

Chapter 26

Sourcing the Fungal Endophytes: A Beneficial Transaction of Biodiversity, Bioactive Natural Products, Plant Protection and Nanotechnology

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Abstract Endophytes are the group of microorganisms that reside to internal and healthy tissues without causing negative symptoms to their host plant. Endophytes are extremely diverse and range from fungi, bacteria and actinomycetes. Development of drug resistance to pathogenic forms of bacteria, fungi and other microbes, emergence of lethal viruses, the perpetuating epidemics in developing and under developing countries, and multifold fungal infection, enhancement in human population globally, all shows our inability to overcome these biomedical problems. In addition to this, we are also unable to assure people towards enough food security in specific regions of the earth due to infestation of different plant diseases. Since the fungal endophytes are relatively less studied group of microbial flora, but are responsible for several prospects such as biodiversity, ecology, bioactive metabolites (metabolomics) and nanotechnology, may enable us to overcome the above mentioned problems. Fungal endophytes represent a dependable source of specific secondary metabolites and can be manipulated both physicochemically and genetically to increase yield of desired compounds and to produce novel analogues of active metabolites. In this chapter, we have discussed several bioactive compounds and classified them in to different classes as per their properties such as antifungal, antibacterial, antiviral, antimalarial, anticancer, antioxidants, antidiabetic and immunosuppressive agents derived from fungal endophytes with their hosts and made the chemical structures

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for 73 compounds using chemdraw 3D ultra version 7.0. These bioactive products are related to human health with MIC/EC/IC₅₀ values less than 50 µg/mL. This article also discusses nematicidal, some antimicrobial volatile compounds (VOCs) that are related to plant protection and faecal disposal. Therefore, this chapter is not very specific and covers almost prospects of fungal endophytes which could be useful in biodiversity, agrochemicals, biotechnology, biomedical and nanotechnology in ecofriendly manner.

Keywords Endophytic fungi • Antimicrobial • Anticancer • Antioxidants • Mycodiesel • Metals nanoparticles

26.1 Introduction

Originally, the term endophyte was introduced by de Bary (1866) and was assigned to all those microbes that reside inside the living healthy tissues of the plants. Later, this term was expanded as fungi and bacteria including actinomycetes, which spend the whole or at least a part of their life cycle colonizing inter- or intra-cellularly, inside the healthy living tissue of the host plant, typically causing no apparent symptoms of disease. Many workers define the endophytes in different ways, but Bacon and White (2000) gave a conclusive and widely accepted definition of endophyte as 'microbes that colonize living, internal tissues of plant without causing any immediate, overt negative effect'. Endophytes are extremely diverse and range from fungi Carroll and Carroll (1978), Petrini (1986), Rajagopal and Suryanarayanan (2000), Gond et al. (2007), to bacteria Hallmann et al. (1997) including actinomycetes Verma et al. (2009a), but the fungi are most studied group of endophytes and among fungi the best studied endophytes are intercellular symbionts from ascomycetous family Clavicipitaceae in the grasses of temperate zone. The presence of endophytes was observed from algae to angiosperm studied till date Aly et al. (2010). The literature suggests that endophytes augment resistance in their hosts against herbivores Brem and Leuchtman (2001), pathogenic fungi, bacteria, viruses, insects, nematodes Gond et al. (2010), illness Clay (1990), reduced seed production Rice et al. (1990), temperature and salinity Redman et al. (2002) and also against drought and minerals Malinowski et al. (1997).

Development of drug resistance to pathogenic forms of bacteria, fungi and other microbes, emergence of lethal viruses, the perpetuating epidemics in developing and under developing countries, and multifold fungal infection, enhancement in human population globally, all shows our inability to overcome these biomedical problems. In addition to this, we are also unable to assure people towards enough food security in specific regions of the earth and in India too, to support the local human population. Environmental degradation, loss of biodiversity and spoilage of land and water also added to the problems facing mankind.

The access of new disease causing agents like AIDS, SARS, Ebola and already epidemic like malaria, leishmania and encephalitis requires the discovery and development of new therapeutic drugs that target them specifically within the cellular metabolism. Due to safety and the environmental problems, many synthetic agricultural agents have been and currently are being targeted for removal from the market, which creates a need to find the alternative ways to control farm pests and pathogens. In search of these new and lead molecule/or drug, we have to find out new and alternative resources, and endophytic fungi are one of them which could be explored for this purpose Kharwar and Strobel (2011). After the discovery of taxol (billon dollar anticancer drug) from an endophytic fungus *Taxomyces andreanae*, research related to fungal endophytes got a great attention for rich and novel alternative source of natural bioactive compounds Stierle et al. (1993). The number of research publications only related to secondary metabolites (115) from fungal endophytes during period of 2000–2009, itself indicates the attention of researchers to this field of study Aly et al. (2010). Since the fungal endophytes are relatively less studied group of microbial flora, but are responsible for several prospects such as biodiversity, ecology, bioactive natural products (metabolomics) and nanotechnology. Therefore, this chapter is not very specific and covers almost prospects of fungal endophytes which could be useful in biodiversity, agrochemicals, biotechnology, biomedical and nanotechnology in ecofriendly manner.

26.2 Biodiversity and Ecology of Endophytic Fungi

Endophytic fungi are hidden, highly diverse and potential entities of microbial world as they reside in a unique biotope. They have successfully been isolated ranged from host of highly water stressed desert Bashyal et al. (2005), cold stressed arctic Fisher et al. (1995), antarctic Rosa et al. (2009), ocean Wang et al. (2006), geothermal soils Redman et al. (2002), highly diverse rain forests Strobel (2002), dry deciduous and coastal forests Suryanarayanan et al. (2003, 2005) and mangrove swamps Lin et al. (2008). The significant presence of endophytes were observed in all green biota ranging from algae Kralj et al. (2006), Wang et al. (2006), Pontius et al. (2008), bryophytes Silvia et al. (2008), pteridophytes Swatzell et al. (1996), gymnosperms Hoffman and Arnold (2008) and to angiosperms Gond et al. (2007) including underground root to all aerial parts of host Kharwar et al. (2008). Our earth harbors all most 300,000 higher plants species and each species represents either one or plethora of endophytic community and it is well proved by the various studies of higher plants fungal endophytes Strobel (2002). Out of 300,000 higher plants that exist on the earth, only a few dozens, have been studied related to their endophytic biology.

It is expected that except bacteria and fungi, other microbial entity may also exist in plants as endophytes such as archbacteria, streptomycetes, mycoplasmas and rickettsia. Actually, non culturable behaviour of these microbes and lack of precise techniques of isolation may be the reasons for not reporting them frequently from plants except a few reports (Verma et al. 2009a). The most frequently isolated endophytes are the fungi. However, at outset, it is important to note that the vast majority of plants have not been studied for any endophytic association. Thus, enormous opportunities exist for the recovery of novel fungal forms, including genera, biotypes, as well as species in the myriad of plants yet to be studied in different settings and ecosystem. Hawksworth and Rossman (1987) estimated there may be as many as 1.5 million different fungal species, while only about 100,000 have been described. As more evidence accumulates, estimates keep rising as to the actual number of fungal species. For instance, Dreyfuss and Chapela (1994) have estimated at least one million species of endophytic fungi alone.

It seems obvious that endophytes are a rich and reliable source of genetic diversity and may represent many previously undescribed species. Among endophytic fungi, ascomycetous members of family clavicipitaceae were excellently recovered and studied from poaceae Clay (1989), however, the members of coelomycetes, hyphomycetes and mycelia-sterila Pereira et al. (1999), Tejesvi et al. (2005), Chareprasert et al. (2006), Gond et al. (2007) were also frequently isolated while members of basidiomycetes Santos et al. (2003) and zygomycetes Spurr and Welty (1975), are not frequently isolated and studied.

26.3 Metabolomics

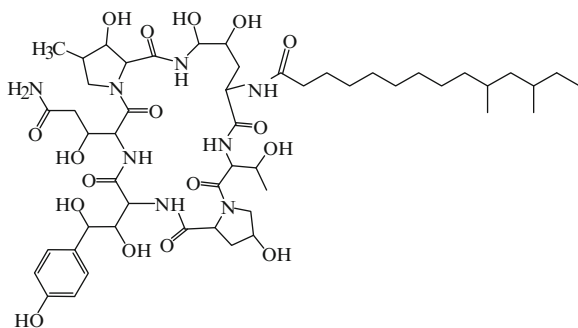
In the course of ongoing study about antimicrobials, it was found that with the evolution of antibiotics, the pathogenic bacteria and fungi also start the development of multidrug resistance, and the conventional antibiotics become failure to cure the diseases. Therefore, to overcome the problem medical sciences carry out the research for new and effective antibiotics from new sources, and in this respect fungal endophytes give the rays of hope and may fill some gaps as endophytic researches indicate that 51% of bioactive substances isolated from endophytic fungi were previously unknown compared to 38% novel compounds from soil fungi Schulz et al. (2002), and this data induce scientists to explore the possibility of antimicrobial compounds of novel entity from this relatively hidden repertoire.

26.3.1 Role of Endophytic Fungal Metabolites in Human Health

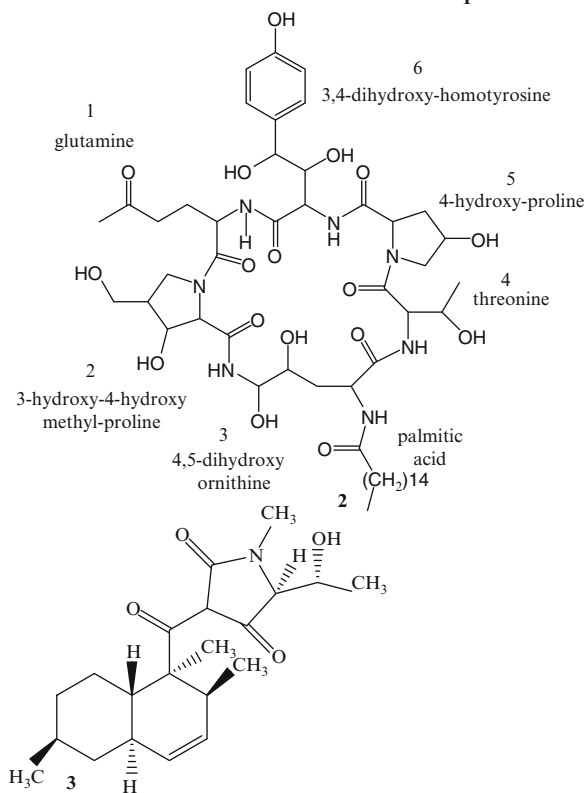
The metabolites received from fungal endophytes could be categorized in the following groups based on their bioactivity either against particular group of pathogens or against diseases.

26.3.1.1 Antifungal Agents

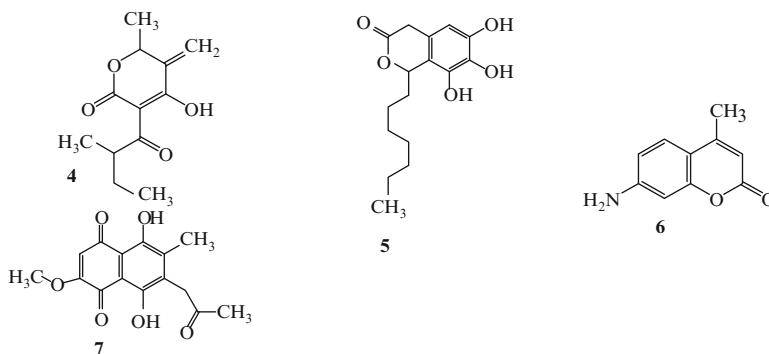
Undoubtedly, fungi are major causal organisms of various diseases in humans and there are a number of chemical (synthetic) fungicides are in use to protect the humans, but these chemicals also make an adverse impact on environment Strobel et al. (2002). Literatures suggest that 52.3% of endophytic metabolites display growth inhibition activity to at least one or more than one pathogenic microbes Gond et al. (2010). There are a huge number of antifungal compounds that have been isolated from endophytic fungi, but here we are mentioning only those having MIC value equal to or less than 50 µg/mL. An echinocandin (antifungal agent L-671,329) (**1**) was isolated from endophytic *Cryptosporiopsis* sp. of *Pinus sylvestris* and *Fagus sylvatica* found to have activity against *Candida albicans* and *Saccharomyces cerevisiae* Noble et al. (1991). An endophytic fungus *Cryptosporiopsis* cf. *quercina* recovered from stem of *Tripterogeum wilfordii*, produces a potent antimycotic, cryptocandin (**2**) (lipopeptide) and cryptocin (**3**). Cryptocandin showed activity against human pathogenic fungi *Trichophyton rubrum* (ATCC 28188), *Trichophyton mentagrophytes* (ATCC 28185), *Candida albicans* (ATCC 90028), *Candida parapsilosis* and *Histoplasma capsulatum* Strobel et al. (1999). CR377 (**4**), a novel pentaketide possess anticandida activity extracted from endophytic fungus CR377 (*Fusarium* sp.) inhabited the internal tissue of *Selaginella pallescens* Brady and Clardy (2000). Cytosporones D (**5**) is trihydroxybenzene lactone that has been reported from two endophytic fungal strains, CR 200 (*Cytospora* sp.) and CR 146 (*Diaporthe* sp.) which were isolated from the tissue of *Conocarpus erecta* and *Forsteronia spicata* plants, respectively possess significant anticandida activity Brady et al. (2000). 7-amino-4-methylcoumarin (**6**) isolated from endophytic sp., residing inside *Ginkgo biloba* exhibit antifungal activity against *Candida albicans*, *Penicillium expansum* and *Aspergillus niger*, including the antibacterial activity Liu et al. (2008). *Chloridium* sp. isolated as an endophyte from *Azadirachta indica*, produced the compound javanicin (**7**) a strong antibacterial naphthaquinone had also inhibited the growth of several fungal pathogens at MIC below 20 µg/mL Kharwar et al. (2009).



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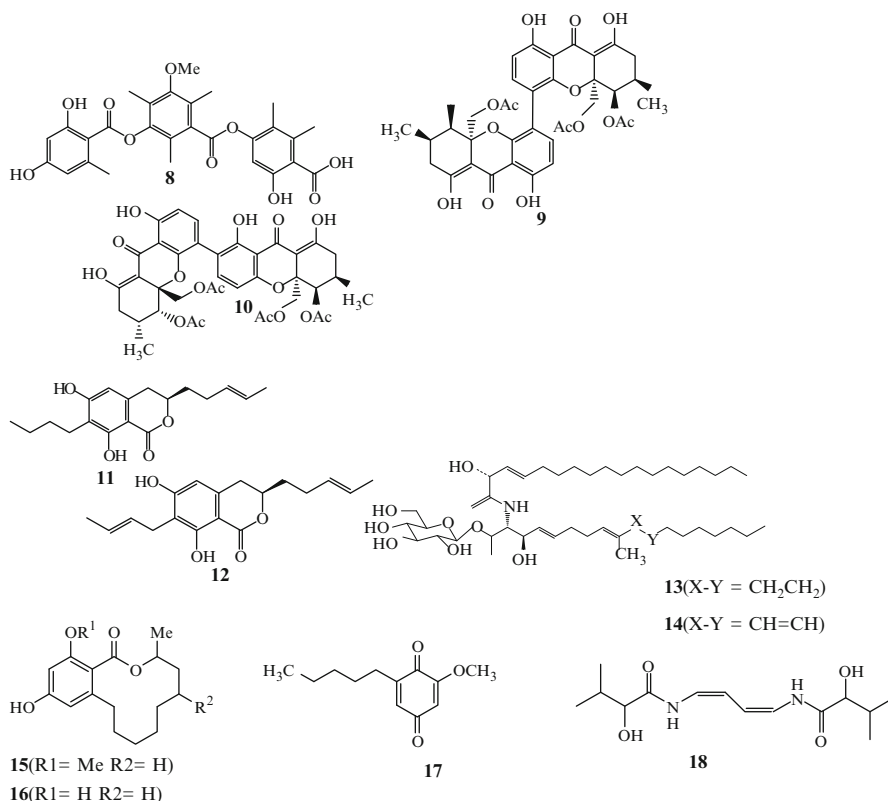
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26.3.1.2 Antibacterial Agent

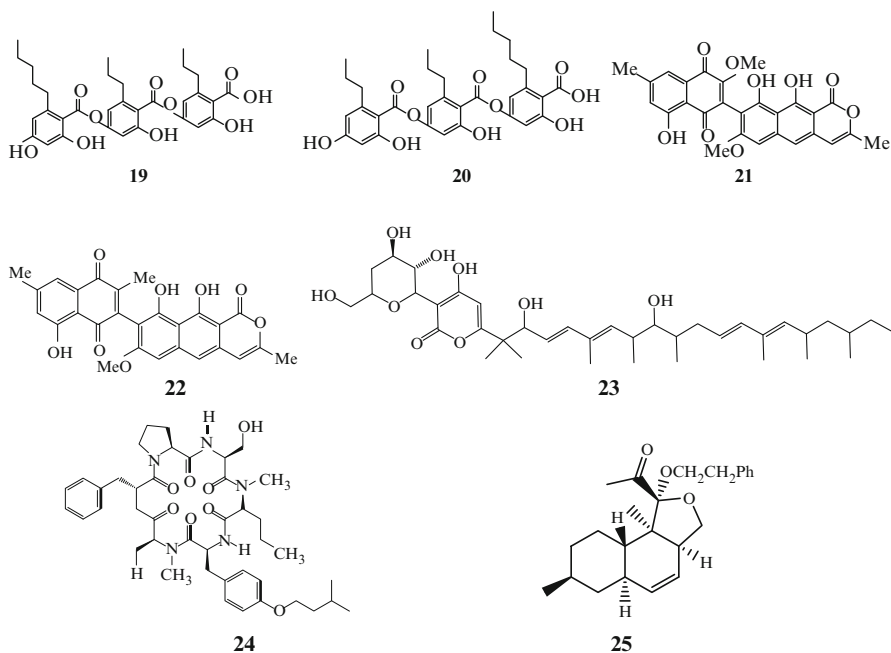
A considerable number of effective and potential antibacterial compounds have been isolated from endophytic fungi against a range of gram +ve and gram -ve bacteria. A novel secondary metabolite colletotric acid (**8**) was isolated from *Colletotrichum gloeosporioides* colonizing the tissues of *Artemisia mongolica* and inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina leutea* Zou et al. (2000). Phomoxanthenes A (**9**) and B (**10**) are novel xanthone dimers that were isolated from the endophytic fungus *Phomopsis* sp. BCC 1323 showed antituberculosis activity Isaka et al. (2001). Cytosporones D (**5**) described earlier as anti-fungal also exhibited the significant activity against *Staphylococcus aureus*, and *Enterococcus faecalis*, respectively Brady et al. (2000). 7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one (**11**) and 7-butyl-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one (**12**) extracted from endophytic *Geotrichum* sp. which inhabited *Crassocephalum crepidioides*. Both the compounds had shown anti-TB (tuberculosis) effect Kongsaree et al. (2003). Two cerebrosides (glycosphingolipids) cerebroside (**13**) and fusaruside (**14**), the later being new were isolated from *Fusarium* sp. IFB-121, an endophytic fungus of *Quercus variabilis*. Both the compounds were recorded as antibacterial in respect to *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas fluorescens* Shu et al. (2004). Two known lactones lasiodiplodin (**15**), and de-*O*-methylasiodiplodin (**16**) isolated from an endophytic fungus (No. ZZ36) of brown alga, possess considerable activity against *Staphylococcus aureus* (ATCC27154) and *Bacillus subtilis* (ATCC6633) while activity against *Salmonella enteritidis* only possessed by later Yang et al. (2006). The endophytic fungus *Botrytis mamane* PSU-M76 was isolated from the interior of *G. mangostana* reported to produce a known metabolite primin (**17**) which exhibits prominent antibacterial activity against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* SK1 Pongcharoen et al. (2007). Another compound phomoenamides (**18**) was isolated from an endophytic fungus *Phomopsis* sp. PSU-D15 an inhabitant of *Garcinia dulcis* leaf, showed the prominent activity in opposition to *Mycobacterium tuberculosis* H37Ra Rukachaisirikul et al. (2008). Javincin (**7**)

described earlier also, exhibits strong antibacterial activity against *Bacillus sp.*, *Escherichia coli*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* Kharwar et al. (2009).



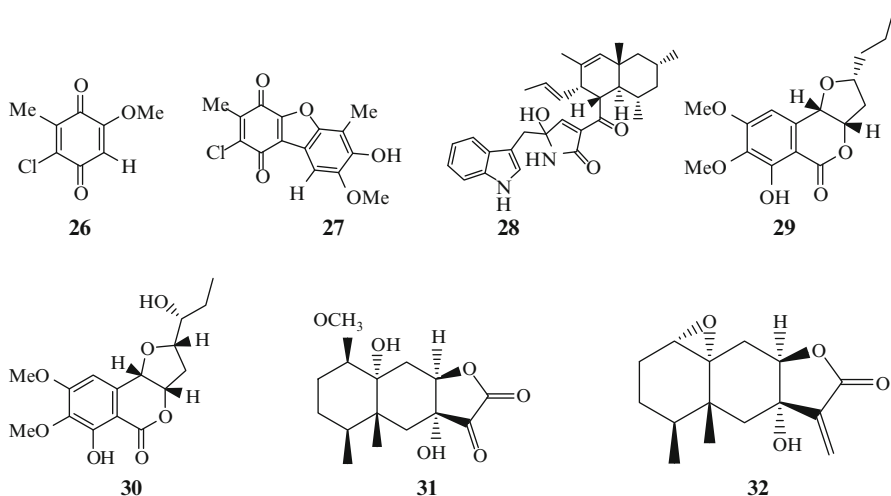
26.3.1.3 Antiviral Agent

Two novel human cytomegalovirus protease inhibitors, cytonic acids A (**19**) and B (**20**) isolated from *Cytospora sp.*, an endophyte of *Quercus sp.* against human cytomegalovirus (hCMV) Guo et al. (2000). Endophytic fungus *Penicillium chrysogenum* of unidentified tree in Peru found to produce xanthoviridicatin E (**21**) and F (**22**), inhibited the cleavage reaction of HIV-1 integrase Singh et al. (2003). Metabolite S 39163/F-I (**23**) extracted from an isolate of endophytic fungus strain NRRL 15684 was isolated from the leaf of *Buxus sempervirens* L., showed better activity against herpes viruses in addition to antifungal activity Tschertter et al. (1988) and Gunatilaka (2006). Pullularin A (**24**) possesses activity against herpes simplex virus type 1, isolated from endophytic fungus *Pullularia sp.* BCC 8613 of *Culophyllum sp.* Isaka et al. (2007). Oblongolide Z (**25**) (hexaketide γ -lactone) has been isolated from *Phomopsis sp.* BCC 9789 associated with *Musa acuminata* (wild banana) as an endophyte possesses activity as anti-herpes simplex virus type 1 Taridaporn et al. (2010).



26.3.1.4 Antimalarial Agent

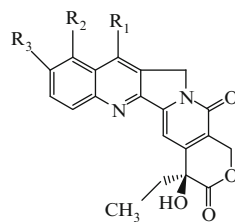
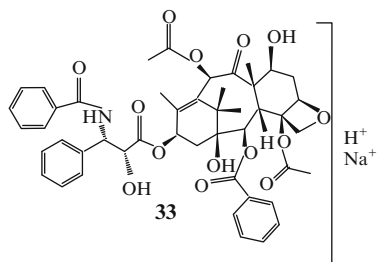
Several anti-malarial compounds have also been isolated from this group of microbes and a few of them are mentioned here as representatives. Phomoxanthones A (**9**) and B (**10**) isolated from an endophytic *Phomopsis* sp., are known to display antimalarial activity Isaka et al. (2001). Endophytic *Geotrichum* sp. collected from *Crassocephalum crepidioides* have found to produce 7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one (**11**) and 7-butyl-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one (**12**) Kongsaree et al. (2003). The above mentioned four anti malarial compounds are also antibacterial which have already been described in previous section. 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**26**) and xylariaquinone A (**27**) are novel benzoquinones that were isolated from an endophytic *Xylaria* sp. Tansuwan et al. (2007). Codinaeopsin (**28**) is another antimalarial agent, isolated from an endophytic fungal isolate CR127A (98% identical to *Codinaeopsis gonytrichoides*) collected from *Vochysia guatemalensis* (a white yemeri tree) Kontnik and Clardy (2008). Monocerin (**29**) and its new analogue 11-hydroxymonocerin (**30**) were isolated from cultures of *Exserohilum rostratum*, a fungal strain endophytic in *Stemona* sp. Sappapan et al. (2008). Two new eremophilane-type sesquiterpenoids, eremophilanolides 2 (**31**) and 3 (**32**) were isolated from an endophytic fungus BCC 21097 of *Licuala spinosa* Isaka et al. (2010). All 12 compound showed *in vitro* activity against *Plasmodium falciparum* K1.



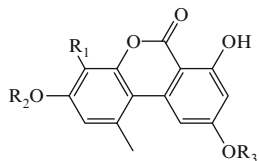
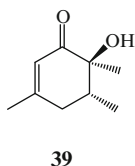
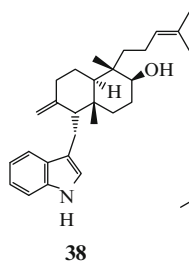
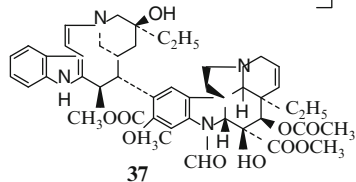
26.3.1.5 Anticancerous Agent

About 100 anticancer compounds have been isolated from endophytic fungi yet, and among them 57% are novel, while rest are previously known Kharwar et al. (2011). Taxol (33) is highly functionalized diterpene and is the first compound possessing the taxane ring, isolated from the bark of the *Taxus brevifolia* (Pacific Yew) for the first time by Wani et al. (1971) and named it as 'taxol'. Its effectiveness against ovarian and breast cancers make the drug highly valuable. For the first time in history, Stierle et al. (1993) isolated this important compound from a fungus *Taxomyces andreanae* endophytically associated with *Taxus brevifolia*. After this discovery, a number of scientists isolated Taxol from different endophytic fungi associated with different hosts such as *Bartalinia robillardoides* and *Pestalotiopsis terminaliae*, the endophytic fungi of *Aegle marmelos* and *Terminalia arjuna*, Gangadevi and Muthumary (2008, 2009). Camptothecin (34), another important anticancer compound previously known to be isolated from wood of *Camptotheca acuminata* plant was isolated from an endophytic fungus of *Nothapodytes foetida*, *Entrophosphora infrequens* and *Neurospora* sp. for the first time by Puri et al. (2005) and Rehman et al. (2008), respectively. This very compound with two of its

analogues, 9-methoxycamptothecin (**35**) and 10-hydroxycamptothecin (**36**) also isolated from *Fusarium solani* an endophytic fungus of *Camptotheca acuminata* Kusari et al. (2009). Additionally, 9-methoxycamptothecin and 10-hydroxycamptothecin do not have the therapeutic drawbacks as it found in plant camptothecin even after showing the similar activity. The similar nature of compound is also reported with vincristine (**37**) produced by *Fusarium oxysporum*, an endophyte of *Catharanthus roseus* Zhang et al. (2000), Tung et al. (2002). Emindole DA (**38**) isolated from *Emericella nidulans* var. *acristata* an endophyte of a Mediterranean green alga showed the antitumor activity against 36 human tumor cell lines Kralj et al. (2006). Leptosphaerone C (**39**) and penicillenone are the novel anticancer polyketides isolated from *Penicillium* sp. JP-1, an endophytic fungus associated to the *Aegiceras corniculatum* (mangrove plant). Leptosphaerone C showed activity against A-549 cells, whereas penicillenone exhibits cytotoxicity against P388 cells Lin et al. (2008). An endophyte *Alternaria* sp., isolated from *Polygonum senegalense* (Egyptian medicinal plant) had produced three lactone compounds, alternariol (**40**), alternariol 5-*O*-sulfate (**41**), alternariol 5-*O*-methyl ether (**42**), and a phenol derivative altenusin (**43**) bearing cytotoxic activity against L5178Y cells Aly et al. (2008). Phomoxanthonones A (**9**) and Phomoxanthonones B (**10**) describe activity against KB, BC-1, Vero cell lines Isaka et al. (2001). Merulin A (**44**) (nor-chamigrane endoperoxide) and C (**45**) (chamigrane endoperoxides) are two new sesquiterpenes produced from an endophytic fungi XG8D, member of class basidiomycetes isolated from mangrove plant, *Xylocarpus granatum* König (Meliaceae). Both the compounds showed significant cytotoxicity against human breast (BT474) and colon (SW620) cancer cell lines Chokpaiboon et al. (2010). *Phomopsis longicola*, an endophytic fungus of the *Dicerandra frutescens* (endangered mint) produces three cytotoxic compounds dicerandrols A (**46**) B (**47**) and C (**48**) against two human cancer cell lines, A-549 and HCT-116 Wagenaar and Clardy (2001). Two new, Oblongolide (hexaketide γ -lactone) Y (**49**) and Z (**25**) have been isolated from *Phomopsis* sp. BCC 9789 associated with *Musa acuminata* (wild banana) as an endophyte. Oblongolide Y showed cytotoxic activity against BC line while Oblongolide Z had shown cytotoxic activities against KB, BC, NCI-H187, and nonmalignant (Vero) cell lines Taridaporn et al. (2010). Six noble benzofuranone-derived γ -lactones, photinides A-F (**50–55**) isolated from a single endophytic fungus *Pestalotiopsis photiniae* resides inside *Roystonea regia*. All six noble γ -lactones exhibit cytotoxicity against MDA-MB-2311 (human tumor cell lines) Ding et al. (2009).

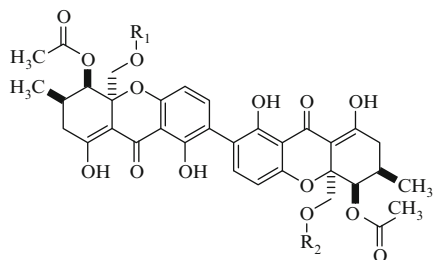
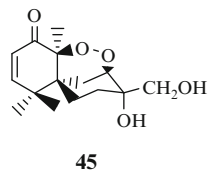
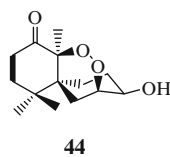
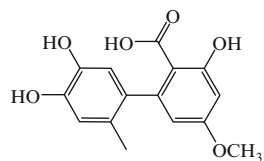


35 ($R_1, R_3 = H; R_2 = OCH_3$)
36 ($R_1, R_2 = H; R_3 = OH$)



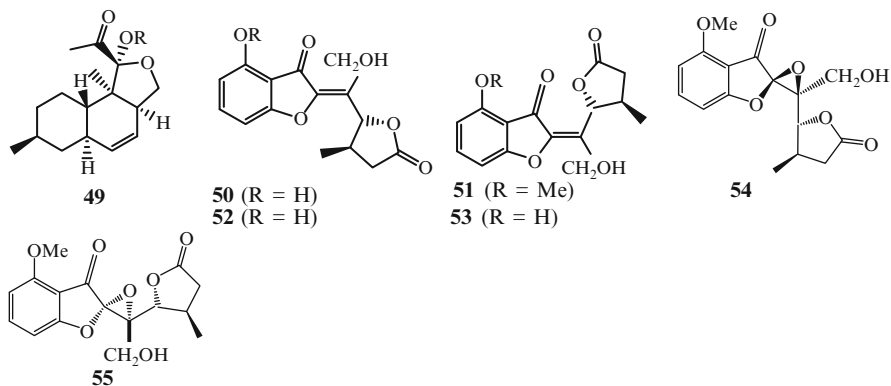
41 ($R^1, R^2 = H$ and $R^3 = SO_3H$)

42 ($R^1, R^2 = H$ and $R^3 = CH_3$)



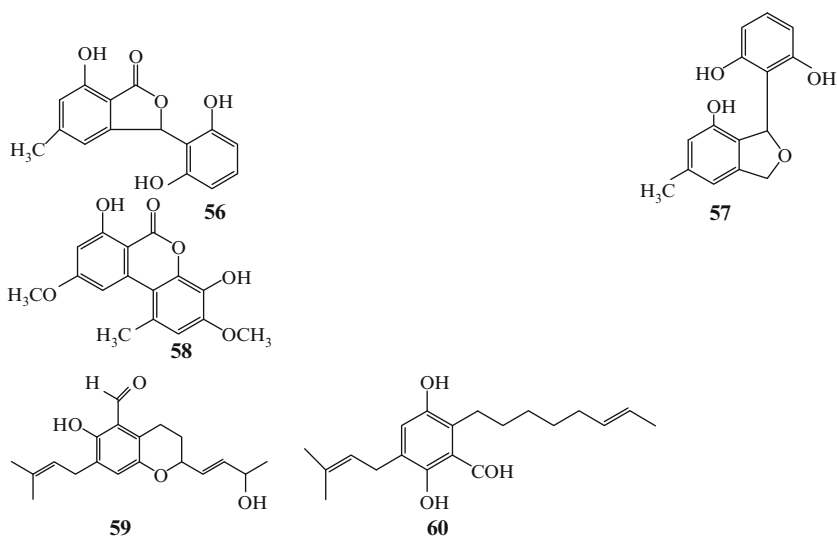
47 ($R_1 = Ac, R_2 = H$)

48 ($R_1 = R_2 = Ac$)



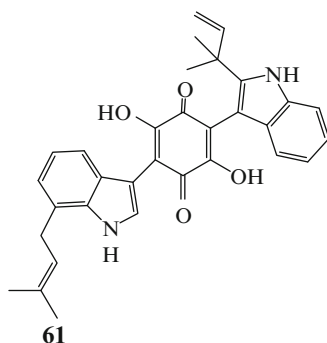
26.3.1.6 Antioxidant Agent

Antioxidant agents are the free radical-scavenging molecules that reduce the possibilities of aging, cancer, coronary heart problems, and Alzheimer's disease. These agents also maintain the quality of food grains during storage. Pestacin (**56**) and isopestacin (**57**) obtained from endophytic *Pestalotiopsis microspora* from interior of *Terminalia morobensis* display an antioxidant activity Strobel et al. (2002) and Harper et al. (2003). *Cephalosporium* sp. IFB-E001, an endophytic fungus harbored in *Trachelospermum jasminoides* produces graphislactone A (**58**) shows significant antioxidant activity Song et al. (2005). Chaetopyranin (**59**) and isotetrahydroauroglucin (**60**) isolated from an endophytic fungus *Chaetomium globosum*, associated with *Polysiphonia urceolata* possesses antioxidant activity. The former compound also exhibit anticancerous activity Wang et al. (2006).



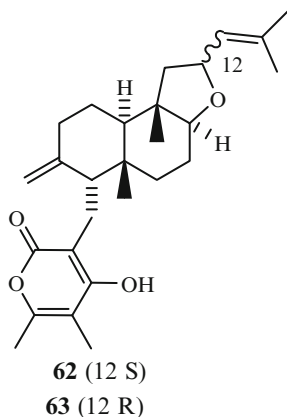
26.3.1.7 Antidiabetic Agent

The two impressive antidiabetic agents namely L783281 (**61**) are the nonpeptidal fungal metabolites isolated from endophytic *Pseudomassaria* sp. The compound acts as an insulin mimetic, but without destroying the digestive tract Zhang et al. (1999).



26.3.1.8 Immunomodulator and Immunosuppressive Agents

Immunomodulator agents play a key and potential role in the treatment of patients suffering from AIDS, cancer and organ transplant. One of the dominant fungal genus of endophyte *Pestalotiopsis leucothes*, isolated from *Tripterygium wilfordii* produces three compounds designated as BS, GS, and YS. These compounds have uneven effects on T- and B-cells and monocytes, and they may partially show the immunosuppressive activity particularly, against human immune mediated diseases Kumar et al. (2005). The rejection of organ/s is the major difficulty in organ transplanted patients and therefore, there is a need of immunosuppressive drugs until transplanted organ totally adopted by the body. An endophytic fungus *Fusarium subglutinans* from *Tripterygium wilfordii* produces the immunosuppressive, but non cytotoxic diterpene pyrones subglutinol A (**62**) and B (**63**) Lee et al. (1995). Collutellin A, a novel peptide with antimycotic activity isolated from *Colletotrichum dematium* is another endophytic fungus recovered from interior of *Pteromischum* sp. Collutellin A, inhibits IL-2 indicates the potential immunosuppressive activity of this compound at such a lower concentration than other previously known compound cyclosporin Ren et al. (2008).

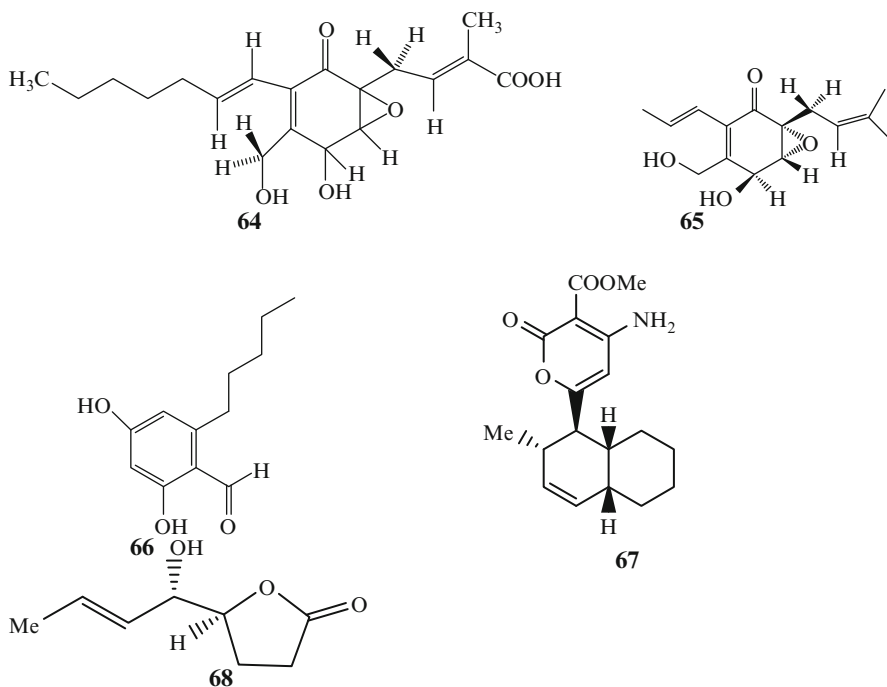


26.3.2 Endophytic Fungi and Plant Protection

26.3.2.1 Anti Fungal Agent

The role of endophytic fungi is well known in plant protection and disease control as many of their isolates have potential to produce effective antifungal compounds that have inhibited fungal pathogens growth successively. Cryptocin (**3**) possesses antifungal activity against phytopathogens like *Pythium ultimum*, *Phytophthora cinnamomi*, *Phytophthora citrophthora*, *Sclerotinia sclerotiorum*, *Pyricularia oryzae*, *Rhizoctonia solani*, *Geotrichum candidum*, *Fusarium oxysporum* Li et al. (2000). *Colletotrichum gloeosporioides* isolated as an endophyte from *Artemisia mongolica* produced colletotric acid (**8**) showed antibacterial and antifungal activity against *Helminthosporium sativum* Zou et al. (2000). Ambuic acid, (**64**) a highly functionalized cyclohexene isolated from endophytic *Pestalotiopsis* sp. and *Monochaetia* sp. showed significant antifungal property against several pathogens like *Fusarium solani*, *F. cubense*, *Helminthosporium sativum*, *Diploidia natelensis*, *Cephalosporium gramineum* and *Pythium ultimum* Li et al. (2001). Endophytic fungus *Pestalotiopsis jesteri* isolated from *Fragariaea bodenii* has produced jesterone (**65**), a highly functionalized novel cyclohexenone epoxides exhibits antifungal ability in opposition to *Pythium ultimum*, *Aphanomyces* sp., *Phytophthora citrophthora*, *P. cinnamomi*, *Rhizoctonia solani* and *Pyricularia oryzae* Li and Strobel (2001). Pestacin (**56**) and Isopestacin (**57**) are isobenzofurans isolated from *Pestalotiopsis microspora* obtained as an endophyte from *Terminalia morobensis*. Both compounds exhibit antioxidant and antifungal activity against pathogenic fungus *Pythium ultimum* Strobel et al. (2002), Harper et al. (2003). 2,4-dihydroxy-6-[(1'E,3'E)-penta-1', 3'-dienyl]-benzaldehyde (**66**) has been isolated from *Periconia atropurpurea*, endophytically obtained from the leaves of *Xylopia aromatic* showed the acute antifungal activity against *Cladosporium sphaerospermum* and *C. cladosporioides* at concentration of 1.0 and 2.5 µg/mL respectively Teles et al. (2006). A novel

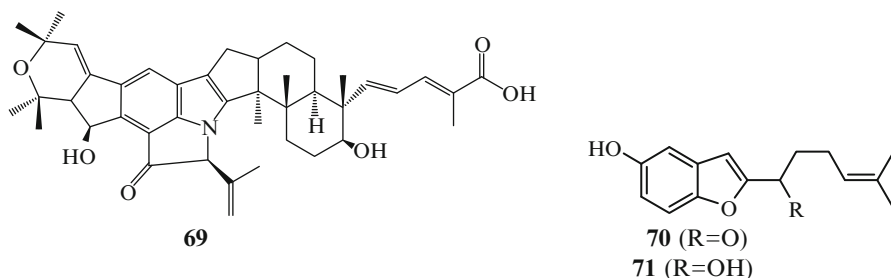
solanapyrone N (**67**) along with a known compound, nigrosporalactone (**68**) were isolated from the fermentation culture of *Nigrospora* sp., YB-141, an endophytic fungus of *Azadirachta indica*. Solanapyrone N exhibits activity against *Botrytis cinerea* and *Penicillium islandicum* while nigrosporalactone showed activity in opposition to *Botrytis cinerea* Wu et al. (2008). *Chloridium* sp. isolated as an endophyte from *Azadirachta indica*, produced javanicin (**7**) a highly functionalized naphthaquinone inhibited the growth of *Cercospora arachidicola*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Verticillium dahliae* Kharwar et al. (2009).



26.3.2.2 Insecticidal Agent

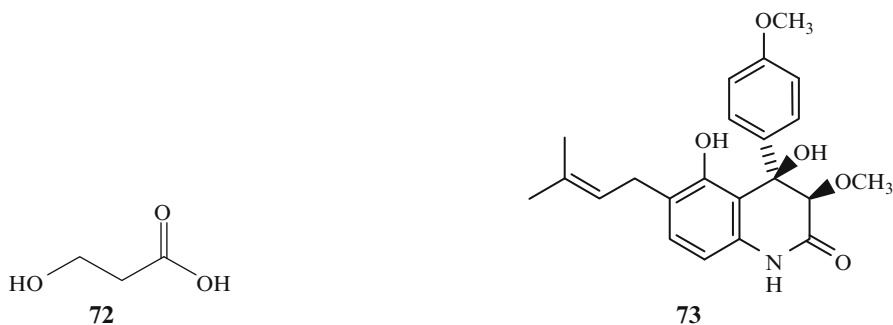
The potent insecticidal agent nodulisporic acid A (**69**), an indole terpene was isolated from fermentation of an endophytic fungus *Nodulisporium* sp., of *Bontia daphnoides*. Nodulisporic acid A was active against the larvae of the blowfly and mosquito at sub-part-per-million levels Ondeyka et al. (1997). Another two novel compounds 5-hydroxy-2-(1-oxo-5-methyl-4-hexenyl) benzofuran (**70**) and 5-hydroxy-2-(1-hydroxy-5-methyl-4-hexenyl) benzofuran (**71**) were isolated from unidentified endophytic fungus of *Gaultheria procumbens*. The later one exhibits toxicity to *Christoneura fumiferana* (spruce budworm cells) only, whereas the former was toxic to larvae also Findlay et al. (1997). 12 fractions were obtained from secondary metabolites of *Penicillium* sp., an endophytic inhabitant of *Derris elliptica*,

and out of these, fraction D, E and J showed the significant toxicity against adult turnip aphid, *Lipaphis erysimi* Hu et al. (2005).



26.3.2.3 Nematicidal Agents

The compound 3-Hydroxypropionic (72) acid was isolated from *Phomopsis phaseoli* endophytically present to *Betula pendula* and *B. pubescens* showed selective nematicidal activity against the plant-parasitic nematode *Meloidogyne incognita* Schwarz et al. (2004). The nematicidal alkaloid peniprequinolone (73), has been extracted from an endophytic fungus *Phomopsis janczewskii* of Chilean gymnosperm *Prumnopitys andina* with significant activity, however, initially the compound was also reported from a soil fungus *Penicillium* cf. *simplicissimum* Schmeda-Hirschmann et al. (2005).



26.3.2.4 Endophytic Fungi and Abiotic Stresses

Excess or lowering of temperature, water, metals, salts and pH cause stresses on survival of plants and literatures suggest that endophytes are also involved in protection of plants against these abiotic stresses. Redman et al. (2002) concluded that *Dichanthelium lanuginosum* infected with endophytic *Curvularia protuberate* was able to grow at temperatures as high as 57°C in the geothermal soils of Yellowstone National Park (YNP) and Lassen Volcanic National Parks (LVNP). However, the

refined research indicates that this effect is not only due to endophytic *Cuvularia*, but was also due to a *Curvularia* thermal tolerance virus (CThTV) as a third partner, which parasitized on *C. protuberate* Marquez et al. (2007). Hutton et al. (1996), observed in south-west Australia that in drought condition, the water availability could be maintained at higher levels in endophytes infected tall fescue compared to enophytes free plants. Elbersen and West (1996), Buck et al. (1997), Liu et al. (1996), observed the aluminium tolerance in endophyte infected fine fescues (*Festuca* spp.) was greater compared to non-infected plants. Malinowski and Belesky (1999) concluded that endophytes infected root of tall fescue increases the pH of limed and acidic soil faster than non infected one.

26.4 Antimicrobial Volatiles Compounds (VOCs)

Muscodor albus I-41.3a, isolated as an endophyte from interior of unidentified vine (plant I-41) produced a number of volatiles with antimicrobial property. The VOC's included tetrahydrofuran, 2-methylfuran, 2-butanone, aciphyllene, and much amounts of an unusual azulene derivatives. The fungus showed interesting activity against *Stachybotrys charatarum* and other phytopathogen Atmosukarto et al. (2005). A volatile oil with trans-1,2,3,3a,4,7a-hexohydro-7a-methyl-5H-inden-5-one (73.1%), 2-methylene-4,8,8-trimethyl-4-vinyl bicycle [5.2.0] nonane (12.0%), and 2,6-dimethyl-6-(4-methyl-3-pentenyl) bicycle [3.1.1] hept-2-ene (4.5%) isolated from endophytic *Fusarium tricinctum* of Chinese medicinal herb *Paris polyphylla* var. *yunnanensis* exhibit anti fungal (*Candida albicans* and *Magnaporthe oryzae*) and anti bacterial activities (*E. coli*, *Salmonella*, *Bacillus* etc.) Zhang et al. (2010). The artificial mixture of volatile compounds may also have usefulness in treating seeds, fruits and plant parts in storage and while being transported. In addition, *M. albus* is already in a limited market for the treatment of human wastes. Its gases have both inhibitory and lethal effects on such faecal-inhabiting organisms as *Escherichia coli* and *Vibrio cholera* Kharwar and Strobel (2011).

26.5 Mycodiesel

Gliocladium roseum was isolated as an endophyte residing inside *Eucryphia cordifolia*, produced a series of volatile hydrocarbons and hydrocarbon derivatives. The hydrocarbon series isolated showing similarities with diesel fuel and this fungus could be used as a novel biofuel source and due to fungal source this fuel was named as a mycodiesel by Strobel et al. (2008). The another recent and interesting discovery appeared when eucalyptol, a rare compound only previously known to be found in eucalyptus bark was isolated from an endophytic fungus. Surprisingly, Dr. Strobel, has run his Honda motorbike using this eucalyptol and therefore, it could be a good and safe alternative of gasoline as its potential is much better Tomsheck et al. (2010). The emphasis is being given to drive this kind of research world wide to solve the

problem of fuel using non conventional sources, so that pressure could be reduced from conventional ores, and Dr. Strobel and his group was given a huge amount from funding agency to precise his research.

26.6 Endophytic Fungi and Nanotechnology

Nanotechnology is one of highly important and cutting edge area of scientific research refers to nano scale synthesis of metals with novel properties. Now these days, the nanotechnology shows valuable scope in communication, energy, electronics, instruments, optical engineering, defence and security, cosmetics, bioengineering, nanofabrics, agri food industry, biomedical and drug delivery. The nano food market is the fastest growing sector where this technology could be used in the production, processing and packaging. A nanocomposite coating process may improve food packaging by placing antimicrobial agents directly on the surface of the coated film. Further, the research is carried out to the detection of chemical and biological substances for sensing biochemical changes in foods quality. As per the survey of Helmut Kaiser consultancy the nanofood market will surge from 2.6\$ billion to 20.4\$ billion by 2011 Verma et al. (2009b). There are many chemical and physical approaches for synthesis of nano particles, but they are highly costly and hazardous to environment. Therefore, scientific community looks for ecofriendly, cost effective and an alternative technology, and the one step biosynthesis or green synthesis is one of them, which synthesizes nanoparticles of useful metals (Au, Ag, Cd, Pt, Ti, Zn and Pd) via biological tools such as Bacteria Yong et al. (2002), fungi and actinomycetes Sastry et al. (2003), Verma et al. (2010), algae Brayner et al. (2007) and plants Narayanan and Sakthivel (2008). Recently, a number of fungi including endophytic such as *Fusarium semitectum*, *Verticillium* sp., *Aspergillus*, *Fusarium* and *Penicillium fellutanum* were successfully employed for synthesis of metal nano particles (NPs). Endophytic *Aspergillus clavatus* isolated from *Azadirachta indica* collected from Banaras Hindu University was efficiently able to synthesize the silver NPs of 5–55 nm. These NPs showed the antifungal (*Candida albicans*) and antibacterial (*E. coli* and *Pseudomonas fluorescens*) activity Verma et al. (2010). *Verticillium* sp. isolated from *Taxus* sp., when challenged with ions of silver and gold led to biofabrication of silver and gold nanoparticles within the fungal biomass Sastry et al. (2003). These examples are enough to prove the use and role of endophytic fungi in different aspects of nanotechnology.

26.7 Conclusions

Historically, the study of endophytes has been limited more or less to botanists to observe their ecology, diversity, distribution and biologics. However, in recent years, the discovery of novel chemical compounds produced by endophytes has opened

the field to structural chemists, biochemists, and medical and industrial scientists as well. The endophytes based bioactive compounds/drugs can only be achieved through interdisciplinary cooperation of research including botanists, microbiologist, molecular biologists, structural chemist, biochemist and pharmacologists. Before dealing the endophytic research we must take care of the basics, such as (i) Location: It is one of the most important criteria as tropical and subtropical rain forests contain the largest diversity of plant species, and can be utilized to discover diverse species of endophytes for the identification of novel anti-fungal and anti-bacterial natural products. In addition to these habitats, researcher should also consider other habitats such as those in the desert and high elevation Alpine passes. (ii) History: The plants with ethanobotanical history and their use by indigenous people for healing and medicinal purposes may provide guidance for further the study. The discovery of "Taxol" a cytotoxic compound used in cancer therapy is made by both the fungal endophyte and the host plant (pacific yew), and also the discovery of vincristine and vinblastine from fungal endophytes of *Catharanthus roseus* are good examples of the potential bioactive compounds that may be obtained based on plant history. (iii) Communication: Very little, if any, is known about the consequences of fungal/fungal, bacterial/bacterial and fungal/bacterial associations and interactions, especially *in planta*. In some preliminary studies, it was found that the presence of one or more than one endophytes facilitate the production of bioactive compounds. Obviously, more efforts must be initiated in this area of research to be able to optimally harnessing the potential of endophytes. (iv) Distribution and abundance: Studies have shown that in some systems, the presence of endophytes is ubiquitous while in some systems very few endophytes colonize the tissues such as roots and stems. So, these studies beg one to as the question of "What are the regulatory aspects involved in the differential *in vivo* and *in planta* distribution(s) observed?" (v) Production: Aspects affecting the stimulation and subsequent production of bioactive compounds is an important variable to determine. Studies have indicated that the production of compounds is affected by the immediate environment. For example, when grown under *in vitro* conditions, an endophyte may successfully and constantly produce bioactive materials for a time, after which, production ceases. Additional research addressing the regulatory factors involved is required to determine how to induce the endophytes to produce the compound(s) in a steady fashion for successful commercialization to become a reality.

The past history of endophytic research in India especially with fungi is not very encouraging. It seems that investigators that have started this research in India are still actively involved in advancing their research manifesto with this 'under studied' group of microbes and yet, have not advanced the field significantly. An exception to this statement only a few people are engaged and pursuing their research in this area consistently such as Prof. T. S. Suryanarayanan (Chennai), Prof Umashaankar (Karnataka), T. Amna and Sanjana Kaul (Jammu and Kashmir), Prof. H. S. Prakash, and K. R. Sridhar (Mangalore, Karnataka) and R. N. Kharwar (BHU, Varanasi, Uttar Pradesh). Their group is actively searching the various habitats of the countryside in van attempt to investigate microbe/plant associations. They have initiated the biodiversity and distribution patterns of fungal endophytes with some medicinal

plants in India and have published several papers along this line. They have also isolated some bioactive compounds from different endophytic fungi inhabited to different hosts. Some other groups have also started research with fungal endophytes, but overall situation in India compared to countries such as China and Brazil, for exploration of bioactive compounds is still minimal. However, recently, several research groups have started displaying more attention and efforts towards the potential importance of endophytic microbes, especially fungi. About two dozen research groups throughout India are presently engaged in vigorous studies centered on biodiversity and hence natural products discovery from this untapped alternative resource. It has become apparent to many researchers that endophytic microbes have enormous potential for addressing and solving many problems of mankind. With the discovery of new compounds, collectively as a society, we may find new options to protect our interests in agriculture, medicine and nanotechnology. Actually, the status of total published work by Indian researchers hardly approaching up to hundred, which shows a small representative potential of the possibilities that exists in this realm of study. If serious efforts were to be explored in India, with its vast and enormous plant diversity, and/or worldwide with all the collective habitat potentials, it boggles the mind in the possibilities we as scientists may unravel. As such, studies of this nature truly need to be supported not only by the scientific community, but society at large that will surely reap the benefits.

Due to great variation in plant biodiversity and seasonal changes in India, we may have better opportunity to collect/isolate various types of promising endophytic fungi, especially from rainforests and mangrove swamps, which may be able to produce an enormous variety of potential bioactive natural compounds. The bioactive compounds to be obtained that are of interest are those that have activity against important plant and human pathogens/parasites (such as those causing malaria, tuberculosis, leprosy and encephalitis, etc.), which have either become drug resistant, and/or lack drug treatment (i.e., AIDS). In recent years, India has experienced an increase in AIDS and immuno-compromised patients. A search for safe and effective drugs to enhance the resistance capacity of patients would be of great benefit and add quality to life. Discovery of novel antimicrobials against disease that can be used as weapons against virulent pathogens such as *Bacillus anthracis* would be greatly beneficial. Unfortunately, in recent times, a high incidence of scientifically advanced and remote-operated terrorist activities in countries like India, USA, UK, Germany, USSR and France is a reality that needs to be urgently addressed. Required are the developments of compounds that can effectively nullify the potential adverse effect of biochemical attacks. Yet another concern is the inordinately huge population in India (more than one billion strong), all who require a safe, reliable food source for sustenance. Such goals may only be achieved once production of disease free crops is obtainable. Management of human fecal matter is yet another alarming issue if left unmanaged will result in unhygienic and potentially lethal conditions, especially in slums and localities of poorer people. India unfortunately is still suffering from several severe human diseases (tuberculosis, malaria and leprosy, etc.) that have been successfully eradicated from other "first world" countries. One of the primary reasons these disease persist is due to the development of

disease resistance in these microorganisms against particular drug(s). Therefore, to overcome these problems, India as well as the world in general, needs to obtain a variety of novel antimicrobial compounds from biological sources to begin to address these serious issues. In general, fungi as a group hold enormous potential as a promising source of antimicrobials and tool for nanotechnology. Our studies indicate that this group of organisms reside inside healthy plant tissue (endophytes) without causing any detectable symptoms, and are capable of producing powerful natural bioactive compounds, biofuels and nanomaterials. Therefore, we strongly feel that India, as well as the world, needs to make serious efforts to address the potential of endophytes for isolation and synthesis of natural products, biofuel and nanoparticles, and in so doing, help facilitate the development of options and ultimately, the rescue of humanity from annihilation.

26.8 Future Perspectives

Endophytes are extremely diverse group of microbes that range from fungi to bacteria including actinomycetes, but the fungi are most studied group of endophytes, and among fungi the best studied endophytes are intercellular symbionts from ascomycetous family Clavicipitaceae in the grasses of temperate zone. The presence of endophytes was observed from algae to angiosperm studied till date. As per studies made world wide, endophytes augment resistance in their hosts against herbivores, pathogenic fungi, bacteria, viruses, insects, nematodes illness, reduced seed production, temperature and salinity and also against drought and minerals as well. The day by day development of drug resistance to pathogenic forms of bacteria, fungi and other microbes, emergence of lethal viruses, the perpetuating epidemics in developing and under developing countries, and multifold fungal infection, enhancement in human population globally, all shows our inability to overcome these biomedical related problems. In addition to this, we are also unable to assure people towards enough food security in specific regions of the earth and in India too, to support the local human population. Environmental degradation, loss of biodiversity and spoilage of land and water also added to the problems facing mankind. The access of new disease causing agents like AIDS, SARS, Ebola and already epidemic like malaria, leishmania and encephalitis requires the discovery and development of new therapeutic drugs that target them specifically within the cellular metabolism.

Although many products can be produced synthetically, but natural bioactive products remains as an important alternative used heavily in modern medicine and agricultures. Approximately 60% of the new drugs produced during the period 1985–2005 were anticancer, antimigraine and antihypertensive agents derived from either natural products or based on natural products structure. In some instances, endophytic microorganisms have developed the biochemical ability to produce compounds similar or identical to those produced by their host plants as a result of gene recombination during the evolutionary process. Bioactive natural products from endophytic microbes have enormous potential as a source for novel medicinal

Table 26.1 Fungal endophyte's derived bioactive compounds with their hosts, activity and MIC/IC₅₀ values

Host	Endophyte	Compound	Activity	Pathogen/cell line	MIC/IC ₅₀
<i>Pinus sylvestris</i>	<i>Cryptosporiopsis</i> sp.	Echinocandin	Antifungal	<i>Candida albicans</i>	0.015 µg/mL
<i>Fagus sylvatica</i>				<i>S. cerevisiae</i>	2.0 µg/ml
<i>Tvipterigeum wilfordii</i>	<i>Cryptosporiopsis quercina</i>	Cryptocandin	-do-	<i>Trichophyton rubrum</i> (ATCC 28188)	0.07 µg/mL
				<i>T. mentagrophytes</i> (ATCC 28185)	0.07 µg/mL
				<i>Candida albicans</i> (ATCC 90028)	0.035 µg/mL
<i>Selaginella pallescens</i>	<i>Fusarium</i> sp.	CR377	-do-	<i>C. parapsilosis</i>	2.5 µg/mL
<i>Conocarpus erecta</i> & <i>Forsteronia spicata</i>	<i>Cytospora</i> sp. & <i>Diaporthe</i> sp.	Cytosporones D	-do-	<i>Histoplasma capsulatum</i>	0.01 µg/mL
<i>Gingo biloba</i>	<i>Xylaria</i> sp.	7-amino-4-methylcoumarin	-do-	<i>Candida albicans</i>	30 µg
				<i>C. albicans</i>	4 µg/mL
<i>Azadirachta indica</i>	<i>Clitoridium</i> sp.	Javanicin	-do-	<i>Candida albicans</i>	40 µg/mL
<i>Artemisia mongolica</i>	<i>Colletotrichum gloeosporioides</i>	Colletotric acid	Antibacterial	<i>Penicillium expansum</i>	40 µg/mL
				<i>Aspergillus niger</i>	25 µg/mL
				<i>Bacillus subtilis</i>	25 µg/mL
<i>Tectona grandis</i>	<i>Phomopsis</i> sp.	Phomoxanthones A & B	-do-	<i>Staphylococcus aureus</i>	50 µg/mL
				<i>Sarcina lutea</i>	50 µg/mL
<i>Conocarpus erecta</i> & <i>Crassocephalum crepidioides</i>	<i>Cytospora</i> sp. & <i>Geotrichum</i> sp.	Cytosporones D	-do-	<i>Mycobacterium tuberculosis</i>	0.50 and 6.25 µg/mL
		7-butyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one	-do-	<i>Staphylococcus aureus</i>	8.0 µg/mL
				<i>Enterococcus faecalis</i>	8.0 µg/mL
				<i>Mycobacterium tuberculosis</i>	25 µg/mL

(continued)

Table 26.1 (continued)

Host	Endophyte	Compound	Activity	Pathogen/cell line	MIC/IC ₅₀
		7-but-15-enyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one	-do-	-do-	50 µg/mL
<i>Quercus variabilis</i>	<i>Fusarium</i> sp. IFB-121	Cerebroside 1 & fusamide 2	-do-	<i>Bacillus subtilis</i> <i>Escherichia coli</i> <i>Pseudomonas fluorescens</i> <i>Bacillus subtilis</i> (ATCC6633)	7.8 and 3.9 µg/mL 3.9 and 3.9 µg/mL 7.8 and 1.9 µg/mL 25 and 50 µg/mL
<i>Sargassum</i> sp.	ZZF36	Lasiodiplodin & de-O-methylasiodiplodin	-do-	<i>Staphylococcus aureus</i> (ATCC27154) Salmonella enteritidis (ATCC 13076) <i>S. aureus</i> (ATCC 25923)	6.25 and 12.5 µg/mL 12.5 µg/mL 8 µg/mL
<i>Garcinia mangostana</i>	<i>Botryosphaeria mamane</i>	Primin	-do-	<i>S. aureus</i> SK1 <i>Mycobacterium tuberculosis</i>	8 µg/mL 6.25 µg/mL
<i>Garcinia dulcis</i>	<i>Phomopsis</i> sp. PSU-D15	Phomoenamide	-do-	<i>Bacillus</i> sp. <i>Escherichia coli</i>	40 µg/mL 40 µg/mL
<i>Azadirachta indica</i>	<i>Clitoridium</i> sp.	Javanicin	-do-	<i>Pseudomonas fluorescens</i> <i>Pseudomonas aeruginosa</i> hCMV HIV-1	2 µg/mL 2 µg/mL 43 and 11 µmol* 6 and 5 µM*
<i>Quercus</i> sp. Unidentified tree	<i>Cytospora</i> sp. <i>Penicillium chrysogenum</i>	Cytionic acids A & B Xanthoviridicatins E & F	Antiviral -do-	Herpes simplex I -do- -do-	3 µg/mL* 3.3 µg/mL* 14 µM*
<i>Buxus sempervirens</i> <i>Cudophyllum</i> sp. <i>Musa acuminata</i>	NRRL 15684 <i>Pullularia</i> sp. <i>Phomopsis</i> sp. BCC 9789	S 39163/F-1 Pullularin A Oblongolide Z	-do- -do- -do-		

<i>Tectona grandis</i>	<i>Phomopsis</i> sp.	Phomoxanthones A & B	Antimalarial	<i>Plasmodium falciparum</i>	0.11 and 0.33 µg/mL*		
<i>Crassocephalum crepidioides</i>	<i>Geotrichum</i> sp.	7-butyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one	-do-	-do-	4.7 µg/mL*		
<i>Sandoricum koejape</i>	<i>Xylaria</i> sp.	7-butyl-6,8-dihydroxy-3(R)-pentylisochroman-1-one -do-2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione	-do-	-do-	2.6 µg/mL*		
<i>Cudophyllum</i> sp.	<i>Pullularia</i> sp.	Xylariaquinone A	-do-	-do-	6.68 µM*		
<i>Vochysia guatemalensis</i>	CR127A	Pullularin A	-do-	-do-	3.6 µg/mL*		
<i>Stemona</i> sp.	<i>Exserohilum rostratum</i>	Codinaeopsin	-do-	-do-	2.3 µg/mL*		
<i>Licuala spinosa</i>	BCC 21097	Monocerin & 11-hydroxymonocerin	-do-	-do-	0.68 and 7.70 µM*		
<i>Taxus brevifolia</i>	<i>Taxomyces andreanae</i>	Eremophilanolides 2 & 3	-do-	-do-	8.1 and 13.0 µM*		
<i>Terminalia arjuna</i>	<i>Pestalotiopsis terminaliae</i>	Taxol	Anticancer	BT220, H116, Int407	0.005–5 µM*		
<i>Aegle marmelos</i>	<i>Bartalinia robillardioides</i>	Taxol	-do-	HL251 and HLK210			
<i>Nothapodytes foetida</i>	<i>Neurospora</i> sp & <i>Entrophospora infrequens</i>	Camptothecin	-do-				
<i>Camptotheca acuminata</i>	<i>Fusarium solani</i>	Camptothecin 9-methoxycamptothecin 10-hydroxycamptothecin	-do-				
<i>Catharanthus roseus</i>	<i>Fusarium oxysporum</i>	Vincristine	-do-				

(continued)

Table 26.1 (continued)

Host	Endophyte	Compound	Activity	Pathogen/cell line	MIC/IC ₅₀
Mediterranean green alga	<i>Emericella nidulans</i> var. <i>acristata</i>	Emindole DA cell lines	-do-	36 human tumor	5.5 µg/mL*
<i>Aegiceras corniculatum</i>	<i>Penicillium</i> sp.	Leptosphaerone C	-do-	A-549	1.45 µM*
				P388	1.38 µM#
				P388	1.38 µM*
<i>Polygonum senegalense</i>	<i>Alternaria</i> sp.	Penicillenone	-do-	L5178Y	1.7 µg/mL*
		Alternariolone	-do-	-do-	7.8 µg/mL*
		Alternariol 5- <i>O</i> -methyl ether	-do-	-do-	4.5 µg/mL*
		Alternariol 5- <i>O</i> -sulfate	-do-	-do-	6.8 µg/mL*
		Altenusin	-do-	-do-	0.99, 0.51, 1.4 µg/mL*
<i>Tectona grandis</i>	<i>Phomopsis</i> sp.	Phomoxanthones A & B	-do-	KB, BC-1, Vero	4.1, 0.70, 1.8 µg/mL*, 4.98 and 4.84 µg/mL*
<i>Xylocarpus granatum</i>	XG8D (basidiomycetes)	Merulin A & C	-do-	BT474, SW620	1.57 and 4.11 µg/mL*
<i>Dicerandra frutescens</i>	<i>Phomopsis longicolla</i>	Dicerandrols A	-do-	A549, HCT-116	7.0 µg/mL*
		Dicerandrols B	-do-	-do-	1.8 µg/mL*
		Dicerandrols C	-do-	-do-	1.8, 7.0 µg/mL*
<i>Musa acuminata</i>	<i>Phomopsis</i> sp.	Oblongolide Z	-do-	KB, BC, NCI-H187	37.0, 26.0 µM*
				BC	32.0 µM#
<i>Roystonea regia</i>	<i>Pestalotiopsis</i> <i>photiniae</i>	Oblongolide Y	-do-	MDA-MB-2311	48 µM*
		Photinides A-F	-do-		10 µg/mL*
<i>Terminalia morobensis</i>	<i>Pestalotiopsis</i> <i>microspora</i>	Pestacin & isopestacin	Antioxidant	hydroxyl free radicals	1.7 and 0.22 mM

<i>Trachelospermum jasminoides</i>	<i>Cephalosporium</i> sp.	Graphis lactone A	-do-	DPPH	2.9 µg/mL*
<i>Polysiphonia urceolata</i>	<i>Chaetomium globosum</i>	Chaetopyranin	-do-	DPPH	35 µg/mL*
Unidentified tree	<i>Pseudomassaria</i> sp.	Isotetrahydroauroglaucin L-783,281	-do-	DPPH	26 µg/mL*
<i>Tripterygium wilfordii</i>	<i>Pestalotiopsis leucothes</i>	BS, GS, YS	Antidiabetic	—	
<i>Tripterygium wilfordii</i>	<i>Fusarium subglutinans</i>	Subglutinol A & B	Immunomodulator		
<i>Pteromischum</i> sp.	<i>Colletotrichum dematium</i>	Collutellin A	Immunosuppressive		0.1 µM* 167.3 ± 0.38 nM
Endophytic Fungi and Plant Protection					
<i>Tripterygium wilfordii</i>	<i>Cryptosporiopsis cf. quercina</i>	Cryptocin	Antifungal	<i>Pythium ultimum</i>	0.78 µg/mL
			-do-	<i>Phytophthora cinnamomi</i>	0.78 µg/mL
			-do-	<i>Phytophthora citrophthora</i>	1.56 µg/mL
			-do-	<i>Sclerotinia sclerotiorum</i>	0.78 µg/mL
			-do-	<i>Pyricularia oryzae</i>	0.39 µg/mL
			-do-	<i>Pyricularia oryzae</i>	0.39 µg/mL
			-do-	<i>Rhizoctonia solani</i>	6.25 µg/mL
			-do-	<i>Geotrichum candidum</i>	1.56 µg/mL
			-do-	<i>Fusarium oxysporum</i>	1.56 µg/mL
			-do-	<i>Helminthosporium sativum</i>	50.0 µg/mL
<i>Artemisia mongolica</i>	<i>Colletotrichum gloeosporioides</i>	Colletotric acid	-do-	<i>Pythium ultimum</i>	7.5 µg/mL
Rain forest trees	<i>Pestalotiopsis</i> spp.	Ambuic acid	-do-	<i>Pythium ultimum</i>	25.0 µg/mL
<i>Fragaria bodenii</i>	<i>Pestalotiopsis jester</i>	Jesterone	-do-	<i>Aphanomyces</i> sp.	6.5 µg/mL

(continued)

Table 26.1 (continued)

Host	Endophyte	Compound	Activity	Pathogen/cell line	MIC/IC ₅₀
<i>Terminalia morobensis</i>	<i>Pestalotiopsis microspora</i>	Pestacin	-do-	<i>Phytophthora citrophthora</i>	25.0 µg/mL
<i>Xylopi aromatic</i>	<i>Periconia atropurpurea</i>	Isopestacin	-do-	<i>Phytophthora cinnamomi</i>	6.5 µg/mL
		2,4-dihydroxy-6-[(10E,30E)-penta-10,30-dienyl]-Benzaldehyde	-do-	<i>Rhizoctonia solani</i>	25.0 µg/mL
<i>Azadirachta indica</i>	<i>Nigrospora</i> sp. YB-141	Solanapyrones N	-do-	<i>Pyricularia oryzae</i>	25.0 µg/mL
		nigrosporalactone	-do-	<i>Pythium ultimum</i>	10.0 µg/mL
<i>Azadirachta indica</i>	<i>Chloridium</i> sp.	Javanicin	-do-	<i>Pythium ultimum</i>	40 µg/mL
<i>Bontia daphnoides</i>	<i>Nodulisporium</i> sp.	Nodulisporic acid A	Insecticidal	<i>C. sphaerospermum</i>	1.0 µg/mL
<i>Betula pendula</i>	<i>Phomopsis phaseoli</i>	3-Hydroxypropionic	-do-	<i>C. cladosporioides</i>	25.0 µg/mL
			Nematicidal	<i>Botrytis cinerea</i>	31.25 µg/mL
				<i>Botrytis cinerea</i>	31.25 µg/mL
				<i>Cercospora arachidicola</i>	5.0 µg/mL
				<i>Fusarium oxysporum</i>	20.0 µg/mL
				<i>Rhizoctonia solani</i>	10.0 µg/mL
				<i>Verticillium dahliae</i>	10.0 µg/mL
				<i>Aedes aegypti</i> larvae	0.5 ppm
				<i>Lucilia sericata</i>	0.3 ppm
				<i>Meloidogyne incognita</i>	12.5–15.0 µg/mL

* represent IC₅₀ values

and agricultural product development. Thus, there is an urgent need to facilitate the identity of appropriate natural products and the subsequent development of drugs based on them. Such needs add further credence to the importance of the need to preserve natural habitats, and in so doing, preserve plant and endophyte biodiversity. A concerted effort involving multiple organizations needs to be spearheaded in order to begin the daunting task of collection and cataloguing of endophytic microorganisms throughout the world. A better understanding of the biosynthetic pathways involved in the production of bioactive endophytic compounds by chemical and biochemical means is essential. Recent progress in the field of molecular biology of secondary metabolites has given us better insights about how the genes encoding for these bioactive compounds are organized. Investigation of these microorganisms and their relationship to the host requires improved quantitative analysis.

Interestingly, 02 biofuels of fungal endophytes origin have been discussed which certainly provide an alternative to gasoline in future and an integrated approach is required to explore this source of biofuel. Role of fungal endophytes especially against abiotic stresses has also 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 promising area under niche of nanotechnology as it has lower environmental impact than 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. Therefore, we may conclude that this group of microbes, in addition to other usages, may also play a crucial role in this cutting edge technology.

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References

- A.H. Aly, R.A. Edrada-Ebel, I.D. Indriani, V. Wray, W.E.G. Muller, F. Totzke, U. Zirrgiebel, C. Schachtele, M.H.G. Kubbutat, W.H. Lin, P. Proksch, R. Ebel, J. Nat. Prod. **71**, 972–980 (2008)
- A.H. Aly, A. Debbab, J. Kjer, P. Proksch, Fungal Divers. **41**, 1–16 (2010)
- I. Atmosukarto, U. Castillo, W.M. Hess, J. Sears, G. Strobel, Plant Sci. **169**, 854–861 (2005)
- C.W. Bacon, J.F. White, *Microbial Endophytes* (Marcel Dekker, New York, 2000)
- B.P. Bashyal, E.M.K. Wijeratne, S.H. Faeth, A.A.L. Gunatilaka, J. Nat. Prod. **68**, 724–728 (2005)
- S.F. Brady, J. Clardy, J. Nat. Prod. **63**, 1447–1448 (2000)
- S.F. Brady, M.M. Wagenaar, M.P. Singh, J.E. Janso, J. Clardy, Org. Lett. **2**, 4043–4046 (2000)
- R. Brayner, H. Barberousse, M. Harnadi, J. Nanosci. Nanotechnol. **7**, 2696–2708 (2007)
- D. Brem, A. Leuchtmann, Oecologia **126**, 522–530 (2001)
- G.W. Buck, C.P. West, H.W. Elbersen, in *Neotyphodium/Grass Interactions: Endophyte Effect on Drought Tolerance in Diverse Festuca Species*, ed. by C.W. Bacon, N.S. Hill (Plenum Press, New York, 1997), pp. 141–143

- G.C. Carroll, F.E. Carroll, *Can. J. Bot.* **56**, 3034–3048 (1978)
- S. Chareprasert, J. Piapukiew, S. Thienhirun, J.S.A. Whalley, P. Sihanonth, *World J. Microbiol. Biotechnol.* **22**, 481–486 (2006)
- S. Chokpaiboon, D. Sommit, T. Teerawatananond, N. Muangsin, T. Bunyapaiboonsri, K. Pudhom, *J. Nat. Prod.* **73**, 1005–1007 (2010)
- K. Clay, *Mycol. Res.* **92**, 1–12 (1989)
- K. Clay, *Ecology* **71**, 558–570 (1990)
- A. De Bary, *Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten*. Hofmeister's Hand Book of Physiological Botany, vol. 2 (W. Engelmann, Leipzig, Germany, 1866)
- G. Ding, Z. Zheng, S. Liu, H. Zhang, L. Guo, Y. Che, *J. Nat. Prod.* **72**, 942–945 (2009)
- M.M. Dreyfuss, I.H. Chapela, in *The Discovery of Natural Products with Therapeutic Potential*, ed. by V.P. Gullo (Butterworth-Heinemann, Boston, 1994), pp. 49–80
- H.W. Elbersen, C.P. West, *Grass Forage Sci.* **51**, 333–342 (1996)
- J.A. Findlay, S. Buthelezi, G. Li, M. Seveck, *J. Nat. Prod.* **60**, 1214–1215 (1997)
- P.J. Fisher, F. Graf, L.E. Petrini, B.C. Sutton, P.A. Wookey, *Mycologia* **87**(3), 319–323 (1995)
- V. Gangadevi, J. Muthumary, *World J. Microbiol. Biotechnol.* **24**, 717–724 (2008)
- V. Gangadevi, J. Muthumary, *Biotechnol. Appl. Biochem.* **52**, 9–15 (2009)
- S.K. Gond, V.C. Verma, A. Kumar, V. Kumar, R.N. Kharwar, *World J. Microbiol. Biotechnol.* **23**(10), 1371–1375 (2007)
- S.K. Gond, V.C. Verma, A. Mishra, A. Kumar, R.N. Kharwar, in *Management of Fungal Plant Pathogens*, ed. by A. Arya, A.E. Perelló (CAB International, Cambridge, UK, 2010), pp. 183–197
- A.A.L. Gunatilaka, *J. Nat. Prod.* **69**, 509–526 (2006)
- B. Guo, J. Dai, S. Ng, Y. Huang, C. Leong, W. Ong, B.K. Carte, *J. Nat. Prod.* **63**, 602–604 (2000)
- J. Hallmann, A. Quadt-Hallmann, W.F. Mahaffee, J.W. Kloepper, *Can. J. Microbiol.* **43**, 895–914 (1997)
- J.K. Harper, A.M. Arif, E.J. Ford, G.A. Strobel, J.A. Porco, D.P. Tomer, K.L. O'Neill, E.M. Heider, D.M. Grant, *Tetrahedron* **59**, 2471–2476 (2003)
- D.C. Hawksworth, A.Y. Rossman, *Phytopathology* **87**, 888–891 (1987)
- M.T. Hoffman, A.E. Arnold, *Mycol. Res.* **112**, 331–344 (2008)
- M.Y. Hu, G.H. Zhong, Zh.T. Sun, G. Sh, H.M. Liu, X.Q. Liu, *J. Appl. Entomol.* **190**, 413–417 (2005)
- B.J. Hutton, K. Sivasithamparam, K.W. Dixon, J.S. Pate, *Ann. Bot.* **77**, 399–404 (1996)
- M. Isaka, A. Jaturapat, K. Rukseree, K. Danwisetkanjana, M. Tanticharoen, Y. Thebtaranonth, *J. Nat. Prod.* **64**, 1015–1018 (2001)
- M. Isaka, P. Berkaew, K. Intereya, S. Komwijit, T. Sathitkunanon, T. Sathitkunanon, *Tetrahedron* **63**, 6855–6860 (2007)
- M. Isaka, P. Chinthanom, T. Boonruangprapa, N. Rungjindamai, U. Pinruan, *J. Nat. Prod.* **73**, 683–687 (2010)
- R.N. Kharwar, G. Strobel, in *Natural Products in Pest Management*, ed. by N.K. Dubey (CAB International, Wallingford, Oxfordshire, UK, 2011), pp. 218–241
- R.N. Kharwar, V.C. Verma, G. Strobel, D. Ezra, *Curr. Sci.* **95**, 228–233 (2008)
- R.N. Kharwar, V.C. Verma, A. Kumar, S.K. Gond, J.K. Harper, W.M. Hess, E. Lobkovsky, C. Ma, Y. Ren, G.A. Strobel, *Curr. Microbiol.* **58**, 233–238 (2009)
- R.N. Kharwar, A. Mishra, S.K. Gond, A. Stierle, D. Stierle, *Nat. Prod. Rep.* **28**, 1208–1228 (2011)
- P. Kongsaree, S. Prabpai, N. Sriubolmas, C. Vongvein, S. Wiyakrutta, *J. Nat. Prod.* **66**, 709–711 (2003)
- R. Kontnik, J. Clardy, *Org. Lett.* **10**, 4149–4151 (2008)
- A. Kralj, S. Kehraus, A. Krick, E. Eguereva, G. Kelter, M. Maurer, A. Wortmann, H.H. Fiebig, G.M. König, *J. Nat. Prod.* **69**, 955–1000 (2006)
- D.S.S. Kumar, C.S. Lau, J.M.F. Wan, D. Yang, K.D. Hyde, *Life Sci.* **78**, 147–156 (2005)
- S. Kusari, S. Zuhlke, M. Spittler, *J. Nat. Prod.* **72**, 2–7 (2009)
- J.C. Lee, E. Lobkovsky, N.B. Pliam, G. Strobel, J. Clardy, *J. Org. Chem.* **60**, 7076–7077 (1995)

- J.Y. Li, G.A. Strobel, *Phytochemistry* **57**, 261–265 (2001)
- J.Y. Li, G. Strobel, J. Harper, E. Lobkovsky, J. Clardy, *Org. Lett.* **2**, 767–770 (2000)
- J.Y. Li, J.K. Harper, D.M. Grant, B.O. Tombe, B. Bashyal, W.M. Hess, G.A. Strobel, *Phytochemistry* **56**, 463–468 (2001)
- Z. Lin, T. Zhu, Y. Fang, Q. Gu, W. Zhu, *Phytochemistry* **69**, 1273–1278 (2008)
- H. Liu, J.R. Heckman, J.A. Murphy, *J. Plant Nutri.* **19**, 677–688 (1996)
- X. Liu, M. Dong, X. Chen, M. Jiang, X. Lv, J. Zhou, *Appl. Microbiol. Biotechnol.* **78**, 241–247 (2008)
- D.P. Malinowski, D.P. Belesky, *J. Plant Nutri.* **2**, 835–853 (1999)
- D. Malinowski, A. Leuchtmann, D. Schmidt, J. Nösberger, *Agronom J.* **89**, 673–678 (1997)
- L.M. Marquez, R.S. Redman, R.J. Rodriguez, M.J. Rossinck, *Science* **315**, 513–515 (2007)
- K.B. Narayanan, N. Sakthivel, *Mat. Lett.* **62**, 4588–4590 (2008)
- H.M. Noble, D. Langley, P.J. Sidebottom, S.J. Lane, P.J. Fisher, *Mycol. Res.* **95**, 1439–1440 (1991)
- J.G. Ondeyka, G.L. Helms, O.D. Hensens, M.A. Goetz, D.L. Zink, A. Tsipouras, W.L. Shoop, L. Slayton, A.W. Dombrowski, J.D. Polishook, D.A. Ostlind, N.N. Tsou, R.G. Ball, S.B. Singh, *J. Am. Chem. Soc.* **119**, 8809–8816 (1997)
- J.O. Pereira, M.L. Carnerio Vieira, J.L. Azevedo, *World J. Microbiol. Biotechnol.* **15**, 43–46 (1999)
- O. Petrini, in *Microbiology of the Phyllosphere*, ed. by N.J. Fokkema, J. van den Heuvel (Cambridge University Press, Cambridge, 1986), pp. 175–187
- W. Pongcharoen, V. Rukachaisirikul, S. Phongpaichit, J. Sakayaroj, *Chem. Pharm. Bull.* **55**, 1404–1405 (2007)
- A. Pontius, A. Krick, S. Kehraus, R. Brun, G.M. Konig, *J. Nat. Prod.* **71**, 1579–1584 (2008)
- S.C. Puri, V. Verma, T. Amna, G.N. Qazi, M. Spitteller, *J. Nat. Prod.* **68**, 1717–1719 (2005)
- K. Rajagopal, T.S. Suryanarayanan, *Curr. Sci.* **78**, 1375–1378 (2000)
- R.S. Redman, S.B. Kathy, S.G. Richard, R.J. Rodriguez, J.M. Henson, *Science* **298**, 1581 (2002)
- S. Rehman, A.S. Shawl, A. Kour, R. Andrabi, P. Sudan, P. Sultan, V. Verma, G.N. Qazi, *Appl. Biochem. Microbiol.* **44**, 203–209 (2008)
- Y. Ren, G.A. Strobel, J.C. Graff, M. Jutila, S.G. Park, S. Gosh, D. Teplow, M. Condrón, E. Pang, W.M. Hess, E. Moore, *Microbiology* **154**, 1973–1979 (2008)
- J.S. Rice, B.W. Pinkerton, W.C. Stringer, D.J. Undersander, *Crop Sci.* **30**, 1303–1305 (1990)
- L.H. Rosa, A.B.M. Vaz, R.B. Caligiorme, S. Campolina, C.A. Rosa, *Polar Biol.* **32**, 161–167 (2009)
- V. Rukachaisirikul, U. Sommart, S. Phongpaichit, J. Sakayaroj, K. Kirtikara, *Phytochemistry* **69**, 783–787 (2008)
- R.M.G. Santos, E. Rodrigues-Fo, W.C. Rocha, M.F.S. Teixeira, *World J. Microbiol. Biotechnol.* **19**, 767–770 (2003)
- R. Sappapan, D. Sommit, N. Ngamrojanavanich, S. Pengpreecha, S. Wiyakrutta, N. Sriubolmas, K. Pudhom, *J. Nat. Prod.* **71**, 1657–1659 (2008)
- M. Sastry, A. Ahmad, M.I. Khan, R. Kumar, *Curr. Sci.* **85**, 165–170 (2003)
- G. Schmeda-Hirschmann, E. Hormazabal, L. Astudillo, J. Rodriguez, C. Theoduloz, *World J. Microbiol. Biotechnol.* **21**, 27–32 (2005)
- B. Schulz, C. Boyle, S. Draeger, A.K. Rommert, K. Krohn, *Myc. Res.* **106**, 996–1004 (2002)
- M. Schwarz, B. Kopcke, R.W.S. Weber, O. Sterner, H. Anke, *Phytochemistry* **65**, 2239–2245 (2004)
- R.G. Shu, F.W. Wang, Y.M. Yang, Y.X. Liu, R.X. Tan, *Lipids* **39**, 667–673 (2004)
- P. Silvia, L. Roberto, J.G. Duckett, E.C. Davis, *Am. J. Bot.* **95**, 531–541 (2008)
- S.B. Singh, D.La Zink, Z. Guan, J. Colladob, F. Pelaezb, P.J. Felock, D.J. Hazudac, *Helv. Chim. Acta* **86**, 3380–3385 (2003)
- Y.C. Song, W.Y. Huang, C. Sun, F.W. Wang, R.X. Tan, *Biol. Pharm. Bull.* **28**, 506–509 (2005)
- H.W. Spurr, R.E. Welty, *Phytopathology* **65**, 417–422 (1975)
- A. Stierle, G. Strobel, D. Stierle, *Science* **260**, 214–216 (1993)

- G.A. Strobel, *Crit. Rev. Biotechnol.* **22**, 315–333 (2002)
- G.A. Strobel, R.V. Miller, C. Martinez-Miller, M.M. Condrón, D.B. Teplow, W.M. Hess, *Microbiology* **145**, 1919–1926 (1999)
- G. Strobel, E. Ford, J. Worapong, J.K. Harper, A.M. Arif, D.M. Grant, P.C.W. Fung, R.M.W. Chau, *Phytochemistry* **60**, 179–183 (2002)
- G.A. Strobel, B. Knighton, K. Kluck, Y. Ren, T. Livinghouse, M. Griffin, D. Spakowicz, J. Sears, *Microbiology* **154**, 3319–3328 (2008)
- T.S. Suryanarayanan, G. Venkatesan, T.S. Murali, *Curr. Sci.* **85**, 489–493 (2003)
- T.S. Suryanarayanan, S.K. Wittlinger, H.F. Stanley, *Mycol. Res.* **109**, 635–639 (2005)
- L.J. Swartzell, M.J. Powell, J.Z. Kiss, *Int. J. Plant. Sci.* **157**, 53–62 (1996)
- S. Tansuwan, S. Pornpakakul, S. Roengsumran, A. Petsom, N. Muangsin, P. Sihanonta, N. Chaichit, *J. Nat. Prod.* **70**, 1620–1623 (2007)
- B. Taridaporn, Y. Seangaroon, S. Prasert, S. Kitlada, L. Saisamorn, *J. Nat. Prod.* **73**, 55–59 (2010)
- M.V. Tejesvi, B. Mahesh, M.S. Nalini, H.S. Prakash, K.R. Kini, V. Subbiah, S.S. Hunthrike, *World J. Microbiol. Biotechnol.* **21**, 1535–1540 (2005)
- H.L. Teles, R. Sordi, G.H. Silva, I. Castro-Gamboa, B.V. da Silva, L.H. Pfenning, L.M. de Abreu, C.M. Costa-Neto, M.C.M. Young, A.R. Araujo, *Phytochemistry* **67**, 2686–269 (2006)
- A.R. Tomscheck, G.A. Strobel, E. Booth, B. Geary, D. Spakowicz, B. Knighton, C. Floerchinger, J. Sears, O. Liarzi, D. Ezra, *Microb. Ecol.* **60**(4), 903–914 (2010)
- H. Tschertter, H. Hofmann, R. Ewald, M.M. Dreyfuss, U.S. Patent No. 4,753,959, 1988
- C.Y. Tung, D.B. Yang, M. Gou, J. Chuxiong Norm. Univ. **6**, 3–41 (2002)
- V.C. Verma, S.K. Gond, A. Kumar, A. Mishra, R.N. Kharwar, A.C. Gange, *Microb. Ecol.* **57**, 749–75 (2009a)
- V.C. Verma, R.N. Kharwar, A.C. Gange, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 4, No. 026, 2009b
- V.C. Verma, R.N. Kharwar, A.C. Gange, *Nanomedicine* **5**, 33–40 (2010)
- M.M. Wagenaar, J. Clardy, *J. Nat. Prod.* **64**, 1006–1009 (2001)
- S. Wang, X. Li, F. Teuscher, D. Li, A. Diesel, R. Ebel, P. Proksch, B. Wang, *J. Nat. Prod.* **69**, 1622–1625 (2006)
- M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, A.T. McPhail, *J. Am. Chem. Soc.* **93**, 2325–2327 (1971)
- S.H. Wu, Y.W. Chen, S.C. Shao, W. Li-Dong, Y. Yu, Z. Li, Y. Li-Yuan, S. Li, R. Huang, *Chem. Biodivers.* **6**, 79–85 (2008)
- R. Yang, C. Li, Y. Lin, G. Peng, Z. She, S. Zhou, *Bioorg. Med. Chem. Lett.* **16**, 4205–4208 (2006)
- P. Yong, N.A. Rowsen, J.P.G. Farr, I.R. Harris, L.E. Macaskie, *Biotechnol. Bioeng.* **80**, 369–79 (2002)
- B. Zhang, G. Salituro, D. Szalkowski, Z. Li, Y. Zhang, I. Royo, D. Vilella, M. Dez, F. Pelaez, C. Ruby, R.L. Kendall, X. Max, P. Griffin, J. Calaycay, J.R. Zierath, J.V. Heck, R.G. Smith, D.E. Moller, *Science* **284**, 974–981 (1999)
- L.B. Zhang, L.H. Gou, S.V. Zeng, *Chin. Tradit. Herb. Drug* **11**, 805–807 (2000)
- Y. Zhang, J. Zhao, J. Wang, T. Shan, L. Zhou, J. Wang, *Nat. Prod. Commun.* **5**, 1–5 (2010)
- W.X. Zou, J.C. Meng, H. Lu, G.X. Chen, G.X. Shi, T.Y. Zhang, R.X. Tan, *J. Nat. Prod.* **63**, 1529–1530 (2000)