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Review

Diversity of endophytic fungi in some tropical medicinal plants – A report

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Endophytic fungi are a taxonomically and ecologically heterogenous group of organisms; mainly belong to ascomycetes, coelomycetes and hypomycetes. The abundance of endophytic fungi in tropical regions has been studied. However, scant attentions have been given to medicinal plants of tropics. Synthesis of Indole acetic acid and metabolites showing antiviral, volatile metabolites, anticancer agents, anti-oxidants, insecticidal activities, antidiabetic and immunosuppressive compounds are widespread among the endophytic fungi. Now, more concentration has been given to the chemistry and bioactive compounds of endophytic fungi, mycodiversity and their ecological role. This review deals with the brief survey of the endophytic fungi of some medicinal hosts and their bioactive compounds characterized.

Key words: Ascomycetes, coelomycetes, ecology, hypomycetes, secondary metabolites, and tropical.

INTRODUCTION

The term endophyte was first coined by De Bary in 1866 (Wilson, 1995). Since then it has undergone many subtle changes.

Endophyte: Circumscription

The term endophyte itself evolved to accommodate our increasing knowledge regarding this group of fungi. Ainsworth (1971) defined endophyte as “a plant living inside another organism”. Carroll (1986) confined the use of the term to organisms that cause asymptomatic infections within plant tissue; this definition excluded pathogenic fungi. Petrini (1991) widened the scope of this definition to include all organisms inhabiting plant organs that, at some time in their life, can colonize internal plant tissues without causing apparent harm to the host. This circumscription would include latent pathogens and epiphytes that are found as endophytes during some stages of their life. Thus, some endophytes residing in plant tissues become pathogenic when their hosts are weakened (Rajagopal, 1998). Wilson (1995) proposed that endophytes are fungi or bacteria that, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues but cause no symptoms of disease. The fossil record indicates that plants have been associated with endophytic (Krings et al., 2007) and mycorrhizal (Redecker et al., 2000) fungi for > 400 million years. Almost all the plant species (~400,000) harbor one or more endophytic organisms (Tan and Zou, 2001). Dreyfuss and Chapela (1994) estimated that endophytic fungi from the 270,000 species of plants existing on this planet could account for 1.38×10^6 unique fungal sp. The enormity of this estimation is because endophytic fungi have been isolated from every plant species examined to date and endophytes are important components of fungal diversity (Arnold et al., 2000; Kumaresan and Suryanarayanan, 2002; Suryanarayanan and Rajagopal, 1998).

The presence of endophytic fungi in plant tissues was discovered more than 75 years ago when Sampson (1935) reported such fungi from *Lolium* grass. The con-temporary resurgence of research on endophytic fungi began when Bernstein and Carroll (1977) reported the presence of endophytes in needles of *Pseudotsuga*

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menziesii. The study of endophytic fungi apart from shedding light on diversity of fungal kingdom, offers a promising digression since some endophytes produce novel metabolites of pharmaceutical and agricultural value (Rajagopal et al., 2010).

Biodiversity and ecology of endophytic fungi of medicinal plants

Fungal endophytes are ubiquitous. Almost all vascular plant species examined to date were found to harbor endophytic bacteria and or fungi (Sturz et al., 2000; Arnold et al., 2000). They have been isolated from such diverse groups of plants as marine algae (Cubit, 1974), mosses and ferns (Petrini, 1986), coniferous trees (Carroll and Carroll, 1978; Johnson and Whitney, 1992), angiosperms such as *Dalbergia latifolia*, *Azadirachta indica*, Grasses (Clay, 1990; Suryanarayanan and Rajagopal, 2000).

Medicinal plants are reported to harbor endophytes (Strobel, 2002), which in turn provide protection to their host from infectious agents and they are unexpected producers of metabolites useful to pharmaceutical and agricultural industries (Petrini et al., 1992b). Research on species composition of endophyte communities of many hosts has shown that a large number of fungal taxa could be isolated from a single host species (Petrini et al., 1989). However, usually one or a few fungal species dominate in a host plant (Rajagopal et al., 2010; Suryanarayanan et al., 1998). The distribution of endophytes in a host is influenced by environmental factors and age of the host species or tissue. The number of endophytic species that can be recovered from host decreases with extreme climates (Suryanarayanan et al., 1998). Several studies have shown that the frequency of colonization increases with age of the tissue colonized (Espinosa-Gracia and Langenheim, 1990; Rajagopal, 1998). Similarly, the endophyte community of a plant is affected by the quality of air (Petrini, 1991) and leaf chemistry (Rajagopal et al., 2010). The endophytes occupy a unique niche. Though they may not be exposed to the vagaries of environment as the phylloplane fungi are, they have to encounter the defense reactions of the host. Hence, their life strategies are likely to be different from those of other fungi. Some of the medicinal host for endophytic fungi, host plant and chemical compound reported tabulated in (Tables 1, 2 and 3).

Physiology of endophytes

The production of extracellular enzymes such as cellulases, pectinases, esterases, amylases, proteases etc., by some endophytic fungi has been studied by substrate utilization test and isozyme analysis (Sieber et al., 1991; Rajagopal, 1998). Production of the growth hormone indole acetic acid has been demonstrated for fungal endophytes such as *Aureobasidium pullulans* and *Epicoccum purpurascens* (Pugh et al., 1971, 1972) *A. indica* (Rajagopal, 1998). Carroll and Petrini (1983) investigated the ability of endophytes to tolerate or metabolize phenolics and other defense chemicals of the host tissues. Suryanarayanan and Rajagopal (1998) reported that endophytic fungi are known to produce compounds that interfere with plant cell division.

Endophytes have recently been shown to be key elements in plant symbiosis, affecting host tolerance in stressful conditions (Redman et al., 2002; Rodriguez et al., 2004; Marquez et al., 2007) plant defense (Omacini et al., 2001; Bailey et al., 2006) plant growth (Ernst et al., 2003) plant community biodiversity (Clay and Holah, 1999) melanin synthesis (Rajagopal et al., 2011). However the diversity, geographic distribution and host specificity of endophytes remain largely unknown (Arnold et al., 2001, Ganley et al., 2006, Otero et al., 2002, 2007, Arnold and Lutzoni, 2007, Higgins et al., 2007). Recent estimation of fungal diversity (Hawksworth, 1991, 2001; Schmit and Mueller, 2007) suggest that more than 90% of fungal species are not described and there are no specific estimates of the number of existing endophytes (Hyde and Soytong, 2007, Hyde et al., 2007).

Biotechnological prospective of endophytic fungi of medicinal plants

Endophytic fungi are one of the major potential sources for new, useful metabolites (Dreyfuss and Chapela, 1994). There has been a great interest in endophytic fungi as potential producers of novel, biologically active products (Schulz et al., 2002; Strobel and Daisy, 2003; Tomita, 2003; Urairaj et al., 2003; Wildman, 2003).

Tan and Zou (2001) reported that endophytic fungi are rich source of functional metabolites which include alkaloids, amines, amides, indole derivatives, pyrrolizidines, steroids, terpenoids, sesquiterpenes, diterpenes, isocoumarin derivatives, quinines, flavonoids, phenyl propanoids, lignans, peptides, phenol, phenolicacids, aliphatic compounds, chlorinated metabolites etc.

Endophytic fungi colonize very special and often very hostile habitats and were increasingly recognized as a group of organisms that are likely to be sources of new metabolites useful in biotechnology and agriculture (Bills and Polishook, 1992). Many endophytic fungi elaborate antibiotic compounds in culture that are active against human and plant pathogens. Fisher et al. (1984a) showed that more than 30% of the endophytic fungi that they tested possess antifungal and antibacterial activity. Fisher et al. (1984b) have isolated a broad-spectrum antibiotic from an endophyte of *Vaccinium* sp. Endophytes such as *A. pullulans* and *E. purpurascens* influenced seed germination by producing phyto-

Table 1. Host plants surveyed.

S/N	Host plants	S. No	Host plants	S. No	Host plants
1.	*Abies spectabilis*	13.	*Collicarpa tomentosa*	25.	*Podocarpus netifolius*
2.	*Adhatoda zeylanica*	14.	*Crataeva magna*	26.	*Saussurea involucrate*
3.	*Aegle marmelos*	15.	*Eucalyptus globules*	27.	*Stipa grandis*
4.	*Azadirachta indica*	16.	*Garcinia mongostana*	28.	*Taxus cuspidate*
5.	*Bauhinia phoenicea*	17.	*Garcinia pravifolia*	29.	*Taxus muirei*
6.	*Butea monosperma*	18.	*Holarrhena antidysenterica*	30.	*Taxus wallichiana*
7.	*Calotropis procera*	19.	*Leucas aspera*	31.	*Terminalia arjuna*
8.	*Camptotheca acuminate*	20.	*Lobelia nicolinifolia*	32.	*Terminalia chebuba*
9.	*Catharanthus roseus*	21.	*Madhuka nerifolia*	33.	*Tridax procumbens*
10.	*Cedrus deodara*	22.	*Naregamia alata*	34.	*Tsuga dumosa*
11.	*Clerodendron serratune*	23.	*Ocimum basilicum*	35.	*Vitex negundo*
12.	*Coleus aromatics*	24.	*Ocimum sanctum*	36.	*Withania somnifera*

Table 2. Endophytic fungi genera reported.

S/N	Endophytic fungi	S/N	Endophytic fungi	S/N	Endophytic fungi
1.	*Chaetomium*	13.	*Phomopsis*	25.	*Fusarium*
2.	*Glomerella*	14.	*Phyllosticta*	26.	*Myrothecium*
3.	*Guignardia*	15.	*Sphaeropsis*	27.	*Nigrospora*
4.	*Pyrenospora*	16.	*Acremonium*	28.	*Paecilomyces*
5.	*Xylaria*	17.	*Alternaria*	29.	*Penicillium*
6.	*Botryosphaeria*	18.	*Aspergillus*	30.	*Pithomyces*
7.	*Colletotrichum*	19.	*Aurobasidium*	31.	*Rhizoctonia*
8.	*Cytospora*	20.	*Bipolaris*	32.	*Sporidesmium*
9.	*Monochaetia*	21.	*Cladosporium*	33.	*Stenella*
10.	*Pestalotia*	22.	*Curvularia*	34.	*Trichoderma*
11.	*Pestalotiopsis*	23.	*Cylindrocarpon*	35.	*Verticillium*
12.	*Phoma*	24.	*Drechslera*	36.	*Mucor*

hormones (Petrini et al., 1992a). In developed countries, fungal endophytes are viewed, as biocontrol agents for fungal plant diseases (Clay, 1989; Petrini, 1991). They are readily selectable component of the fungus-plant system and are ideal for experimental manipulations. Endophyte represents natural genomes vested with useful attributes that could be selected and introduced into host plants for desirable traits.

Alternatively, endophytes could be used as vectors of genes to be introduced artificially into a population of host plants (Petrini et al., 1992a).

Endophyte association offer greatest potential for biocontrol programmes because these fungi are integrated into host systems (Clay, 1989). Dewan and Sivasithamparam (1989) reported that a fungal endophyte isolated from wheat provides significant protection to the host from infection by “take all” fungus. Endophytic fungi of conifer needles produce chemicals that ward off insect pests. The best example for endophyte-mediated antagonism towards an insect pest is that of Douglas fir. The needles of this plant harbour *Rhabdocline parkeri* as an endophyte, and this fungus controls the gall midge, *Contarina* sp. (Carroll and Carroll, 1978). Thus, some endophytes are potential candidates for biocontrol programme (Dorworth and Callan, 1996). Host specific endophytic fungi could be used as a vector for introducing foreign genes into the host. Endophytic fungi could be genetically manipulated to produce novel compounds in a host- a kind of indirect genetic engineering of plants (Clay, 1989).

Endophytes are being explored by both pharmaceutical and agricultural industries (Monaghan et al., 1995) as they represent an untapped pool of secondary metabolites (Dreyfuss and Chapela, 1994). Merck Research Laboratories, USA, is collaborating with Costa Rica in INBIO project to screen fungal endophyte from forest trees for novel antibiotics (Bills and Polishook, 1994; Sanders et al., 1995). Recently Li et al. (1998) reported that an endophyte occurring in the bark of *Taxus wallichiana* growing in Nepal Himalayas produces taxol, an anticancer drug. Souvik et al. (2008) reported that an endophyte from *Hypericum perforatum* produced

Table 3. Compounds extracted from endophytic fungi.

S/N	Chemical compounds reported	S/N	Chemical compounds reported
1.	Taxol	38.	Cyclohexane, 4, methyl
2.	Asperfumin	39.	Decane 3, 3, 6-trimethyl
3.	Cryptocin,	40.	Undecane, 4, 4-demethyl
4.	Cryptocandin,	41.	Alkanes including those of pentyl, hexyl, heptyl, octyl, sec-octyl and decyl alcohols
5.	Jesterone	42.	Decane, 3, 3, 5-trimethyl
6.	Oocydin	43.	Chaetomugilin A(2)
7.	Isopestacin	44.	Chaetoglobosins A(3) Chaetoglobosins C (4) Polyhydroxylated C_{29}
8.	Pseudomycins	45.	Sterol, 25ε- methyl 22-homo-5α-cholest- 7
9.	Ambuic acid	46.	22-diene-3β, 6β, 9α- triol
10.	Ergovaline	47.	Designated globosterol (1) Tetrahydroxylated ergosterol 22E, 24R-ergosta-7,
11.	Peramine	48.	22-diene-3β, 5α, 6β- 9α-tetrol (2)
12.	2-methoxy-4-hydroxy-6-methoxymethyl-benzaldehyde	49.	7-hydroxypthalide [4]
13.	Cryptosporiopsin and 4-epi-ethiosolide	50.	4- hydroxypthalide 5
14.	loline (1-aminopyrrolizidines)	51.	5-methoxy-7- hydroxypthalide[6]
15.	Fumonisius	52.	5,7, dihydroxypthalide [7] (3R,4R)-cis-4-hydroxymellein [8]
16.	6-isoprenylindole-3-carboxylic acid	53.	(3R,4R)-cis-4-hydroxy-5-methylmellein [9]
17.	3β, 5α -dihydroxy-6β -acetoxy-ergosta 7, 22-diene	54.	Ergosterol [10]
18.	Cytosporones D and E	55.	5α, 8α- epidioxyergosterol [11]propanoic acid
19.	Trihydroxybenzene laotones	56.	2-methyl-methyl ester
20.	Phomoxanthone A and B	57.	2-methyl- 1-butanol
21.	(-)-(2S,5Z)-2-(1-methylethyl)-4-oxo-5-(phenylmethylene)-1	58.	3-methyl 1-butanol
22.	3-dioxalane-2 carboxylic acid	59.	3-methyl, acetate
23.	Munumbicins A, B, C and D,	60.	2-methyl- 2-methylbutyl ester Ethanol
24.	Bicyclic fusidilactones A (1) and B(2)	61.	Diethylpthlate
25.	Fusidilactone C (4)	62.	Ethyl alcohol
26.	Taxoids	63.	Ethyl ether
27.	Naphthalene 1, 1'- oxybis	64.	Tricyclic sesquiterpenoids Brasilamides A-D
28.	(2'S)-2-(Propan 2'-01)-5-hydroxy-benzopyran-4-one(2)	65.	Pinthunamide
29.	2, 3-dihydro-2 methyl-benzopyran-4,5-diol (4):	66.	Brasilamide (A-D)
30.	Benzopyrans; 2-methyl-5-methoxy-benzopyrone-4-one(1)	67.	Diketopiperazine (DKP) Colletopiperazine,
31.	(2R) -2, 3-dihydro-2-methyl 5-methoxy-benzopyran-4-one (3)	68.	Fusapepazine C and E
32.	Hypericin	69.	Sesquiterpenoids
33.	Naphthoquinone spiroketals	70.	Polyketide
34.	Palmarumycin	71.	Eremophilane
35.	Preussomerin	72.	Mairetolide F (6)
36.	Undecane-2,6-dimethyl	73.	Eremophilanolides α-methylene-γ-lactone (1-3)

Table 3. Contd.

37.	Decane-3,3,5-trimethyl	74.	Butanedioic acid

Hypericin (Tables 1, 2 and 3).

Endophyte represents natural genomes vested with useful attributes that could be selected and introduced into host plants for desirable traits. Alternatively, endophytes could be used as vectors of genes to be introduced artificially into a population of host plants (Petrini et al., 1992a).

Conclusion

Endophytic fungi of medicinally important host are poorly investigated group of microorganisms that represent untapped pool of bioactive and novel chemical compounds which can be exploited in a variety of agriculture, pharmaceutical and chemical industries in near future. Some species of endophytic fungi have been identified as source of anticancer, antidiabetic, insecticidal and immuno-suppressive compounds are widespread among endophytic fungi. All aspects of the biology and interrelation of endophytic with the respective hosts are largely under investigation. More information about host plant and their internal microbiology would be exceedingly useful in developing the search for novel bioactive com-pounds. A great deal of uncertainty also exists between what the endophyte produces in culture and what it may produce in nature. This may be especially true if the bioactive product of the endophyte is unique to it and the host does not produce it. Even though studies on isolation, characterization and extraction bioactive compounds from endophytic fungi from medicinal plants have just started and use of this enormous mycoresourse is poorly understood. More discoveries of endophytes and their products from this field hold exciting promise. This is amply supported by the identification of wide variety of endophytic fungi and products reported so for. To get more benefit from these groups of organisms natural forests must be protected in countries like India, that have different forest types. Hence, more studies on these groups of organisms are required. Joint effort is needed between chemists and mycologists to understand fungal biology, ecology and the mycologist will have the opportu-nity to gain more insight into the multifarious diversity of the fungal kingdom. The chemists could discover new chemical compounds including proteins or enzymes.

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