically active molecules were ascertained as 3, 6, 9-trihydroxy-7-methoxy-4, 4-dimethyl-3,4-dihydro-1H-benzo[g]isochromene-5,10-dione, fusarubin **(8)**, 3-O-methylfusarubin **(9)**, and javanicin **(10)** (Fig. 4). All the four metabolites inhibited the growth of various strains of pathogenic bacteria with MIC values ranging between 1 and 256 μg mL-1. Fusarubin showed proficient activity against *Mycobacterium tuberculosis* strain H37Rv with MIC value of 8 μg mL-1, whereas 4-dimethyl-3,4-dihydro-1H-benzo[g]isochromene-5,10-dione, 3-O-methylfusarubin, and javanicin exhibited moderate activity with MIC values of 256, 64, 32 μg mL-1, respectively [25].

3.1.4 Purpurester B and Pestaphthalides A

Czapek's medium inoculated endophytic fungus *Aspergillus* sp. led to the production of four new metabolites, aspergifuranone, isocoumarin derivatives, demethylpurpurester A, collectively known purpurester B **(11)** and pestaphthalide

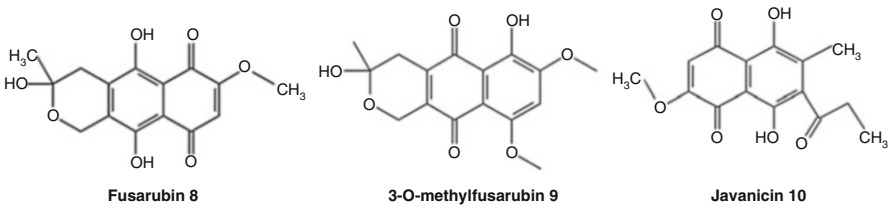


Fig. 4 Chemical structures of fusarubin, 3-O-methylfusarubin, and javanicin

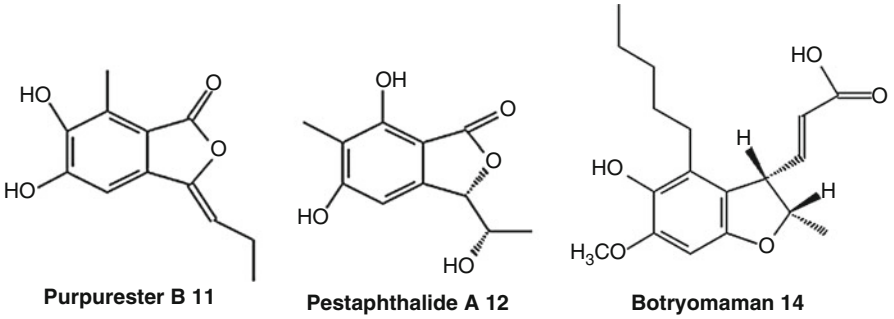


Fig. 5 Chemical structures of purpurester B, pestaphthalide A, and botryomaman

A **(12)** (Fig. 5). All isolated compounds were screened for their inhibitory activities against α-glucosidase, and aspergifuranone showed significant inhibitory activity with IC50 value of 9.05 ± 0.60 μM. Kinetic analysis showed that aspergifuranone acted as noncompetitive inhibitor against α-glucosidase while isocoumarin exhibited moderate inhibitory activity [26].

Endophytes receive nutrition from the plant and exchange various benefits to the plant for stress tolerance, pest resistance, and protection from grazing animals. Identification of the potential endophytic fungi and their screening for production of newer and efficacious drugs may fulfill the growing demand worldwide for new drug discovery. Surprisingly, more than 100 anticancer compounds have been isolated from the endophytic fungi after 1993 [27].

Javanicin **(10)**, a naphthaquinone compound isolated from endophytic fungus *Chloridium* sp. showed potent antibacterial activity (2 μg mL-1) against *Pseudomonas* spp., pathogens to both humans and plants. This fungus was isolated from *Azadirachta indica*. Javanicin **(10)** showed comparatively higher MIC values against human pathogenic bacteria *Escherchia coli* and *Bacillus* sp. which were 20 and 40 μg mL-1 while it also displayed the same MIC value against pathogenic fungus *Fusarium oxysporum*. Javanicin activity was also screened antimicrobial for *Verticillium dahliae* and *Rhizoctonia solani* at 10 μg mL-1 and 5 μg mL-1 against *Cercospora arachidicola* [28, 29].

Cytosporone A, an octapeptide, has been isolated from endophytic fungus *Cytospora* sp., of CR 200 strain and *Diaporthe* sp. of CR146 strain [30, 31].

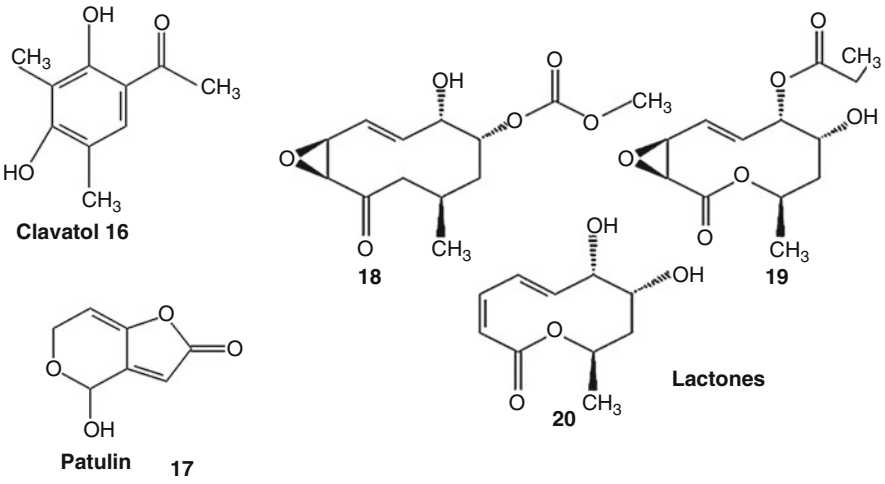


Fig. 6 Chemical structures of clavatol, patulin, and lactones

Botryomaman **(14)** (Fig. 5), derivative of dihydrobenzofuran, was isolated from endophytic fungus *Botryosphaeria mamane* PSU-M76. Both compounds showed the potent antibacterial activity against methicillin-resistant *S. aureus* SK1 and *Staphylococcus aureus* ATCC 25923 with MIC value μg mL-1.

3.2 Antifungal Compounds

An endophytic fungus *Aspergillus clavatonanicus* isolated from *Torreya mairei* produces clavatol **(16)** (2′,4′-dihydroxy-3′,5′- dimethylacetophenone), patulin **(17)** (Fig. 6), (2-hydroxy- 3,7-dioxabicyclo [4.3.0] nona-5,9-dien-8-one) confirmed by EI-MS, NMR, and X-ray crystallography. Both compounds showed antifungal activity against *Didymella bryoniae*, *Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pythium ultimum* [32].

An endophytic fungus *Phomopsis* sp. YM 311483 isolated from *Azadirachta indica* produces new members of lactone **(18–20)** (Fig. 5). They showed weak antifungal activity against *Helminthosporium maydis*, *Fusarium avenaceum*, *F. moniliforme*, *Ophiostoma minus,* and *Penicillium islandicum*, with the highest MIC value lying in the range of 31.25–500 μg mL-1 [33].

3.3 Antiviral Metabolites of Endophytes

The endophytic fungus *Pullularia* sp. BCC 8613 produced pullularins A–D **(21–24)** (Fig. 7), new cyclohexadepsipeptides. Pullularin A was found effective against *Plasmodium falciparum* K1 (IC50 3.6 μg mL-1) as well as herpes simplex virus type 1 (HSV-1; IC50 3.3 μg mL-1. Weak cytotoxic activity was also screened against

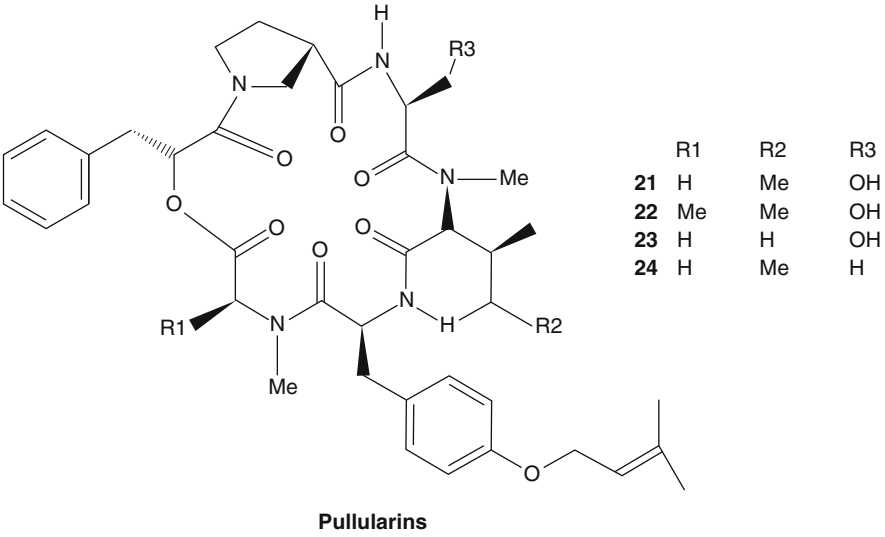


Fig. 7 Common chemical structure of pullularins

vero cells (IC50 36 μg/mL1). *Cytonaema* sp., an endophytic fungus, produces cytonic acids A and B, known as protease inhibitors of human cytomegalovirus [34].

3.4 Nematicidal and Insecticidal Metabolites

Geotrichum sp. strain AL4, an endophytic fungus isolated from the leaf of *Azadirachta indica* (neem tree), produced two new chlorinated epimeric 1,3 oxazinane derivatives **(25–26)** (Fig. 8). Both compounds were screened for their nematicidal activity against the nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus* [35].

3.5 Cytotoxic Products of Endophytes

3.5.1 Anthraquinone Derivatives (27–29)

SZ-685C compounds were identified as anthraquinone derivatives (Fig. 9) extracted from the mangrove endophytic fungus *Halorosellinia* sp. (No. 1403). SZ-685C showed anticancer and tumor suppressive properties against pituitary adenoma, and it was also screened for growth inhibition of primary human NFPA cells, MMQ cell lines of rat prolactinoma normal pituitary cells (RPCs). It showed increased expression levels of phosphate, caspase 3, and tensin homolog (PTEN) that were predicted by Western blotting. SZ-685C treated cells displayed increased rate of apoptosis which was identified by double staining, Hoechst 33342 dye/propidium iodide (PI), and fluorescein isothiocyanate-conjugated Annexin

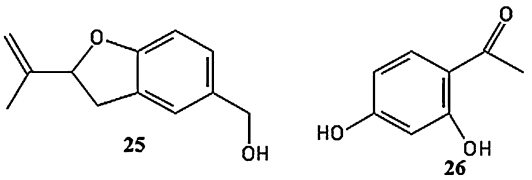


Fig. 8 Chemical structure of 1,3 Oxazinane derivatives

1,3 Oxazinane derivatives

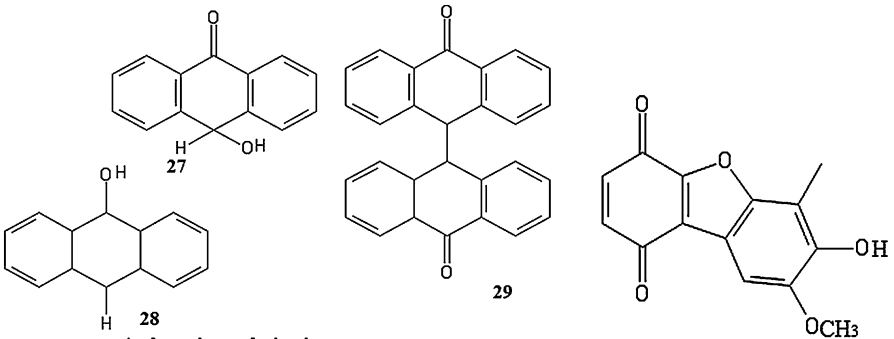


Fig. 9 Chemical structures of anthraquinone derivatives and xylariaquinone A

V/PI (Annexin V-FITC/PI) apoptosis assays. Notably, SZ-685C treated cells showed decrease in protein level expression of Akt gene. The assay indicated that SZ-685C inhibited the Akt pathway that induces apoptosis of human NFPA cells. Property of this endophytic compound to induce apoptosis gives clue of targeted therapies that can cause death in cancer cells [36].

Xylariaquinone A **(30)** (Fig. 9) and 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione are two novel benzoquinone derivatives isolated from endophytic fungus *Xylaria* sp. which showed cytotoxic activity against kidney fibroblasts cells of African green monkey with IC50 values of 1.35 and >184 μM, respectively [37]. Tetramic acid derivatives penicillenols A1, A2, B1, B2, C1, and C2 **(31–36)** (Fig. 10) with phenol A dihydrocitrinin, citrinin, and phenol A acid were isolated from endophytic fungus *Penicillium* sp. GQ-7 of *Aegiceras corniculatum* plant. The tetramic acid derivatives penicillenol A1 and B1 was screened for cytotoxic activity by the MTT bioassay against HL-60 cell line and their IC50 values were calculated as 0.76 mM and 3.20 mM, respectively [38].

3.6 Host Mimetic Compounds Produced by Endophytic Fungi

Vinca alkaloid vincristin **(37)** (Fig. 11) was isolated from a member of the family Apocyanaceae, *Catharanthus roseus*. Vincristin inhibits the polymerization of

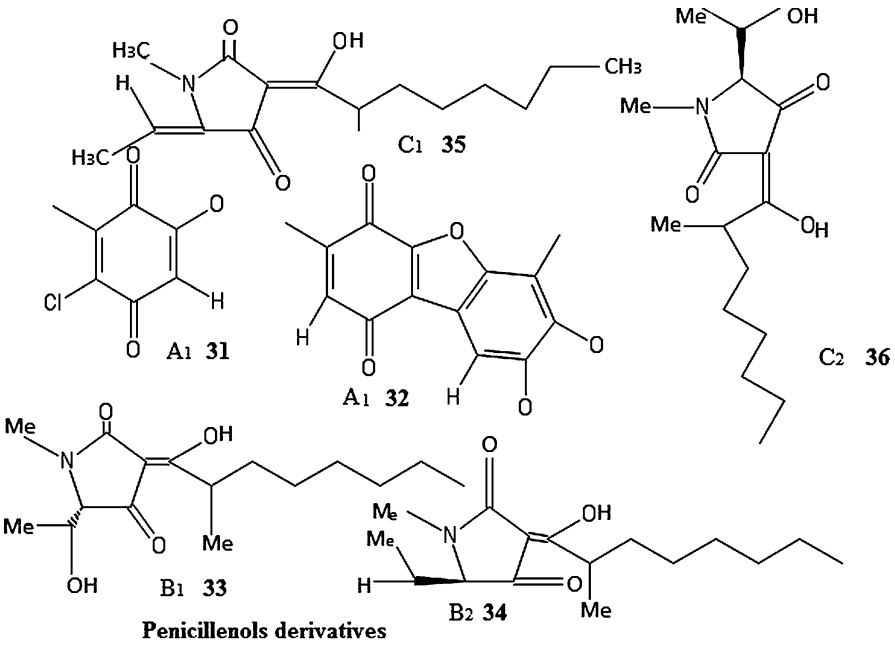


Fig. 10 Chemical structures of penicillenols derivatives

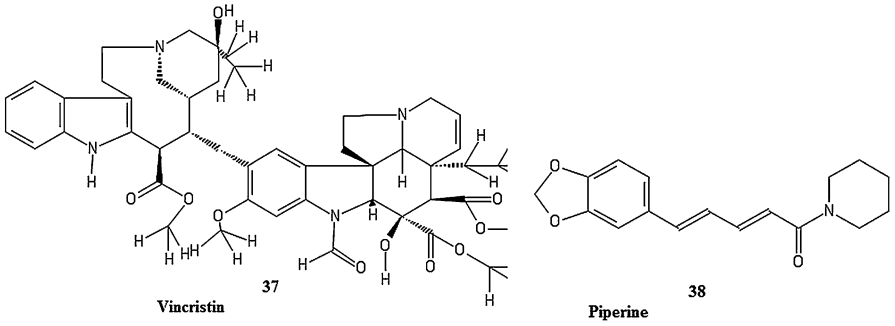


Fig. 11 Chemical structures of vincristin and piperine

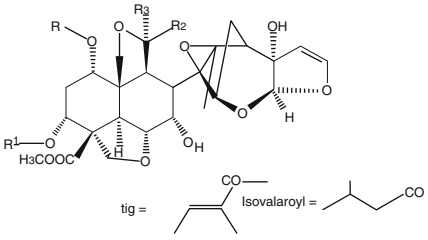
microtubules as well as checks the formation of spindle protein in the synthesis phase of cell cycle. Action of vincristin on microtubule consequently inhibits the spindle formation, so arrest the tumor cell in metaphase stage of cell cycle. Vinca alkaloids are originally isolated from *Catharanthus roseus* [39], but it has also been isolated from *Fusarium oxysporum*, an endophytic fungus of *Catharanthus roseus* [40].

The endophytic fungus *Periconia* sp., isolated from *Piper longum*, produces piperine **(38)** (Fig. 11) in liquid broth culture, and (5-(3, 4-methylenedioxyphenyl)-1-piperidinopent-2, 4-dien-1-one) piperine showed strong activity against

Mycobacterium smegmetis and *M. tuberculosis* with MIC values 1.74 and 2.62 l μg mL-1, respectively. This compound has potent cytotoxic activity also. It is a host mimetic compound, so this can increase the principle bioactive compound content in the plant [41].

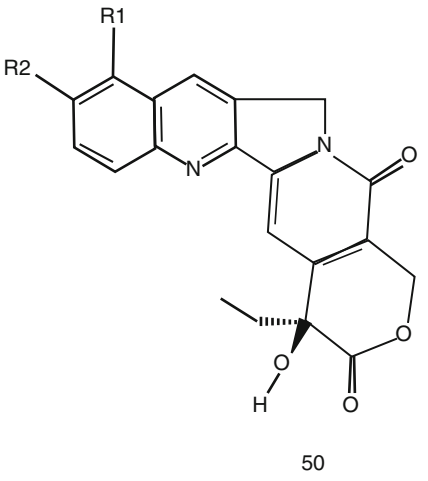
Endophytic fungus *Eupenicillium parvum* produces azadirachtin A **(39)** and B **(40)** (Fig. 12), a complex tetranortriterpenoid limonoids have been isolated from the Indian neem tree (*Azadhiracta indica*) seeds. Compound identification of the fungal endophyte was done using LCHRMS^n [42]. Azadirachtin **(39–49)** (Fig. 12) action was reported highly active against insects that could be used in improving human and plant health [43].

Camptothecin **(50)** (Fig. 13), exhibiting potent antitumor and antileukemic activities in animals, was first isolated from wood of *Camptotheca acuminata*. This plant is native of China called "xi shu" or the "happy tree." Camptothecin (CPT) inhibit dissociation of the DNA–topoisomerase I complex during replication



		R	R1	R2	R3
39	Azadirachtin A	tig	Ac	OH	CO_2CH_3
40	Azadirachtin B	H	tig	H	CO_2CH_3
41	11 alpha Azadirachtin H	tig	Ac	H	OH
42	11 bita Azadirachtin H	tig	Ac	OH	H
43	Azadirachtin L	tig	Ac	H	OAc
44	Azadirachtin M	tig	Ac	H	CO_2CH_3
45	Azadirachtin N	H	tig	H	OH
46	Azadirachtin O	Isovalaroyl	Ac	H	CO_2CH_3
47	Azadirachtin P	Isovalaroyl	Ac	H	OH
	Azadirachtin Q 48	Ac	Ac	H	CO_2CH_3
49	Azadirachtol	H	H	H	CO_2CH_3

Fig. 12 Chemical structure of Azadirachtin A and substituent groups of its analogues



R1,R2=H Camptothecin : **50**
R2=H; R1=OCH_3 9-Methoxycamptothecin **51**
R1=H; R2=OH 10 Hydroxycamptothecin **52**

Fig. 13 Chemical structure of camptothecin and substituent groups of its analogues

[44, 45]. Topoisomerase I lacking mutant cells of yeast were immune to the cytotoxic effect of these alkaloids [46]. CPT neither binds to topoisomerase I independently nor to DNA, and it only bind with the Topo I complex when it cleaves DNA. It is interesting to note that this camptothecin and its analogue 9-methoxycamptothecin have been reported from cell suspension cultures and inner bark of *Nothapodytes foetida* native of India and Sri Lanka [47].

Initially, camptothecin **(50)** was isolated in 2005 from a fungal endophyte of the inner bark of *Nothapodytes foetida* plant; however, few years later; CPT was identified from a *C. acuminata* seed endophyte, *Neurospora crassa* [48]. Both isolated CPT were tested against human cancer cell lines HEP-2 (liver cancer), A549 (lung cancer), and OVCAR-5 (ovarian cancer) with effective results [48]. Recently, camptothecin and its two analogues, 9-methoxycamptothecin 3 and 10-hydroxycamptothecin 4, were isolated from endophytic fungus *Fusarium solani*, isolated from *Camptotheca acuminata* [49]. These analogues are more water soluble in comparison to camptothecin and more potent inhibitors of DNA topoisomerase I [49].

4 Stimulation of Cryptic Metabolites in Fungi by Epigenetic Modulators

4.1 Way of Modification in Genome

Modifications in genome lead to the activation of cryptic gene(s) product and changes at proteome and transcriptome level. The modification may be at genome level including epigenetic modulation (methylation, acetylation, sumoylation, phosphorylation, etc.), exchange of promoter, gene knockout, etc. Modulation at transcriptome level includes regulation of transcription factors (either over expression or suppression) and altering the metabolomics [50].

Modification in gene(s) through epigenetic modulators may take place either by the activation of new gene or by the suppression of previously active genes involved in natural metabolites biosynthesis (Table 1). For example, tricyclazole, a fungicide,

Table 1 Some studies on treatment of epigenetic modulators on endophytic fungi and observed effects

S.No.	Endophytic fungus	Epigenetic modulator	Induced/enhanced secondary metabolites	References
1.	*Colletotrichum gloeosporioides*	Resveratrol and curcumin	Increases antibacterial and antioxidant properties	[54]
2.	*Eupenicillium* sp. LG41	Nicotinamide	Eupenicinicols C and D	[55]
3.	*A. nidulans*	SAHA, 5-azacytidine	Cladochromes, calphostin B	[57]
4.	*Muscodor yucatanensis* Ni30	SAHA, 5-azacytidine	Ergosterol xylaguaianol C	[58]

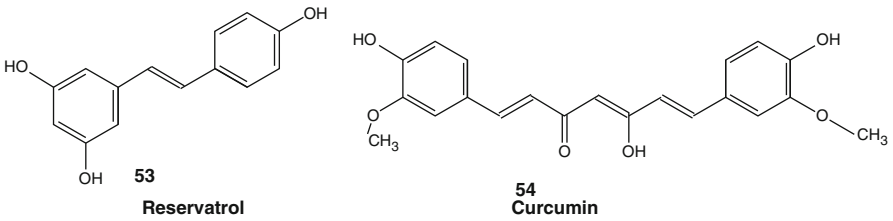


Fig. 14 Chemical structures of resveratrol and curcumin

induces the production of sphaerolone and dihydrosphaerolone and simultaneously inhibits the production of 1, 8-dihydroxynaphthalene in some members of Sphaeropsidales [51, 52]. Many modulators have been identified that show reduced growth of the targeted microorganism and their metabolites production. Such approach can be considered as untargeted with unpredictable results [53].

4.2 Epigenetic Modulations in Endophytic Fungi

4.2.1 Resveratrol and Curcumin

Resveratrol **(53)** and curcumin **(54)** (Fig. 14) are the major compounds from the grapes skin and turmeric extracts, respectively. These compounds have been used to treat the *Colletotrichum gloeosporioides,* an endophytic fungus of *Syzygium cumini,* for the modification in gene or gene cluster. The extracts of turmeric and grape skin treated fungal cultures produced higher amount of crude metabolites 174.32% and 272.48%, respectively as compared to the untreated control. Total crude was tested against human pathogenic bacterium *Aeromonas hydrophila* IMS/GN11 and its growth was found to be inhibited significantly in comparison to untreated crude. Treated crude compounds obtained from turmeric extract showed significant DPPH free radicals scavenging activity (86.46% inhibition) and grape skin treated cultures (11.80% inhibition) while the control cultures was (1.92% inhibition). The treated crude compound had both the activities higher – the antibacterial and antioxidant in comparison to the control. After analysis of crude compounds by HPLC, it was found that turmeric extract and grape skin treated cultures showed an expression of 20 and 14 cryptic compounds in the crude extract, respectively [54].

4.2.2 NAD^+ Inhibitor of Histone Deacetylase

Nicotinamide is an epigenetic modulator which functions as NAD^+ dependent histone deacetylase (HDAC) inhibitor. An endophytic fungus *Eupenicillium* sp. LG41, isolated from *Xanthium sibricum*, was treated with nicotinamide which resulted in the activation of new gene and production of two known compounds eujavanicol A **(55)** and eupenicinicol A **(56)** along with two new decalin-containing compounds, eupenicinicol C **(57)** and D **(58)** (Fig. 15). The eupenicinicol D was found active against *Staphylococcus aureus* with an MIC of 0.1 μg mL-1 and

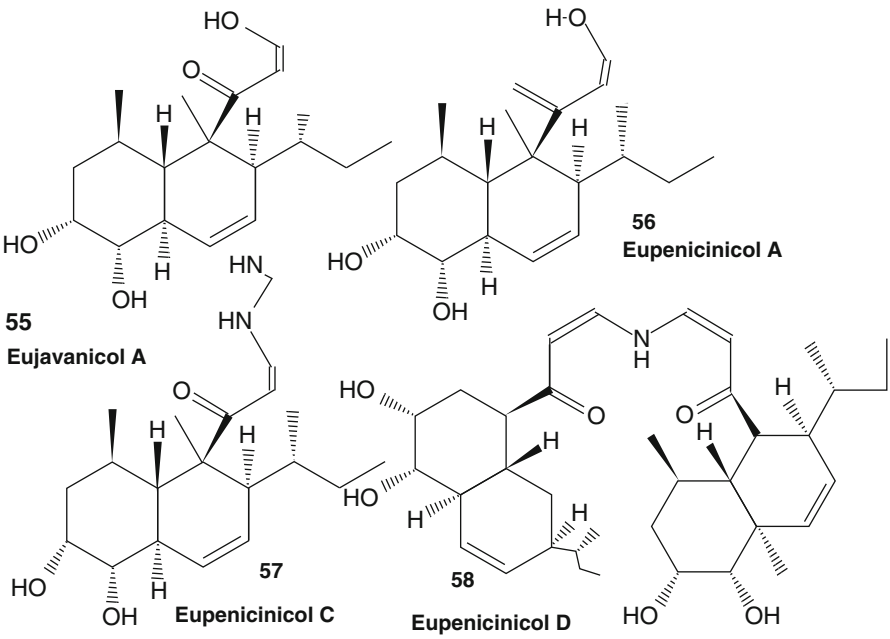


Fig. 15 Chemical structures of eujavanicol and eupenicinicol

displayed noticeable cytotoxicity against the human acute monocytic leukemia cell line (THP-1) [55].

4.2.3 Suberoylanilide Hydroxamic Acid (SAHA) and 5-Azacytidine as Epigenetic Modulators

Suberoylanilide hydroxamic acid (SAHA) is an inhibitor of histone deacetylase enzyme. Treatment with SAHA enhanced the production of new cladochromes in *A. nidulans* and of calphostin B **(59)** in *Cladosporium* sp. [56]. The outcomes of SAHA treatment were very similar to the consequence of direct physical contact of *A. nidulans* with particular actinomycete strains resulting in orsellinic acid production was induced [57]. In another experiment, the epigenetic modifiers (SAHA and 5-azacytidine) (Fig. 16) treatment induced the overexpression of PKS genes in *Muscodor yucatanensis* Ni30. Treated organism varies greatly from the wild type in appearance in morphological as well as metabolites production level. Endophyte produced a separate set of VOCs different from the wild type, and various VOCs including methyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl) hexane-2, 4-diol and 2-carboxymethyl-3-n-hexylmaleic appeared new in the strains. The bioactive extrolite brefeldin A **(60)** (Fig. 16) was extracted and analyzed from the wild type [58].

An endophytic *Hypoxylon* sp. (strain CI-4) produced 1, 2, 4-tris (methylene), cyclohexan 1,8-cineole, 1-methyl-1,4-cyclohexadiene, and these compounds are volatile in nature. Most of the compounds produced by this endophyte has high

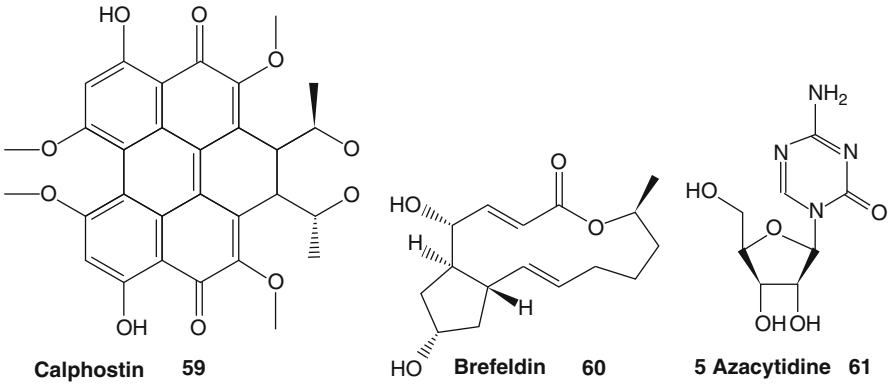


Fig. 16 Chemical structures of calphostin, brefeldin, and 5-azacytidine

energy densities as like mycodiesel fuel. Treatment of this fungus by epigenetic modulator 5-azacytidine AZA **(61)**, a methyltransferase inhibitor (DNMT), and suberoylanilide hydroxamic acid SAHA, a histone deacetylase inhibitor (HDAC), caused variation in pigmentation, cultural changes, growth rates, and odor with significant variation in volatile compounds. Changes were observed in treated fungus compound for terpenes (α-thujene, sabinene, γ-terpinene, α-terpinolene, and β-selinene) with secondary alkanes, alkenes, and benzenes derivatives as compared to the control fungus. Treatment of Strain CI4-B endophyte with 100 μM SAHA caused changes in the metabolites as compared to the control but removal of SAHA from the culture medium did not revert to the wild type phenotype [59].

5 Contribution of Endophytic Fungi in Stress Tolerance in Plant

5.1 Water Deficit Stress

An investigation was carried out to determine the effects of endophytic fungus *Neotyphodium coenophialum* on metabolite levels in shoot and root tissues of genetically similar clone pairs of tall fescue with endophyte (E+) and without endophyte (E−) under water stress condition. Endophyte free clones (E−) were generated after treating E+ plants with fungicide. Water availability was controlled (stopped) for 0–5 days, and during that time, sugar alcohols, free sugars, and amino acids levels were found elevated along with the levels of some major fungal metabolites. When plants were rewatered after 2–3 days of withholding, the survival rate of plants was found significantly greater for E+ survival and tillering than E− clones. Within 2–3 days of controlled water condition, increased levels of free glucose, trehalose, sugar alcohols, proline, fructose, and glutamic acid were noticed in shoot and root. Increased metabolites in plant were induced by endophyte's mannitol and loline alkaloids under water deficit condition. Thus this result suggest

that symbiotic *N. coenophialum* associated with plant support in survival and revival of tall fescue plants from water deficit and induces fast accumulation of the compatible solutes soon after providing the stress [60].

5.2 Response of Endophytic *Aspergillus flavus* Against Abiotic Stress

Endophytic fungus *Aspergillus flavus* production of secondary metabolites was altered in response to stress. Its ecological niche changed every time when it was grown under four different conditions that mimic saprophytic growth to parasitism. The analysis at genetic and phenotypic levels revealed expression changes in over 800 genes of *A. flavus*. The amazing transcriptional change was found between saprophytic and parasitic growth. The adaptive changes in conidia also took place, which brought differences in their ability to utilize carbon sources [61].

5.3 Salt Stress Resistance in *Aspergillus flavus* CHS1

Aspergillus flavus CHS1, an endophytic fungus, was isolated from the roots of *Chenopodium album*. It showed plant growth promoting traits such as phosphate solubilization, production of indole acetic acid, gibberellins, and siderophore production. Culture extract of CHS1 contained different types of GAs and IAA, after the salt stress treatment to improve the plant growth, chlorophyll content, root shoot length and significantly promoted the biomass production in plant. CHS1 potential was also checked for the resistant of the plant against NaCl stress up to 400 mM. It was also found to improve the growth of the soybean plant growth under NaCl stress by downregulating ABA and Jasmonic acid biosynthetic pathways. Further, it also improved antioxidant activity enzymes catalase, superoxide dismutase, and peroxidase, etc. in inoculated salt-stressed plants as compared to noninoculated ones [62].

5.4 Abiotic Stress Tolerance in *Piriformospora indica*

5.4.1 Salt Stress

The endophytic fungus *Piriformospora indica*, residing in the roots of barley plants, was found to increase plant adaptation and tolerance to abiotic stress. In order to recognize the genes and metabolic regulatory system behind the salt tolerance in *P. indica* colonized barley plants, ionomics, metabolomics, and transcriptomic data was studied. Control (0 mM NaCl) and severe salt stress (300 mM NaCl) treated *P. indica*-colonized and noninoculated barley plants leaf samples were harvested after fungal inoculation. Its metabolomic analysis revealed 14 metabolites and ions involved in tolerance to salt

stress. Gene expression analysis has led to the recognition of 254 variably expressed genes at 0 mM NaCl stress and 391 genes at 300 mM NaCl stress compared to noninoculated samples. The integration of metabolome and transcriptome analysis specifies that carbohydrate metabolism, nitrogen metabolism, and ethylene biosynthesis pathways might play a role in systemic salt-tolerance in leaf tissues promoted by the root-colonized fungus [63].

5.4.2 Drought Stress

Piriformospora indica, an endophytic fungus of Sebacinales order, was found to increase the resistant to abiotic stress by promoting plant growth and biomass production. Association of *P. indica* and maize roots increases leaf size with surface area of tap root. Presence of this fungus with seedlings of maize increased the drought tolerance ability. Transcriptome of colonized and uncolonized seedlings of maize in respect of *P. indica* was analyzed at 0, 6, and 12 h after drought stress. The number of *P. indica*-responsive genes increased from 464 (no stress at 0 h) to 1337 (6 h drought) and 2037 (12 h drought). Carbon and sulfur metabolisms are major targets of the fungus as revealed by ontological screening of the gene. Association of plant with the *P. indica* improved the root oxidative potential, activated the hormone-related genes like abscisic acid, auxin, salicylic acid, and cytokinins [64].

5.5 Endophytic Fungus Induced Ethylene Response in Plants

Ethylene, a gaseous phytohormone, is involved in plant growth and development as well as in responses to environmental stress. However, there is a little knowledge of ethylene role in endophyte induced secondary metabolite production. *Atractylodes lancea*, a Chinese herb quality, depends on sesquiterpenoid content. An endophytic fungus *Gilmaniella* sp. AL12 was found to induce ethylene production in *A. lancea*. When plantlets were pretreated with ethylene inhibitor, aminooxyacetic acid (AOA), the endophyte induced accumulation of ethylene and sesquiterpenoids was found reduced along with salicylic acid (SA) and jasmonic acid (JA) suggesting the role of ethylene as an upstream signal in the pathway of SA and JA biosynthesis. On the other hand, ethylene was also found to involve in the downregulation of H_2O_2 and NO pathway. Taken together, the above study demonstrated that ethylene is the reason for downstream signal of H_2O_2 and NO signaling pathways while upstream signal of jasmonic acid, salicylic acid, and provide signal in endophytic fungus *Atractylodes lancea* for sesquiterpenoids biosynthesis [65]. All these studies clearly suggest that the secondary metabolite production by endophytic fungi is immensely affected by the environmental stresses and the culture conditions (Table 2). So, alteration in growth conditions or stress generation may be utilized as a strategy for the enhancement of fungal secondary metabolite profile.

Table 2 Abiotic stress induced production/enhancement of secondary metabolites by endophytic fungi

S.No.	Producer organism	Stress	Induced/enhanced secondary metabolites	References
1.	*Neotyphodium coenophialum*	Water deficit stress	Free glucose, trehalose, sugar alcohols, proline, fructose, glutamic acid, mannitol, and loline alkaloids	[60]
2.	*Aspergillus flavus*	Abiotic stress	Saprophyte to parasite	[61]
3.	*Piriformospora indica*	Salt stress	Changes in 14 metabolites	[63]
4.	*Beauveria bassiana*	Entamopathogen	Biocontrol agent	[66]
5.	*Aspergillus flavus CHS1*	Salt stress	ABA, JA, IAA, GA	[62]
6.	*Piriformospora indica*	Abiotic	ABA, IAA, SA, cytokinin	[64]

6 Fungal Endophytes as Biocontrol Agents

Beauveria bassiana and *Metarhizium anisopliae* are entomopathogenic fungal endophytes. Association of these fungi with plant checks the activity of most insect that becomes harmful to the host. This strategy may be used as biocontrol agent without doubt as many scientists have displayed the result successfully. It has been reported that the ability of these fungal pathogens defend their colonized host plants against the primary herbivore pests. Recent findings provided evidence of other possible functions as plant yield promoter, soil nutrient distributor, abiotic stress and drought tolerance enhancer in plants. However, reports on this supplementary major consequence of fungal endophytes on the colonized plants remain brief [66].

Thirty one endophytes were isolated from the rhizome of healthy turmeric (*Curcuma longa* L.) and morpho-molecular analysis was the basis of identification for all endophytes.Total isolates were screened for antagonistic property against *Rhizoctonia solani* Kuhn and *Pythium aphanidermatum* causing leaf blight and rhizome rot disease in *Curcuma longa* L. (turmeric sp.). Out of all, six endophytes showed strong antagonistic activity against *Rhizoctonia solani* and *Pythium aphanidermatum*. Out of six, *T. harzianum* TharDOB-31 shows 76.9% inhibition against *R. solani* and 76% against *P. aphanidermatum* by antagonistic dual culture method. SEM studies of interaction zone of *T. harzianum* TharDOB-31 with of *P. aphanidermatum* and *R. solani* showed in vitro mycelia growth inhibition including unusual breakage and lysis of hyphae and abnormalities in parasitism. Association of endophyte TharDOB-31 with rhizome of turmeric decreases the disease occurrence of rhizome rot and leaf blight by 11.6% and 13.8%, respectively. Treated plant with TharDOB-31 increases fresh rhizome yield/plant and improve

plant height (85 cm). Ethyl acetate extracted secondary metabolites of TharDOB-31 contained higher number of antifungal compounds revealed by liquid chromatography mass spectrometer analysis. This approach indicates that endophyte *T. harzianum* can be exploited as a potential biocontrol agent for controlling rhizome rot and leaf blight diseases in turmeric [67].

7 Role of Fungal Endophytes in Synthesis of Nanoparticles (Nps)

There is an increasing commercial need of metal nanoparticles due to their usefulness in various areas such as energy, electronics, medicine, biomedical, agriculture, textile, food, etc. Among various metal nanoparticles, silver nanoparticles (AgNPs) [68], cobalt oxide nanoparticles [69], ruthenium naoparticles, and gold nanoparticles [70] are the major ones which have been successfully synthesized using endophytic fungi.

7.1 Fungal Endophytes Mediated Synthesis of Nanoparticles

Nanoparticles (NPs) are known to be used for numerous biological, pharmaceutical, and physical applications. Silver nanoparticles have been known as good antimicrobial agents in many public places such as railway stations and elevators in India. Fungal endophytes are an easy source for developing safe, cost-effective, and eco-friendly nanoparticles (Fig. 17) [71, 72].

7.1.1 Antimcrobial Silver Nanoparticles

Silver nanoparticles (AgNPs) are known to have inhibitory and bactericidal effects. Diseases resulting due to fungal infections are a major health problem nowadays. Synthesis of silver nanoparticles (AgNPs) by cell free filtrate (CFF) of *Chaetomium globosum*, an endophytic fungus isolated from *Tectona grandis*, has been reported which were exceptionally stable and showed significant activity against the

Fig. 17 Endophytic fungi mediated synthesis of gold and silver nanoparticles

pathogenic bacteria. The ongoing research provides a new way for the eco-friendly synthesis of AgNPs, and the approach is simple to produce nanoparticles for biomedical applications [73].

AgNPs synthesized using an endophytic *Alternaria alternata* strain was found to increase the antifungal activity of fluconazole against *Phoma glomerata*, *Fusarium semitectum*, *Phoma herbarum, Trichoderma* sp., and *Candida albicans*. Combination of AgNPs and fluconazole showed increase in inhibition zone of flucanozole against *C. albicans* [74].

Endophytic fungus *Cryptosporiopsis ericae* PS4, isolated from the ethnomedicinal plant *Potentilla fulgens* L., synthesized silver nanoparticles (AgNPs) with an average particle size of 5.5 ± 3.1 nm. Antimicrobial property of these AgNPs was screened singly and in combination with the antibiotic/antifungal agent chloramphenicol/fluconazole against five pathogenic microorganisms, S*taphylococcus aureus* MTCC96, *Salmonella enteric* MTCC735, *Escherichia coli* MTCC730, *Enterococcus faecalis* MTCC2729, and *Candida albicans* MTCC 183. It was found that AgNPs at concentrations between 10 and 25 μM suppressed the growth of tested bacteria as well as fungus indicating their bactericidal and fungicidal nature. SEM of AgNPs treated bacteria showed pits and ruptures in cell wall and fragmented bilayer cell membrane [75].

The AgNPs synthesized by endophytic fungus *Aspergillus clavatus* (AzS-275), isolated from sterilized stem tissues of *Azadirachta indica* A. Juss., were polydispersed, polygonal, spherical, or hexagonal in shape with size ranging from 10 to 25 nm. Their antimicrobial property was assessed by disc-diffusion method against pathogens, *Pseudomonas fluorescens*, *Candida albicans*, and *Escherichia coli*. The results showed normal minimum inhibitory concentration at 5.83 μg mL-1 and minimum at 9.7 μg mL-1 against *C. albicans* [76].

7.1.2 Cytotoxic Silver Nanoparticles

Silver nanoparticles (AgNPs) synthesized using cell-free extract of *Pestalotiopsis microspora* VJ1/VS1 isolated from leaf of *Gymnema sylvestre* ranged 2.0–10 nm in size. These AgNPs showed effective cytotoxic property against different cancer cell lines such as A549 (human lung adenocarcinoma, IC50 = 39.83 ± 3.74 μg mL-1), SKOV3 (human ovarian carcinoma, IC50 = 16.24 ± 2.48 μg mL-1), against B16F10 (mouse melanoma, IC50 = 26.43 ± 3.41 μg mL-1), and PC3 (human prostate carcinoma, IC50 = 27.71 ± 2.89 μg mL-1), respectively. Interestingly, eco-friendly synthesized AgNPs were biocompatible for the normal cells with IC50 = 438.53 ± 4.2 μg mL-1 in Chinese hamster ovary cell line. Cytological observations revealed concentration-dependent apoptotic changes in the cancerous cell either by destructive fragmentation of nuclei or cell shrinkage, pyknotic nuclei, and karyorrhexis. These observations indicate development of prospective biomedical applications of AgNPs [77].

7.1.3 Cytotoxic Gold Nanoparticles

Cell-free extract of endophytic *Fusarium oxysporum* isolated from *Azadirachta indica* A. Juss., reacted with gold chloride (HAuCl4) solution resulted in the

biosynthesis of well distributed gold nanoparticles of 10–40 nm with a typical size of 22 nm. These gold nanoparticles showed antiproliferative property against breast cancer cell line (ZR-75-1), human burkitt's lymphoma cancer, and normal human peripheral blood mononuclear cells (PBMC) and showed less than 0.1% hemolytic activity on human cells, suggesting their safe nature. A moderate level of antibacterial and antifungal activity was also screened for these gold nanoparticles. Such types of eco-friendly and biocompatible nanomaterials could have varied applications such as in drug delivery, in therapeutics, theranostics, and so on [78].

8 Conclusions

Fungi are a group of microbes which have been the important source of bioactive compounds since long back in addition to species diversity. Overall, it has been estimated that only about 8% of fungal species are yet known, and in this regard, "fungal endophytes" are being considered as a new and alternative source of diversity and natural bioactive products. Since endophytes grow in healthy plant tissues and live in a unique biotope, therefore this environment may lead to produce novel bioactive compounds. Presently, the study on endophytic fungi is confined only to some regions especially to temperate and cold, whereas from India, only fragmentary reports are available in this line. Viewing India's rainforests and great biodiversity, the idea to isolate the endophytic fungi from Indian medicinal plants and screening their abilities to produce bioactive compounds may be of reasonably great interest.

Endophytic fungi have been found promising in producing natural bioactive compounds having new mechanism of action within the cellular metabolism. Although many products can be produced synthetically, natural bioactive products remain as an important alternative used heavily in the modern medicine and agriculture. Approximately 60% of the new drugs produced during the period 1985–2012, whether anticancer, antimicrobial and antihypertensive agents, were derived from either natural products or based on natural products structure. Interestingly, some cases have been reported where endophytic microorganisms have developed the biochemical ability to produce the host mimetic compounds as a result of either gene recombination during the evolutionary process or by the help of precursor molecule. Thus, there is an urgent need to facilitate the identity of appropriate natural products and the subsequent development of drugs based on them.

A better understanding of the biosynthetic pathways involved in the production of bioactive endophytic compounds by chemical and biochemical means is essential. The approach in genome survey has made feasible the recognition and identification of secondary metabolites gene/or gene cluster in microbial system with the help of which the possible secondary metabolic profile of microbe can be predicted. Future strategies involve induction of these silent genes for the activation of encoded product. However, lack of information of the exact procedure that activate the cryptic gene/or gene cluster makes the research tough task, which needs interdisciplinary approach to produce the total metabolites variation existing in endophytic

fungi. Approaches like epigenetic modulation, stress effect, change in culture media, as well as co-culturing of the endophytic fungi have been successfully tried for the activation of cryptic metabolites, but further work is required for exact and proficient knowledge for easy and reliable expression of gene(s)/gene cluster for metabolites production.

Discovery of endophytes as a biocontrol agent may be a key role for replacement of synthetic pest because of eco-friendly nature. Role of fungal endophytes especially against abiotic (water, drought, and salt) stresses has been discussed, and this may be used as tool in plant growth promotion. Using the endophytic microbes, biofabrication of nanoparticles (NPs) of noble metals is also one of the challenging and the promising areas under the niche of nanotechnology as it has lower environmental impact than the other techniques available. Several fungal endophytes have been identified and used as potential microbes for biosynthesis (green Synthesis) of various metals nanoparticles with different properties. Thus, we may conclude that this group of microbes, in addition to other usages, may also play a crucial role in drug delivery and cutting edge technology.

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Endophytes as a Source of High-Value Phytochemicals: Present Scenario and Future Outlook

21

Vijay Lakshmi Jamwal and Sumit G. Gandhi

Contents

Abstract

Endophytes, a group of microorganisms that reside within plants, are promising eco-friendly source of high-valued bioactive phytochemicals that are produced by their host. Some of the well-known examples of phytochemicals produced by endophytes are Taxol, camptothecin, azadirachtin, podophyllotoxin, vinca alkaloids, *cinchona* alkaloids rohitukine, and many others. The molecular machinery for production of phytochemicals in endophytes is likely acquired from the host plant. After growing in axenic conditions for a few generations, the endophyte generally undergoes attenuation, and the production of phytochemical may reduce to a great extent or stop completely. Genome sequencing of several endophytes revealed that complete biosynthetic pathways for production of phytochemicals may not be present or if present the genes may not be homologous to the plant genes. Other possible reasons for attenuation as well as experimental methods through which the issue of attenuation may be addressed have also been discussed in the chapter.

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Keywords
Attenuation · Bioactive · Biosynthetic pathway · Mutualism · Plant-microbe interactions · Secondary metabolism · Symbiosis

1 Introduction

After years of incessant exploration by pharmaceutical as well as agricultural industries for novel products, natural assortment has been established to be superior to combinatorial chemistry for discovering new substances that have the potential to be flourished into new industrial and pharmaceutical products [1]. Plant secondary metabolites are chemical compounds which are not involved in the development, primary growth, or reproduction but involved in the defense mechanism, communication, attraction of pollinators, etc., by furnishing the plants with their color, flavor, and smell [2]. These chemicals are mostly responsible for the medicinal properties of a plant, such as anticancer, antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, hepatoprotective, cardioprotective, etc. (Fig. 1). On the basis of their structure, these compounds can be categorized into different groups such as alkaloids, terpenoids, phenolics, phytosteroids, flavonoids, etc.

Plants are sourced for phytochemicals used by pharmaceutical industries for preparation of drugs and medicines. But excessive deforestation and mass exploitation of some important plants resulted in many of them becoming endangered, putting at risk, the availability of important medicinal compounds produced by them [3, 4]. Apart from being an eco-hazard, it is also very laborious, costly, and time-consuming to extract these chemicals from plants. Thus, it is desirable to explore other eco-friendly sources of high-value phytochemicals.

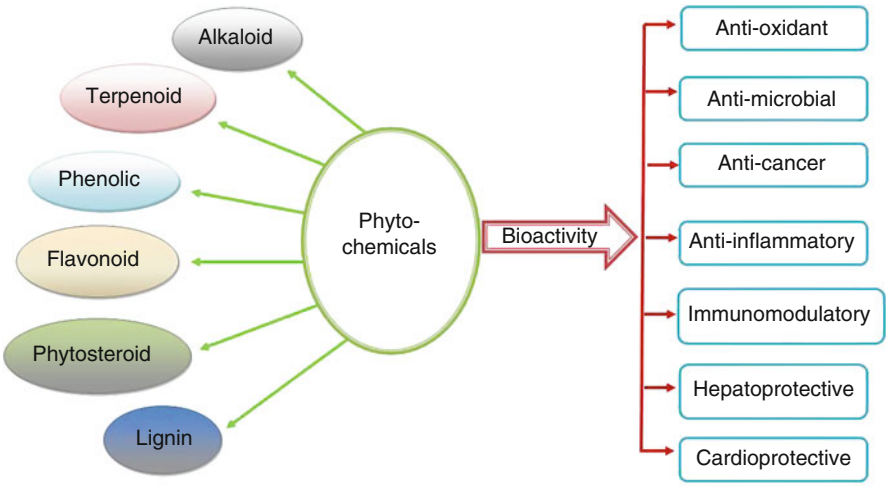

Fig. 1 Phytochemicals and their bioactivities

In 1866 the term "endophyte" was first projected for the organisms which reside within the plants without harming them [5]. Endophytes were earlier defined as mutualists that infect the plant asymptomatically [6]. In 1991 Petrini defined endophytes as "all organisms inhabiting plant organs that at some time in their life cycle can colonize internal plant tissues without causing apparent harm to the host" [7]. All organisms that occupy the living tissues of the host plant during any period of their life cycle without causing any symptoms are included in an all-encompassing topographical term "endophyte" [8]. The subsistence of endophytic fungi within the plant parts has been acknowledged since the end of the nineteenth century [9]. In the history of endophyte research, discovery of the endophytic fungus *Neotyphodium coenophialum* which causes a syndrome "fescue toxicosis," to the cattle fed in pastures of *Festuca arundinacea* grass, was a milestone [10]. Endophytes are allied with plants in diverse forms, counting bacteria or fungi that colonize within the parts of the plant. On the basis of the host range, transmission colonization pattern and ecological functions endophytes are subdivided into four classes [11]. Endophytes that belong to class I are limited to a few monocot hosts, whereas endophytes that belong to class II–IV have a much broader host range including both dicots and monocots [11]. Class II endophytes can grow in plant tissues, both below and above the ground, while class III endophytes are limited to grow only in aboveground tissues [12]. Surprisingly, investigation of the natural product chemistry of endophytes showed their potential to synthesize phytochemicals: chemicals that are normally produced by the host plant [13–17]. Endophytes are now well-known to fabricate different phytochemicals, many of which are noted antimicrobial and anticancer compounds.

Study of plant-microbe interaction is imperative as it lucids the reason why and how endophytes which reside within plants become an important source of phytochemicals. There are three major types of favorable interactions amid endophytes and their host plants: (1) augmentation of the growth of host plants, (2) enhancement in the resistance of the host plants to stresses (biotic and abiotic), and (3) accretion of high-value phytochemicals (secondary metabolites), counting bioactive compounds used as drugs, which are originally produced by the plants. The colossal biological diversity of endophytes united with their potential to biosynthesize phytochemicals has provided the thrust for the study of endophytes. Endophytes which fabricate host plant secondary metabolites with therapeutic value have been discovered; some examples include paclitaxel [18], podophyllotoxin [19, 20], deoxypodophyllotoxin [21], camptothecin [22–24], hypericin and emodin [25, 26], azadirachtin [27], etc. These discoveries have imperative matter-of-fact implications for producing plant natural product drugs in an eco-friendly and consistent manner.

In this chapter, through some of the examples of endophytes that are reported to produce phytochemicals, we will attempt to address issues such as why endophytes produce these chemicals, how these endophytes gained the ability to produce phytochemicals, reasons why the production of phytochemical reduces to a great extent or completely stops after culturing in artificial medium outside the plant and what are the future possibilities and prospects for exploiting endophytes as commercial source for phytochemicals.

2 Phytochemicals Produced by Endophytes

Taxol, a multibillion dollar anticancer drug, is used in treatment of advanced and drug refractory breast cancer [28] and ovarian cancer [29–31]. Taxol was originally isolated from pacific yew tree (*Taxus brevifolia*) [32]. Later, it was reported to be produced by numerous other species of the genus *Taxus*, such as *T. baccata, T. wallichiana, T. cuspidata, T. yunnanensis, T. floridana, T. sumatrana, T. canadensis, T. mairei,* and *T. chinensis* [33]. The geographical distribution of these plants is isolated, and they are slow-growing. Taxol was originally extracted by the removal of the bark of the tree which ultimately results in tree death [34], and later an agrotechnology for extraction of Taxol from yew needles was developed [35]. The path-breaking discovery of production of Taxol by an endophytic fungus, *Taxomyces andreanae* [18], is the most famous example of phytochemical produced by an endophyte and was an important foundation stone. Since this discovery, several studies have reported endophytes that produce similar kind of metabolites as produced by the host. Paclitaxel, an anticancer metabolite, is reported to be produced by many endophytic fungi such as *Pestalotiopsis guepinii* [36]; *Pestalotiopsis terminaliae* [37]; *Phyllosticta spinarum* [38]; *Alternaria* sp. [39]; *Phyllosticta dioscoreae* [40]; *Aspergillus fumigatus* [41]; *Phyllosticta citricarpa* [38]; *Pestalotiopsis pauciseta* [42]; *Botryodiplodia theobromae, Fusarium lateritium, Monochaetia* sp., and *Pestalotia bicilia* [43]; *Fusarium solani* [44]; *Fusarium solani, Metarhizium anisopliae,* and *Mucor rouxianus* [45, 46]; *Ozonium* sp., *Alternaria alternata, Botrytis* sp., *Ectostroma* sp., *Fusarium mairei, Papulaspora* sp., and *Tubercularia* sp. [47–49]; *Alternaria* sp., *Aspergillus niger* var. *taxi, Botrytis* sp., *Fusarium arthrosporioide,* and *Pestalotiopsis microspora* [39]; *Cladosporium cladosporioides* [50]; *Pithomyces* sp. [51]; *Taxomyces* sp. [52]; and *Pestalotiopsis microspora* [53]. Few examples of important phytochemicals produced by endophytes are listed in Table 1.

Alternaria sp. was isolated from *Catharanthus roseus* and reported for the production of vinblastine (vinca alkaloid) [56]. Another endophytic fungus *Fusarium oxysporum* was isolated from *Catharanthus roseus* and shown to produce vincristine (vinca alkaloids) [57]. Vinca alkaloids are used in anticancer therapies against various human tumors. Rohitukine which is an anticancer chromone alkaloid was initially isolated from *Amoora rohituka* [87] and later reported from the trunk bark of *Dysoxylum binectariferum*. An endophyte *Fusarium proliferatum* isolated from *D. binectariferum* was shown to produce minute quantity of rohitukine [65]. An antineoplastic drug camptothecin is used against uterine, colon, ovarian, and cervical cancer [88]. Camptothecin-producing endophytic fungi such as *Entrophospora infrequens* and *Fusarium solani* have been isolated from host plants *Nothapodytes foetida* and *Apodytes dimidiata* [22, 24]. Similarly, another anticancer compound podophyllotoxin was reported to be produced by endophytic fungi *Fusarium oxysporum* and *Alternaria* sp. isolated from *Juniperus recurva and Sinopodophyllum emodi*, respectively [58, 59]. Podophyllotoxin is used as a precursor for semisynthesis of anticancer agents etoposide, teniposide, and etoposide phosphate [89].

Table 1 Examples of phytochemicals produced by endophytes

Metabolite	Host plant	Endophytic fungus	References
Paclitaxel	*Taxus brevifolia*	*Taxomyces andreanae*	[18]
	Wollemia nobilis	*Pestalotiopsis guepinii*	[36]
	Terminalia arjuna	*Pestalotiopsis terminaliae*	[37]
	Cupressus sp.	*Phyllosticta spinarum*	[38]
	Ginkgo biloba	*Alternaria* sp.	[39]
	Hibiscus rosa-sinensis	*Phyllosticta dioscoreae*	[40]
	Podocarpus sp.	*Aspergillus fumigatus*	[41]
	Citrus medica	*Phyllosticta citricarpa*	[40]
	Cardiospermum helicacabum	*Pestalotiopsis pauciseta*	[42]
	Taxus baccata	*Botryodiplodia theobroma, Fusarium lateritium, Monochaetia* sp., *Pestalotia bicilia*	[43]
	Taxus celebica	*Fusarium solani*	[44]
	Taxus chinensis	*Fusarium solani, Metarhizium anisopliae, Mucor rouxianus*	[45, 46]
	Taxus chinensis	*Ozonium* sp., *Alternaria alternata, Botrytis* sp., *Ectostroma* sp., *Fusarium mairei, Papulaspora* sp., Tubercularia sp.	[47, 49]
	Taxus cuspidata	*Alternaria* sp., *Aspergillus niger* var. *taxi, Botrytis* sp., *Fusarium arthrosporioide, Pestalotiopsis microspora*	[39]
	Taxus media	*Cladosporium cladosporio*	[50]
	Taxus sumatrana	*Pithomyces* sp.	[51]
	Taxus wallachiana	*Pestalotiopsis microspora, Sporormia minima, Trichothecium* sp.	[54]
	Taxus yunnanensis	*Taxomyces* sp.	[52]
	Torreya grandifolia	*Periconia* sp.	[55]
	Taxodium distichum	*Pestalotiopsis microspora*	[53]
Vinblastine	*Catharanthus roseus*	*Alternaria* sp.	[56]
Vincristine	*Catharanthus roseus*	*Fusarium oxysporum*	[57]
Camptothecin	*Nothapodytes foetida*	*Entrophospora infrequens*	[22]
	Camptotheca acuminate	*Fusarium solani*	[21]

(*continued*)

Table 1 (continued)

Metabolite	Host plant	Endophytic fungus	References
	Apodytes dimidiate	*Fusarium solani*	[24]
Azadirachtin	*Azadirachta indica*	*Eupenicillium parvum*	[27]
Podophyllotoxin	*Juniperus recurva*	*Fusarium oxysporum*	[58]
	Sinopodophyllum emodi	*Alternaria* sp.	[59]
	Diphylleia sinensis	*Penicillium implicatum*	[60]
	Sinopodophyllum hexandrum	*Penicillium* sp., *Phialocephala fortinii, Trametes hirsuta, Alternaria neesex*	[61]
Hypericin	*Hypericum perforatum*	*Chaetomium globosum*	[25]
Huperzine A	*Huperzia serrata*	*Acremonium* sp.	[62]
	Phlegmariurus cryptomerianus	*Blastomyces* sp., *Botrytis* sp.	[63]
	Lycopodium serratum	*Penicillium chrysogenum*	[64]
Rohitukine	*Dysoxylum binectariferum*	*Fusarium proliferatum*	[65]
Quinine	*Cinchona ledgeriana*	*Diaporthe* sp.	[66]
Cinchonidine	*Cinchona ledgeriana*	*Diaporthe* sp.	[66]
Quinidine	*Cinchona ledgeriana*	*Diaporthe* sp.	[66]
Cinchonine	*Cinchona ledgeriana*	*Diaporthe* sp.	[66]
Berberine	*Phellodendron amurense*	*Alternaria* sp.	[67]
Sipeimine	*Fritillaria ussuriensis*	*Cephalosporium corda*	[68]
Chlorogenic acid	*Eucommia ulmoides*	*Sordariomycete* sp.	[69]
Cajaninstilbene acid	*Cajanus cajan*	*Fusarium oxysporum, Neonectria macrodidym, F. solani, F. proliferatum*	[70]
Borneol	*Cinnamomum camphora*	*Cochliobolus nisikadoi*	[71]
Ginkgolide B	*Ginkgo biloba*	*Fusarium oxysporum*	[72]
Peimisine and imperialine-3β-D-glucoside	*Fritillaria unibracteata* var. *wabuensis*	*Fusarium redolens*	[73]
Piperine	*Piper nigrum*	*Colletotrichum gloeosporioides*	[74]

(*continued*)

Table 1 (continued)

Metabolite	Host plant	Endophytic fungus	References
Ergosterol, Cerevesterol	*Ocimum basilicum*	Unidentified	[75]
Radicicol	*Ephedra fasciculata*	*Chaetomium chiversii*	[76]
Isoflavonoids	*Erythrina crista-galli*	*Phomopsis* sp.	[77]
Caryophyllene, phenylethyl alcohol, 2-phenylethyl ester, bulnesene	*Guazuma ulmifolia*	*Muscodor albus*	[78]
Cochlioquinone A, Isocochlioquinone A	*Piptadenia adiantoides*	*Cochliobolus* sp.	[79]
7-amino-4-methylcoumarin	*Ginkgo biloba*	*Xylaria* sp.	[80]
Terpenoid	*Plumeria acutifolia*	*Phomopsis* sp.	[81]
Sesquiterpenes	*Chinese holly*	*Trichoderma harzianum*	[82]
Asarone	*Cinnamomum camphora*	*Muscodor tigerii*	[83]
Camphor	*Lagerstroemia loudoni*	*Nodulisporium* sp.	[84]
Limonene	*Lactuca sativa*	*Wickerhamomyces anomalus*	[85]
Pinane	*L. loudoni*	*Nodulisporium* sp.	[84]
Oxylipin	*Alternanthera brasiliana*	*Bacillus* sp.	[86]

Endophytes have not only been reported to produce anticancer phytochemicals, rather there are several examples of other bioactive phytochemicals produced by endophytes. For instance, hypericin was reported to be synthesized by *Chaetomium globosum* which was isolated as an endophytic fungus from *Hypericum perforatum* [25]. A natural phytochemical insecticide azadirachtin originally produced by *Azadirachta indica* has also been reported to be produced by *Eupenicillium parvum* [27]. *Cinchona* alkaloids are used as antimalarial drugs from ancient time [90]. The endophytic fungus *Diaporthe* sp. harbored in *Cinchona ledgeriana* has gained enormous interest due to the discovery of antimalarial drug quinine and other *Cinchona* alkaloids including quinidine, cinchonine, and cinchonidine obtained from it [66]. An antibiotic berberine was reported to be isolated from endophytic fungus *Alternaria* sp. which resides within *Phellodendron amurense* [67]. Borneol which is an antioxidant and anti-inflammatory agent, originally produced by *Cinnamomum camphora*, was reported to be obtained from an endophytic fungus *Cochliobolus nisikadoi* [71].

Although most examples cited here are of phytochemicals produced by endophytic fungi, few bacterial endophytes have also been reported to produce similar compound as their host plant. Extracts prepared from the stem of *Alternanthera*

brasiliana contain compounds from oxylipin family which are antimicrobial in nature. Endophytic bacteria belonging to genus *Bacillus* isolated from *Alternanthera brasiliana* have also been reported to produce same antimicrobial oxylipins as their host plant [86].

3 Why and How the Endophytes May Be Producing Phytochemicals?

There is a long evolutionary history of association between plants and endophytes, and their specific associations are referred to as mutualism or symbiosis [91]. The fossilized tissues of plant have revealed that the mutualistic association between host and endophytes dates back to the origin of vascular plants and became the evidence of plant-microorganisms' relationship [92, 93]. A number of endophytes emerge in the host only for some duration of their life cycle. Endophytes with larger genomes may be able to survive in variable environments, whereas those with smaller genomes size may possibly survive in the environment which is stable, and these are generally transmitted vertically [94]. There are two modes of transmission of endophytic fungus: horizontal and vertical. Horizontal transmission is the transmission of the fungus by sexual or asexual spores, whereas vertical transmission is the transmission of the systemic fungus from plant to offspring via host seeds [95]. It was first explained in Poaceae family (grasses). Endophytes have adapted themselves to their microenvironment during their long coevolution with plants [96]. It is possible that there could be an intergeneric genetic exchange between the endophytes and their host which leads to a symbiotic relationship between them. The gradual genetic variation may allow endophytes to probably take up some host genome fragments into their own genome [93]. This may have resulted in the capability of certain endophytes to biosynthesize a few phytochemicals which are originally related with the host [18, 64, 97] (Fig. 2). Also this coevolution might have led to development of similar compounds in both the host and the endophyte, with similar effect on the other competing organisms (Fig. 2). The possibility of integration of host genes or gene fragments in the endophytic genome has remained controversial, and it has been contradicted by some studies. This indicates that there is less substantiation for the horizontal transfer of genes coding for secondary metabolites among endophytes and their host [98].

Usually, it is observed that endophytes synthesize a wide range of similar metabolites as host plant as both may use common precursors. Archaea and eubacteria are proficient in synthesizing phytochemicals such as quinones, phytohormones, isoprenoids precursors, and countless other secondary metabolites which play a very crucial role in communication and defense [99]. Several chemical, as well as physical barriers, must be overcome to establish an association between a plant and microbe. Endophyte manages to survive within its host without causing visible manifestations of disease or infection by avoiding activation of the host defense system through "balanced antagonism" [100, 101]. The plant-endophyte interaction might not be just equilibrium among virulence and resistance, rather a

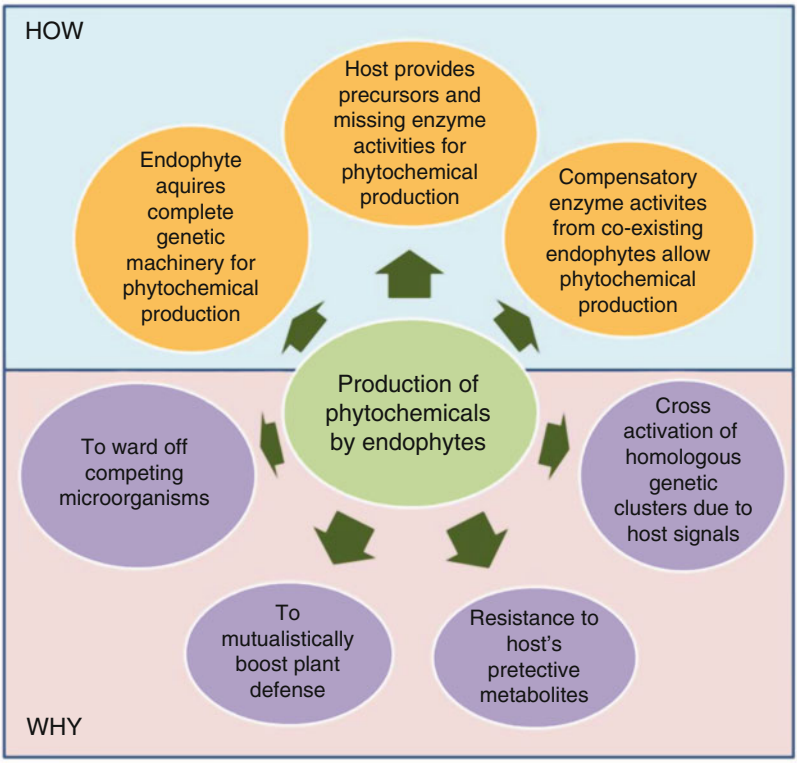

Fig. 2 Possible reasons and mechanisms through which endophytes produce phytochemicals

more intricate and precisely controlled interaction (Fig. 2). *Camptotheca acuminata* is known for the production of an anticancer alkaloid camptothecin which inhibits topoisomerase I [17]. An endophyte (*Fusarium solani*) isolated from the inner bark of the plant which also produces camptothecin, ensures defense from its own as well as host camptothecin through particular amino acid residue modifications in the camptothecin-binding and catalytic domains of its topoisomerase I [102].

According to a hypothesis, microbes have the ability to sense any sort of stress-induced molecules from homologous gene clusters present in its host and under stressful circumstances; microbes may get cross-activated [103]. Endophytes act as biochemical factories within plants which secrete phytochemicals and are not toxic for higher organisms [104] (Fig. 2). Under evolutionary selective pressures, endophytes have developed abilities to sense signaling and stress-induced molecules from the host, according to the "xenohormesis" hypothesis [103]. As per this hypothesis, stress occurs in one organism, and the beneficiaries include other organisms that evolved to sense the stress-induced chemical cues. It is possible that some of the phytochemicals which are supposed to be produced by plants could, in fact, be the biosynthetic products of their endophytes. The ability of heterotrophs to

biosynthesize phytochemicals might gradually be lost, but they regain this capacity again on sensing stress-induced chemical cues in plants [103].

Phytochemicals are synthesized by the plant through specific biosynthetic pathways, and these pathways are regulated by genes which are usually clustered. The first plant secondary metabolite pathway cluster was DIMBOA cluster which was discovered in maize [105]. In case of endophytes also, gene clusters are involved in the synthesis and regulation of secondary metabolites. It may be possible that endophytes produce similar secondary metabolites because of the cross-talk between the two gene clusters. The recent discovery of plasmids isolated from *Streptomyces* species which are known to produce antibiotics has shown that these plasmids carry secondary metabolite gene clusters [106]. Similarly, three polyketide synthase (*PKS*) clusters and a carotenoid biosynthetic gene cluster are possessed by pSLA-2 plasmid of *S. rochei* [107]. Even though it does not instantly lucid the reason why these plasmids carry the secondary metabolite genes clusters, it is believed that they may contribute to horizontal transfer of antibiotic production [106].

4 Possible Reasons for Attenuation

Although many studies have reported the synthesis of similar secondary metabolites as host produce, few have shown the way to recognize endophytes as a feasible source of phytochemicals [108]. On culturing the endophytic fungi in artificial conditions outside the host plant, it undergoes attenuation (Fig. 3), a process in which endophytes have a tendency to lose their ability to produce secondary metabolites. Li (1998) explained the attenuation of Taxol production by endophytic fungi *Periconia* sp. isolated from *Torreya grandifolia* [55].

In endophytes the regulation of phytochemicals might be significantly different within and outside the host [13]. Lack of information of the host selection pressure upon the related endophytes might be one of the main reasons why it has not been possible so far to maintain biosynthetic stability of endophytes capable of producing plant compounds on successive subculturing (Fig. 4). Thus after subculturing

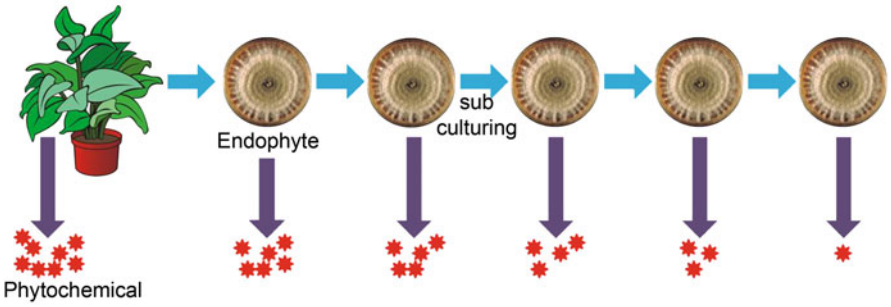

Fig. 3 Attenuation of phytochemical production by endophytes in axenic culture

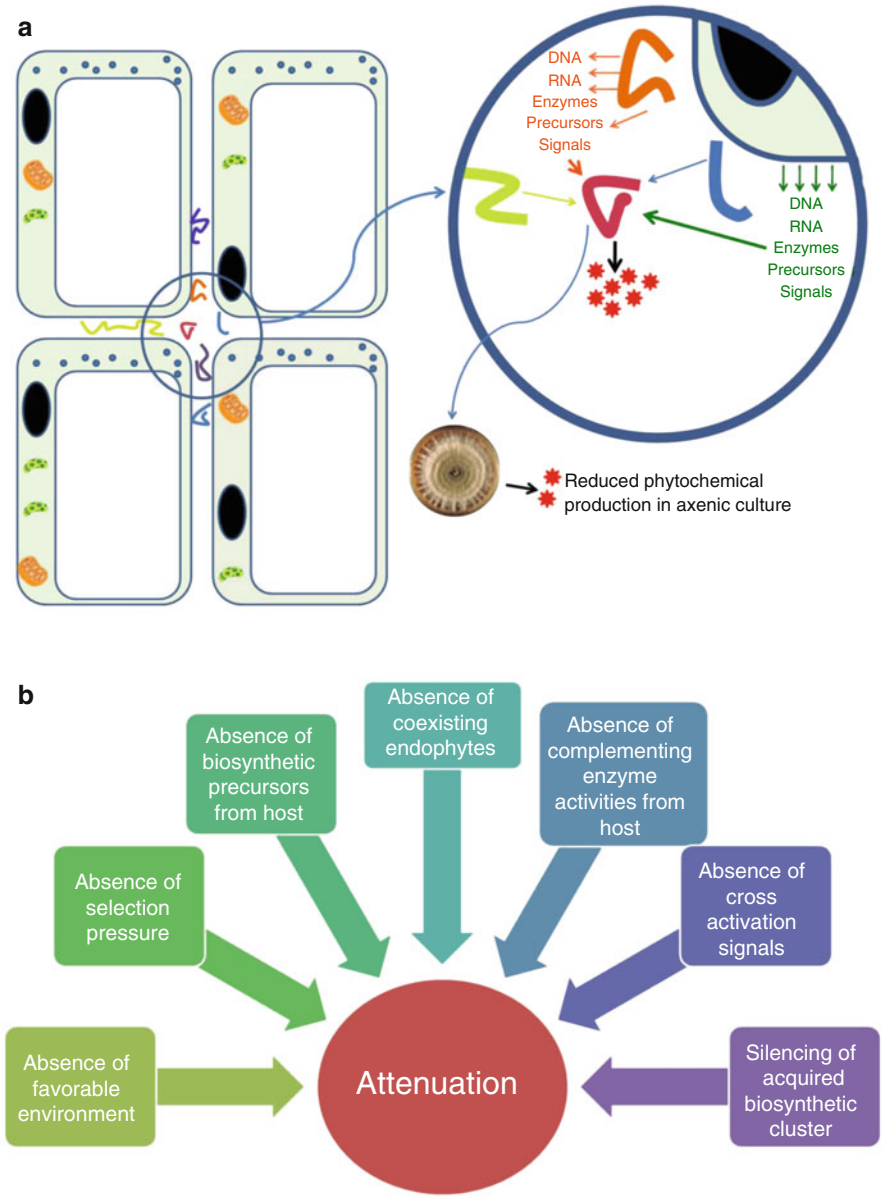

Fig. 4 Possible reasons for attenuation in endophytes

endophytes in axenic conditions for a few generations, they undergo attenuation, and the production of phytochemical reduces to a great extent or completely stops, possibly due to differences in the environmental factors such as carbon and nitrogen sources, temperature, light, and pH [109] (Fig. 4).

Recently it was shown that a camptothecin-producing endophyte (*Fusarium solani*), isolated from the inner bark of *C. acuminata* [23], might indigenously produce the precursors of camptothecin. However, strictosidine synthase enzyme, which catalyzes a key step in camptothecin production, was found to be absent in the fungus [110]. When the fungus was subcultured under axenic conditions, it resulted in drastic reduction of camptothecin production. It is possible that an enzyme which is functionally analogous but not homologous in sequence may be catalyzing the biosynthetic step, and hence it was skipped from sequence-based discovery of genes involved in phytochemical biosynthesis. And in this case, as previously mentioned, the pathway may not be functioning because of absence of signals or certain precursors from the host (Fig. 4). Another possibility is that inside the host, the partial biosynthetic pathways of phytochemicals in the endophytic genomes and the compensatory enzymatic activities of the host may collaboratively increase the phytochemical production (Figs. 2 and 4). However, once the endophyte is cultured outside the host, the compensatory enzymatic activities that catalyze the missing biosynthetic steps may no longer be present, and hence entire biosynthetic pathway may not operate, resulting in the loss of phytochemical production (Fig. 4).

Diverse types of endophytic microorganisms are observed in plants. It is impossible that a host has only one type of endophyte. These various types of endophyte may directly or indirectly interact with each other within the host, and it is possible that their interaction may affect the production of chemicals produced by them (Figs. 2 and 4). These encounters may involve miniature, diffusible signaling molecules, which may activate or silence the biosynthetic pathways [111–113]. This may also be one of the reasons why outside the host, in the absence of other coexisting endophytes, the culture may stop producing the phytochemicals.

According to spin-off hypothesis, the endophytes residing within the host plants may acquire gene clusters found in extrachromosomal elements liable for the production of similar secondary metabolites [114]. However outside the host, in the absence of selection pressure, these externally acquired genes may be treated as selfish genetic elements by the cellular defense machinery resulting in their silencing and thus attenuation of phytochemical biosynthesis [24] (Fig. 4).

5 Conclusion and Future Perspectives

Considering endophytes as a source of high-value phytochemicals, it is important to bioprospect them in order to discover huge variety of associated phytochemicals with therapeutic value. It is also very important to study the kinetics and production pattern over a number of successive subculture generations to unambiguously authenticate whether an endophyte indigenously produces a compound or only its precursors. This is critical to understand if it is even feasible to use an endophyte on a commercial scale.

The possibility of potent endophytes coordinating synergistically with the other associated endophytes under *in planta* selection pressure must be investigated at the molecular level. Recent examples of microbial interspecies cross talk [115, 116]

point to the prospect and necessity of further similar research on endophytes. This may help to identify factors that may help to alleviate the issue of attenuation.

Addition of plant extracts and plant-based elicitors has been shown to restart the production of phytochemical by previously attenuated endophytic cultures [25]. Reversal of epigenetic silencing of phytochemical biosynthetic pathway of endophyte, by treatment with DNA methyl transferase inhibitor, has also been demonstrated [117]. Addition of ethanol was shown to reverse attenuation in *Fusarium solani*, thereby increasing the camptothecin production by more than tenfolds [118]. Greater understanding of the epigenetic aspects of phytochemical biosynthetic pathways of endophytes would help to tackle attenuation and help in exploitation of endophyte resource for phytochemical production.

The availability of inexpensive genome sequencing techniques and easy-to-use bioinformatic tools have enabled microbial genomes to be mined for the identification of candidate biosynthetic gene clusters [119]. Study of metabolic gene clusters is crucial for overcoming the drawbacks of using endophytes as a source of phytochemicals. Most of the metabolic gene clusters that have been reported in plants to date have been discovered by chance, using a combination of genetics and biochemical studies. However, it is now becoming feasible to make use of genome sequence information for the discovery of new metabolic gene clusters in host plants as well as the endophytes [120–122]. The genomics-based discovery of candidate metabolic gene clusters is only the starting point. Biochemical and chemical analyses will be critical in order to define these new predicted pathways and identify their end products. It is necessary to be aware of how amenable these metabolic gene clusters are to engineering. After knowing the possible candidate genes from the genome sequence information, it will become possible to clone and express the missing genes in the endophyte leading to a fully functional phytochemical biosynthetic pathway. This also necessitates that transformation procedures are developed for several endophytic fungi species, for which such protocols do not already exist.

Synthetic biology approaches for production of plant-derived specialized metabolites by metabolic engineering have so far been carried out primarily in yeast (*Saccharomyces cerevisiae*) and *Escherichia coli* [123–125]. The potential of yeast for production of plant specialized metabolites has been highlighted by the genetic engineering of strains that provide the precursor of artemisinin, a major antimalarial drug, on an industrial scale [126, 127]. Synthetic biology, though provides much greater control and surgical precision, is cumbersome and time-consuming, especially for production of complex phytochemicals requiring multiple biosynthetic steps. In contrast, synthetic biology tools can be helpful to initiate/enhance the production of phytochemicals in endophytes, where most of the genetic components required for phytochemical biosynthesis may already be present and only a missing step needs to be added or minor optimization is required. Such molecular tools (gene overexpression, random mutagenesis, genome shuffling) have been used in some fungal isolates, known to produce Taxol. In *Ozonium* sp. EFY-21 isolated from *Taxus chinensis*, overexpression of *Taxus* TS gene driven by a promoter which is fungal specific resulted in an increase in the production of Taxol by fivefold compared to the control [128]. By multiple mutagenesis of *Nodulisporium*

sylviforme, three genetically stable strains of NCEU-1 were obtained which led to an increase in Taxol yield by 31%, 64%, and 45% over the control [129].

Presently there is no success story of production of a phytochemical by an endophyte at an industrial scale, and no such process has yet been commercialized. However, it is clear from numerous published studies that endophyte-mediated production of phytochemicals holds serious potential, and in coming time, they may become eco-friendly sources of such plant-derived natural products. More basic research into the mechanisms by which an endophyte gains the ability to produce phytochemicals and the reasons for loss of such ability on repeated sub-culturing in artificial media would most likely show the way forward for their viable exploitation.

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The Interaction Between Plants and Bacterial Endophytes Under Salinity Stress

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Abstract

Salinity leads to a decline in agricultural production and an increase in the percentage of salinity-affected land which exceeds 20% of the world's cultivated land. Endophytes are a class of endosymbiotic microorganisms widely distributed among plants and colonize intercellular and intracellular spaces of all plant compartments and do not cause any apparent infection or significant morphological change. Furthermore, endophytes have many beneficial effects on host plants including adaptation to biotic and abiotic stress such as salinity through different activities including the production of scavengers like reactive oxygen species that are produced in plants when exposed to salinity, production of ACC deaminase enzyme which is responsible for lowering the levels of ethylene in the plant, nitrogen fixation, production of compatible solutes, antibiotics, and phytohormones. The use of endophytic microbes is of particular interest in the development of agricultural applications that ensure improved performance of crops under salinity stress.

Keywords

Endophytes · Endophyte-plant interaction · Salinity stress · Salt tolerance · Plant adaptation · Phytohormones · ACC deaminase

Abbreviations

ABA	Abscisic acid
ACC	1-amino cyclopropane-1-carboxylate
EC	Electrical conductivity
ECe	Electrical conductivity of the saturation extract
ESP	Exchangeable sodium percentage
IAA	Indole-3-acetic acid
ISR	Induced systemic resistance
ROS	Reactive oxygen species
SAR	Sodium absorption ratio

1 Introduction

Salinity stress has been significantly affecting the fertile lands and therefore creating a huge impact on agriculture and economy. Approximately, 5.2 billion hectares of agricultural land are affected by erosion, salinity, and soil degradation [1]. Different characteristics of plants such as physiological, biochemical, and genetic are affected under stress condition [2]. Endophytes are microorganisms including bacteria, fungi, and actinomycetes that survive within healthy plant tissues and promote plant growth under stress. The property of endophytes to induce stress tolerance in plants can be applied to increase crop yields [3]. The adverse effects of salinity can be ameliorated with the application of plant growth-promoting endophytic bacteria (PGPEB) [4]. Therefore, the evaluation of plant growth-promoting abilities of new

and beneficial endophytic microorganisms is a significant area of research for the improvement of plant health and stress resistance [5]. Symbiotic endophytes conferred abiotic stress tolerance to plants via at least two mechanisms: (1) activation of host stress response systems soon after exposure to stress allowing the plants to avoid or mitigate the impacts of the stress and (2) biosynthesis of anti-stress biochemical by endophytes [3]. Endophytes can ameliorate the impact of salinity on plants through production of reactive oxygen species (ROS) scavengers such as catalase, ascorbate, and glutathione which are involved in the removal of ROS [6]. ACC deaminase is another endophytic enzyme that improves stress tolerance by cleaving plant ethylene precursor ACC [7]. Nitrogen fixation, production of phytohormones, and compatible solutes are other endophytic activities involved in alleviation of salinity stress in plants [8, 9, 10].

In this chapter, we provide an overview on endophytes and their role in ameliorating the impact of salinity on plants through various mechanisms such as the production of phytohormones, antioxidants, and compatible solutes as well as a brief description of salinity, its definition, types, and characteristics of soils affected by salinity.

2 Soil Salinity

Soil salinization occurs when water-soluble salts accumulate in the soil to a level that impacts on agricultural production, environmental health, and economics. In the early stages, salinity affects the metabolism of soil organisms and reduces soil productivity, but in advanced stages, it destroys all vegetation and other organisms living in the soil, consequently transforming fertile and productive land into barren and desertified lands [11, 12].

A saline soil is generally defined as one in which the electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeds 4 dS m^{-1} (approximately 40 mM NaCl) at 25 °C and has an exchangeable sodium of 15%. The yield of most crop plants is reduced at this ECe, though many crops exhibit yield reduction at lower ECs [13, 14]. It is a major factor contributing to the loss of productivity of cultivated land. Although difficult to estimate accurately, the area of salinized soils is increasing, and this phenomenon is especially intense in irrigated soils. It was estimated that about 20% (45 million ha) of irrigated land, producing one-third of the world's food is salt-affected [15].

Soil salinity affects an estimated one million hectares in the European Union, mainly in the Mediterranean countries, and is a major cause of desertification. For example, in Spain about 3% of the 3.5 million hectares of irrigated land is severely affected markedly reducing its agricultural potential, while another 15% is under serious risk [16]. In the Mediterranean region, land degradation associated with soil alkalization may worsen at increasing rates in the coming decades owing to the expected increase in irrigated areas and the increasing scarcity of good quality water [17].

The first reason for soil salinity was natural or primary salinity which occurred due to the long-term natural accumulation of salts in the soil or surface water. This is a natural process which is caused mainly by weathering of parent materials containing soluble salts through breakdown of rocks containing Cl^{-}, Na^{+}, Ca^{2+}, and Mg^{2+} and sometimes SO_4^{2-} and CO_3^{2-}. In addition, deposition of sea salt carried by wind and rain is also a reason which varies with the type of soil, while the second reason for soil salinity was that secondary or human-induced salinity occurs due to anthropogenic activities that disrupt the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops (transpiration) [13, 18].

3 Types and Properties of Salt-Affected Soils

Salt-affected soils are classified as saline, sodic, and saline-sodic based on electrical conductivity (EC), sodium absorption ratio (SAR), or exchangeable sodium percentage (ESP) and pH [19, 20].

3.1 Saline Soils

Saline soils are characterized by high concentration of soluble cations such as sodium (Na^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}), and anions such as chloride (Cl^{-}), sulfate ($SO4^{-2}$), carbonate ($CO3^{-2}$), and bicarbonate ($HCO3^{-}$) in the soil solution [21]. Saline soils are characterized by electrical conductivity in the saturated soil extract (ECe) > 4 dS m^{-1}, SAR < 13, or ESP < 15 and pH < 8.5) [20]. Salinity can be caused by Ca^{+2} salts [22]. However, in Australia, the majority of saline soils are dominated by Na^{+} and Cl^{-}, and thus 50–80% of total soluble salt is NaCl [23]. Saline soils are flocculated as a result of high ion concentration in soil solution. The high salt concentration causes low osmotic potential, ion toxicity, and ion imbalance which have adverse effect on soil biota and plant growth [24].

3.2 Sodic Soils

Sodicity is expressed as sodium absorption ratio (SAR) or exchangeable sodium percentage (ESP).

Sodium absorption ratio of the soil water extract is calculated by the following equation: $SAR = [Na^{+}]/ [(Ca^{+2} + Mg^{+2})/2]^{1/2}$.

where the concentrations of Na^{+}, Ca^{+2}, and Mg^{+2} are in mmol L^{-}.

Exchangeable sodium percentage is calculated as:

$ESP = (Na_{ex}/CEC) \times 100$

where Na_{ex} = concentration of exchangeable sodium (cmol kg^{-1}). CEC = cation exchange capacity (cmol kg^{-1}).

The determination of SAR in the laboratory is easier than ESP; therefore, SAR is more widely used than ESP to determine sodicity [19]. Sodic soils have ECe < 4, SAR > 13 or ESP >15, and pH > 8.5. In Australia, a soil is considered sodic when it has an ESP > 6 [25], instead of ESP >15 as classified by USDA. The lower ESP in Australian sodic soils is due to low content of soluble salts particularly Ca^{+2} which causes soils to disperse at lower percentages of Na. Furthermore, in Australia most work has been conducted using soils with fine texture, whereas soils with coarse texture were used in the USA [26, 27].

Sodic soils are characterized by a high percentage of Na^{+} at the cation exchange sites of soil particles compared to Ca^{+2} and Mg^{+2} which causes (i) ion toxicity and nutrient imbalance (nutrient deficiency) that reduce the growth of plants and microorganisms and (ii) deterioration of soil structure as result of slaking, swelling, and dispersion of clay particles. Moreover, poor drainage and aeration can increase crusting and erosion as well as reduce the water available to plants, seeding emergence, and root penetration [26, 28, 29, 30].

3.3 Saline-Sodic Soils

Saline-sodic soils have an ECe greater than 4 and SAR greater than 13 or ESP higher than 15. These soils are characterized by high concentration of both neutral and sodium salts, and thus high electrolyte concentrations in the soil solution leads to flocculation of soil particles [30, 31]. Therefore, these soils have a good structure, aeration, and drainage.

4 Impact of Soil Salinity on Plants

Salinity has a wide impact on agricultural crops which include low agricultural productivity, affects soil physicochemical properties and ecological balance of the area, and in addition causes soil erosion and lowers economic returns [32].

Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes including seed germination, seedling growth, vegetative growth, enzyme activity, water uptake, DNA, RNA, protein synthesis, and mitosis [33, 34]. Salinity has a profound effect on reproductive development in plants by inhibiting microsporogenesis and stamen filament elongation, enhancing programed cell death in some tissue types, ovule abortion, and senescence of fertilized embryos. Wheat plants stressed at 100–175 mM NaCl showed significant reduction in number of spikelets per spike, delayed spike emergence, and reduced fertility [35]. Because many salts are also plant nutrients, high salt concentrations in the soil can upset the nutrient balance in the plant or interfere with the uptake of some nutrients (N, Ca, K, P, Fe, Zn) causing nutrient deficiency. Soil salinity significantly reduces P uptake because P ions precipitate with Ca ions [36].

Soil salinity leads to ion toxicity which results from replacement of K^+ by Na^+ in biochemical reactions. K^+ acts as cofactor for several enzymes and required in high concentration for binding tRNA to ribosomes during protein synthesis. Na^+ and Cl^- induce conformational changes in proteins [37]. Soil salinity causes osmotic stress which results in loss of turgidity, cell dehydration, and ultimately death of cells. Ion toxicity and osmotic stress cause metabolic imbalance, which in turn leads to oxidative stress [38].

Photosynthesis is adversely affected by soil salinity through reduction of leaf area, chlorophyll content, stomatal conductance, and decreased photo system II efficiency [39]. In addition, salinity might result in impairment of the supply of photosynthetic assimilates or hormones to the growing tissue [38].

Salinity stress arrests the cell cycle transiently by reducing the expression and activity of cyclins that results in fewer cells in the meristem, thus limiting growth. The activity of cyclin-dependent kinase is also diminished by posttranslational inhibition during salt stress [40].

To assess the tolerance of plants to salinity stress, growth or survival of the plant is measured because it integrates with the upregulation or downregulation of many physiological mechanisms occurring within the plant [38].

5 Signaling During Biotic and Abiotic Stresses

Plants have developed several mechanisms to realize stress signals and survive under adverse environmental conditions. Signaling during biotic or abiotic stress involves an interactive regulatory network with frequent interchange between individual pathways and signal molecules/cofactors [41]. Reactive oxygen species (ROS) such as Ca^{2+}, NO_2, systemin, inositol phosphates, and phytohormones serve as signaling molecules. Drought exerts osmotic stress, while salt stress has both osmotic and ionic or ion toxicity effects on cells. The accumulation of phytohormone and abscisic acid (ABA) induces adaptive responses in plants during drought and salt stresses [37]. The ROS generation was proposed to be a basic process common to biotic and abiotic stress responses. Recent studies have reported that molecular entities such as Ca^{2+} and NO have a significant impact on the cross talk of stress response pathways via hormone signals. Both Ca^{2+} and nitric oxide signaling play important roles in plant defense responses, ABA-dependent stomata movements, and drought stress responses [42]. Mitogen-activated protein (MAP) kinase (MAPK/MPK) cascades are highly conserved regulators of diverse cellular processes such as differentiation, proliferation, growth, death, and stress responses. MAPK cascade plays a crucial role in various biotic and abiotic stress responses. Heat shock proteins are also expressed in plants during heat stress which prevent protein denaturation and maintain protein homeostasis [43].

6 Endophytes

Endophytes are microorganisms including bacteria, fungi, and actinomycetes that survive within healthy plant tissues and promote plant growth under stress [3]. These microorganisms showing endophytic lifestyles play crucial roles in plant development, growth, fitness, and diversification [8]. Endophytic bacteria have been shown to have several beneficial effects on their host plant including growth-promoting activity, modulation of plant metabolism, and phytohormone signaling that leads to adaptation to environmental abiotic or biotic stress. The use of endophytic bacteria presents a special interest for development of agricultural applications that ensure improved crop performance under cold, drought, salinity, or contaminated soil stress conditions or enhanced disease resistance [3, 44].

7 Abiotic Stress Alleviation by Bacterial Endophytes

More than 20% of agricultural soil faces increase in salinity problems [45], and nearby 50% of the agriculturally important land will be affected by salinity stress by the year 2050 [46]. Endophytic bacteria give particular attention to improved crop adaptation to stress as they are relatively protected from the harsh environment of the soil under draft, high salt, or other stress conditions [47].

8 Responses to Osmotic Stress

Plants exhibited a wide range of responses against osmotic stress at the molecular, cellular, and whole-plant level such as inhibition of shoot growth and enhancement of root growth, modification in ion transport, and metabolic changes. Some of these responses are trigged by the primary osmotic stress signals, while others may result from secondary stresses/signals caused by the primary signals. These secondary signals are ROS, phytohormones (e.g., ABA, ethylene) and intracellular second messengers (e.g., phospholipids). Some of these secondary signals may not be confined to the primary stress sites such as the root. Root-derived ABA can ascend with transpiration flow to regulate stomatal aperture in leaves under drought [48].

Endophytic bacteria have been found to be more active in inducing strong defense responses against stresses than the other rhizospheric or soil microbes [49, 50].

Endophytes conferred abiotic stress tolerance to plants by two mechanisms (i) activation of host stress response systems soon after exposure to stress, allowing the plants to avoid or mitigate the impacts of the stress [51] and (ii) biosynthesis of anti-stress biochemicals by endophytes [52].

9 Salinity and Alkalinity Tolerance

In addition to enhanced growth properties, modulation of plant metabolism and phytohormone signaling by the endophytic bacteria enhances adaptation to environmental abiotic or biotic stress. Endophytic bacteria present a special interest for improved crop adaptation to stress as they have the advantage of being relatively protected from the harsh environment of the soil under drought, high salt, or other stress conditions [47].

10 The Main Benefits of Endophytes to Reduce Salinity Effects on Plants

10.1 Plant Antioxidant Status

Reactive oxygen species formed in plants on the onset of osmotic and salt stress. Oxidation of membrane proteins, lipids, or DNA is prevented by scavenging enzymes including catalase, superoxide dismutase, and ascorbate peroxidase. Microorganisms use similar approaches to cope with oxidative stress. Recently, **Hamilton and colleagues in 2012** reported the production of reactive oxygen species in plants by fungal endophytes [53]. Earlier studies suggested the correlation between tolerance of plants to salt stress and alleviation of antioxidant enzymes [54]. The ROS scavengers include the enzymes, superoxide dismutases (SOD), catalases (CAT), ascorbate or thiol-dependent peroxidases (APX), glutathione reductases (GR), dehydroascorbate reductases (DHAR), and monodehydroascorbate reductases (MDHAR), in addition to glutathione, ascorbate, and tocopherol [6]. These are involved in the removal of ROS either directly (SOD, CAT, APX) or indirectly via regeneration of ascorbate and glutathione in the cell. **Rodriguez et al. 2008** reported that nonsymbiotic plants of *Leymus mollis* (dune grass) on constant exposure to 500 mmol l^{-1}NaCl solution became severely wilted and desiccated within 7 days and were dead after 14 days [55]. While, symbiotic plants infected with *Fusarium culmorum* did not show wilting symptoms until they were exposed to 500 mmol l^{-1}NaCl solution for 14 days. Endophytic *P. indica* induces salt tolerance in barley by increasing the levels of antioxidants [56].

10.2 ACC Deaminase

Endophytes produce ACC deaminase enzyme that has no function in bacteria but contributes to plant growth promotion and improves stress tolerance by cleaving the ethylene precursor ACC [7]. This enzyme is responsible for lowering the levels of ethylene in the plant by cleaving the plant-produced ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) to ammonia and 2-oxobutanoate, preventing ethylene signaling [57]. Ethylene is an important plant hormone which acts

during the germination of seeds and in response to various stresses, and it is the key regulator of colonization of plant tissue by bacteria [58]. Stress-induced accumulation of ethylene is usually deleterious to plant growth and health [59]. Apart from stress alleviation, ACC deaminase supports colonization of a number of bacterial endophytes. When the ACC deaminase gene of *Burkholderia phytofirmans* PsJN was inactivated, the endophyte lost the ability to promote root elongation in canola seedlings [60]. Another study performed on cut flowers indicated that bacterial endophytes can colonize the shoot and that ACC deaminase delays flower senescence [61].

10.3 Phytohormone Production

Auxin production, specially indole-3-acetic acid (IAA), is considerably found in growth-promoting endophytes [9]. Auxins play a key role in elevation of root growth and development and act against ethylene. So, managing auxin production in halophytic plants by endophytic microbes might be an important tool in conferring salt tolerance. IAA production has been found in (i) species of *Bacillus*, *Brevundimonas*, *Exiguobacterium*, *Halobacillus*, *Oceanobacillus*, *Serratia*, *Staphylococcus*, and *Vibrio* originating from four halotolerant plants from a Chinese coastal sandbank [62] and (ii) salt-tolerant rhizobacteria (*Arthrobacter* sp., *Bacillus pumilus*, *Halomonas* sp., *Nitrinicolalacis aponensis*, and *Pseudomonas mendocina*) isolated from highly saline habitats [63]. Some researchers proposed that IAA, a member of the auxin class, increase colonization efficiency [64] possibly via interference with the host defense system [65], and production of this compound or related compounds may be an important property for plant colonization by endophytes. Production of IAA, ABA, and gibberellins has also been identified in yet unclassified bacterial strains isolated from the rhizosphere of halophytic weeds from the Pakistani Khewra salt range [66] as well as from the halophyte *Prosopis strombulifera* [67]. ABA is the phytohormone critical for plant growth and development, and its levels are known to rise under stress condition. Main function of ABA seems to be the regulation of plant water balance and osmotic stress tolerance [68]. **Tiwari and colleagues (2011)** demonstrated an increase in the fitness of wheat plants grown in salt-affected soil when they were inoculated with salt-tolerant IAA producing rhizobacteria [63].

Cytokinin production is commonly observed in endophytes, but on one occasion in a root-colonizing fungal strain of *Piriformospora indica*, cytokinin biosynthesis was demonstrated, and mutational deletions in cytokinin biosynthesis genes resulted in abortion of any plant growth-promoting effect [69]. Mycorrhizal fungi exert impact on nearly all phytohormones, and this has been shown for jasmonate and abscisic acid [70, 71]. The role of phytohormones for increasing salt tolerance has, however, neither been analyzed for mycorrhizal nor for other root endophytic fungi [72].

10.4 Nitrogen Fixation

Beneficial effects of endophytes resulting from nitrogen fixation, pathogen suppression, phytohormone production, and supply of nutrients also account for the alleviating effects of microorganisms when host plants encounter unfavorable environmental conditions [72]. Several root endophytes fix nitrogen (e.g., *Acetobacter diazotrophicus*, *Herbaspirillum* spp., and *Azoarcus* spp.). However, the nitrogen fixation efficiency in free-living endophytes is much lower than that found in the root nodules of leguminous plant rhizobium interactions. One exception is the relatively high nitrogen fixation capacity observed in endophytic strains of *Gluconacetobacter diazotrophicus* in symbiosis with sugarcane plants. Nitrogen fixation contributes to the fitness of the host plant especially in nitrogen poor environments. Even if the quantities of fixed nitrogen measured in single nitrogen-fixing species are low, it remains to be clarified if the fixed nitrogen is for the endophytes' own demands and/or for provision to the host plant. The endophyte *Paenibacillus* P22 strain found in poplar trees could fix nitrogen, contributed to the total nitrogen pool of the host plant, and induced metabolic changes in the plant [8].

10.5 Production of Antibiotics and Secondary Metabolites

Bacterial endophytes also produce antimicrobial compounds. For example, the endophyte *Enterobacter* sp. strain 638 produces antibiotic substances, including 2-phenylethanol and 4-hydroxybenzoate [73]. Generally, endophytic actinomycetes are the best-known examples of antimicrobial compound producers, and compounds discovered so far include munumbicins, kakadumycins [74, 75], and coronamycin [76].

Secondary metabolites are biologically active compounds that are an important source of antifungal, anticancer, antioomycete, antibacterial, antiviral, antioxidant, immunosuppressive, antidiabetic, insecticidal, and nematicidal agents [8]. In addition to secondary metabolites that are involved in mechanisms of signaling, defense and genetic regulation of the establishment of symbiosis [77]. Endophytes are also able to influence the secondary metabolism of their plant host [78]. This was elucidated in strawberry plants inoculated with a *Methylobacterium* species strain in which the inoculant strain influenced the biosynthesis of flavor compounds such as furanones in the host plants [79].

10.6 Compatible Solutes

If Na^+ and Cl^- are sequestered in the vacuole of the plant cell, organic solutes that are compatible with metabolic activity even at high concentrations (hence "compatible solutes") must accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole. The most commonly accumulated compounds are proline, glycine betaine, and sucrose [46].

Accumulation of organic solutes is important mechanism of facing osmotic stress, and this has been also found in halophytes [80]; the amino acid proline has been the subject of research into understanding increases in salt tolerance after colonization of plants with endophytes. However, results with arbscularmycorrhizal fungi have been variable and suggest that proline accumulation is, in most cases, not the cause but the effect of salt tolerance [10]. Osmoregulation can be also achieved with sugars and with betaines. Both were increased in mycorrhizal plants and were suggested to be involved in salt tolerance [81, 82]. Endophytic bacteria *Pseudomonas pseudoalcaligenes* were shown to stimulate accumulation of higher concentrations of glycine betaine-like compounds leading to improved salinity stress tolerance in rice [83].

10.7 Induced Systemic Resistance (ISR)

Endophytes may induce plant defense reactions so-called induced systemic resistance (ISR) leading to a higher tolerance to pathogens [84, 85]. At the initial stages, there are enough evidences that prove interactions between beneficial microorganisms and plants stimulate immune response in plants similar to the responses against pathogens, but later, mutualists escape host defense responses and are able to successfully colonize plants. These include the bacterial strains of the genera *Pseudomonas* and *Bacillus* [86], although ISR induction is not exclusive to these groups [87, 88]. Antibiotics, N-acyl homoserine lactones, salicylic acid, jasmonic acid, siderophores, volatiles (e.g., acetoin), and lipopolysaccharides are bacterial factors responsible for ISR induction [88, 89]. The ISR primes plant defense mechanisms and protects nonexposed plant parts against a future attack by pathogenic microbes and herbivorous insects. Although several plant-associated bacteria have been reported to induce a salicylic acid-mediated type of induced systemic resistance, the plant hormones jasmonic acid (JA) and ethylene (ET) play a major regulatory role in the network of interconnected signaling pathways involved in ISR induction [90]. The protection of cucumber plants against cucumber anthracnose induced by *Pseudomonas fluorescens* strain 89B-61 was the first case demonstrating that endophytic bacteria could elicit ISR in plants [86, 91]. The shoot endophyte *Methylobacterium* sp. strain IMBG290 was shown to induce resistance against the pathogen *Pectobacterium atrosepticum* in potato in an inoculum-density-dependent manner. The observed resistance was accompanied by changes in the structure of the innate endophytic community. Endophytic community changes were shown to correlate with disease resistance indicating that the endophytic community or just fractions thereof can play a role in disease suppression [87].

In contrast to bacterial endophytes, fungal endophytes have less frequently been reported to be involved in protection of their hosts via ISR [92, 93, 94]. Fungal endophytes are better known for their potential to produce compounds that have growth-inhibitory activities toward plant pathogens and herbivores. These compounds comprise alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, and chlorinated compounds [95, 96, 97]. Several reports have been

published on the production of antiviral, antibacterial, antifungal, and insecticidal compounds by fungal endophytes, and most of these endophytes are transmitted horizontally causing local infections in their hosts [95, 98].

11 Conclusions

Crop yield decreases when salt concentration exceeds the threshold salinity level due to salt affecting the development of reproductive structures or translocation of nutrient reserves. Salinity impairs plant growth by causing osmotic imbalance and ion toxicity. It is well-understood that environmental adaptations and genetic traits regulate salinity tolerance in plants, but the knowledge gained regarding crop improvement remains arduous. Harnessing the potential of phytobeneficial microbes, particularly endophytes, such as fungi and bacteria is an alternative strategy for improving plant stress tolerance. Microbe-mediated stress tolerance in plants is an eco-friendly approach for better crop yield. In addition, plant growth-promoting endophytes can live under high concentration of saline stress and potentially synthesize and release different plant growth hormones and regulators that significantly promote plant growth under such conditions. The beneficial effects of endophytes involve production of ROS scavengers such as catalase and superoxide dismutase enzymes. ACC deaminase is another important endophytic enzyme which has important role in modulation of the plant hormone ethylene. Furthermore, other extracellular secretions of endophytes function as compatible solutes such as proline and glycine betaine that can balance the osmotic stress. Endophytic symbionts improve ISR and improve plant growth through nitrogen fixation and production of various phytohormones as IAA and ABA. Application of endophytic inoculants is a promising measure to combat salinity in agricultural fields thereby, increasing global food production.

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Endophytes as Pollutant-Degrading Agents: Current Trends and Perspectives

23

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Contents

Abstract

Bioremediation is based on biological systems, bacteria, fungi, and plants. They are effective systems to treat a polluted site because they are able to modify the chemical structure of the contaminant into less hazardous end products. Investigations regarding the theme have immensely accelerated during the last years, what originated a great number of articles involving the terms "phytoremediation" and "bioremediation." Initially the term phytoremediation was defined as being the use of plants for the degradation of polluting hazardous chemicals. However, the discovery that healthy plants could be containing endosymbiotic groups of microorganisms, often bacteria or fungi, led to the notion that these microorganisms could be, partly at least, responsible for the degradation of

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the pollutants. This review focuses on this proposed partnership in the bioremediation process, taking into account investigations conducted during the last 5 years.

Keywords
Bioremediation · Endophytes · Pollutant-degrading agents · Phytoremediation · Xenobiotics

1 Introduction

Industrial processes, agricultural practices, and the use of chemicals in many areas of our daily lives result in the deliberate or accidental release of potentially toxic chemicals into the environment. Environmental chemicals of particular concern include petroleum hydrocarbons, halogenated solvents from industrial sources, polycyclic aromatic hydrocarbons, endocrine-disrupting agents, pharmaceutical and personal care products, explosives, agricultural chemicals, and heavy metals, among others [1, 2].

The impact of hazardous xenobiotic residues on the environment has led to the necessity of finding feasible technologies to remediate these sites. The conventional remediation methods use physical and chemical processes, such as incineration, adsorption on resins and UV irradiation [3]. These methods generally result in excellent contaminant removal. However, from an ecological viewpoint, they are not friendly because they produce unwanted by-products and hazardous residues, besides generating the danger of human exposure to contaminants. An innovative technology for complementing or substituting the conventional methods and which presents the same or an even improved efficiency is bioremediation. By definition, bioremediation is the use of biological processes to clean up polluted sites. Such biological methods have the potential of being less expensive and more eco-friendly than physical and chemical treatments [4, 5]. Bioremediation is based on biological systems, bacteria, fungi, and plants. They are effective systems to treat a polluted site because they are able to modify the chemical structure of the contaminant into less hazardous end products [6]. Investigations regarding the theme have immensely accelerated during the last 10 years, what originated a great number of articles involving the terms “phytoremediation” and “bioremediation,” 10.441 and 20.560, respectively (data obtained from Web of Science, May 2018). Originally the term phytoremediation was defined as being the use of plants for the degradation of polluting hazardous chemicals. However, the discovery that healthy plants could be containing endosymbiotic groups of microorganisms, often bacteria or fungi, led to the idea that these microorganisms could be, partly at least, responsible for the degradation of the pollutants. The present review focuses on this proposed partnership in the bioremediation process, considering mainly experimental results published in the last 5 years.

2 Endophytes as Promising Pollutant-Degrading Agents

Endophytes are defined as fungi or bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within the plant tissues, but no symptoms of disease [7]. It is also important to note that plants can contain a mixture of colonizing endophytes, and not just a single species. In cases of both fungal endophyte- and bacterial endophyte-plant interactions, positive effects reported for plants involve overall biomass and growth enhancement, as well as enhanced biotic and abiotic stress tolerance [8–10]. In recent years, many studies demonstrated that endophytes are helpful in the remediation of contaminated soil, improve plant growth, and generate higher levels of soil activity. Since 2007, there has been a 15-fold increase in the number of publications addressing the theme of phytoremediation assisted by endophytic microorganisms (Fig. 1). Phytoremediation by plant–endophyte partnership is, consequently, an emerging, efficient, and eco-friendly technology, which consists in the use of plants and their associated microbes to clean up pollutants from the soil, water, and air [11, 12].

Being an area of active current investigation, novel efficient pollutant hyperaccumulators are being constantly prospected for utilization in phytoremediation and phytomining. In addition, molecular tools are being applied to improve knowledge on the mechanisms of xenobiotic uptake, translocation, sequestration, and tolerance in plants [3]. In the past years, several investigations have documented the endophyte-assisted phytoremediation as a promising approach for in situ bioremediation of contaminated areas [11–15].

Endophytes improve bioremediation processes through diverse ways, as they minimize heavy metal stress to plants [9], degrade toxicant components and metabolites released by plants [6], eliminate greenhouse gases from air [15], and finally control plague development in plant hosts [16]. They make the adaptation of plants to contaminated areas viable by providing the host with the required degradation pathways and metabolic abilities for diminishing phytotoxicity while enhancing plant growth through nitrogen fixation, mineral solubilization, and generation of phytohormones and siderophores, utilizing 1-aminocyclopropane-1-carboxylic acid as N source and via nutrient transformation [2, 6]. Moreover, this biodegradation strategy can also have a role in reducing the residual concentration of potentially toxic compounds in food crops, thus contributing to food safety [4].

In cases where genetic engineering of a xenobiotic degradation pathway is requested, bacteria are easier to manipulate than plants. Moreover, quantitative gene expression of pollutant catabolic genes within the endophytic populations could be a convenient tracking tool for evaluating the remediation efficiency. The special niche of the interior plant environment allows the pollutant degrader microorganism to achieve larger population sizes owing to the reduced competition [17]. Finally, an additional benefit of using endophytic pollutant degraders is that any intoxicant xenobiotics absorbed by the plant may be broken down in planta, thus

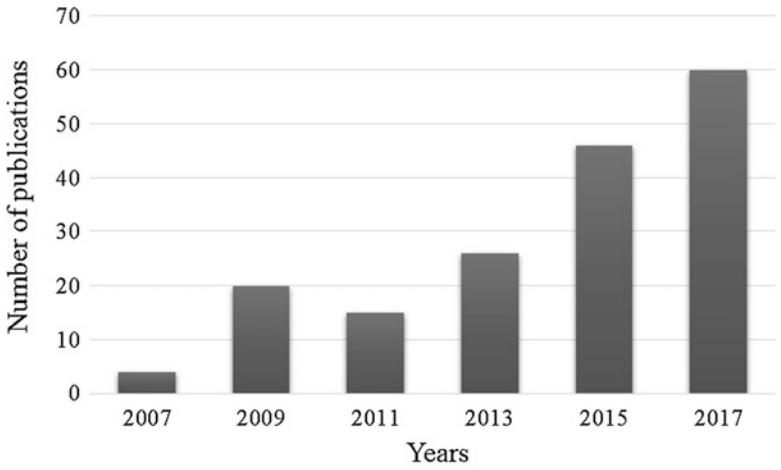

Fig. 1 Number of research articles and reviews published in the period from 2007 to 2017 regarding both "endophytic" and "phytoremediation" issues (obtained from Web of Science, May 2018; keywords restricted to the topics: endophytic and phytoremediation)

minimizing phytotoxic effects and suppressing any harm to the fauna surrounding the contaminated areas [17].

Many endophytic microorganisms display a natural competence for xenobiotic degradation or may operate as vectors to insert degradative traits [4]. Such capacity to resist to heavy metals and antimicrobial agents and disintegrate organic compounds likely stems from their exposure to distinct compounds in the plant/soil niche. In the past 5 years, endophytes' natural ability to degrade xenobiotics was approached in some review papers. The majority of the reviews in this field prioritized prospection and the advances in the exploitation of endophytic bacteria to assist the phytoremediation of pollutants [4–6, 11–14], whereas only few papers focused on the use of fungal endophytes for this purpose [10, 18, 19].

2.1 Endophytic Bacteria

Plant–endophytic bacteria partnerships have been prospected for boosting the phytoremediation capacity of plants growing in areas infected with diversified organic compounds. There are several reports on the successful phytoremediation of polycyclic aromatic hydrocarbon (PAHs)-polluted sites using the plant-endophytic bacteria approach [20–22]. For instance, it has been found that the inoculation of willow and grass clones with the endophytic bacterial strain *Pseudomonas putida* PD1 caused a substantial reduction in the phytotoxicity of phenanthrene while promoting root and shoot growth [20]. Furthermore, it improved the

removal (up to 40%) of phenanthrene from soil by host plants when compared to the uninoculated controls. Additionally, endophytic bacteria have been effectively applied to assist the phytoremediation of plants/lands contaminated with other organic compounds, such as pesticides like chlorpyrifos [2, 16], petroleum hydrocarbons [12, 23, 24], and toluene [25] (Table 1).

Constructed wetlands (CW) are sustainable eco-friendly systems employed for treating diverse kinds of effluents (varying from domestic to industrial toxicant wastewaters) which exploit the capacity of plants, together with their associated microorganisms, in clearing up organic compounds and metals from the water. In parallel with the biological processes, complex physical and chemical processes occur in the system, boosting the elimination of contaminants [40]. Shehzadi et al. [31] investigated, in a vertical flow CW reactor, the effects of the inoculation of two textile endophytic strains, *Microbacterium arborescens* TYSI04 and *Bacillus pumilus* PIRI30, on the detoxification efficiency of the wetland plant *Typha domingensis*. According to the authors, the combined plant-bacteria approach promoted, within a period of 72 h, significant reductions in chemical oxygen demand (79%), biological oxygen demand (77%), total dissolved solids (59%), and total suspended solids (27%) of four assessed textile effluents. Moreover, *T. domingensis* growth was improved, and there was a reduction in the effluent's mutagenicity. Syranidou et al. [35] studied, for the first time, the potential of endophytic bacteria in upgrading the efficiency of wetland helophyte *Juncus acutus*. They reported positive results with respect to its capacity of removing emerging organic contaminants together with metals in simulated wetland systems. Very recently, Ashraf et al. [39] assessed the potential of a consortium of endophytic bacteria for bioaugmentation (application of indigenous or allochthonous wild type or genetically modified microorganisms to polluted hazardous waste site in order to accelerate the removal of undesired compounds) in a vertical flow CW vegetated with *Leptochloa fusca*. CW vegetated with only *L. fusca* indeed remediated the tannery effluent. However, bioaugmentation with *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* sp. HU38 not only stimulated the plant growth but also enhanced the removal of both organic and inorganic pollutants from the effluent, thus reducing its toxicity. Authors concluded that plant-endophyte partnerships make constructed wetlands a more powerful technique for the removal of organic and inorganic xenobiotics from wastewater than the plants employed alone.

Heavy metals cause serious toxic outcomes in plants, animals, and human health; thence, their remediation is mandatory. In the midst of the miscellaneous approaches that were employed, phytoremediation stands out as a modern, effective, and extremely safe tool for this end [41]. According to Ma et al. [11], endophytic bacteria ameliorate plant development in metal-contaminated soils via two means: (1) directly, through the generation of plant growth favorable substances, comprising solubilization/transformation of mineral nutrients and production of phytohormones, siderophores, and specific enzymes and (2) indirectly, by the biocontrol of pathogens or by inducing in plant hosts a systemic resistance against pathogens. In addition,

Table 1 Past 5-year experimental reports regarding bacterial endophyte-assisted phytoremediation of polluted sites and/or industrial effluents

Endophytic bacteria Plant host	Degraded pollutant main findings
Bacillus thuringiensis GDB-1 *Alnus firma Siebold & Zucc.*	Arsenic (As), cadmium (Cd), copper (Cu), zinc (Zn), and nickel (Ni): GDB-1 enhanced the growth of *A. firma* seedlings by virtue of 1-aminocyclopropane-1-carboxylic acid deaminase activity, indole acetic acid, and siderophore production, besides phosphorus solubilization. Inoculating *A. firma* with a GDB-1 strain alleviated the metabolic perturbations and stress induced by high concentrations of heavy metals and enhanced biomass as well as metal accumulation by the plant [26]
Rahnella sp. JN6 *Brassica napus L.*	Cd, Pb, and Zn: JN6-inoculated plants presented significantly higher dry weights, enhanced concentrations, and increased uptake of Cd, Pb, and Zn in both above-ground and root tissues when compared to non-inoculated controls and when growing in soils amended with Cd (25 mg kg^{-1}), Pb (200 mg kg^{-1}), or Zn (200 mg kg^{-1}) [27]
Achromobacter xylosoxidans F3B *Chrysopogon zizanioides (L.) Roberty*	Toluene, an aromatic hydrocarbon that can cause severe neurological harm: The strain F3B enhanced the degradation of toluene in vetiver, what resulted in a decrease in the phytotoxicity of the compound and a 30% reduction of its evapotranspiration through the leaves. Importantly, *Achromobacter xylosoxidans* F3B was able to maintain a stable population in plant roots without greatly interfering with the diversity of native endophytes [25]
Burkholderia sp. SaZR4, *Burkholderia* sp. SaMR10, *Sphingomonas* sp. SaMR12, *Variovorax* sp. SaNR1 *Sedum alfredii Hance*	Zn and Cd: SaMR10 exhibited the smallest total population in plant's tissues and minor impact on *S. alfredii* growth and phytoextraction, whereas SaZR4 significantly upgraded Zn-extraction, however, not Cd-extraction. SaMR12 and SaNR1 significantly enhanced plant growth on substrates supplemented with Zn or Cd as well as the phytoextraction of Zn and Cd [28]
Pseudomonas sp. Lk9 *Solanum nigrum L.*	Cd, Zn, and Cu: Inoculation of *S. nigrum* with Lk9 enhanced the phytoextraction of Cd, Zn, and Cu. It improved soil's Fe, P mineral nutrition supplies, as well as soil Cd, Zn, and Cu bioavailability. Moreover, Lk9 tolerated high levels of metal pollution and produced biosurfactants, siderophores, and organic acids [29]
Pseudomonas monteilii PsF84, *Pseudomonas plecoglossicida* PsF610 *Pelargonium graveolens L'Hér.* (rose scented geranium)	Hexavalent chromium [Cr(VI)], a toxic and mobile form of the metal: Considering the biomass and Cr(VI) uptake in *P. graveolens* tissues, the total metal uptake in plant tissues per pot was notably superior in endophyte-inoculated plants when compared to the non-inoculated ones [30]
Pseudomonas putida PD1 *Salix purpurea L. and Salix discolor Muhl.; Lolium* spp.	Phenanthrene, a polycyclic aromatic hydrocarbon (PAH) compound: The inoculation of two different willow clones and a grass with PD1 allowed a substantial reduction in the phytotoxicity of phenanthrene while promoting root and shoot growth. Furthermore, it improved the removal (25–40%) of phenanthrene from soil by the tested host plants, when compared to the uninoculated controls [20]
Microbacterium arborescens TYSI04	Textile effluents: The combined plant-bacteria approach promoted, within 72 h, significant reductions in chemical oxygen demand (79%),

(*continued*)

Table 1 (continued)

Endophytic bacteria Plant host	Degraded pollutant main findings
and *Bacillus pumilus* PIRI30 *Typha domingensis Pers.*	biological oxygen demand (77%), total dissolved solids (59%), and total suspended solids (27%) of four assessed textile effluents [31]
Pseudomonas sp. Ph6-*gfp* *Lolium multiflorum Lam.*	Phenanthrene, a polycyclic aromatic hydrocarbon (PAH) compound: Strain Ph6-*gfp* inoculation diminished the risk of PAH contamination in plant's shoots and roots, thus showing its capacity of resisting to phenanthrene in planta [21]
Pseudomonas sp. J4AJ *Scirpus triqueter L.*	Diesel, a toxic mixture of paraffin, cyclic alkenes, and aromatic compounds: The soils planted with *S. triqueter* and inoculated with J4AJ exhibited the highest diesel removal ratio (more than 54%) after 60-day experiment. However, the removal ratio of J4AJ-treated soils was near to 39%. The plant height and stem biomass in the J4AJ-inoculated soils significantly increased. The synergistic effect of *S. triqueter* and J4AJ also improved the activities of catalase and dehydrogenase in the soil [23]
Pseudomonas koreensis AGB-1 *Miscanthus sinensis Andersson*	As, Cd, Cu, Pb, and Zn: *M. sinensis* inoculation with AGB-1 incremented heavy metal availability in the rhizosphere, lessened plant's stress to metals and therefore its growth, and finally boosted metal uptake. AGB-1-inoculated plants phytostabilized and phytoremediated mine site soil [32]
Azospirillum spp. and *Pseudomonas stutzeri* *Dactylis glomerata L.*	Anthracene, phenanthrene, and pyrene, all PAHs, and diesel: The authors reported a statistically important increase in the physical properties of soils polluted with PAHs and diesel fuel compared with the control and a significant decrease in the content of PAHs and heavy metals in soils inoculated with *Azospirillum* spp. and *P. stutzeri* after *D. glomerata growth* [22]
Bacillus pumilus E2S2, *Bacillus* sp. *E1S2*, *Bacillus* sp. E4S1, *Achromobacter* sp. E4L5, and *Stenotrophomonas* sp. E1L *Sedum plumbizincicola X.H. Guo et S.B. Zhou ex L.H. Wu*	Cd and Zn: The tested endophytic bacterial strains increased the water extractable Cd and Zn concentrations in soil. E2S2 bettered the performance and metal uptake of *S. plumbizincicola*, likely through the generation of growth-promoting metabolites and production of metal-mobilizing enzymes. The isolated endophytes enhanced the phytoextraction capacity of *S. plumbizincicola* [33]
41 bacteria belonging to *Bacillus*, *Microbacterium*, and *Halomonas* genera *Typha domingensis* Pers., *Pistia stratiotes* L., *Eichhornia crassipes* (Mart.) Solms	Textile effluent: Among the strains demonstrating maximum efficiency of textile effluent degradation, eight of them displayed plant growth-promoting characteristics, namely, production of indole-3-acetic acid and siderophore, presence of 1-amino-cyclopropane-1-carboxylic acid deaminase, and solubilization of inorganic phosphorous. *T. domingensis* not only exhibited superior growth in textile effluent but also hosted the utmost number of endophytic bacteria [34]

(*continued*)

Table 1 (continued)

Endophytic bacteria Plant host	Degraded pollutant main findings
Sphingomonas sp. U33, *Bacillus* sp. R12, *Ochrobactrum* sp. R24 *Juncus acutus L.*	Emerging organic contaminants (EOCs) and metals (Zn, Ni, Cd): The advantageous outcome of bioaugmentation with selected endophytes was more expressive in the exposure to high contamination, where most of the inoculated plants degraded the uppermost percentages of xenobiotics in shorter periods when compared to the control plants [35]
Bacillus pumilus DSKP8; 43 As-resistant bacteria, from Proteobacteria and Actinobacteria phyla *Pteris vittata L.*	As: Strain DSKP8 can enhance growth as well as the uptake of arsenic by plants and may be exploited for cleaning up arsenic contaminated sites together with hyperaccumulators such as *P. vittata*. In the presence of 10 mM arsenate, six endophytic bacterial strains had greater growth than the control, thus indicating a stimulated development [36, 37]
Sphingomonas sp. HJY *Allium tuberosum Rottler ex Spreng.*	Chlorpyrifos, toxic synthetic pesticides: Marked with the *gfp* gene, strain HJY successfully colonized *Allium tuberosum* diverse tissues and improved the degradation of chlorpyrifos inside the plants. Later, strain HJY displayed potential for reducing chlorpyrifos residues in *A. tuberosum* [2, 16]
Rhodococcus erythropolis, *Ensifer adhaerens*, *Variovorax paradoxus, Phyllobacterium myrsinacearum* *Betula celtiberica (Rothm. & Vasc.)*	As: *Betula celtiberica* inoculation with *R. erythropolis* and *E. adhaerens* promoted an in vitro increase in total nonprotein thiols content in roots, indicating a detoxification mechanism via phytochelatin complexation. Furthermore, *E. adhaerens* inoculation boosted plant growth, while inoculation with the consortium comprising *V. paradoxus* and *P. myrsinacearum* improved As accumulation in the host roots [38]
26 hydrocarbon-degrading strains from *Rhizobium*, *Pseudomonas*, *Stenotrophomonas*, and *Rhodococcus* genera *Lotus corniculatus* L., *Oenothera biennis* L.	Petroleum hydrocarbons: All assessed strains displayed at least one plant growth-promoting trait and possessed genes encoding for the hydrocarbon degradation enzymes. The endophytes were capable to develop in the presence of crude oil, diesel oil (more than 90% of the bacteria), and n-hexadecane (20% of the strains) [24]
Pantoea stewartii ASI11, *Microbacterium arborescens* HU33, *Enterobacter* sp. HU38 *Leptochloa fusca (L.) Kunth*	Tannery effluent: Constructed wetlands, vegetated only with *L. fusca*, indeed remediated tannery effluent; however, augmentation with endophytic bacteria not only efficiently stimulated the plant growth but also enhanced the removal of both organic and inorganic pollutants from the tannery effluent, also reducing its toxicity [39]

they can shift the metal accumulation ability in plants by excreting metal-immobilizing extracellular polymeric substances and/or metal-mobilizing organic acids and biosurfactants.

Babu et al. [32] investigated the potential of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to bioremediate mining site soil contaminated with arsenic, cadmium, copper, lead, and zinc. According to the authors, the

inoculation of *M. sinensis* with the AGB-1 strain incremented heavy metal availability in the rhizosphere, lessened plant's stress to metals and therefore its development, and finally boosted metal uptake. More recently, Xu et al. [37] assessed the potential of 43 arsenic-resistant endophytic bacteria isolated from *Pteris vittata*, an arsenic hyperaccumulator plant. In the presence of 10 mM arsenate, six bacterium endophytes had greater growth than the control, thus indicating arsenic-stimulated development. Results demonstrated that arsenic-resistant endophytes might improve *P. vittata* growth, thus enhancing its phytoextraction activity in arsenic-contaminated sites. Last but not least, endophytic bacteria can be engineered to improve heavy metal resistance/degradation systems and to remove organic toxic compounds present in soil [4, 11].

In the past 10 years, autofluorescent protein (AFP) techniques have figured as fundamental tools for investigating processes such as endophytes–plant interactions and biofilm formation [17]. These methodologies have been applied to detect and count microorganisms in situ on the plant exterior and in planta. One of the AFP's strategies, the green fluorescent protein (GFP) gene marker, has been largely applied to visualize and monitor the colonization patterns of bacterial strains within inoculated plants, allowing a visual phenotype for investigating microorganisms' population dynamics within vegetable tissues [20, 21]. Sun et al. [21] isolated the endophytic bacterium *Pseudomonas* sp. Ph6 from clover (*Trifolium pratense*) grown in PAH-contaminated soil and tagged it with the green fluorescent protein (GFP) gene in order to investigate its colonization and performance on PAH uptake by ryegrass. The authors could directly visualize, for the first time, its colonization and distribution in plant roots, stems, and leaves of ryegrass.

Despite the aforementioned evidence, the relevance of plant–endophyte synergisms for the removal of xenobiotics is presently undervalued. As many endophytic bacteria present pollutant-degrading, plant growth-promoting potentialities, and commonly both attributes, unravelling the mechanisms involved in these activities is a mandatory step to improve the phytoremediation of organic pollutants present in soil and water and expand its use in practice [4].

Babu et al. [26] found that the endophytic *Bacillus thuringiensis* GDB-1 had removal capacities of 77% for lead, 64% for zinc, 34% for arsenic, 9% for cadmium, 8% for copper, and 8% for nickel, throughout the growth cycle in a medium composed of heavy metal-amended mine tailing extract. Govarthanan et al. [43] when investigating the in vitro potential of the bacterium strain *Paenibacillus* sp. RM isolated from the roots of *Tridax procumbens* for the bioremediation of metals found that it was significantly resistant to copper, zinc, lead, and arsenic. Moreover, in batch experiments, the endophytic bacteria removed substantial amounts of copper (59%) and zinc (51%).

Shi et al. [42] identified and characterized an acid-stable bacterial laccase (Lac4) produced by the endophyte *Pantoea ananatis* Sd-1 cultured in rice straw. Lac4, which also presented interesting lignin degradation potential, was able to decolorize various synthetic dyes. It displayed a superior decolorization efficiency with Aniline Blue (47%) and Congo red (89%) when compared to that with RBBR (35%) after 4 h

in the presence of a mediator. With Congo red, the decolorization reached 60% after 2 h, in the absence of a mediator.

Recently, Feng et al. [16] assessed the chlorpyrifos-degrading potential of the strain *Sphingomonas* sp. HJY isolated from Chinese chives. Nearly 96% of 20 mg L^{-1} chlorpyrifos was removed by the endophyte at the end of a 15-day liquid culture experiment using a minimum salts medium. Authors determined the optimal conditions for chlorpyrifos removal and proposed, for the first time, a metabolic pathway for the degradation of chlorpyrifos by an endophytic bacterium of the genus *Sphingomonas* (Fig. 2).

Other examples of recent investigations addressing the potential of endophytic bacteria alone as xenobiotic degraders are shown in Table 2.

2.2 Endophytic Fungi

In comparison to bacteria, most fungi display a filamentous growth trend, which allows to follow both explorative and exploitative growth strategies and to form linear organs of aggregated hyphae to safeguard fungal translocation. This capacity of translocating nutrients through the mycelia network is a relevant feature in colonizing heterogeneous environments [10]. In addition, the low specificity of their catabolic enzymes and their independence from utilizing xenobiotic compounds as growth substrates makes them strong candidates for bioremediation agents [1].

Endophytic fungi possess the biochemical and ecological capacity to degrade or solubilize organic, mineral, and metal pollutants, either by chemical modification (directly, by enzymatic action) or by influencing chemical bioavailability. The latter is accomplished, for example, through the excretion of metabolites and varied mechanisms, including acidolysis, complexolysis, redoxolysis, and metal accumulation in biomass [1, 19].

Although knowledge on the role of endophytic fungi in phytoremediation is limited, some recent papers have addressed their potential use in bioremediation processes (Table 3).

Tong et al. [55, 57] performed a study on native grass species infected by endophytic fungi in a copper tailings dam through progressive years of phytoremediation. Authors not only found that the endophytic infection frequency raised over the years but also highlighted that the infection rates of *Bothriochloa ischaemum* and *Festuca rubra* were positively related to the cadmium pollution levels. Moreover, endophytic fungi colonizing *Imperata cylindrical* and *Elymus dahuricus* became tolerant to lead. Structure and relative ampleness of the bacterial communities had small fluctuations over the period; however, there was a marked variation in soil fungi species.

Chen et al. [47] demonstrated that the endophytic fungus *Phomopsis liquidambari* B3 was capable to promote in vitro the litter release of $NH4^{+}$–N from plant litter to soil, thus enhancing soil inorganic N contents. This increment in $NH4^{+}$–N, on its turn, boosted the soil ammonia-oxidizing bacteria community and

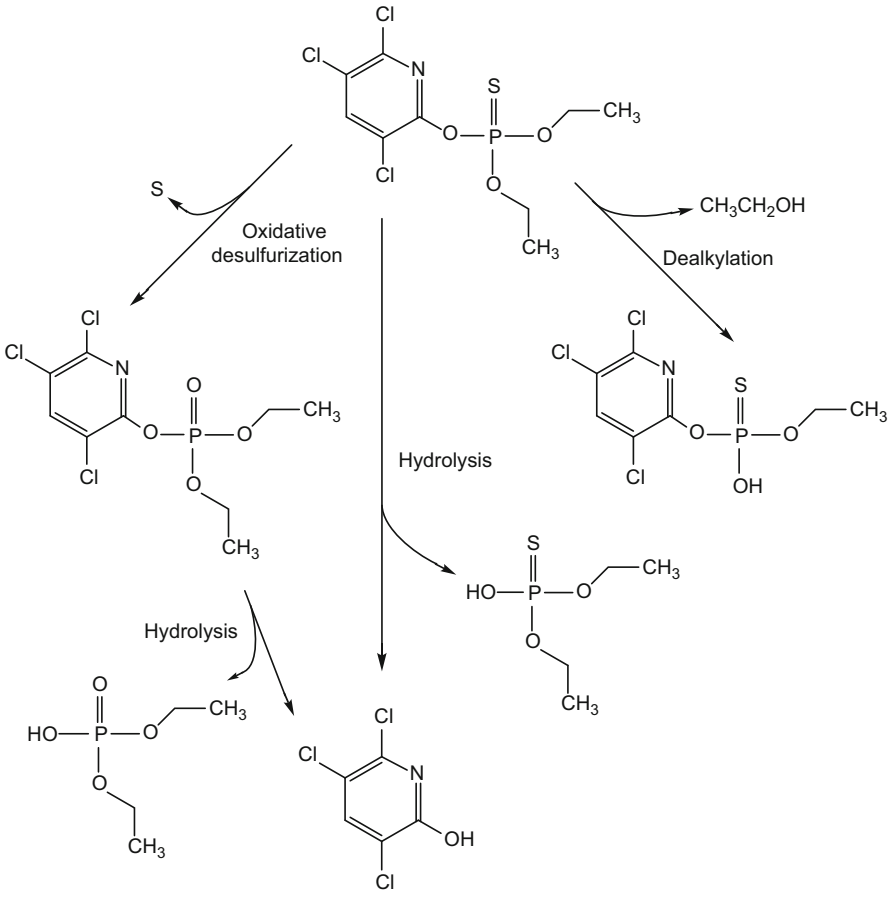

Fig. 2 Possible metabolic pathways for chlorpyrifos degradation by *Sphingomonas* sp. strain HJY, as proposed by Feng et al. [16]. HPLC coupled to time-of-flight mass spectrometry analysis indicated that *O*,*O*-diethyl *O*-3,5,6-trichloropyridinol was the major degradation product of chlorpyrifos

enhanced nitrification, leading to an elevation in soil NO^{3-}–N. Posteriorly, the same group investigated the biodegradation of N-heterocyclic indole (at 100 mg L^{-1}) by strain B3 and reported a degradation ratio of almost 42% within 120 h. According to the authors, plant litter supplementation significantly incremented and speeded up the fungal degradation activity. Results obtained in HPLC–MS and nuclear magnetic resonance analyses provided the basis for suggesting a metabolic pathway for indole degradation by strain B3. Two non-specific oxidases induced by plant liter, namely, laccase and LiP, were key enzymes acting in the production of oxindole and transformation of isatin [48].

Xie and Dai [50] investigated the biodegradation of the model allelochemical cinnamic acid by *P. liquidambari* B3, with promising results. As shown in Fig. 3,

Table 2 Past few years major experimental papers reporting the in vitro potential of endophytic bacteria as pollutant-degrading agents

Endophytic bacteria Plant host	Degraded pollutant main findings
Bacillus thuringiensis GDB-1 *Alnus firma Siebold & Zucc.*	As, Cu, Pb, Ni, and Zn: GDB-1's removal capacity was about 77% for Pb, 64% for Zn, 34% for As, 9% for Cd, 8% for Cu, and 8% for Ni throughout the growth cycle in medium composed of heavy metal-amended mine tailing extract [26]
Pseudomonas koreensis AGB-1 *Miscanthus sinensis Andersson*	As, Cd, Cu, Pb, and Zn: AGB-1 inoculation enhanced heavy metal(loid) solubilization in vitro. The isolated endophyte presents arsB, ACR3(1), aoxB, and bmtA marker genes for heavy metal resistance [32]
Rahnella sp. JN6 *Polygonum pubescens Blume*	Cd, Pb, and Zn: Strain JN6 displayed notable Cd, Pb, and Zn tolerance and competently solubilized $CdCO_3$, $PbCO_3$, and $Zn_3(PO_4)_2$ in culture solution [27]
Paenibacillus sp. RM *Tridax procumbens (L.) L.*	Cu, Zn, Pb, and As: Strain RM displayed a significant resistance to all tested heavy metals. In batch experiments RM performed high removal of Cu (59%) followed by Zn (51%) [43]
8 isolates from *Bacillus*, *Enterobacter*, *Stenotrophomonas*, and *Rhizobium* genera *Pteris vittata* L.	As: All endophytic isolates displayed tolerance to arsenic up to 1000 mg L^{-1}, among which five isolates were indole acetic acid positive (highest production up to 60 mg/L). Presence of aox gene was confirmed in two strains and arsB gene in six isolates. The isolated strain named E4 was a good indole acetic acid producer as well as arsenic-tolerant [45]
Pseudomonas sp. Ph6-*gfp* *Lolium multiflorum Lam.*	Phenanthrene, PAH compound: Ph6-*gfp* consumed more than 80% of phenanthrene in a culture solution (50 mg/L) within 15 days, evidencing its capacity to resist against phenanthrene in vitro [21]
Pseudomonas sp. J4AJ and *Bacillus subtilis* U-3 *Scirpus triqueter*	Diesel: J4AJ significantly degraded the n-alkane component of diesel, especially the short-chain hydrocarbons. Addition of the surfactant sodium lauroyl sarcosine to the system effectively improved the removal ratios of such compounds. The biosurfactant produced by the U-3 strain could also improve the removal ratios of most diesel's n-alkanes [44]
Pantoea ananatis Sd-1 *Oryza* sp.	Congo red, Remazol Brilliant Blue R (RBBR), and Aniline Blue: A novel microbial laccase (Lac4) produced by Sd-1 displayed superior decolorization efficiency for Aniline Blue (47%) and Congo red (89%) than for RBBR (35%) after 4 h in the presence of a mediator. For Congo red, the decolorization reached 60% after 2 h in the absence of a mediator [42]

(*continued*)

Table 2 (continued)

Endophytic bacteria Plant host	Degraded pollutant main findings
Stenotrophomonas sp. and *Pseudomonas* sp. *Conyza canadensis L. Cronquist* and *Trifolium pretense L.*	Mixed polycyclic aromatic hydrocarbons (PAHs): Both *Stenotrophomonas* sp. and *Pseudomonas* sp. were able to utilize PHAs as their exclusive sources of carbon and energy. In biodegradation studies, *Stenotrophomonas* sp. was able to consume 98% naphthalene, 83% fluoranthene, 87% phenanthrene, 14% pyrene, and 2% benzo(α) pyrene, while *Pseudomonas* sp. removed 95% naphthalene, 88% fluoranthene, 90% phenanthrene, and 7% pyrene, both after 7 days of inoculation [46]
Sphingomonas sp. HJY *Allium tuberosum Rottler ex Spreng.*	Chlorpyrifos, toxic synthetic pesticides: Authors investigated the degradation gene and proposed a metabolic pathway for the degradation of chlorpyrifos by HJY (Fig. 2), which was able to metabolize 96% of 20 mg L^{-1} chlorpyrifos during 15 days in liquid minimal salts medium [16]

cinnamic acid was initially transformed into styrene, which was further broken down sequentially into benzaldehyde, benzoic acid, 4-hydroxybenzoic acid, and protocatechuic acid, involving phenolic acid decarboxylase, laccase, hydroxylase, and protocatechuate 3,4-dioxygenase.

Wang et al. [52] also studied the remediation properties of *P. liquidambari* B3, this time its potential of degrading the phytoestrogen luteolin. The authors found that the optimum concentration for luteolin metabolization by strain B3 was 200 mg L^{-1}. Further, they suggested that the compound was metabolized via caffeic acid and phloroglucinol into protocatechuic acid and hydroxyquinol, which were subsequently disjointed by dioxygenases (Fig. 4). Later, Xie et al. [54] assessed the potential of *P. liquidambari* B3 for transformation and biodegradation of the recalcitrant pollutant sinapic acid, a typical methoxy phenolic pollutant found in industrial wastewaters.

Besides reporting an in vitro degradation rate of almost 99% within 48 h by the strain B3, authors tentatively proposed the complete sinapic acid degradation pathway (Fig. 5). The degrading enzyme activities, along with their gene transcription levels, notably varied throughout the degradation course and displayed a "cascade induction" response with the dynamics of substrate and metabolite concentrations.

3 Concluding Remarks and Future Prospects

The past 5 years investigations on the role of endophytes in the bioremediation of contaminated soils and waters reveal highly positive and promising prospects for future investigations. Most papers published over the period of the last 5 years

Table 3 Past 5-year major papers reporting the in vitro potential of endophytic fungi as pollutant-degrading agents

Endophytic fungi Plant host	Degraded pollutant main findings
Phomopsis liquidambari B3 *Atractylodes lancea (Thunb.) DC.*	Indole, a typical N-heterocyclic compound: The addition of B3 to soil significantly promoted mineral N release by changing the distribution of soil organic nitrogen. Authors investigated its indole biodegradation potential at 100 mg L^{-1}. The attendance of plant litter significantly incremented and speeded up fungal degradation activity. HPLC–MS and NMR analysis indicated the metabolic pathway: indole was first oxidized to oxindole and isatin and subsequently broke down the C–N position in the pyridine ring [47, 48]
Phomopsis liquidambari B3 *Bischofia polycarpa (H.Lév.) Airy Shaw*	Ferulic acid, a high-priority environmental pollutant: B3 was capable of using ferulic acid as its unique carbon source, efficaciously degrading the compound in mineral salt medium and soil. Authors proposed a degradation pathway: ferulic acid was firstly decarboxylated to 4-vinyl guaiacol and next oxidized to vanillin and vanillic acid, followed by demethylation to protocatechuic acid, which was further broken down through the *β*-ketoadipate pathway. Fungal laccase had a key role in the biodegradation process [49]
Phomopsis liquidambari B3 *Bischofia polycarpa (H.Lév.) Airy Shaw*	Cinnamic acid, a phenolic allelochemical: Strain B3 was able to effectively decompose cinnamic acid in mineral salt medium and soil, and the proposed metabolic pathway for the allelochemical degradation is shown in Fig. 3. The generation of laccase significantly enhanced the biodegradation process [50]
Cunninghamella echinulata, *Pestalotiopsis* sp., *Hypoxylon anthochroum*, *Paecilomyces lilacinus*, *Aspergillus* sp., and *Lasiodiplodia theobromae* *Psychotria flavida Talbot* and *Humboldtia brunonis Wall.*	Gamma-irradiated low-density polyethylene (common plastic polymer) and polypropylene (thermoplastic polymer): Reductions on intrinsic viscosity and average molecular weight of gamma-irradiated semicrystalline low-density polyethylene strips inoculated with *Aspergillus* sp. and *Paecilomyces lilacinus* (both from *H. brunonis*) and *Lasiodiplodia theobromae* (from *P. flavida*) showed fungal effectiveness in plastic transformation. This study suggests that higher doses of gamma rays could increase plastics' sensitivity toward microorganisms instead of guaranteeing sterilization of the material [51]
Phomopsis liquidambari B3 *Bischofia polycarpa (H.Lév.) Airy Shaw*	Luteolin, a common phytoestrogen: The optimum concentration for luteolin metabolization by B3 was 200 mg L^{-1}, and the proposed degradation pathway is shown in Fig. 4. Genes encoding protocatechuate 3,4-dioxygenase and hydroxyquinol1,2-di-oxygenase enzymes were successfully cloned. Reverse-transcription quantitative polymerase chain reaction assays revealed the important role of these genes in catalyzing the ring fission during the biodegradation process [52]
Penicillium sp. FT2G59 and *P. columnaris* FT2G7 *Dysphania ambrosioides (L.) Mosyakin & Clemants*	Pb, Zn, and Cd (heavy metals): In in vitro tolerance assays, FT2G59 tolerated Pb, Zn, and Cd with the MIC of 30–50, >680, 20–30 mmol/l, respectively, while FT2G7 tolerated Cd with the MIC of 30–50 mmol/l. Therefore, these endophytic strains displayed potential for phytoremediation of metal-contaminated sites [53]

(*continued*)

Table 3 (continued)

Endophytic fungi Plant host	Degraded pollutant main findings
Phomopsis liquidambari B3 *Bischofia polycarpa (H.Lév.) Airy Shaw*	Sinapic acid, one of the most representative methoxy phenolic pollutants: Both in flasks and in the soil, almost 99% of the added sinapic acid (at the optimum concentration for biodegradation of 200 mg L^{-1}) was consumed within 48 h by strain B3. The complete sinapic acid metabolic pathway was tentatively proposed for the first time (Fig. 5) [54]
Neurospora intermedia DP8–1 *Saccharum sp. (sugarcane)*	Diuron, a phenylurea herbicide classified as a priority hazardous substance: In biodegradation studies in liquid media, DP8-1 degraded up to 99% diuron within 3 days under the optimal degrading conditions. Moreover, it was able to utilize other phenylurea herbicides, namely, fenuron, monuron, metobromuron, isoproturon, chlorbromuron, linuron, and chlortoluron, as substrate for its growth. The main diuron metabolization pathway by strain DP8-1 consisted in sequential N-dealkylations [55]
Penicillium oxalicum B4 *Artemisia annua L.*	Triclosan, a widely used antimicrobial and preservative agent: The triclosan metabolization degree by strain B4 reached more than 97% at 1 h in liquid medium. Yet in non-sterile synthetic wastewater, only 2 h were required for the complete removal process. Compared to other microbial degraders, B4 presented a higher efficiency in removing triclosan [56]

addressed prospection and use of endophytic bacterial strains for enhancing phytoremediation. The potentiality of endophytic fungi, in contrast, in spite of the proposition of several metabolization mechanisms, has been uncovered for only a limited number of species. This is a fact duly taken into account in the series of future directions that we are proposing and that are also summarized in Fig. 6.

1. Referring to the study of bacterial endophytes in bioremediation, further investigations should prioritize the assessment of the remediation potential of the bacteria alone (away from the plant environment). Moreover, the identification of the genes involved in their degradation abilities and the elucidation of the degradation pathways are needed.
2. Notably, the capacity of endophytic fungi as degraders of xenobiotics is still underexploited, and the discovery of new species, together with the detection of genes involved in such abilities seems to be of general interest.
3. Further investigations should consider to investigate the interaction and possible synergistic action of fungi and endophytic bacteria in phytoremediation.

Lastly, despite the past two decade's significant advances, our knowledge regarding the potential of endophytes as pollutant-degrading agents is still incomplete. Hopefully in the future, the full potential of these microorganisms can be exploited for environmental and agricultural purposes.

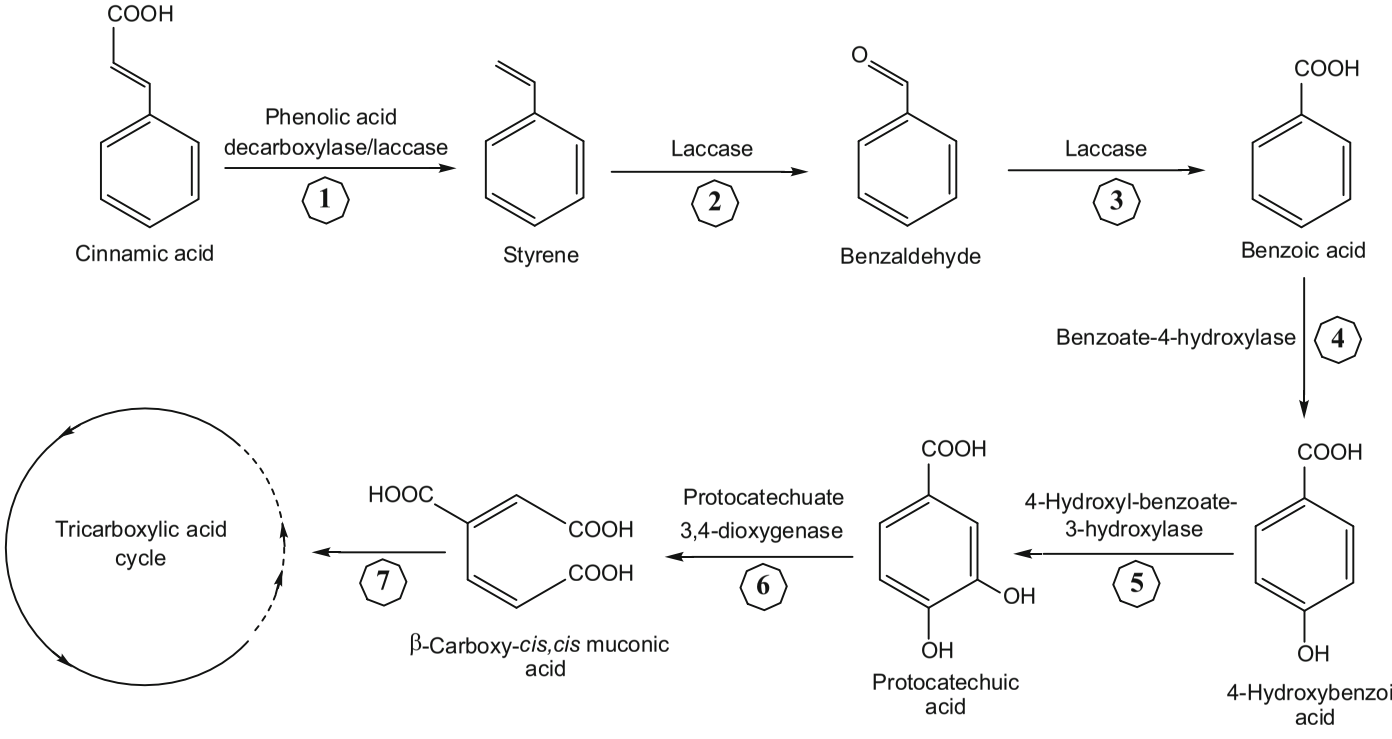

Fig. 3 Cinnamic acid biodegradation pathway by *Phomopsis liquidambari*, as proposed by Xie and Dai [50]. (1) Cinnamic acid decarboxylation; (2) styrene oxidation; (3) benzaldehyde oxidation; (4) benzoic acid hydroxylation; (5) hydroxybenzoic acid hydroxylation; (6) protocatechuic acid ring fission; and (7) entry into the TCA cycle

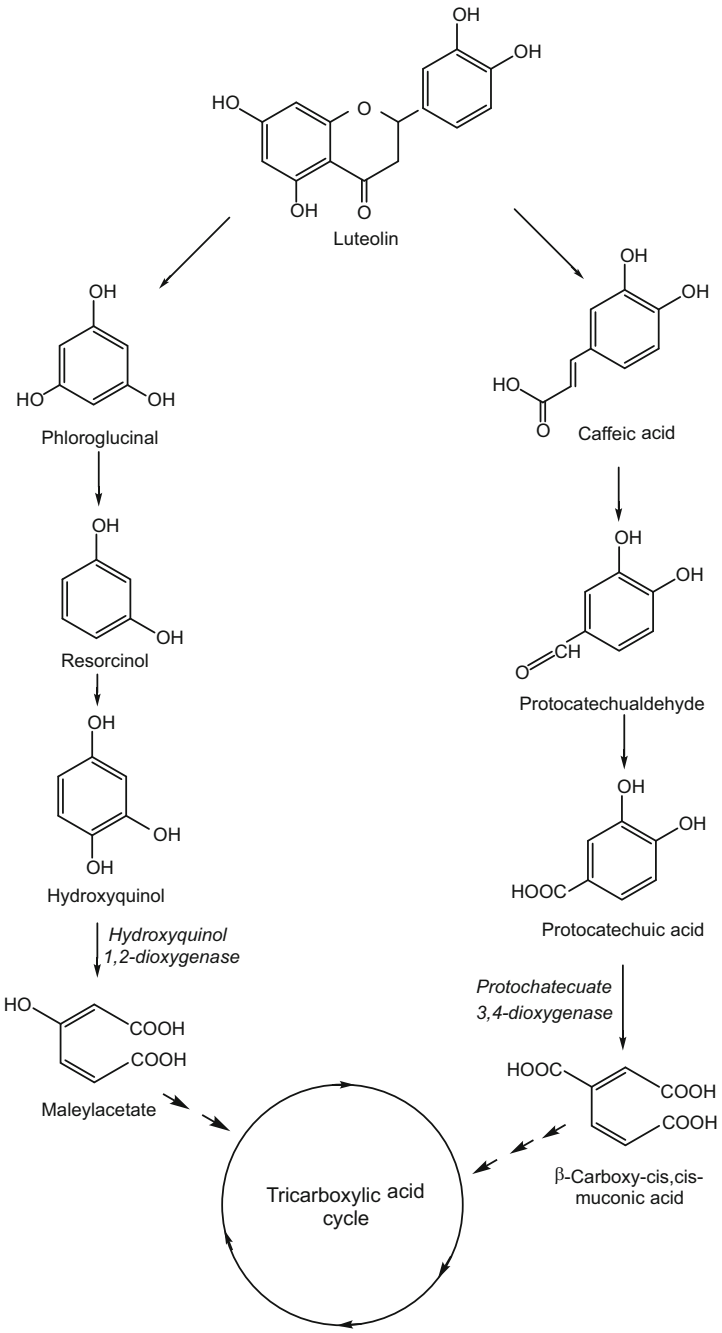

Fig. 4 The likely degradation pathway of luteolin by *Phomopsis liquidambari*, as proposed by Wang et al. [52]. Maleylacetate and β-carboxy-cis,cis-muconic acid are proposed intermediates and have not been isolated

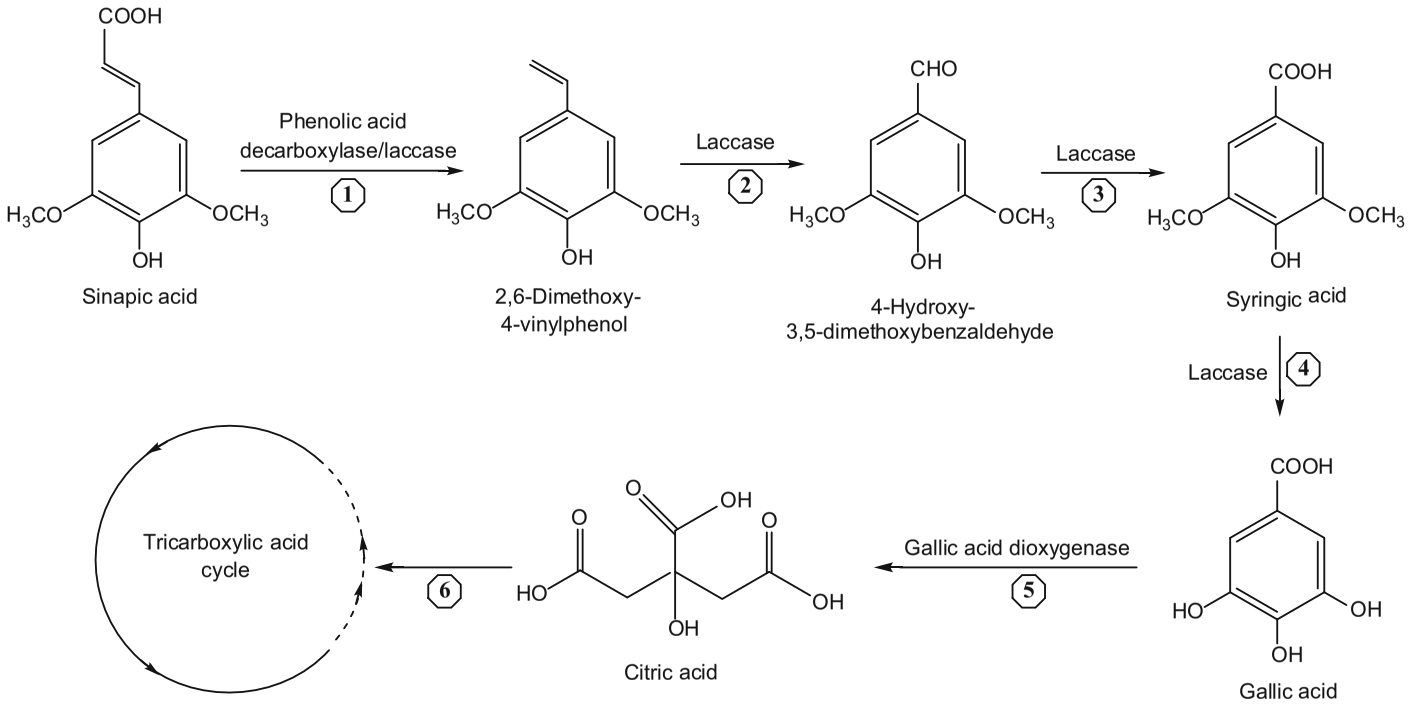

Fig. 5 The biodegradation pathway of sinapic acid in *Phomopsis liquidambari*, according to Xie et al. [54]. (1) Sinapic acid decarboxylation; (2) 2,6-dimethoxy-4-vinylphenol oxidation; (3) 4-hydroxy-3,5-dimethoxybenzaldehyde oxidation; (4) syringic acid demethylation; (5) gallic acid ring fission; (6) citric acid goes into the citric acid cycle

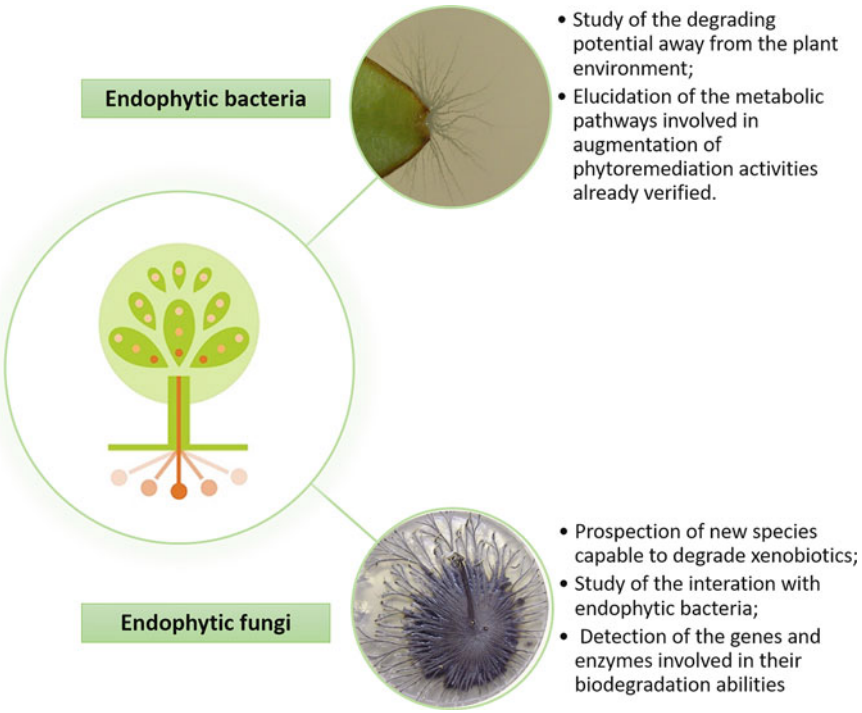

Fig. 6 Perspective of new studies of endophytes in bioremediation

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Fungal Endophytes: Rising Tools in Sustainable Agriculture Production

24

Hemraj Chhipa and Sunil K. Deshmukh

Contents

Abstract

Endophytes are the microorganisms that lived inside the plant during their life cycle and develop a mutualistic or symbiotic relationship with the host plant. In mutualistic relation, the plant provides nutrition to endophyte, and in return endophyte supports the plant growth and induces immunity in the host by producing secondary metabolites. These secondary metabolites play a significant role in the inhibition of plant pathogen and pest by inducing plant defense. Some of them take part in the induction of salicylic acid, jasmonic acid, and ethylene pathways which are responsible for plant defense. Different microbes like nitrogen-fixing bacteria and mycorrhizal fungi have been explored for decades in sustainable agricultural practices; some of them are being used at a commercial level. But the role of endophytes in plant stress tolerance (biotic and abiotic) and

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their commercial utilization is not much explored, and researchers are only screening endophytic microbial potentials in bio-fertilizer and bio-pesticide application at lab scale. The role of bioactive compounds from fungal endophytes in sustainable agriculture is least explored. Exploration of natural phenomena of such fungal endophytes and their compounds in crop production and protection is the need of present scenario which is facing problems of pollution with synthetic chemicals and their detrimental impacts on the environment. In the current chapter, we reviewed the role of fungal endophytes and their bioactive compounds in crop production and protection. Detailed analysis of endophytes and their bioactive compounds in plant protection (antibacterial, antifungal, insecticidal, and nematicidal) and growth promotion under different abiotic stress has been presented. The challenges and limitations in commercial agricultural product development of fungal endophytes are also discussed in the chapter.

Keywords
Endophytes · Fungi · Sustainable agriculture · Bioactive compounds

1 Introduction

Endophytes are the microorganisms that are isolated from surface sterilized plant tissues, and they don't harm to the host during their life cycle in the plant. They have been isolated from plants growing in temperate to the tropic ecosystems, hot deserts, arctic tundra, mangroves, grasslands, savannahs, and cropland ecosystems [1–3]. Initially, De Bary [4] in the year 1866 used the term endophyte in the nineteenth century for fungus residing inside the plants. Endophytes produce secondary metabolites which act as plant protectant or induce plant immunity. Endophytes also play an essential role in plant growth, fitness, and development [5]. In general, an endophyte genome contains 5–15 terpenoid synthase, 8–21 nonribosomal, and 7–29 polyketide synthase genes, which are responsible for bioactive compounds diversity in endophytes [6, 7]. Different forms of microbes including archaea, bacteria, fungi, and unicellular eukaryotes have been reported as endophytes [8, 9]. Among endophytic microorganisms, *Ascomycetes* and fungi imperfectly grouped represent the largest endophytic fungal groups containing 1.5×10^6 species and are sources of untapped biologically active small molecular natural products. In the case of fungi, *Glomeromycota* has been found to be the most dominant division in endophytic fungi followed by *Ascomycota*, *Basidiomycota*, *Zygomycota*, and unidentified phyla (Fig. 1). Further, in the divisions *Glomeromycota*, endophytic fungi from genera *Glomus* and *Rhizophagus*; in *Ascomycota*, class *Dothideomycetes* and *Sordariomycetes*; and in *Basidiomycota*, endophytes from *Agaricomycetes* were the found dominant groups (Fig. 1) [10].

Azevedo et al. [11] reported that xylariaceous *Ascomycetes* are the most dominant endophytes in tropical region. They found high endophytic diversity in the tropical plants in comparison to the temperate ones. Temperate region endophytes showed host specificity, while tropical region endophytes are less host specific.

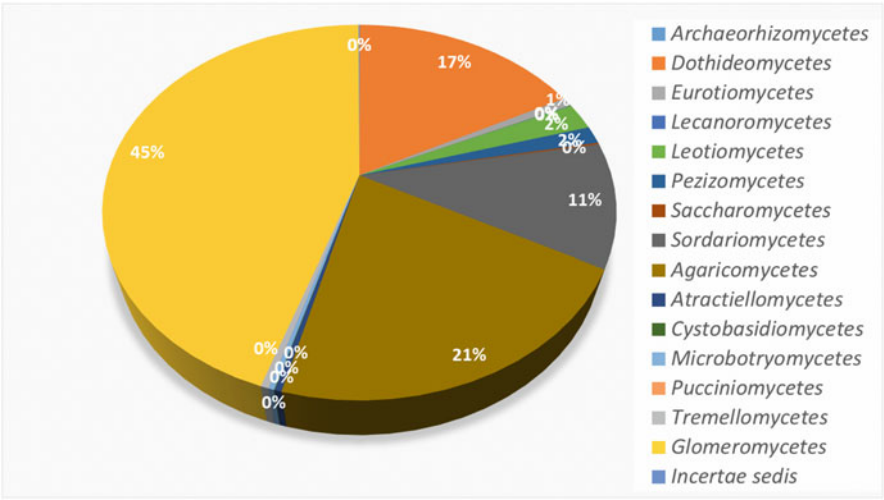

Fig. 1 Dominance of endophytic fungi. (Data source: Hardoim et al. 2015 [10])

Fungal endophytes have been divided on the basis of their colonization into different groups such as endophytes that colonize aerial parts or belowground plant parts, which can be transmitted vertically or horizontally, some are restricted to aerial tissue and transmitted horizontally, and the remaining required dark to colonize in plants and restricted to plant roots for colonization [12]. The study of endophytes is required to understand their role in enhancing plant growth, nutrient use efficiency for the host plant, abiotic stress tolerance, and disease resistance for sustainable agriculture production. Busby et al. [13] identified different research areas to understand plant-microbe interactions as given below, which would be helpful in identifying effective endophytic consortia for sustainable crop production:

1. Host-microbiome systems development to understand associated microbial culture collections and reference genomes in crop plants and non-crop plants
2. Define core microbiomes and metagenomes in these model systems
3. Elucidation of the rules of synthetic, functionally programmable microbiome assembly
4. Determination of functional mechanisms of plant-microbiome interactions
5. Characterization and refinement of plant genotype-by-environment-by-microbiome-by-management interactions

2 Mutualistic Relationship

Plant and endophytes lived in mutualistic relationship and benefit from each other. The plant provides nutrition to the endophytes, while in return endophytes help in adaption to abiotic conditions like nutrients limitation, salination and extreme pH,

drought, temperature variation, and protection from pathogens, insects, and nematodes. In addition to mutualistic relation, endophytes also show detrimental effects on the plant under specific conditions [14]. Beneficial microbes can be harmful to other species, such as bacteria that live inside the plant that can be harmful to humans [15]. Genre et al. [16] reported that colonization process is initiated when endophyte hyphal tip and barley root hair come into contact of each other; subsequently, strigolactone 5-deoxy-strigol (strigolactones are a group of sesquiterpene lactones) compound triggered hyphal branching and facilitates colonization in rhizosphere of barley roots. The colonization of endophyte induces the plant defense but not in full-blown defense response, so the relationship developed without harming each other, and plant recognizes endophyte as a friendly intruder which is sensed by kinase-mediated transmembrane signaling [17]. The endophyte produced different enzymes like polyphenol oxidases, cellulases, and laccases which help them in an entry into root cells by degradation of cellulose, hemicellulose, and pectin in a limited manner. Secondary metabolites and plant hormones also play a significant role in endophytic colonization. Plant hormone – auxin, abscisic acid, and gibberellin – regulation suppresses the plant innate immune system during endophytic colonization [18].

3 Role of Endophytes in Sustainable Agriculture

Fungal endophytes help plants in resilience and adaptation to the new ecosystem by increasing plant immunity to suppress biotic and abiotic stress [19, 20]. Endophytes induce different mechanistic approaches for plant growth promotion such as biological nitrogen fixation, phosphate solubilization, phytohormone production, and inhibition of ethylene biosynthesis and induce resistance in the plant to prevent pathogenic attacks by the release of secondary metabolites such as enzymes, siderophore, and antibiotics. Fungal endophytes proved them as a good source of phytohormones like auxin, and gibberellin which promote the plant growth. Endophytic colonization inhibits the phytopathogens by inducing plant defense system. Endophytes also significantly increase plant antifungal and antibacterial compounds, secondary plant metabolites like phenylpropanoids, and oligomeric proanthocyanidins in crops [21]. In addition endophytes also induce yield and plant biomass increase in grass species [22], rice [23], and barley [18].

Endophytes also play their role in plant growth promotion by interference in carbon fixation and photosynthesis and induction of phytohormones. Endophytes produce auxin, gibberellin, cytokinin, adenine, adenine ribosides, indole-3-butyric acid, acetoin, 2,3-butanediol, and polyamines which are helpful in promotion of plant growth [24–28]. Many reports have been published on the role of endophytes in plant growth promotion (Table 1). The detailed information has been reviewed by M. Rai et al. [29]. Production of auxin hormone indole acetic acid (IAA) has been reported by endophytes *Chaetomium globosum*, *Fusarium* sp., *Fusarium oxysporum.*, *Phomopsis* sp., *Phoma glomerata*, *Penicillium verruculosum*, *Penicillium funiculosum*, *Penicillium* sp., and *Paecilomyces formosus*

Table 1 Plant growth-promoting endophytes in different host plants [29]

S. No	Endophyte	Host plant	References
1	*Aspergillus fumigatus*	*Glycine max*	Khan et al. 2011 [46]
2	*Aspergillus* sp.	*Monochoria vaginalis*	Nadeem et al. 2010 [5]
3	*Aspergillus ustus*	*Solanum tuberosum*	Marina et al. 2011 [58]
4	*Chaetomium globosum*	*Capsicum annuum*	Khan et al. 2012 [59]
5	*Chrysosporium pseudomerdarium*	*Glycine max*	Hamayun et al. 2009 [38]
6	*Cladosporium* sp.	*Cucumis sativus*	Hamayun et al. 2010 [42]
7	*Cladosporium sphaerospermum*	*Glycine max*	Hamayun et al. 2009 [37]
8	*Exophiala* sp.	*Cucumis sativus*	Khan et al. 2011 [46]
9	*Fusarium oxysporum*	*Sesamum indicum*	Hasan 2002 [30]
10	*Fusarium oxysporum*	*Musa* sp.	Machungo et al. 2009 [60]
11	*Fusarium oxysporum*	*Ipomea batatas*	Hipol 2012 [61]
12	*Fusarium* sp.	*Euphorbia pekinensis*	Dai et al. 2008 [31]
13	*Fusarium* sp.	*Dendrobium loddigesii*	Chen et al. 2010 [62]
14	*Gliomastix murorum*	*Elymus mollis*	Khan et al. 2009 [45]
15	*Helminthosporium velutinum*	*Sorghum bicolor*	Diene et al. 2010 [63]
16	*Metarhizium anisopliae LHL07*	*Glycine max*	Khan et al. 2012 [28]
17	*Paecilomyces formosus*	*Cucumis sativus*	Khan et al. 2012 [59]
18	*Penicillium citrinum*	*Ixeris repenes*	Khan et al. 2008 [43]
19	*Penicillium simplicissimum*	*Zoysia tenuifolia*	Hossain et al. 2007 [64]
20	*Penicillium* sp.	*Suaeda japonica*	You et al. 2012 [48]
21	*Penicillium* sp.	*Chrysanthemum coronarium*	Hamayun et al. 2010 [41]
22	*Penicillium* sp.	*Cucumis sativus*	Waqas et al. 2012 [35]
23	*Penicillium* sp.	*Monochoria Vaginalis*	Nadeem et al. 2010 [5]
24	*Penicillium verruculosum*	*Potentilla fulgens*	Bhagobaty and Joshi 2009 [32]
25	*Phoma glomerata*	*Cucumis sativus*	Waqas et al. 2012 [35]
26	*Phoma herbarum*	*Glycine max*	Hamayun et al. 2009 [65]
27	*Phoma* sp.	*Cucumis sativus*	Hamayun et al. 2010 [42]
28	*Pyrenochaeta* sp.	*Dendrobium loddigesii*	Chen et al. 2010 [62]
29	*Scolecobasidium tshawytschae*	*Glycine max*	Hamayun et al. 2009 [37]
30	*Sebacina vermifera*	*Ziziphus nummularia*	Dolatabadi et al. 2012 [66]
31	*Trichoderma hamatum*	*Theobroma gileri*	Bae et al. 2009 [55]

[28, 30–36]. Similarly, gibberellin (GA) has been produced by many endophytes such as *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Arthrinium phaeospermum*, *Chrysosporium pseudomerdarium*, *Cladosporium* sp., *Cladosporium sphaerospermum*, *Exophiala* sp., *Fusarium* sp., *Fusarium oxysporum*, *Chaetomium globosum*, *Gliomastix murorum*, *Penicillium* sp., *Penicillium corylophilum*,

Penicillium citrinum, *P. cyclopium*, *P. funiculosum*, *Phoma* sp., *Phoma herbarum*, *Phoma glomerata*, *Paecilomyces formosus*, *Scolecobasidium tshawytschae*, and *Rhizopus stolonifer* [5, 28, 30, 31, 33, 34, 36–48]. In contrast, the production of abscisic acid is reported in a less number of endophytes. Dai et al. [31] reported production of abscisic acid by *Phomopsis* sp. isolated from *B. polycarpam* plant. Previously, many scientist also reported that different species of *Penicillium* and *Aspergillus* genera were also identified as gibberellin producers, such as *Aspergillus flavus*, *A. niger*, *Penicillium corylophilum*, *P. cyclopium*, *P. funiculosum*, *Penicillium* sp., and *P. citrinum* [30, 33, 41], which also induce the production of defense hormone salicylic and jasmonic acid.

Endophytic fungi are also helpful to induce the ISR (induced systematic resistance) in the plant after pathogen attack and triggered the PR genes [49, 50]. Fungal endophytes induce the phytoalexins in plants against plant pathogens [51]. Waqas et al. [52] studied the role of endophytes *Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5 on *Helianthus annuus* plant growth in time-dependent manner and their role in plant hormone regulation in plant protection against stem rot disease. They found that both fungal endophytes are able to relive the biotic stress by induction of salicylic and jasmonic acid content, which are responsible for plant defense. They found *Penicillium citrinum* is more active in comparison to *Aspergillus terreus* in *Helianthus annuus* plant growth promotion and triggers the systematic acquired resistance in the plant against *Sclerotium rolfsii*.

In addition to biotic stress, endophytes also help to plant intolerance of abiotic stress such as drought, salt, and heat stress. *Neotyphodium* sp. increased drought tolerance in grass plant by osmo- and stomatal regulations and protected plants in water stress and nitrogen starvation [53]. Similarly, *Trichoderma* and *Piriformospora indica* showed drought and salt tolerance in cacao, barley, and Chinese cabbage plant [54–56]. Murphy et al. [57] isolated different endophytes from wild barley species and found endophyte *Penicillium brevicompactum* was helpful in drought tolerance improvement of barley plant in drought condition. *Penicillium brevicompactum* also increased the germination index of the barley seed and also increased the yield by suppressing the seed-borne infectious organism.

Endophytes are also used as seed coating material and realized their true potential in seed germination. Murphy et al. [67] found a significant increase in the seedling length of barley by endophytic induction after 28 days of seedling growth. They used the mixture of endophytes and showed similarity to *Penicillium glabrum*, *Penicillium brevicompactum*, uncultured *Cladosporium*, and uncultured *Metarhizium* in BLAST results.

Piriformospora indica is another endophytic fungus and has shown beneficial symbiosis with xerophyte plants of Thar Desert [68]. The plant's root colonized with *P. indica* showed tolerance in different abiotic stresses like extreme temperature, salinity, drought, heavy metals, and biotic stress of foliar and root pathogen. *P. indica* modulates the phytohormones of plant growth and development and enhanced the nutrient uptake. *P. indica* also showed their role in protection of plants from *Fusarium* crown rot disease and induced local and systemic resistance to other viral and fungal plant diseases via signal transduction. Colonization of *P. indica*

controlled various plant diseases such as powdery mildew, eyespot, *Rhizoctonia* root rot, *Fusarium* wilt, black root rot, yellow leaf mosaic, *Verticillium* wilt, cyst nematode, and leaf blight in barley, wheat, maize, tomato, and *Arabidopsis* plants [69]. The detailed review on *P. indica* and its role in yield and tolerance to biotic and abiotic stresses in crop plants has been published by Johnson et al. [69].

Endophytes *Epichloe* sp. of *Festuca rubra* is a plant growth-promoting endophyte which increase the plant growth with high uptake of nutrients [70]. It has also been reported that plant-fungal interaction enhances the temperature tolerance to host plant by inducing heat shock protein expression. Redman et al. [71] reported that endophytic fungi *Curvularia* sp. isolated from grass *Dichanthelium lanuginosum* of Volcanic and Yellowstone National Parks confer thermos tolerance to grasses. Further, this endophyte also provided thermos tolerance ability to other plants tomato, watermelon, and wheat [72]. The application of endophytes in increasing plant resistance against abiotic and biotic stress, seed coating for increased germination, and induction of ISR makes them important tools of sustainable agriculture production.

4 Bioactive Compounds for Plant Protection

The bioactive compounds produced by endophytic fungi have shown their potential in controlling plant pest and pathogen and can be an alternate of chemical pesticide in the near future [73]. The production of novel bioactive compounds depends on the organism found in the unique biotope. In a unique biotope, organisms lived in optimized condition and have constant interaction with the surrounding communities which resulted in higher production of bioactive compounds [74]. Recently, Lugtenberg et al. [75] have reported a detailed review of the endophytic bioactive compounds and their application in crop pest management. Fungal endophytes produce compounds that showed growth inhibitory activity toward plant pathogens and herbivores. These compounds include alkaloids, chlorinated compounds, flavonoids, peptides, polyketides, quinols, steroids, and terpenoids (Table 2).

It has been reported that after colonization, fungal endophytes produce lytic enzymes such as β-1,3-glucanases, chitinases, and cellulases which directly degrade the cell wall of the plant pathogen and control their pathogenesis [51].

It has been reported that the presence of endophytes *Epichloe* in temperate grass produce bioactive compounds in host plant which works as a deterrent to herbivores and pests. *Epichloe festucae* produces bioactive compounds mainly ergovaline, lolitrem B, epoxy-janthitrems, and peramine which act as a neurotoxin, vasoconstriction agent, and pest deterrent [91, 94, 121–123]. *Piriformospora indica* showed as a biocontrol agent against plant pathogen in maize, tomato, wheat, and barley [124–127]. *Piriformospora indica* showed the reduced severity of *Verticillium* wilt by 30% in tomato, caused by *Verticillium dahlia*, and increased leaf biomass by 20% [125]. It also reduced the density of Pepino mosaic virus of tomato which has been reported in the greenhouse of South and North America, China, and many European countries. *Epicoccum nigrum* is also known for its biocontrol potential

Table 2 Endophytes as source of bioactive compounds for sustainable agriculture

S. No	Endophyte	Host plant	Compounds	Activity	Reference
1	*Acremonium coenophialum*	*Festuca arundinacea*	Quitinases	Nematicide activity against *Festuca arundinacea*	Roberts et al. 1992 [76]
2	*Acremonium zeae*	*Zea mays*	Pyrrocidines A and B	Antifungal activity against *Aspergillus flavus* and	Wicklow et al. 2005 [77]
3	*Alternaria* sp.	*Salvia miltiorrhiza*	Alternariol-9-methyl ether	Antibacterial, anti-sporulatating and nematicidal agent	Lou et al. 2016 [78]
4	*Alternaria* sp.		Altersetin	Active against Gram-positive, Gram-negative bacteria and pathogenic yeasts	Hellwig et al. 2002 [79]
5	*Ampelomyces* sp.	*Urospermum picroides*	3-*O*-methylalaternin, altersolanol A	Antibacterial activity against *Staphylococcus aureus*, *S. epidermidis, Enterococcus faecalis*	Aly et al. 2008 [80]
6	*Aspergillus fumigatus*		Fumigaclavine A, fumigaclavine B, and fumigaclavine C	Plant protection against herbivores	Cavaglieri et al. 2004 [81]
7	*Aspergillus* sp. *KJ-9*	*Melia azedarach*	Asperpyrone A, asperazine, rubrofusarin B, and (*R*)-3-hydroxybutanonitrile	Antifungal and antibacterial	Xiao et al. 2014 [82]
8	*Aspergillus terreus*	*Helianthus annuus*	Malic, quinic, and succinic acid	Antifungal activity against *Alternaria alternata* and plant growth promoting agent	Waqas et al. 2015 [83]
9	*Chaetomium globosum*	*Triticum aestivum*		Resistance to *Pyrenophora tritici-repentis* infection	Istifadah and McGee 2006 [84]
10	*Colletotrichum* sp.	*Artemisia annua*	Colletonoic acid	Antibacterial, antifungal, and anti-algal	Hussain et al. 2014 [85], Zou et al. 2000 [86]

11	*Cordyceps dipterigena*		Cordycepsidone A	Anti-fungal activity against *Gibberella fujikuroi*	Varughese et al. 2012 [87]
12	*Cryptosporiopsis quercina*	Hardwood species	Cryptocandin	Anti-fungal activity against *Sclerotinia sclerotiorum* and *Botrytis cinerea*	Strobel et al. 1999 [88]
13	*Cryptosporiopsis quercina*		Cryptocin	Anti-fungal activity against *Pyricularia oryzae*, *Fusarium oxysporum*, *Geotrichum candidum*, *Rhizoctonia solani*, *S. sclerotiorum*, *Py. ultimum*, *Phytophthora cinnamomi,* and *Ph. citrophthora*	Li et al. 2000 [89]
14	*Curvularia protuberata*	*Oryza sativa*		Tolerance of abiotic stresses	Redman et al. 2011 [23]
15	*Daldinia concentrica*	Olive tree	Volatile organic compounds	Postharvest control: protects peanuts against *Aspergillus niger*, oranges and tomatoes against *Penicillium digitatum*, and grapes against *Botrytis cinerea*	Liarzi et al. 2016 [90]
16	*Epichloe*		Peramine	Feeding deterrent against the insect pest Argentine stem weevil	Rowan 1993 [91], Johnson et al. 2013 [92]
17	*Epichloe coenophialum*		Ergovaline	Pesticidal effect	Popay et al. 1990 [93], Rowan et al. 1990 [94]
18	*Epicoccum nigrum*	*Saccharum*	Epicorazines A-B	Biocontrol agent	Baute et al. 1978 [95]

(*continued*)

Table 2 (continued)

S. No	Endophyte	Host plant	Compounds	Activity	Reference
19	*Epicoccum nigrum*		Flavipin	Biocontrol agent	Bamford et al. 1961 [96], Brown et al. 1987 [97]
20	*Epicoccum nigrum*		Epipiridones and epicocarines	Plant protectant	Wangun and Hertweck 2007 [98]
21	*Eupenicillium parvum*	*Azadirachta indica*	Azadirachtin A and B	Insecticidal	Kusari et al. 2012 [99]
22	*Fusarium oxysporum*	*Solanum lycopersicum*	–	Nematicidal activity against *Radopholus similis*	Shahasi et al. 2006 [100]
23	*Fusarium*, *Trichoderma*, *Chaetomium*, *Acremonium*, *Paecilomyces*, and *Phyllosticta*	*Cucumis sativus*	–	Nematicidal activity against *Meloidogyne incognita*	Hallmann et al. 1998 [101]
24	*Gliocladium* sp.	*Eucryphia cordifolia*	Annulene	Plant protectant	Stinson et al. 2003 [102]
25	*Muscodor albus*	*Cinnamomum zeylanicum*	Volatile compounds from, namely, alcohols, esters, ketones, acids, and lipid class	Growth inhibition of Gram-positive and Gram-negative bacteria and plant-pathogenic fungi and oomycetes	Worapong et al. 2001 [103], Ezra, et al. 2004 [104]
26	*Muscodor albus*	Tropical tree	Tetrahydrofuran, 2-methylfuran, 2-butanone, aciphyllene	*Stachybotrys chartarum*	Atmosukarto et al. 2005 [105]

27	*Muscodor crispans*	*Ananas ananassoides*	Propanoic acid, 2-methyl-, methyl ester; propanoic acid, 2-methyl-; 1-butanol, 3-methyl-;1-butanol, 3-methyl-, acetate; propanoic acid, 2-methyl-, 2-methylbutyl ester; and ethanol	Anti-fungal activity against *Pythium ultimum*, *Alternaria helianthi*, *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *Phytophthora cinnamomi*, *Ph. palmivora*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *and Verticillium dahliae.* Also the plant pathogenic bacterium *Xanthomonas axonopodis* pv. citri	Mitchell et al. 2010 [106]
28	*Muscodor vitigenus*	Liana	Naphthalene	Insect repellent: wheat stem sawfly *Cephus cinctus*	Daisy et al. 2002 [107]
29	*Nodulisporium* sp.	*Bontia daphnoides*	Nodulisporic acids	Insecticidal properties against the larvae of the blowfly	Demain et al. 2000 [108]
30	*P. microspora*	*Terminalia morobensis*	Pestacin and isopestacin	Antimicrobial	Strobel and Daisy 2003 [73]
31	*Penicillium citrinum*	*Helianthus annuus*	Gibberellins and siderophore	Plant growth promotion and antifungal activity	Waqas et al. 2015 [83]
32	*Periconia* sp.	*Taxus cuspidata*	Fusicoccane diterpenes	*Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*	Kim et al. 2004 [109]
33	*Pestalotiopsis jesteri*	*Fagraea bodenii*	Jesterone and hydroxyjesterone	Anti-oomycetes activity	Li and Strobel 2001 [110]
34	*Pestalotiopsis microspora*		Ambuic acid	Anti-fungal activity against *Fusarium* species and *Pythium ultimum*	Li et al. 2001 [111]

(*continued*)

Table 2 (continued)

S. No	Endophyte	Host plant	Compounds	Activity	Reference
35	*Phialocephala scopiformis*	*Picea glauca*	Rugulosin	Kills spruce budworm *Choristoneura fumiferana* (an anti-insect compound)	Sumarah et al. 2008 [112], Miller et al. 2009 [113]
36	*Phomopsis cassiae*	*Cassia spectabilis*	Cadinane sesquiterpenes, 3,11,12-trihydroxycadalene	Anti-fungal activity against *Cladosporium sphaerospermum*, *C. cladosporioides*	Silva et al. 2006 [114]
37	*Phomopsis phaseoli*	*Betula pendula*	3-Hydroxypropionic acid	Nematicidal activity against *Meloidogyne incognita* and *Caenorhabditis elegans*	Schwarz et al. 2004 [115]
38	*Phomopsis spp.*	*Erythrina crista-galli*	Phomol	Antifungal, antibacterial	Weber et al. 2004 [116]
39	*Sebacina vermifera*	*Hordeum vulgare*		Resistance to *Blumeria graminis f.* sp. *hordei* infection	Schafer and Kogel 2009 [117]
40	*Verticillium* sp.	*Rehmannia glutinosa*	Massariphenone, ergosterol peroxide	Antifungal against *Pyricularia oryzae* P-2b	You et al. 2009 [118]
41	*Macrophomina phaseolina*	*Ocimum sanctum*	2H-pyran-2-one, 5, 6-dihydro-6-pentyl	Antifungal	Chowdhary and Kaushik 2015 [119]
42	*Acremonium* sp.	*Mentha piperita*	1-Heptacosanol and 1-nonadecane	Antifungal	Chowdhary and Kaushik 2018 [120]

against bacterial and fungal plant pathogens. The isolate of sugarcane has shown biocontrol activity against fungal pathogen *Sclerotinia sclerotiorum* in sunflower and *Pythium* in the cotton crop. It has also demonstrated antibacterial activity against *Phytoplasma* in apple and *Monilinia* sp. in peach fruit [128, 129]. *Penicillium brevicompactum* has been reported to suppress various seed-borne pathogens including *Rhynchosporium*, *Pyrenophora*, *Fusarium*, and *Cochliobolus* and soil-borne pathogen *Gaeumannomyces graminis* var. *tritici* [57, 130]. *Penicillium indica* increased the resistance in barley against root rot causing agent *Fusarium culmorum* and *Blumeria graminis* [127]. Endophyte *Colletotrichum gloeosporioides* isolated from *Theobroma cacao* tissues showed antagonistic activity against black pod rot pathogen *Phytophthora palmivora*, frosty pod rot pathogen *Moniliophthora roreri*, and witches broom pathogen *M. perniciosa* in in vitro and field studies [131]. Vega et al. [132] isolated many coffee endophytes including *Acremonium*, *Beauveria bassiana*, *Cladosporium*, *Clonostachys rosea*, and *Paecilomyces* from Mexico, Puerto Rico, Hawaii, and Colombia. *Beauveria bassiana* and *C. rosea* were found pathogenic to coffee berry borer *Hypothenemus hampei* and worked as entomopathogenic endophytic fungi. *Phialocephala scopiformis*, endophytes from *Picea glauca* (white spruce), produce anti-insecticide compound rugulosin which controls the budworm *Choristoneura fumiferana* which is a severe pathogen of the white spruce tree in Canada and Northern USA [112].

It has also been reported that endophytes also affect the host bioactive compound profiles. Jaber and Vidal [133] observed the effect of *Acremonium strictum* on nectar production in the bean plant. They found increased nectar production and many extrafloral nectaries in plants inoculated with *A. strictum*, which reduce the aphid fecundity in bean. Similarly, endophyte *A. strictum* also changed the volatile compound profile after inoculation in tomato by lowering the emitted quantities of terpene and sesquiterpene and increment in the amount of trans-β-caryophyllene which affected the oviposition of moth *Helicoverpa armigera* [134]. On the other hand, *Acremonium coenophialum* produce quitinases in *Festuca arundinacea* Schreb, which induce resistance against nematode [76]. Further, it has been reported that endophytes *Phomopsis phaseoli* and *Melanconium botulinum* also produced 3-hydroxypropionic acid and 3-nitropropionic acid in some plants which are also responsible for nematicide activity. Recently, Sun et al. [135] reported that endophytic fungi *Aspergillus oryzae* in *Raphanus sativus* enhanced the plant growth and negatively affected the growth of pest diamondback moth *Plutella xylostella. Aspergillus oryzae* contributed to plant resistance against herbivores and diseases. *Aspergillus fumigatus* produces ergot-type alkaloids, fumigaclavine A, fumigaclavine B, and fumigaclavine C, which play a vital role in plant protection against herbivores [81]. Xiao et al. [82] isolated endophytic fungi *Aspergillus* sp. from *Melia azedarach*, which produce dianhydroaurasperone C, isoaurasperone A, fonsecinone A, asperpyrone A, asperazine, rubrofusarin B, and (R)-3-hydroxybutanonitrile compounds and showed activity against different plant pathogen *Gibberella saubinetti*, *Magnaporthe grisea*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Alternaria solani.*

Trichoderma sp. produced different secondary metabolites; some of them, like 6-pentyl-a-pyrone, harzianolide, and harzianopyridone, showed antimicrobial activity at higher concentration and microbial-associated molecular pattern (MAMP) at a lower concentration. These compounds reported as a plant defense activator in canola, pea, and tomato [136]. Similarly, compounds like alamethicin, trichokonin, and trichovirin II have been reported as resistance inducer in plants and elicit jasmonic acid and salicylic acid in lima bean to enhance plant defense [137–139]. Several endophytes have been reported in disease mitigation such as *Acremonium zeae*, *Botryosphaeria ribis*, *Clonostachys rosea*, *Colletotrichum gloeosporioides*, nonpathogenic *Colletotrichum magna*, *Colletotrichum* sp., *Fusarium* sp., *F. verticillioides*, and *Xylaria* sp. [131, 140–143]. The co-inoculation of endophytes showed more positive results in plant protection and plant growth promotion activities. Waqas et al. [83] demonstrated that fungi *Penicillium citrinum* and *Aspergillus terreus* showed control of harmful effects of *Alternaria alternata* in leaf spot and blight disease of sunflower. They found that besides plant protection, endophytes induced the plant promotion by enhancing plant nutrient uptake [144]. *Penicillium citrinum* and *Aspergillus terreus* also produce siderophores which chelate the iron and activate the plant defense mechanism [145]. Endophytes induce the salicylic acid and jasmonic acid pathways and induce the plant defense against a broad range of plant pathogens. The co-inoculation of endophytes also influences the catalase activity, free amino acid production, ascorbate peroxidase, glutathione content, and NADPH oxidases which regulate the programmed cell death system [146, 147]. Cosoveanu et al. [148] isolated endophytes from grapevine cultivars from the Canary Islands and found bioactive endophytic strains such as *Alternaria* sp., *Acremonium strictum*, *Aureobasidium pullulans*, *Bionectria ochroleuca*, and *Chaetomium spirochaete* against targeted phytopathogenic fungi *B. cinerea* and *F. oxysporum* in in vitro bioassays. Cosoveanu et al. [149] found antifungal compounds producing endophytic fungal strains *Penicillium*, *Aspergillus*, *Fusarium*, and *Chaetomium* in *Musa acuminata* "Dwarf Cavendish." They measured antifungal activity against phytopathogens *Fusarium oxysporum* f. sp. *lycopersici*, *F. moniliforme* (Sheldon), *Alternaria alternata* (Fr.) Keissl, *Geotrichum* sp., *Phoma* sp., and *Cladosporium* sp. Previously, Cosoveanu et al. [148] isolated endophytes *Acremonium strictum*, *Alternaria* sp., *Bionectria ochroleuca*, and *Chaetomium spirochaete* in grapevine cultivars from the Canary Islands and found antifungal activity against *Alternaria alternata*, *Fusarium oxysporum*, and *Botrytis cinerea.* Recently, Santos et al. [150] isolated endophytes from passion fruit plants *Passiflora edulis f. flavicarpa* and were identified as *Phyllosticta* sp. and *Cercospora beticola* which produce steroid- and triterpenoid-type secondary metabolites against pathogenic bacteria.

Pestalotiopsis jesteri, an endophyte of *Fagraea bodenii*, produced jesterone and hydroxyjesterone compounds which showed anti-oomycetes activity against fungal pathogen [110]. Similarly, ambuic acid was found in *Pestalotiopsis microspora* which showed activity against the fungal pathogen *Pythium ultimum* [111]. Endophytic fungus *Alternaria* sp. Samif 01 isolated from medicinal plant *Salvia miltiorrhiza* produces alternariol-9-methyl ether (AME), a dibenzo-α-pyrone analog

found active against bacteria, fungus, and nematodes [78]. Endophytes directly inhibit the plant fungal pathogen by direct competition for space and nutrition. Initially, Stierle et al. [151] reported that endophyte *Acremonium strictum* of *Pennisetum* sp. plant showed inhibition of *Alternaria alternata.* Similarly, *Aureobasidium pullulans* showed inhibited *Botrytis cinerea* and *Monilinia laxa* in the postharvested crop [152]. Endophytes also produce compounds responsible for insect repellents such as ergonovine, chanoclavine, ergovaline, ergocristine, and ergocryptine, which affect the development of *Spodoptera frugiperda* [153].

5 Advantages of Endophytes

Fungal endophytes are symptomless microorganisms which are present in all living plant species reported so far. Most of the fungal endophytes develop a symbiotic relationship with their host plants by colonizing in the internal tissues; this symbiotic association made them valuable tool for agriculture in improving crop health. Natural compounds from endophytes and their characteristic as plant growth promoter or antagonistic activity made them an alternate source of chemical pesticide and fertilizers. The infection of endophytes influences the rate of root colonization and induced systematic resistance in the plant and increases disease control capacity. The endophytic infection increases the microbial root colonization in plants by inducing production of root exudates which attract the rhizospheric microbes; therefore it makes convenient to degradation of complex minerals into simpler form and facilitates mineral transportation smooth from soil to plant. In contrast, chemical fertilizer or pesticide makes a detrimental impact on microbes and destroys untargeted microbial communities, which are responsible for mineralization of complex metal compounds and disturb the balance of biogeochemical cycles in soil environment.

The use of endophytes with a chemical pesticide can provide a synergistic effect in crop protection. The rapid action of chemical pesticide can control the vast disease instantaneously which can further control by endophytic action continuously. The use of endophytes in integrated pest management can reduce the cost of crop protection. The role of endophytes in plant growth, stress tolerance, plant protection, seed germination, environmental balance, and pest resistance makes them a valuable agri-asset for sustainable agriculture practices.

6 Challenges in Commercialization of Endophyte-Based Agriproducts

The research on endophyte isolation and screening for bioactive compounds has been going on for many decades. Some endophytes have been shown to handle potential as medicine and for plant protection. The yield of bioactive compounds for commercial production is a major subject to be addressed for commercialization. The yield of bioactive compounds from endophytes depends on many factors like

endophytic habitat and other surrounding communities present nearby the endophytes which play a significant role in the production of bioactive compounds such as pestacin, hydroxyjesterone, cytosporone B, phomoxanthone A, etc. The process development for these bioactive compounds' production using fermentation technology is in infant stage [154]. Most of the fermentation processes have failed in constant production of bioactive compounds from endophytic fungi due to repetitive subculturing under an axenic condition which affects the continuous expression of genes. Repetitive subculturing under an axenic condition reduces the substantial production of bioactive metabolites which is the key challenge in front of the biotech industry. Still, more research is needed to understand the mechanism of bioactive compound production in endophytes. The activity of endophytes was also found to be site and host specific and requires appropriate niche for their optimized production and activity. Different types of cross talks among endophytic bacteria, virus, fungi, and host plant cell are required for optimized production of bioactive metabolites in their niche. Correct identification of endophytic fungus is also a major problem due to their pleomorphic nature, which means many endophytes occur in two different phases in plant and outside the plant. Some of the endophytes were found in anamorph and teleomorph stage in planta and in vitro, respectively. In addition to habitat, some legal issue also arises for commercial production and import-export of endophyte-based products. The patent on the endophyte *Piriformospora indica* (application no WO 99/29177) restricted the commercial production of this endophyte without technology transfer in different countries [155].

7 Conclusion

Endophytes play a significant role in plant growth and plant protection. Endophytes reside in the plant tissue during their life cycle and show a mutualistic relation with their host. They produce many bioactive compounds which interact with plant defense pathway and induce resistance against pest/pathogen. It has been proven that endophytes are capable of inducing systemic resistance in the plant. In addition to biotic stress, endophytes also confer plant resistance to different abiotic stress such as temperature, salinity, waterlogging, nutrient limitation, and extreme pH conditions. The use of endophytes as biocontrol agents can provide sustainable solution for pest resistance problem. The use of endophytes in agriculture would be helpful to maintain environmental balance by protecting untargeted organisms, which is destroyed by uncontrolled use of chemical fertilizers and pesticide. The use of endophytes in agriculture retains soil fertility by promoting rhizosphere and phyllosphere communities, but, still, their commercial production required further research to increase the production of bioactive compounds at the industrial level, which is dependent on circumvent network of microorganism and host plant. Translation of multistep cascade pathway of endophytic secondary metabolites from the laboratory to successful industrial process is somewhat challenging.

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A Thorough Comprehension of Host Endophytic Interaction Entailing the Biospherical Benefits: A Metabolomic Perspective

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Contents

Abstract

Endophytism is the phenomenon of *in planta* residency and mutualistic association of microbes with hosts without causing any disease symptoms. The

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multifaceted attributes of endophytes include plant growth promotion as well as resistance of the host to several forms of abiotic or biotic stresses. Moreover, endophytes are reported to manipulate the rhizospheric microbiota as well as the microbiota present within plants so as to amplify the host beneficial mechanisms. Endophyte mediated host beneficial traits become far more significant owing to the differential recruitment of endophytes by host under varying root exudate profile, host's age, as well as host-endophyte compatibility. However, in spite of such beneficial attributes, our understanding of endophytes is still quite limited and inadequate. Thus, the true potential of endophytes can be particularly harnessed when we gain a thorough insight on the molecular mechanisms responsible for mutualistic host-endophyte interaction. In this chapter, we present an exhaustive investigation of endophyte-plant interaction, beginning from chemotactic attraction of the supposed endophytic microflora from soil to establishment of endophytism. We will also focus on the endophyte-directed metabolite biosynthesis aiding in effective host functioning.

Keywords
Endophytes · Host-microbe interaction · Secondary metabolites · Endophytic diversity · Pharmaceutical benefits

1 Introduction

An incessant upsurge in global population requires an augmented agricultural productivity. However, a gradual decrease in arable land and poor land management practices has led to the search for plausible alternatives [1]. The use of plant growth-promoting rhizobacteria (PGPR) as probable biofertilizers is a potentially promising technique to ensure food security [2–4]. However, resistance posed by the indigenous rhizospheric microflora as well as environmental stress factors lead to difficulty in colonization of the inoculum *in planta*. In this context, a thorough understanding of host-microbe interaction is required for better implementation of microbes in farming as well as enhancement of sustainable agriculture.

Endophytes may be classified as a subgroup of PGPB living within plant tissues for certain parts of their life cycle. Endophytes during the course of their colonization systemically prime the host immune system so as to mount an augmented defense response upon any form of biotic or abiotic stress [5]. A mutualistic association exists between the host and their existing endophytes with the endophytic partner gaining nutrient access from the host while the latter gaining an augmented immunity from the colonized endophytic community [6, 7]. One example of an enhanced immune system includes the production of host secondary metabolites in an integrated manner with the residing endophytic community, possessing not only an amplified biotic resistance capability but also with other interesting bioactive properties [8]. In this context, harnessing the potential of endophytes for synthesis of secondary metabolites with several biotechnological applications has garnered significant focus [9]. Though there is an abundance of literature discussing the ability of

endophytes to synthesize host metabolites, yet a comprehensive detail entailing the entire process of endophytism to metabolite production needs to be thoroughly comprehended.

In the current chapter, we aim to encompass the entire process of endophytism, initiating from the screening of endophytic microbes and the traits allowing successful invasion and endophytic colonization into heterogenous communities. A further comprehension of the endophytic ability to overcome host defense responses would be taken up in detail followed by the adjustments made by the host to accommodate the endophytic community. Finally, the diversity of endophytes will be studied in detail followed by the various uses of endophytic microbe synthesized secondary metabolites.

2 Recruitment of Endophytes by Host

Release of root exudates by plants acts as chemotactic signals for recruitment of endophytes. Phytoexudates act as quorum-sensing signals for inter and intraspecies signaling within the rhizospheric region. Acyl homoserine lactones act as the autoinducer of gram-negative bacteria, while peptide pheromones act as the signaling molecule for gram-positive bacteria [10]. Recruitment of endophytic microbes is a discerning process wherein the hosts engage in a screening mechanism for the process. According to [11, 12], the initial screening commences at the rhizospheric region wherein the presence of carbon-rich molecules and antimicrobial compounds in root exudates differentially stimulate the proliferation of beneficial microbes while simultaneously suppressing the harmful ones. Microbes surpassing the initial screening are further assessed on their ability to bind to the rhizoplane region [13]. During this procedure, the microbes finally capable of binding to the root surface region are further allowed access to the host interior [12]. Prior to attachment, host-microbe recognition is performed by a type III protein secretion system released into the host cells [14]. Following recognition, type IV pili are primarily responsible for attachment of endophytic bacteria to the host surface. In case of gram-negative bacteria, exopolysaccharides (EPS) and lipopolysaccharides (LPS) act as surface components for attachment and colonization.

Finally, post-colonization of the host endosphere region, the phyto-immune system decides upon the fate of residence of the colonized microbes in the plant interior [15]. For instance, according to [16], a comparative increase in the concentration of gammaproteobacteria was observed in the host endosphere with a subsequent decline in the population of *Acidobacteria*, *Gemmatimonadetes*, and Archaea. The principle behind the screening process may be explained as the microbial competition or cooperation processes presiding under distinct pH, nutrient conditions, and oxygen concentration in the root endosphere [17, 18]. Another prominent factor affecting the initial stage of screening process is the production of indole acetic acid by rhizospheric microbes which not only enables enhanced root colonization but also weakens defense mechanism of the host thereby facilitating *in planta* colonization. Further, according to [19], presence of secreted IAA leads to

augmented biofilm formation due to enhanced trehalose, lipopolysaccharide (LPS), and exopolysaccharide production (EPS), which not only enables higher host colonization and biofilm formation by the proposed endophytes but also an augmented host resistance against various environmental factors.

3 What Makes a Microbe an Endophyte?

Genes responsible for endophytic colonization coincide with that of the rhizospheric microbiota since both the lifestyles share a variety of mechanisms. In one of the studies by [20], the set of common genes between both the types were subtracted from the endophytic variety thereby leaving the genes particularly responsible for endophytic behavior. One of the basic set of genes responsible for endophytic colonization is the resistance nodulation and cell division family (RND) efflux system membrane-associated genes. While Type I (TSI) and Type II (TS2) are commonly prevalent in majority of the endophytic bacteria, Type III and Type IV are mostly found in cultures with pathogenic traits. However, [21] reported the presence of the Type III and Type IV genes in eleven endophytic bacterial strains. In yet another report by [22], Type III and IV gene products were found to be essential for colonization by *Bradyrhizobium* sp. SUTN9-2. In this context, [16] hypothesized silencing of the genes responsible for pathogenic traits in the endophytic colonizers thereby facilitating host acceptance. For instance, the flagellin-sensing system flg22-Flagellin Sensing 2 (FLS2) present in grapevine recognizes and differentiates the flagellin-derived epitopes of beneficial endophytic bacterium from that of a pathogen [23].

Apart from the genes responsible for colonization, secretory genes, such as transporter proteins (ATP-binding cassette) transporters and MFS transport systems, are responsible for uptake of carbohydrates and proteins from the external environment [24]. Further, resistance nodulation and cell division family (RND) efflux system genes are also required for endophytic colonization. According to [24], the MFP subunit of the RND system was reported to play an active role in colonization of the endophyte *Enterobacter* spp. 638.

Post colonization, endophytic microbes typically encode proteins, such as glycoside hydrolases (e.g., trehalases) which aids in its sugar utilization [24]. In this context, [25] reported that members of the endophytic niche encode the protein cupin which aids in modification of host cell wall carbohydrates for enhanced utilization of the substrates responsible for host growth and development. However, interestingly, microbial endophytes were not found to possess enzymes related to cell wall degrading enzymes, such as cellulases/hemicellulases.

Another set of genes particularly conserved in endophytic genome are the various transcriptional regulators, such as AraC, FrmR, AsnC, LrgB, LysR, DeoR, and WrbA. While the AsnC regulator operates in presence of branched chain amino acids, AraC is primarily involved in metabolic pathway regulation and defense management [26]. The LysR family of proteins regulate overall bacterial metabolic pathway as well as quorum sensing and motility toward the chemo attractants [27].

FrmR family of proteins is however still not fully characterized. Hyeon et al. [28] reported FrmR proteins as a transcriptional regulator aiding in negative regulation of carbohydrate metabolism. Similarly, DeoR was reported to possess an analogous function to that of FrmR [29]. Though the exact role of the transcriptional regulators in host-endophyte interaction is still not clear, yet the regulators are reported to express their significant assistance during adjustment of the endophyte within the host interior. All in all, the regulators intricately modulate the host defense response that might pose a threat to the colonizing endophyte, thereby enhancing their effective colonization [20].

The host-stimulated defense mechanism involving production of reactive oxygen species upon colonization of endophyte as well as the oxygen-rich environment of the host interior is countered by a protective enzyme system pathway of the microbe, involving glutathione synthesis and reductase-related genes [24].

Apart from the aforementioned genes, dehydrogenases are also reported in endophytic genome which aid in transfer of protons from substrates during their transfer across membranes as well as for maintenance of the membrane redox potential [24].

4 Diversity of Microbial Endophytes

An enormous diversity of endophytic microorganisms exists in environment with only a miniscule portion being explored till date [30, 31]. A plethora of reports suggest presence of diverse bacterial endophytes in agronomically important host plants ranging from *Bacteroidetes*, *Firmicutes*, *Actinobacteria* to *Proteobacteria* [32, 33]. However, primarily gram-negative bacteria occupy the bulk portion of the endophytic niche, such as *Pseudomonas putida*, *Azospirillum fluorescens*, and *Azospirillum lipoferum*, and potential nitrogen fixers, such as *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, and *Rhizobium* [34].

Earlier, studies related to endophytic diversity primarily employed cultivation-based methods. However, molecular approaches applied in recent studies unfolded entirely different perspectives of endophytic diversity unknown hitherto [32]. A number of techniques including length-heterogeneity PCR, genus-specific PCR, and taxon-specific real-time PCR are applied to elucidate the presence of bacterial endophytes in host. Romero et al. [33] elucidated the efficiency of 16S-rRNA pyrosequencing approach in studying the endophytic bacterial composition in leaves of *Solanum lycopersicum*. Similarly, assessment of endophytic diversity has unleashed a hyperdiversity in fungal taxa which comprise of a significant and valuable fraction of fungal community biodiversity that affect host metabolism and are of significant therapeutic and economic value [35]. Most of the endophytic fungi belong to the Ascomycetous group and are anamorphic in nature.

Significantly, environmental biodiversity affecting host survival also affects the biodiversity of inhabiting endophytes [36]. For instance, temperate regions uphold large number of endophytic species in comparison to tropical regions [37]. Another feature of marked prominence is the ability of plants surviving harsh ecological

conditions due to their inhabiting endophytes. For instance, 12 different species of endophytes were obtained from mangrove forests surviving in tidal shallow of sea border [38]. Approximately, 347 endophytic fungal strains were isolated from *Rhodiola crenulata*, *R. angusta*, and *R. sachalinensis* which are perennial herbs of Crassulaceae family inhabiting high altitude of Arctic and mountain region of Asia and Europe [39].

Apart from geographical diversity, different portions of the host plant also play a significant role in endophytic diversity. For instance, roots, being the storehouses of metabolites, support inhabitation of diverse endophytes [40, 41]. However, leaves are also reported to exhibit significant diversity of endophytes as they possess less infection barriers compared to other tissues [42]. For instance, while *Salvia* sp. of Lamiaceae family was reported to inhabit 18 strains in roots and 58 strains in leaves, approximately 55 endophytic isolates were isolated from the leaves of *Macleaya cordata* [43]. Apart from roots and leaves, endophytic fungal strains have also been isolated from fresh bulbs of *Fritillaria cirrhosa* [44]. Similarly, [45] reported *Fusarium oxysporum*, *Pestalotiopsis uvicola*, and two other endophytic strains from bark and leaves of *Ginkgo biloba* of Ginkgoaceae family.

5 Adjustment of the Phytomicrobiota

The phytoecosystem represents a perfect example of the intricate relationship between host and its associated microbial community. The initiation of the relationship commences from the rhizospheric region where multiple factors, such as presence of carbon-rich root exudates, rhizospheric pH, etc., determine chemotaxis of the rhizomicrobiota toward the host [46]. Apart from the physical aspects, temporal effects also lead to a resplendent amelioration of the rhizospheric community. For instance, a gradual increment in the sugar, amino acids, and phenolic component of root exudates enhanced the actinobacterial and proteobacterial community in the rhizospheric region [47]. Similarly, loss of ABC transporter gene product aiding in rice root exudation led to a significant change in the rice rhizospheric microbial community [48].

Apart from the host influence, microbial superficial structures also contribute significantly to the fine-tuning of host endomicrobiota. For instance, *Rhizobium* sp. IRBG74 with alterations on its superficial structures is unable to colonize their host, *Sesbania rostrata*. The superficial structures are recognized by LysM receptors present on the host cell membranes, aiding in the recognition of microbial ligands, such as nodulation (Nod) factors of rhizobial species, chitin of fungal microbes, and exopolysaccharides (EPS) of bacterial colonizers.

Post-colonization of the endomicrobiota, adaptation of the host against phytopathogen infection, occurs by a variety of pathways, such as inhibition of access of host secondary metabolites to pathogens. For instance, phosphorylation of sugar transporter 13 (STP13) protein of the host beneficial bacterial

microbiota by BAK1 (Brassinosteroid insensitive 1-associated receptor kinase 1) augments host immunity thereby augmenting uptake of self-synthesized monosaccharides from the apoplastic region to outcompete pathogenic colonization [49].

Amendment of the colonizing beneficial endomicrobiota by the host has been appropriately explained as a process of adaptive gene loss, precisely termed as the "black queen hypothesis" [50, 51], which suggests gradual plausible gene loss as well as modification. In this context, it may be hypothesized that during coexistence in a spatially and temporally homogenous environment, a gradual transition occurs from competition to a dependency relationship. Gradually the selection pressure enables the deletion of genes responsible for individual existence. Also, smaller genome size ensures greater adaptability as compared to the contrary [52]. Further, metabolite synthesis by both the partners is also altered according to the common requirement, probably forming the ultimate basis of endophytism [50].

6 Endophyte-Induced Production of Bioactive Metabolites

Endophytes are reported to produce a wide range of bioactive metabolites with numerous applications including agriculture, medicine, food, as well as cosmetic industries during their course of interaction with the host system [34] (Fig. 1). These bioactive metabolites are low molecular weight natural products of enormous structural diversity [53]. Fungal endophytes produce largest number of secondary metabolites in comparison with any other class of endophytic microorganism [54]. Bioactive metabolites produced by fungal endophytes can be classified according to different structural groups such as alkaloids, terpenoids, steroids, quinones, phenols, coumarins, glycosides, benzopyranones, terpenoids, peptides, chinones, xanthones, phenylpropanoids, isocoumarins, lignans, tetralones, polyketides, flavonoids aliphatic metabolites, lactones, etc. [55]. These metabolites can be produced by endophytic fungi together with host plant as well as alone. Some of the endophytic fungi also produce enormous class of phytochemicals, i.e., secondary metabolites of plants which includes paclitaxol [56], podophyllotoxin [57, 58], deoxypodophyllotoxin [59], camptothecin and structural analogs [60–62], hypericin, emodin [63, 64], and azadirachtin [65]. Sesquiterpenes, diterpenoids, triterpenoid, and polyketide are frequently purified secondary metabolites possessing antimicrobial property. Among a wide variety of structural and chemical diversity of metabolites, some of the secondary metabolites released by endophytic fungi are mentioned below [66]:

- Sesquiterpenes including trichodermin, eremophilane sesquiterpenes, phomenone, 8a-acetoxyphomadecalin C, phomadecalin E, cycloepoxylactone, cycloepoxytriol B, 3,12-dihydroxycadalene(canine sesquiterpenes), and 1a-10a-epoxy-7a-hydroxyeremophil-11-en-12,8-b-olide are structurally related

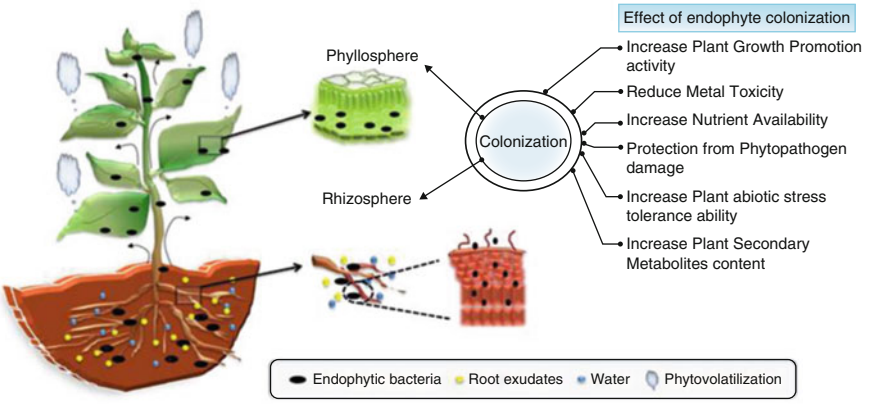

Fig. 1 A brief outline highlighting endophytic colonization in planta followed by release and utilization of secondary metabolites for several phytobenefits

to eremophilanolide sesquiterpenes, heptelidic acid, hydroheptelidic acid ENREF-28, chokols- and benzofuran-carrying normonoterpene derivatives, such as 5-hydroxy-2-(1-oxo-5-methyl-4-hexenyl) benzofuran and 5-hydroxy-2-(1-hydroxy-5-methyl-4-hexenyl) benzofuran.

- Diterpenes including paclitaxol containing taxane ring with a four-membered oxetane ring and a C-13 ester side chain. Fusicoccane diterpenes, periconicin A and B, pimarane diterpene sordaricin (aglycon of sordarin) and diaporthein B (a novel diterpenoid guanacastepene), scoparasin B, tremorgenic indole diterpenes, asporyzin C, and diterpene CJ-14445.
- Triterpenes includes helvolic acid which is a nordammarane triterpenoid.
- Alkaloids including amine and amides such as peramine, ergot alkaloids, phomopsichalasin, phomoenamide, and cryptocin pestalachloride A.
- Indole derivative such as loline alkaloid saturated 1-aminopyrrolizidine with an oxygen bridge.
- Phenolic compounds including 2-methoxy-4-hydroxy-6-methoxymethylbenzaldehyde, p- hydroxyl benzoic acid, p-hydroxyphenylacetic acid, altenusin, tyrosol, p-coumaric acids, colletotric acid, and cytonic acid A.
- Isocoumarin derivatives such as (R)-Mellein.
- Flavonoids such as tricin and related flavone glycosides.
- Lignans such as podophyllotoxin and aryl tetralin lignin.
- Aliphatic compounds including brefeldin A, pestalofone C, pestalofone E, and gamahonolide A and B.
- Polyketides including tetrahydroanthraquinones (e.g., 6-*O*-methylalaternin and altersolanol A), pentacyclic spiroketals, rugulosin (bis-anthraquinoid pigment), nodulisporins, pyrrocidines, isofusidienol, chaetoglobosins, pestalotheol C, pentaketide CR377, xanalteric acids, and pestalachloride B.
- Peptides including leucinostatin A, echinocandin A, and cryptocandin.

7 Applications of Endophyte-Induced Bioactive Metabolites

Evolution of the endophytic microbiome along with the host due to incorporation of genetic information from the latter plays a major role in production of bioactive metabolites [67]. Studies suggest application of endophytic metabolites as immune-suppressants, agrochemicals, antioxidants, antibiotics, antiparasitic, and anticancer agents [54, 67–70]. In various countries, a number of modern registered drugs have been developed by traditional herbal products, and their efficacy has been proved by clinical trials [71–74]. Among the various benefits conferred by the secondary metabolites released by host endophyte association, few well-studied applications in various fields for both are mentioned below.

7.1 Host Immunity Augmentation

Secondary metabolites released by host endophyte interaction play an essential role in environmental adaption and defense system enhancement of the host. Hence, these compounds may also be termed as plant-defensive compounds generated by the host plants in response to pathogens including fungi, bacteria, virus, nematodes, etc., herbivorous insects, mammals, and even abiotic factors [75]. These biologically active molecules specifically target neuroreceptors, neurotransmitters, ion channels and pumps, enzymes involved in various pathways, and even the components of cytoskeleton [53, 76]. In some cases, these secondary metabolites also act as UV protectants, antioxidants, and signal compounds for pollinators and seed dispersing animals [77]. Some metabolites are commonly secreted by all endophytes such as 5, 8 dimethyl quinolone, which form irreversible complex of nucleophilic amino acids thereby hindering the growth of microbes [78]. These endophytes also release phytohormones to face the environmental competition and develop more resistance against biotic and abiotic factors [67].

Accumulations of plant-specific secondary metabolites are favored by some endophytes during stress phase. For instance, production of different bioactive molecules such as alkaloids, diterpenes, flavonoids, and isoflavonoids is particularly promoted during encounter of stress conditions [79]. In this context, *Ralstonia*, *Rhizobium*, *Bacillus*, *Acinetobacter*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, *Enterobacter*, etc. are few endophytes that confer the resistance to the host plant [80]. Endophytes residing on the outer layer of plant such as *Phomopsis* synthesize and deposit DOPA (3,4-dihydroxyphenylalanine), a type of melanin pigment on their hyphal structure which provides assistance to the host to survive under harsh environmental conditions [81]. Likewise, *Fusarium* sp., an endophyte of *Azadirachta indica*, produces tyrosinase, thereby enhancing melanin production, a key factor responsible for wound healing and generation of immune response [82].

7.2 Pharmaceutical Application

Owing to the catastrophic effects of antibiotics on human health, a drastic shift in focus toward phyto-pharmacology, phyto-medicine, and phyto-therapy has resulted in the current era. Plant extracts or their different bioactive compounds are currently employed for treating human disease and also for enhancing animal production and health in an ecofriendly approach [83]. Numerous endophytes such as *Aspergillus niger*, *Macrophomina* spp., *Trichoderma* spp., and *Penicillium* spp. produce a huge range of various bioactive compounds with human health benefits and hence may be utilized as plausible alternatives for production of novel drugs [84] (Fig. 2).

Javanicin, produced by *Chloridium* sp. inhabiting roots of *Azadirachta indica*, expressed antimicrobial, antifungal activity against various human pathogens such as *Candida albicans*, *Escherichia coli*, *Bacillus* sp., *Pseudomonas aeruginosa*, and *P. fluorescens* [85] (Table 1). Similarly, precursors of anticancer drugs topotecan and irinotecan were obtained from endophytic *Fusarium solani* inhabiting *Camptotheca acuminate*plants. These drugs are particularly effective against brain tumors, liver cancer, cancer of gastrointestinal tract, leukemia, and other cancers as they inhibit DNA topoisomerase I [86]. Likewise, *Aspergillus fumigatus* inhibiting the medicinal shrub *Juniperus communis* produce deoxypodophyllotoxin which can be converted into podophyllotoxin or 6-methoxypodophyllotoxin. Podophyllotoxins also is produced by *Fusarium oxysporum* inhabiting *Juniperus recurva* and *Phialocephala fortinii* inhabiting *Podophyllum peltatum.* These compounds act as precursor of numerous important anticancer drugs such as etopophos phosphate, etoposide (VP-16), teniposide (VM-26), GP-7, NK-611, etc. [87, 88]. Wagenaar et al. [89] reported antitumor activity by three new cytochalasins: cytochalasin H, cytochalasin J, and epoxycytochalasin H along with cytochalasin E obtained from

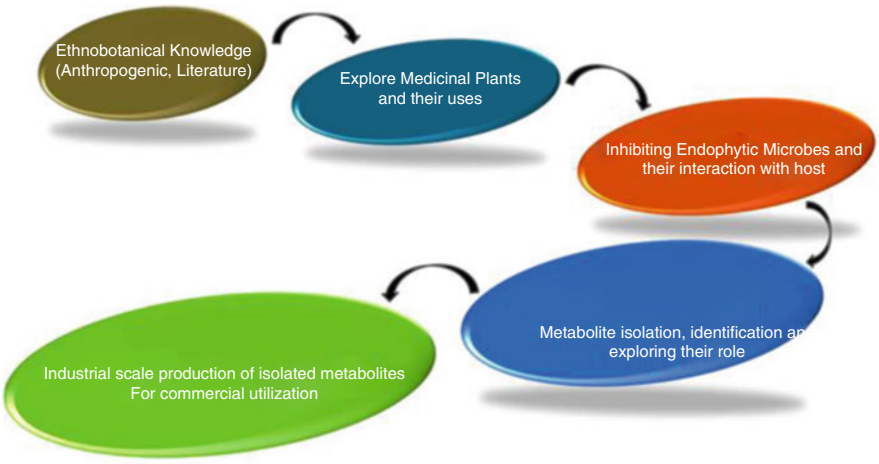

Fig. 2 Scale-up of endophyte mediated metabolite production and their utilization for several biotechnological applications

Table 1 Microbial endophytes as sources of bioactive compounds

S no.	Microbial endophytes	Host plant	Bioactive compound	Reference
1	*Fusarium* spp.	*Catharanthus roseus*	Vincristine, vinblastine	[102]
2	*Fusarium oxysporum*	*Ephedra fasciculate*	Beauvericin	[103]
3	*Fusarium oxysporum*	*Cylindropuntia echinocarpa*	Bikaverin	[103]
4	*Alternaria* spp.	*Polygonum senegalense*	Alternariol	[104]
5	*Xylaria* spp.	*Licuala spinosa*	Eremophilanolides	[105]
6	*Aspergillus* spp.	*Gloriosa superba*	6-methyl-1,2,3-trihydoxy-7,8-cyclohepta-9,10-diene-7-acetamide	[106]
7	*Cladosporium cladosporioides*	*Azadirachta indica*	Tetranor triterpenoids	[107]
8	*Chaetomium* spp.	*Salvia officinalis*	Cochliodinol	[108]
9	*Streptomyces* spp.	*Alpinia galanga*	3-methyl carbazole	[109]
10	*Aspergillus fumigatus*	*Melia azedarach*	12β-hydroxy-13α-methoxyverruculogen TR-2 (6)	[110]
11	*Fusarium granarium*	*Linum album*	Lignan	[111]
12	*Aspergillus tenuis*	*Taverniera cuneifolia*	Saponin	[112]
13	*Piriformospora indica*	*Artemisia annua*	Sesquiterpene lactone	[113]
14	*Trochoderma atroviride*	*Salvia miltiorrhiza bge*	Diterpene	[114]
15	*Aspergillus* spp.	*Cameroonian*	*n*-acetyl-D- glucosamine	[115]
16	*Xylaria* spp.	*Morus cathayana*	Presilphiperfolian sesquiterpene	[116]
17	*Fusarium solani* JK 10	*Chlorophora regia*	7-desmethyl fusarin	[117]
18	*Phomopsis longicolla*	*Dicerandra frustescens*	Dicerandrol A-C	[118]
19	*Muscodor albus*	*Cinnamomum zeylanicum*	1-Butanol 3-methyl acetate	[67]
20	*Penicillium janthinellum*	*Melia azedarach*	Citrinin	[119]
21	*Fusarium* sp.	*Maackia chinensis*	Fusapyridon A	[120]
22	*Alternaria* sp.	*Sonneratia alba*	Altenusin	[121]

(*continued*)

Table 1 (continued)

S no.	Microbial endophytes	Host plant	Bioactive compound	Reference
23	*Aspergillus* sp.	*Bauhinia guianensis*	Fumigaclavin C	[122]
24	*Pestalotiopsis mangiferae*	*Mangifera indica*	4-(2,4,7-trioxabicyclo heptane 3-yl) phenol	[123]

Rhinocladiella sp. inhabiting Chinese medicinal herb *Tripterygium wilfordii.* PTOX, a pivotal lignin produced by *Fusarium* strains inhabiting *Dysosma versipellis*, is an initiatory compound of CPH-82 used for the treatment of rheumatoid arthritis [90]. Taxol, structurally a diterpenoid, is a potent anticancer agent isolated from *Taxomyces andreanae* and *Metarhizium anisopliae* inhabiting *Taxus brevifolia* [91, 92]. Endophytic fungi *Entrophospora infrequens* residing in *Nothapodytes nimmoniana* produce camptothecin, structurally an alkaloid having antineoplastic property [60]. Vincristine obtained from the endophytes of *Catharanthus roseus* is used to treat acute lymphoblastic leukemia and nephroblastoma [93]. Similarly, pestacin, 1,3-dihydro isobenzofuran, and isopestacin, obtained from *Pestalotiopsis microspora* inhabiting *Terminalia morobensis*, are utilized as antioxidant agents due to their ability of scavenging both hydroxyl and superoxide ions [94, 95].

7.3 Biocontrol Activity

Phomopsis sp. YM 311483 was reported to produce five, ten-membered lactones possessing antifungal activity against *Aspergillus niger*, *Botrytis cinerea*, *Fusarium avenaceum*, *Fusarium moniliforme*, *Helminthosporium maydis*, *Penicillium islandicum*, and *Ophiostoma minus* [96]. Similarly, javanicin, isolated from root tissues of *Chloridium* sp. expressed antimicrobial, antifungal activity against various plant pathogens such as *Pythium ultimum*, *Phytophthora infestans*, *Botrytis cinerea*, *Ceratocystis ulmi*, *Fusarium oxysporum*, *Rhizoctonia solani, Verticillium dahliae*, and *Cercospora arachidicola* [85, 97].

Apart from antifungal potential, the endophyte *Geotrichum* sps AL4 inhabiting *Azadirachta indica* was reported to produce novel bioactive compound, such as chlorinated oxazinane derivate, an epimer of the former and two other known compounds with nematicidal activity against *Bursaphelenchus xylophilus* and *Panagrellus redivivus* [98].

Likewise, *Xylaria* sp. YC-10 inhabiting *Azadirachta indica* was found to exhibit insecticidal activity against *Plutella xylostella* due to release of 5-methylmellein, 5-carboxylmellein, hymatoxin C, hymatoxin D, halorosellinic acid, and cerebroside C [99]. Metabolites released from *Alternaria alternata* were reported to confer antifeedant, toxic, and immune-modulatory effects on tobacco caterpillar *Spodoptera litura* [100, 101].

8 Conclusion

Irrespective of the several contributions on endophytes, a thorough comprehension of endophytic establishment *in planta* commencing from invasion, establishment, and exploration of benefits still remains a realm quite unexplored. This chapter is expected to reveal the various facets of endophytic lifestyle as well as highlighting the plausible benefits availed from them. India, being an agriculture-based country in particular, requires the ardent need of plausible alternatives for yield improvement and nutritional benefit. This chapter will be a minor attempt to not only understand the basic fundamentals of endophytic colonization but will also enable the targeting of endophytic strains of high beneficial value with respect to sustainable agriculture and environment.

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Endophyte-Mediated Host Stress Tolerance as a Means for Crop Improvement

26

Satyabrata Nanda, Bijayalaxmi Mohanty, and Raj Kumar Joshi

Contents

Abstract

Plants being sessile are continuously exposed to a wide range of biotic and abiotic stresses that exert adverse effect in their growth and development. Various physiological, biochemical, and molecular machineries are employed by the

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plants to overcome these stresses. Endophytes are mostly the symbiotic fungi and bacteria that reside inside the plant tissue and stimulate plant growth during stress conditions. Endophyte-mediated plant stress tolerance holds significant role in the analysis of plant-microbe interactions. Although still at its infancy, the endophyte-mediated host stress tolerance including drought, salinity, high-temperature stresses, and pathogenic infection has been well described in the recent times. The molecular mechanism governing the endophyte-mediated stress response includes the induction of plant stress genes and regulation of reactive oxygen species. In the present review, we discuss the evidences for bacterial and fungal endophyte-mediated stress tolerance and associated mechanisms. This information from this review will help the scientific community in the development of suitable biotechnological approaches toward usage of endophyte microbes in the improvement of crop yield under multiple stress conditions.

Keywords

Symbiosis · Endophyte · Biotic stress · Abiotic stress · Stress-related genes · Fungi

Abbreviations

ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylate
AHK2	*Arabidopsis* histidine kinase 2
APX	Ascorbate-dependent peroxidases
CDK	Cyclin-dependent protein kinase
CDPK	Calcium-dependent protein kinase
CRE1	Cytokinin response 1
DHAR	Dehydroascorbate reductases
GR	Glutathione reductases
HR	Hypersensitive responses
IAA	Indole acetic acid
IAA	Indole-3-acetic acid
ISR	Induced systemic resistance
JA	Jasmonic acid
MAPK	Mitogen-activated protein kinase
MDHAR	Monodehydroascorbate reductases
PAMP	Pathogen-associated molecular patterns
PRR	Pattern recognizing receptors
ROS	Reactive oxygen species
SA	Salicylic acid
SAKA	Stress-activated mitotic kinase
SAR	Systemic acquired resistance
SOD	Superoxide dismutase
VOC	Volatile organic compounds

1 Introduction

Endophytes are the group of ancestral endosymbionts, primarily consisting of bacteria, fungi, and actinomycetes that reside for at least a part of their life cycle or throughout life without causing any harm or disease in plants across the arctic to tropical regions of the world [1]. They survive in different parts of the plant including healthy stems, roots, twigs, node, internode, petioles, bark, leaves, fruit, flower, and seeds without exhibiting any infection symptoms or severity in the host tissues [2]. Geological time scale has shown that plant and endophyte interactions have originated as early as the origin of the first group of higher plants on earth [3, 4]. The mutualistic symbiotic behavior of the endophytes and plants is demonstrated from the fact that the host plant gives the shelter and nutrients to endophytes while the later increases the survival chance of the host plant by increasing the tolerance effect to insect herbivory as well as biotic and abiotic stresses [5, 6]. Interaction between plants and microbes can be mostly classified as detrimental or neutral. In most of the cases, the interaction is considered as beneficial, because microbes consume the plants organic product for respiration and metabolism and at the same time help in nutrient recycling and tolerance against various stresses. Beneficial microbes encourage plant growth development and inhibit the plant diseases by enhancing different types of the mechanisms which mainly include production of growth regulators, hormones, and pathogen-inhibiting compounds. In rare cases, the endophytic fungi, bacteria, and viruses have detrimental effect on the host plants [7].

Endophytes have received significant importance in the recent times as they could affect the interactions of plants with their environment and alter the course of their interaction with infecting pathogens [8]. They have the ability to adapt in various adverse environments including nutrient-deficient conditions, heavy pollution, and rigid environmental conditions and produce huge amount of bioactive compounds that are essentially used in pharmaceutical industries [9–11]. Endophytic fungi not only inhibit the heat and light-mediated destruction of photosynthetic apparatus, but they also increase the number of photosynthetically active pigments in plants [12, 13]. Bacterial and fungal endophytes facilitate the biological degradation of dead plants which is essential for nutrient recycling and help in phytoremediation of polluted soil and water [14, 15]. Some of the endophytes exhibit a good metal sequestration and chelation system that helps in higher tolerance effect toward the heavy metals [16]. Volatile antibiotics secreted from endophytic fungi inhibit growth of the pathogen through mycofumigation [17, 18]. Endophytes may also increase host fitness and competitive abilities by increasing successful germination and growth rate or enhancing the absorption of nutritional elements by the host [19]. In spite of all these activities, the role of endophytes in crop improvement is still inconspicuous. Recent findings clearly show that endophytes may have an important influence in the regulation of plant growth and stress responses leading to augmentation of productivity. In this chapter, we aim at acquainting the readers with the generation characteristics of endophytes and specifically focus on those facets of endophyte research causing plant improvement through modulation of biotic and abiotic stress responses.

2 Response of Plants to External Stresses

Lack of mobility together with the absence of any specialized immune system in plants brings in numerous challenges for plants in the form of biotic and abiotic stresses. Often, the growth and development of plants gets compromised in overcoming such environmental stresses. With due course of evolution, plants have developed complex regulatory networks, which not only provide an appropriate defense response against specific stresses but also determine the pertinent resource distribution between plant development and defense. Several plants modify their outermost structures such as leaves into trichomes and spines and deposit epidermal layers of lignins, resins, and silica to limit pathogen invasions and discourage herbivory. A diverse repertoire of secondary metabolites further aid in plant defense responses [20].

As a second line of defense, plants can perceive various stress stimuli and can induce local and systemic defense responses [21]. Recognition of pathogens or the pathogen-associated molecular patterns (PAMPs) by the membrane-bound pattern recognizing receptor (PRR) channels activates the plant immune responses through the production of reactive oxygen species (ROS), hypersensitive responses (HR), programmed cell death, tissue reinforcement at the site of infection, and expression of defense-related proteins [21–23]. This kind of immune response is often referred to as the PAMP-triggered immunity (PTI). These induced local defense responses consequently lead to the establishment of immune response throughout the plant known as the systemic acquired resistance (SAR) [21]. Having said that, successful pathogen invasions often break down the PTI immunity in plants by secreting several effectors into plant cells. However, the plant resistance genes (*R* genes), mostly characterized as possessing the nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains meticulously recognize the pathogen effectors and thus trigger another type of immune response known as effector-triggered immunity (ETI) [22].

Plants possess a complex and intertwined networks of signaling pathways consisting of multiple signal molecules, phytohormones, and protein kinases. The earliest possible signaling event includes the perception of abiotic and biotic stresses through the induction of Ca^{2+} influx and ROS accumulations in the affected plants [21, 23]. Stress stimuli and presence of secondary signaling molecules like ROS can lead to the accumulation of several phytohormones including abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), gibberellins (GA), and ethylene (ET) [24]. Besides, a large number of plant kinases play crucial roles in plant defense signaling and provide a highly complex crosstalk network among all the players involved in defense response. Plant kinases including calcium-dependent protein kinase (CDPK), cyclin-dependent protein kinase (CDK), and mitogen-activated protein kinase (MAPK) have been reported in numerous studies to participate, modulate, and confer defense against many biotic and abiotic stresses in plants [25–29]. Thus, plant response to different stresses in their natural habitat involves the maintenance of balance and fine-tuning of these complex repertoire of defense strategies.

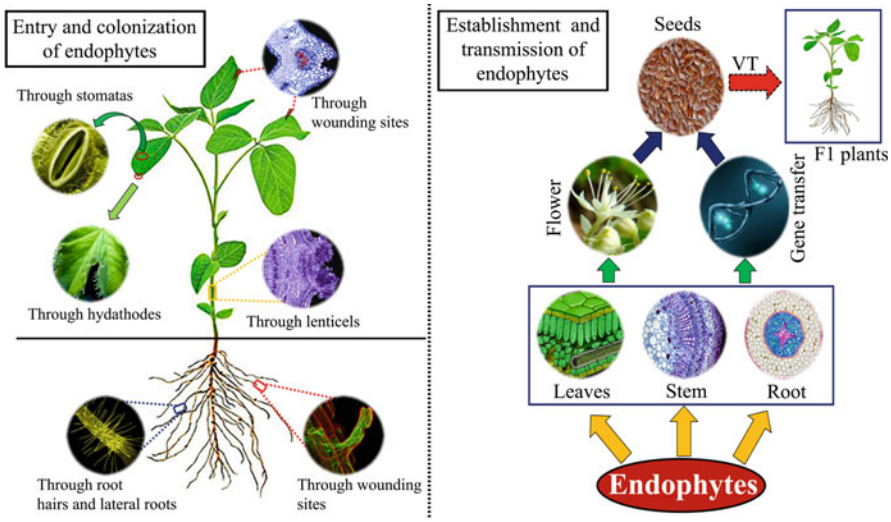

Fig. 1 (**a**) Schematics depicting the mode of entry and colonization of endophytes in host plants. (**b**) Strategies adopted by endophytes to establish in a host plant and transmitted into the host plant progeny. *VT* vertical gene transfer, *F1* first-generation progeny of a host plant

3 Endophytic Symbionts

Symbiosis is defined as the mutualistic, parasitic, or commensalic interactions between two organisms for throughout the life or some part of their life [30]. Endophytes are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and may produce beneficial substances for host [31]. Plants form mutualistic symbioses with a variety of microorganisms including endophytic fungi that live inside the plant and cause no overt symptoms of infection. The microbes enter into the plants through natural pores or wounds created by biotic stress and abiotic stresses and penetrate inside through the secretion of a wide range of cell wall-degrading enzyme such as cellulase, cutinase, protease, pectinase, hemicelluloses, and lignin peroxidase [32] (Fig. 1). The production of ROS and the subsequent activation of multiple cellular mechanisms including programmed cell death, necrosis, and systemic signaling help in maintaining the mutualistic behavior between the plants and the microbes. However, the plants' response to biotic and abiotic stresses due to the result of fungal, plant, or symbiotic metabolism is still poorly understood [33–35]. A fungal endophyte, *Neotyphodium coenophialum*, present in the *Lolium* grasses helps in preventing herbivory by producing different types of secondary metabolites [36]. Orchid mycorrhizal fungi provide necessary nutrients critical to symbiotic seed germination [37]. Continued interaction between plant and endophytes may also result in exchange of genetic material leading to sustained synthesis of microbial-derived bioactive compounds from generation after generation in the plant system [38, 39]. Establishment and

maintenance of this long-term association between plants and fungus is regulated by a group of stress-activated mitotic kinases (SaKAs). Deletion of SaKA gene(s) often converts the mutualistic interaction into pathogenic action. SaKA mutant fungi often result in developmental defects of plants including stunted growth, loss of apical dominance, and premature senescence [40]. MAP kinases (MAPKs) are another group of enzymes that are expressed during plant-microbe interaction. Activation of MAP kinase pathway only results in the accumulation of ROS which have greater role in the maintenance of abiotic stress tolerance and immunity against the invading pathogen and enhancing endophytic growth [41, 42]. Temperature regulation is another important aspect of symbiotic relationship between endophyte and plants. While both the plants and endophyte can tolerate high temperature up to 65 °C during symbiotic growth, they cannot survive above 40 °C under nonsymbiotic existence [43]. Besides, endophytic symbionts also help in crop improvement through production of antifungal, antibacterial, and nematicides which inhibits the pathogenesis in the crop field. Likewise, siderophore produced by the endophytic fungi helps in uptake of iron in low iron habitats and helps in providing nutrient supplements to the plants [44]. Overall, the symbiotic relationship between plants and microbes especially the fungi is highly significant for growth, development, and productivity of crop plants.

4 Endophyte-Mediated Plant Growth

Water, light, and nutrients are integral to the growth, reproduction, and survival of plants under different geographic regions. Often under natural conditions, the plants also face unfavorable and hostile conditions collectively known as abiotic stresses hampering homeostasis and growth. Being sessile, plants are exposed to a broad range of environmental stresses as well as stresses induced by other living and nonliving systems that they cannot escape. Extreme environmental conditions below or above the optimal levels often limit plant growth and development. Drought, low- or high temperature, salt stress and acidic conditions, heavy metal stress, nutrient stress, and starvation are the major abiotic stresses that affect the plants [45]. Biotic stresses include the damage done to plants by bacteria, viruses, fungi, parasites, harmful insects, weeds, and cultivated or native plants. Fortunately, many microorganisms including bacteria, fungi, and protozoa form beneficial or symbiotic association with plants which benefit them not only to overcome various environmental stresses but also support their growth and development. Although extensive studies have been performed in understanding the mutualistic interactions between plants and soil microbes, the interaction between plants and endophytes has only been lately realized. Plant-endophyte interactions have revealed that endophytes help the plants by promoting growth and enhanced defense responses, whereas the plants possess the ability to choose these valuable microbiomes allowing to colonize within its tissues [46, 47]. These endophytes significantly contribute to the regulation of many vital physiological processes and promote the overall plant vigor and growth. For instance, endophytic fungi facilitate the degradation of the cuticular cellulose

resulting in improved carbon uptake and promote seed germination [48]. Endophytes serve the growth and development of host plant by (1) assisting in the production of hormones and compounds, (2) aiding in acquiring essential nutrients, and (3) enhancing plant defense against biotic and abiotic stresses.

4.1 Production of Phytohormones and Other Growth-Inducing Compounds

Phytohormones are the regulatory molecules that are essentially involved in the growth, physiological processes, and defense responses in plants [49–51]. The colonized endophytes can effectively alter the phytohormonal homoeostasis, thereby accelerating the plant growth [52]. Most of the plant endophytes, especially those associated in plant roots, accelerate the plant growth and development via production of the auxin class hormone indoleacetic acid (IAA). Several physiological processes are influenced by IAA homeostasis including cell differentiation, seed germination, development of vascular tissues, root formation and elongation, vegetative growth, pigmentation, and photosynthesis [53]. Colonization of the plant-growth-promoting bacterial endophytes in *Solanum nigrum* and *Nicotiana attenuata* promoted root growth by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and elevated production of indole-3-acetic acid (IAA) in the former, whereas only by IAA accumulation in the later, respectively [52]. On the other hand, production of IAA can result in increased colonization efficiency of the bacterial endophytes, thus maximizing the endophyte-mediated plant growth [54]. In *Populus trichocarpa*, a diazotroph endophytic bacteria *Burkholderia vietnamiensis* produces IAA required for plant growth [55]. Similarly, Bal et al. [56] identified six endophytic bacteria from rice plants grown in the costal fields which produced IAA and ACC deaminase. Further, inoculation of the isolated bacterial strains into an indica rice cultivar "Naveen" resulted in noticeable plant growth enhancements with elongated roots and increased IAA production and ACC deaminase activity. In japonica rice cultivar Dongjin, the association of endophytic fungi *Paecilomyces formosus* conferred enhanced plant growth reflected by improved plant height, biomass, and chlorophyll content compared to plants having no endophytic associations [57]. Passari et al. [58] isolated BPSAC6, an endophytic bacterial strain of *Bacillu*s sp. from *Clerodendrum colebrookianum* which could produce three different phytohormones including IAA, kinetin, and 6-benzyladenine.

Although endophytes can promote the growth kinetics of plants via IAA production, they can also contribute in limiting the production of other phytohormones like GA, JA, and ABA to help the plants overcome various stresses [58, 59]. Often, GA-producing endophytic microorganisms contribute toward improved yield of the host plant. Hamayun et al. [60] isolated an endophytic fungi *Phoma herbarum* from the salt-stressed soybean plant roots, which showed promising plant growth-promoting characters resulting in increased biomass and elevated production of active GAs including GA1, GA3, GA4, and GA7. Similarly, endophytic fungi *Cladosporium* sp. isolated from cucumber have plant growth-promoting properties

through the production of several active and inactive GAs [60]. Further, in *Moringa peregrine*, two fungal endophytes *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13 isolated from the bark tissues were reported to enhance plant growth by producing bioactive GAs [61]. Besides, endophytes play key roles in regulating the cytokine and ET levels in plants. Ethylene has multitude roles in plants and actively participates in plant defense signaling. The elevated level of endogenous ET during stress response often negatively affects the growth and overall plant development [53, 62]. The endophytes having ACC-deaminase activity catabolize the ET precursor molecule ACC to 2-oxobutanoate and NH_3 resulting in a lower level of ET after neutralization of the pathogen attack [63]. The sunflower endophytic bacterial strains SF2, SF3, and SF4 were reported to enhance plant growth under water stress by producing SA [64]. Interestingly, in *Arabidopsis* and its endophytic fungus *Piriformospora indica* association, *P. indica* produces high levels of cytokinins. The trans-zeatin biosynthesis and the cytokinin response 1/*Arabidopsis* histidine kinase 2 (CRE1/AHK2) receptor combinations are essential for *P. indica*-mediated growth in *Arabidopsis*. *Arabidopsis* mutant lines having impaired CRE1/AHK2 receptor combination or lacking cis-zeatin resulted in reduced cytokinin levels even in colonized roots [65].

Endophytes assist their host plants growth by producing other kinds of growth-simulating chemicals apart from the phytohormones. These chemical entities range from metabolites like sugars and polyamines to volatile compounds. The bacterial endophyte *Methylobacterium extorquens* and the fungal endophyte *Rhodotorula minuta* in *Pinus sylvestris* plants produce adenines and adenine ribosides which serve as potential precursor molecules in cytokine biosynthesis and control the morphological attributes of the plant [66]. In *Arabidopsis*, exposure to endophytic volatile organic compounds (VOCs) including acetoin and 2, 3-butanediol resulted in enhanced plant growth and better defense responses as compared to the wild relatives [67, 68]. There are perhaps many more chemicals and bioactive compounds synthesized by the endophytes which remains to be discovered and characterized having significant involvement in plant growth development.

4.2 Role of Endophytes in Nutrient Acquisition

Endophytes play crucial roles in nutrient acquisitions in plants from the natural habitat. Many plants lack the natural machinery to acquire some essential nutrients. For instance, nitrogen is a major requirement for plants for its growth and survival. However, most of the plants can't use the atmospheric nitrogen and heavily rely upon supplies of nitrogenous fertilizers. Others make symbiotic associations with the nitrogen-fixating bacteria, mostly seen in legumes, which help the plants to utilize the atmospheric nitrogen. However, the colonization of endophytes is markedly different than those of rhizobial nitrogen-fixating symbionts. While the rhizobial bacteria colonize within the plant much similar to infections, the endophytes enter into plant roots via root junctions and wounding sites [69]. Plant growth-associated traits provided by different endophytic microorganisms are listed in Table 1. Unlike

Table 1 List of endophyte-plant associations leading to plant growth and development

Endophytes	Type	Host plant	Function
Azoarcus spp., *Azospirillum* spp., *Burkholderia* spp., *Paenibacillus* spp., *Micrococcus* spp., *Enterobacter* spp., *Leclercia adecarboxylata*, *Pantoea* spp., *Staphylococcus epidermidis*, *Pseudomonas* spp., *Bacillus* spp., *Stenotrophomonas maltophilia*, *Ochrobactrum* spp., *Sphingomonas yanoikuyae*, *Flavobacterium* spp., *Curtobacterium* sp., *Frigoribacterium faeni*, *Microbacterium* spp., *Acinetobacter* sp., *Staphylococcus cohnii*, *Sphingomonas* sp., *Rhizobium larrymoorei*, *Bacillus pumilus*, *Kocuria palustris*, *Pantoea ananatis*, *Methylobacterium radiotolerans*, *Methylobacterium fujisawaense*, *Xanthomonas translucens*, *Pantoea ananatis*, *Methylobacterium aquaticum*, *Sphingomonas melonis*, *Sphingomonas yabuuchiae*, *Micrococcus luteus*, *Acidovorax* sp., *Xanthomonas translucens*	Bacteria	*Oryza sativa*	Nitrogen fixation, ACC deaminase synthesis, production of phytohormones, enzyme production, phosphate solubilization, plant growth promotion
Actinobacteria, *Bacillus* spp., *Gammaproteobacteria*, *Paenibacillus* spp., *Firmicutes*, *Pantoea* spp., *Azospirillum lipoferum*, *Klebsiella pneumoniae*	Bacteria	*Triticum aestivum*	Plant growth promotion, IAA production, phosphate solubilization, nitrogen fixation

(*continued*)

Table 1 (continued)

Endophytes	Type	Host plant	Function
Acinetobacter spp., *Cronobacter* spp., *Burkholderia* spp., *Undibacterium*, *Pantoea* spp., *Sphingomonas* spp., *Limnobacter* spp., *Staphylococcus* spp., *Enterobacter* spp., *Escherichia* spp., *Serratia* spp., *Methylobacterium*, *Tsukamurella*, *Alcaligenes*, *Erwinia*, *Microbacterium*, *Rhodococcus* spp., *Bacillus* spp., *Azospirillum lipoferum*, *Klebsiella pneumoniae*	Bacteria	*Zea mays*	Plant growth promotion, ACC deaminase synthesis, IAA synthesis, nitrogen fixation
Acinetobacter, *Aeromonas* spp., *Agrobacterium radiobacter*, *Bacillus* spp., *Chryseomonas luteola*, *Enterococcus*, *Flavimonas oryzihabitans*, *Nocardioides*, *Paracoccus*, *Phyllobacterium*, *Sphingomonas* spp., *Serratia proteamaculans*	Bacteria	*Glycine max*	Plant growth, solubilization of phytate, IAA synthesis, ACC deaminase synthesis, acetoin and 2,3-butanediol synthesis
Tulasnella violea, *Epulorhiza repen*, *Trichosporiella multisporum*, *Beauveria* spp., *Fusarium* spp.	Fungi	*Dendrobium friedericksianum*	Promote seed germination, growth and propagation
Cladosporium sphaerospermum, *Alternaria alternata*, *Colletotrichum* spp., *Aspergillus niger*, *Cladosporium cladosporioides*, *Trichothecium roseum*, *Chaetomium cochliodes*, *Penicillium* sp.	Fungi	*Centaurea cyanus*, *Centaurea nigra*, *Papaver rhoeas*, *Plantago lanceolata*, *Rumex acetosa*, *Senecio vulgaris*	Growth promotion
Epichloë typhina	Fungi	*Dactylis glomerata*	Plant growth and photosynthesis
Diaporthe sp.	Fungi	*Cinchona ledgeriana*	Alkaloid biosynthesis

(*continued*)

Table 1 (continued)

Endophytes	Type	Host plant	Function
Neotyphodium oenophialum	Fungi	*Festuca arundinacea*	Secondary metabolite production
Epichloë festucae	Fungi	*Lolium perenne*	Iron homeostasis

rhizobial bacteria, endophytes also protect the oxygen-sensitive nitrogenase enzyme by adopting different strategies. Oxygen is present in low concentration in the natural rhizobial conditions, and presence of leg hemoglobin assists to scavenge the traces of free oxygen in the nodules. However, the endophytes create a low oxygen environment condition by adopting various biochemical or physical modifications including exopolysaccharides and interior vesicles to exclude free oxygen and compartmentalize the nitrogenase [70]. Carbohydrates produced through photosynthesis are offered to the endophytic symbionts in return of usable nitrogen. The symbionts convert atmospheric nitrogen to ammonia by the help of nitrogenase enzyme and energy. The most studied associations are the rhizobial and actinorhizal plant-bacterial symbioses where several bacterial root endophytes such as *Azoarcus* spp., *Herbaspirillum* spp., and *Acetobacter* spp. fix the atmospheric nitrogen [71]. Such endophytes converting atmospheric nitrogen into the plant usable ammonia or nitrate form are known as "diazotrophs" and found in important crops including rice, sugarcane, sweet potato, maize, and coffee [69].

The endophytic associations between *Gluconacetobacter diazotrophicus* and sugarcane and pines are the well-studied symbiotic associations where the endophyte helps the host plant in nitrogen fixation [71]. In sugarcane, *G. diazotrophicus* facilitate a microaerobic environment to protect nitrogenase enzyme by metabolizing sucrose at an extreme rate to fix atmospheric nitrogen. This phenomenon is known as the respiratory protection, which is peculiar only to endophytes than the rhizobial symbionts [72]. Apart from *G. diazotrophicus*, endophytes from other genera have also been discovered to be associated with the cash crop sugarcane functioning in nitrogen fixation process. Mixture of endophytic bacterial inoculation including *Burkholderia*, *Azospirillum*, and *Herbaspirillum* with *Gluconacetobacter* into sugarcanes resulted in enhanced nitrogen fixation efficiency [73]. The resultant nitrogen fixation in sugarcane was found to double the amount as compared to inoculation of a single bacterial strain. The abundance of *B. vietnamiensis* endophytes in sugarcane was reported to fix nitrogen by producing nitrogenase enzyme and reducing acetylene, an indirect assay for nitrogen fixation [74].

In rice, 13 nitrogen-fixating endophytes from the *Burkholderia* genus were confirmed positive for *nifD* gene and acetylene reduction assay (ARA) [75]. *GUS* assay revealed that *B. vietnamiensis* strain MGK3, an endophyte showing highest ARA activity, entered via the root tips and lateral root junctions and colonized in the intercellular spaces of the root cortex. In maize, *B. unamae* possessing *nif* gene cluster and acetylene reductase activity colonizes in roots and stems [76]. Apart

from *Burkholderia*, endophytes from *Rhizobium*, *Rhanella*, *Pantoea*, *Pseudomonas*, *Azospirillum*, and *Herbaspirillum* genera were also reported to be found in maize which showed potential biological nitrogen fixation capabilities [77]. In another major crop wheat, *Klebsiella pneumoniae* was found to be an entophyte aiding wheat plants to fix atmospheric nitrogen [78]. In addition to these crops, many diazotrophic endophytes have been identified from plants like poplar, sweet potato, coffee, and cottonwood. Poplar endophytic bacteria *Paenibacillus* spp. strain P22 have been reported to help its host via nitrogen fixation and contributing toward overall increase in total nitrogen pool [79]. Another study reported that poplar and willow trees harbor numerous endophytes having putative nitrogen-fixating roles, mainly from genera of *Burkholderia*, *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Herbaspirillum*, *Rahnella*, and *Sphingomonas* [80]. Nitrogen fixation is a vital physiological process in plants that contributes to the overall vigor of the plant, especially for plants growing in low soil-nitrogen concentration areas. Thus, the endophyte-mediated nitrogen fixation serves as a crucial and highly beneficial function for the host plants resulting in proper plant growth and development.

Apart from nitrogen, iron act in many beneficial ways in plant physiology. Several plant endophytes are reported to play role in maintaining the iron homeostasis in plants. Siderophores, produced by the endophytes, are the high-affinity iron-chelating compounds to help in iron uptake [81]. A comparative genomic study of the bacterial endophytes by Mitter et al. [82] revealed that endophytes deficient of the siderophore biosynthesis genes possess additional clusters of membrane receptor encoding genes to facilitate iron transport across the cell membrane. In addition, siderophores help in building and maintaining the mutualistic relationship in some plants and their endophytes. Production of siderophores by *Epichloë festucae* was found to be an essential criterion to maintain the symbiotic relationship with the host grass species *Lolium perenne* [81].

5 Endophyte-Mediated Abiotic Stress Tolerance

Plant growth and development is often compromised by the onset of several environmental stresses as plants prioritize resistance over growth. In this scenario, the endophytes living inside the host plants come in great support in overcoming the challenges. Although endophytes are very short-lived as compared to their host, their shorter life span helps in their rapid evolution in aiding the host toward tackling the diversities. The capacity of different endophytes providing resistance against these environmental stresses are exploited in modern sustainable agriculture [83]. In a plant-endophyte association, the later adopts and employs various strategies to mitigate the abiotic stresses that come from the natural habitat of the host (Fig. 2). Mechanisms like production of growth-promoting hormones and other compounds and nitrogen fixations by plant endophytes, which have been already discussed in this chapter, are actually two major ways of counteracting the deficiency of nutrients in the habitat. Additionally, some endophyte can go a step ahead in providing critical protection to host plants against harsh environmental conditions not only by

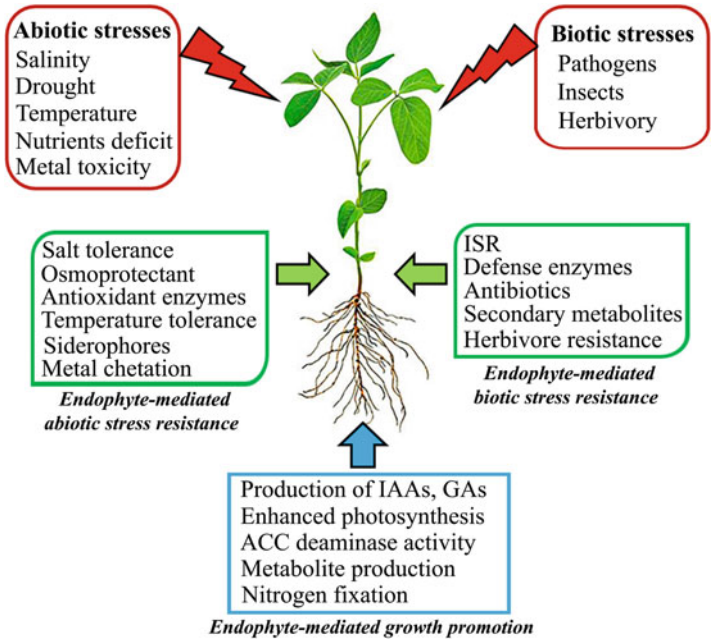

Fig. 2 Mechanisms employed by endophytes in promoting plant growth and mitigating different stress types encountered by plants in their natural habitat

producing the growth-promoting compounds or nutrient acquisition but integrating key genetic elements into the host plant genome which leads to production of stress-responsive molecules in the plant [84].

Endophytes aid in plant stress resistance either by activating the host stress responses or by producing chemical metabolites that act as anti-stress compounds to mitigate the stress effects [85]. For instance, in cucumber, *Penicillium* and *Phoma* spp. not only confer resistance against hyper-sodium toxicity and polyethylene glycol (PEG)-mediated osmotic stress and drought but also positively regulate the growth parameters including plant biomass and nutrition acquisitions [86]. In addition, endophytes are reported to be involved in controlling the gene expressions in host plants with respect to the abiotic stresses [48, 87]. Plants like rice and tomato with beneficial endophytes can survive in water-deficit habitats and show better growth kinetics as compared to the plants lacking these endophytes. The common drought tolerance mechanisms provided by the endophytes include formation of thick cuticles, accumulation of more solutes in plant tissues, and reduction in stomatal transpiration [85]. Removal of *Neotyphodium coenophialum* endophytes from the tall fescue grass caused water intake in plant, nutrient acquisition, and photosynthesis [88]. In rice, the class 2 fungal endophytes originally classified by Rodriguez et al. [89] were reported to confer drought and salinity tolerance in two commercial cultivars originally intolerant to both drought and salinity [90]. Further, the same cultivars exhibited endophyte-mediated improved growth and biomass

accumulation, enhanced yield, and 20–30% reduction in water consumption. Vahid et al. [91] reported the involvement of fungal endophyte *Neotyphodium* spp. in conferring drought resistance in *Lolium perenne* plants reflected by improved growth parameters in terms of higher biomass, plant height, and tiller numbers as compared to the non-infected plants. More recently, bacterial endophytes have been reported as controlling the expression of plant root vacuolar proton pumps [92]. The study reported that bacterial endophyte-mediated manifestation of H+-PPase genes resulted in upregulated expression of the root vacuolar proton pump conferring drought tolerance in *Capsicum annuum.*

Apart from poor irrigation or water-deficit driven drought stress, variation in the salt concentration of the soil is often considered a major threat to global agriculture. High salinity in soil often result in severe crop damage, compromised yield, and at times plant death. Plants possess many complex mechanisms to cope with soil salinity. Regulation of salinity by the mutualistic *in planta* dwellers, the endophytes, has been crucial in rescuing and overcoming such adversities. For instance, *Piriformospora indica*, a fungal endophyte isolated from the rhizosphere of *Prosopis juliflora* and *Ziziphus nummularia*, help its host to adapt the hostile dessert habitat [93]. Inoculation of *P. indica* into barley has resulted in enhanced resistance to biotic stresses and improved salt tolerance [94]. Further, Baltruschat et al. [95] reported that colonization by *P. indica* in the roots of the salt-sensitive barley cultivar "Ingrid" resulted in increased plant biomass and decreased salinity-induced lipid peroxidation, fatty acid desaturation, and metabolic heat efflux in the leaves. Additionally, endophyte-inoculated barley plants displayed elevated ascorbic acid accumulations and escalated activity of the antioxidant enzymes under salt stress. Inoculation of systemic class 2 fungal endophytes in tomato plants conferred salt tolerance in the infected plants [96]. Moreover, the infected plants exhibited higher plant biomass, improved water-use efficiency, and enhanced photosynthetic abilities than the uninfected tomato plants under salinity conditions. More recently, *Aspergillus flavus* CHS1 isolated from *Chenopodium album* has been reported to have high salt tolerance characteristics [97]. In the *in planta* CHS1-soybean interaction study, inoculation of CHS1 resulted in improved plant growth and mitigated the imposed salinity stress by subsiding ABA and JA synthesis. Moreover, activity of the antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase, and polyphenol oxidase was found to be upregulated in compared to endophyte-free soybean plants under salt stress.

Mitigation of drought and salt stresses are usually associated with the accumulation and subsequent scavenging of reactive oxygen species (ROS) [98]. Although reduction in plant ROS levels can be aided by antioxidant compounds like glutathione, ascorbate, and tocopherol, the major scavengers of ROS include SOD, CAT, dehydroascorbate reductases (DHAR), monodehydroascorbate reductases (MDHAR), glutathione reductases (GR), and ascorbate or thiol-dependent peroxidases (APX) [99]. Accumulation of ROS in the plant cell can be toxic, leading to oxidative damage to proteins, lipids, and DNA. Having said that, ROS often act as an early event in the plant stress response signaling cascades [100]. Although production and accumulation of ROS can often be seen in plant-pathogen interactions,

relatively low amount of ROS is also needed in the development of a beneficial plant-endophyte association. For example, the production of hydrogen peroxides or superoxides by *Epichloë festucae* is preconditioned by a mutualistic association between the fungal endophyte and the host *Lolium perenne* grass [34]. While low concentration of ROS is beneficial for the plant growth and signaling, elevated ROS accumulation may cause detrimental effects. Endophytes dwelling inside the plants help their hosts in managing ROS concentrations and thus protecting them from the detrimental effects of ROS. Exposure to high salt conditions caused ROS accumulations in tomato, panic grass, and dunegrass without any endophytes, whereas the endophyte-containing plants didn't show ROS accumulations [101]. Interestingly, some endophytes produce ROS to check the colonization levels in plants and maintain mutualism, while others reduce the ROS levels to nullify the abiotic stress effects on their hosts [34, 43]. Besides, salinity and drought both can disturb the osmotic homeostasis in a plant causing imbalance in solute and ion concentrations within a plant cell. Osmotic stress stimuli can be perceived by the plants as both primary and secondary signals where accumulation of solutes, water loss, and ion influx/efflux belong to the primary signals and accumulation of phytohormones, generation of nitric oxide and phospholipids, and liberation of ROS belong to the secondary signals. In addition, change in the habitat temperature and soil contaminations with heavy metals has arisen as potential abiotic stresses for the plants. While the plant and its intrinsic machinery fail at times to provide protection against high temperature conditions, the colonizing endophytes rescue their host from these hostilities. For example, the fungal endophyte *Curvularia protuberate* in *Dichanthelium lanuginosum* grass helps the host to survive and tolerate soil temperatures as high as 65 °C [12]. Another *Ascomycetes* endophyte isolated from durum wheat (*Triticum turgidum*) conferred significant tolerance to drought and heat in the inoculated plants [102]. Interestingly, the endophyte-free seeds produced from the *Ascomycetes*-inoculated wheat plants under drought and heat stress showed increased germination rate than the non-inoculated wheat plant seeds [102]. Similarly, several plant endophytes, especially the root-associated endophytes, have been reported to support their host in heavy metal tolerance and mitigate metal toxicity. Several strains of *Pestalotiopsis* spp. isolated from the mangrove palm species *Nypa fruticans* conferred significant tolerance against heavy metals including copper (Cu), zinc (Zn), lead (Pp), and chromium (Cr) [103]. In vitro studies revealed that 1 out of the 93 endophytic isolates from the nipa palm could resist heavy metal contaminations to a level as high as 1000 ppm. *Exophiala pisciphila*, a root-associated fungal endophyte in maize, was reported to provide tolerance against soil cadmium (Cd) toxicity [104]. Upon subjection of Cd stress, the *E. pisciphila*-inoculated plants exhibited upregulated expression of maize genes responsible for metal uptake, translocation and chelation, increased Cd accumulation on maize cell walls, conversion of Cd to lesser toxic forms, and upregulated activities of antioxidants and antioxidant enzymes. Moreover, the plants containing endophytes displayed improved growth parameters with a significant decrease in Cd-induced phototoxicity levels as compared to the maize plants lacking the endophytes. *Penicillium funiculosum* LHL06 has been reported to secret gibberellin that alleviated metal toxicity and reprogramed the

growth of soybean plants under Cu and Cd stress [105]. Similarly, endophytes like *Pseudomonas* spp. and *Gigaspora* spp. can improve plant resistance against metal toxicity by altering the endogenous ethylene levels [106]. More recently, Mukherjee et al. [107] reported that inoculation with *Kocuria* spp. and *Enterobacter* spp. isolated from *Lantana camara* into a surrogate host *Solanum nigrum* resulted in enhanced arsenic (As) tolerance and phytoremediation. Under an applied As consortium, endophyte-inoculated *S. nigrum* plants exhibited improved photosynthesis, root-to-shoot As transport, increased As bioaccumulation, and improved plant growth. Elevated ROS levels were also observed in response to As stress, however no ROS-mediated toxicities were experienced by the plants as the inoculated plants exhibited enhanced antioxidant levels. Thus, endophytes efficiently and effectively serve their host plants in overcoming various abiotic stresses there by maintaining a homeostasis in between plant growth and defense response.

6 Endophyte-Mediated Plant Defense Response

Even with the intrinsic and well-evolved defense mechanisms, plants at times require additional sophisticated defense strategies assisted by their microbial alliances to defend and rescue themselves from an array of biotic stresses. During the interaction between plant and endophytes, initially the infection of endophytes triggers the plant defense similar to of a pathogen infection, but subsequently, the endophytes escape these defense and colonize in the host plants [108] (Fig. 2). However, the defense responses induced by the colonization of endophytes in plants act as a priming effect and provide enhanced resistance against other phytopathogen. This phenomenon in plants conferred by the endophytes is known as induced systemic resistance (ISR) and commonly seen in bacteria-plant endophytic associations [108, 109]. In potato plants, inoculation of endophytes from genera *Pseudomonas* and *Methylobacterium* resulted in improved resistance against the necrotrophic pathogen *Pectobacterium atrosepticum* via ISR [110]. However, the extent of resistance conferred by colonization of *Methylobacterium* bacteria was inversely proportional to the amount of inoculum used. The induction of ISR and consecutive-enhanced pathogen defense in host plants have been reported in response to the colonization of *Pseudomonas* and *Bacillus* spp. bacteria [111]. *Bacillus amyloliquefaciens*, a bacterial endophyte isolated from corn plants, exhibited in vitro antifungal activities against multiple phytopathogens including *Aspergillus flavus*, *Colletotrichum gloeosporioides*, and *Fusarium moniliforme*. Further, the pretreatment of *B. amyloliquefaciens* to corn seedlings induced the expression of defense-related genes against pathogen infection as compared to the non-inoculated controls [112].

While bacterial endophytes are the masters of manipulating the plant defense and mimicking a priming defense effect against phytopathogen via ISR, the fungal endophytes commonly don't exhibit ISR-mediated defense responses in their hosts [113, 114]. Conversely, the fungal endophytes produce growth-inhibiting chemical compounds against the invading pathogens and attacking herbivores to protect their host plants. These chemicals include phenols, terpenoids, flavonoids,

alkaloids, quinols, steroids, polyketones, and peptides [71]. Clavicipitaceous fungi of several grass species produce alkaloids which provide defense against herbivory. For instance, indole diterpenoids, also known as lolitrems, are neurotoxic and produced in endophyte-infected grass, which are responsible for intoxication of cattle grazing on them [71]. Endophytic actinomycetes are extensively characterized for producing antimicrobial compound including munumbicins, kakadumycinx, and coronamycin [71]. In *Kandelia candel*, the endophytic *Streptomyces* sp. HKI0595 produces multicyclic indolosesquiterpene which has antibacterial activity [115]. In orchid plants, spoxazomicins A to C having antitrypanosomal activity were produced by the endophyte *Streptosporangium oxazolinicum* K07-0450T [116]. These compounds having different bioactive functions are exploited in clinical or agricultural purposes, whereas their exact roles in plant-microbe interactions are still under investigation [117]. Furthermore, the horizontal transfer of endophytes and their establishment results in enhanced biotic stress tolerance capacities by production of antibacterial, antiviral, antifungal, and insecticidal compounds [118]. However, enhancing the host immunity is not supported by all horizontally transmitted endophytes often due to the lack of opportunity for interaction with plant pathogens; hence, their role in host protection is yet to be characterized. For example, colonization of plant pathogens in the leaves of cacao plants doesn't always result in disease, rather at times it acts as harmless or beneficial endophytes [2, 119]. Moreover, the endophytic-mediated production of antimicrobial compounds can be induced by the presence of a phytopathogen [120].

Apart from production of antimicrobial compounds in their hosts, endophytes code for several defense-related proteins and enzymes that further enhance the host defense responses. In rice, the endophytic bacteria *Paenibacillus polymyxa* confer defense against the rice false smut pathogen *Ustilaginoidea oryzae* by exhibiting antagonistic activity and β-1,3-1,4-glucanase production [121]. In wheat, bacterial endophyte colonizations support the host to overcome infection by *Fusarium graminearum* [122]. Similarly, endophyte colonization of varied bacterial genera in *Zea mays*, *Arachis hypogaea*, and *Cucurbita pepo* conferred resistance to fungal phytopathogens by producing compounds of antifungal nature [123–125]. In *Fraxinus* plants, the endophytes *Pantoea agglomerans*, *Staphylococcus succinus*, and *Aerococcus viridans* produce several antibiotics that help to protect the host from bacterial infections [126]. Recently, inhibition of infection in tomato plants by the fungal pathogen *Phytophthora infestans* was achieved by the inoculation of endophytic *Phoma eupatorii* isolate 8082. The inhibition of *P. infestans* was found to be conferred by the enhanced endophyte-mediated production of anthocyanins [127]. Cosme et al. [128] reported that tolerance to root herbivory in plants can be aided by root colonizing fungal endophytes by manipulating the JA and GA hormonal signaling. In addition to plant defense, endophyte-produced secondary metabolites are involved in mechanisms of genetic regulations, signaling, and the establishment of symbiosis and even influence host secondary metabolism [129, 130]. Inoculation of *Methylobacterium* spp. into strawberry plants resulted in the biosynthesis of flavor compounds, such as

furanones [131]. Another report confirming the regulation of host metabolism by the endophytes reported the discovery of bacterial endophytes and gene transcripts in the vascular tissues of strawberry, the locations where the furanone biosynthesis is carried out in plants [132]. Likewise, inoculation of a fungal endophyte *Paraphaeosphaeria* sp. in the bilberry plant resulted in upregulated biosynthesis and significant accumulation of phenolic acids, flavan-3-ols, and oligomeric proanthocyanidins [133]. Overall, the colonization and establishment of endophytes in plants helps in boosting the host immunity by employing one or more of the above discussed strategies.

7 Conclusions

The sessile nature of the plants makes them to associate with diverse groups of symbiotic microbes exhibiting significant role in plant development and protection. Plants constitute an ecological niche within its inner space for the assemblage and sustenance of multiple microbial symbionts. It appears that a majority of these mutualistic microbes affect in the way plants network with their environment. Myriads of studies have shown that endophytes confer stress tolerance, survivality, and improved growth of the host plant species under extreme environmental conditions including salinity, extreme temperature, drought, and environmental toxicity [101, 134]. Additionally, recent reports also demonstrated that endophytes have good potential for application in plant improvement and disease control [135]. Therefore, it is collectively accepted that plants survive and flourish in the biotic and abiotically stressed environments for the reason that the endophytic symbionts have simultaneously evolved and essentially involved in their adaptation to stressed ecosystems [136].

Multiple endophytes penetrate and infect plants without any symptoms. Further, a few of them are inseparable from the plant system and vertically transmitted from one generation to another [137]. This suggests that endophytes provide a vast reservoir of heritable DNA providing genetic flexibility toward adaptation of the plants to changing environments. The usage of such endophytes in a mechanized agricultural system will be significant in the development of novel germplasms leading to rapid agricultural productions. Besides, the ability of certain endophytes to infect multiple hosts suggests that they could be transferred to native plants and agricultural crops to improve their capabilities in tolerating multiple types of environmental stresses brought about by ecological changes [101]. Although several intellectual property rights and patents have been granted on different aspects of endophytes [138], the commercial applications of endophytic microbes are still at its infancy. It is essential that a large majority of these endophytes are subjected to experimental trials and tests to evaluate their efficiency under natural conditions. Both the plant host and the endophytes have to deal with the natural environment for their survival. As such, a broader understanding and acknowledgment of the symbiotic interaction with the microbes is essential for optimum plant growth and development.

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