3 Diversity and Biopotential of Endophytic Fungal Flora Isolated from Eight Medicinal Plants of Uttar Pradesh, India

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Abstract

Endophytic fungi are hidden diversity mines of microbes that reside in the healthy and symptomless interior of plant tissues without causing any harmful effects. This chapter focuses on fungal endophytic diversity of eight medicinal plants of Uttar Pradesh, India with their biopotential ability. Total of 4,002 (38.38 % CF) endophytic isolates were recovered from 10,425 segments representing 131 endophytic fungal species belonging to different fungal classes. Out of 4,002 isolates, hyphomycetes were more pronounced with 71.43 % recovery followed by coelomycetes 16.61 %, ascomycetes 6.59 %, mycelia sterilia or unidentified 5.32 % and least from zygomycetes 0.020 %. Among total endophytic fungal species isolated, *Cladosporium cladosporioides* (3.39 % CF) was found to be the most dominated taxa followed by *Alternaria alternata* (2.35 % CF), *Curvularia lunata* (2.13 % CF), *Aspergillus niger* (1.95 % CF), *Chaetomium globosum* (1.85 % CF), *Nigrospora oryzae* (1.57 % CF) and *Phoma glomerata* (1.09 % CF). From a total of 131 endophytic species, 101 were tested for their antimicrobial and antioxidant activity. Out of 47 active species, 29.78 % displayed antibacterial activity, 27.65 % showed antifungal activity, 38.29 % exhibited antibacterial and antifungal activity both while only 4.25 % displayed antimalarial as well as antioxidant activity. Twenty-one endophytic fungal species were tested for extracellular production of amylase, xylanase and phosphate solubilization where 76.19 % found to produce amylase, 23.80 % for xylanase and 14.28 % exhibited phosphate-solubilization activity.

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Antimicrobial · Diversity · Enzyme activity · Fungal endophytes · Medicinal plants

3.1 Introduction

Microorganisms are an important component of the environment, they affect their surroundings in various ways and forms, one of them are endophytes. The term endophyte was first introduced by de Bary in 1866 for all those microbes that reside inside the living healthy tissues. Many workers define endophytes in various ways, but the definition given by Bacon and White (2000) was perhaps most acceptable as 'microbes that colonize living, internal tissues of plants without causing any immediate and overt negative symptoms'. This is a topographical term and includes bacteria, fungi, actinomycetes and algae, which spend their whole or a period of life cycle either in symplast or apoplast region of healthy plant tissues without producing any disease or clinical symptoms. On the basis of their nature, endophytes may be categorized in three groups: (1) pathogens of another host that are nonpathogenic in their endophytic relationship, (2) nonpathogenic microbes, (3) pathogens that have been rendered nonpathogenic but are still capable of colonization by selection methods or genetic alteration (Backman and Sikora 2008). Among all the endophytic microbes, fungi are the most studied group so far. Endophytic fungi play an important role in plant/host community health by providing resistance from herbivores (Brem and Leuchtmann 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), heavy metal (Li et al. 2012). Endophytic fungi are also able to produce a considerable number of useful enzymes and this ability can make enzymes cost effective because approximately 60 % of the currently used industrial enzymes are of fungal origin (Østergaard and Olsen 2010). Interestingly, Suryanarayanan and his colleagues observed the number of foliar fungal endophytes associated with trees of forests in the Western Ghats mountain (in Southern India) produced a range of extracellular enzymes including amlyases, cellulases, chitinases, chitosanases, laccases, lipases, pectinases and proteases (Suryanarayanan et al. 2012).

An irrational and irregular use of antibiotics makes pathogen more resistant and it is a serious impediment for microbiologists providing the required demand of antibiotics. To cope with this problem, there is ultimate necessity for an alternative and novel source of effective drugs without destroying biodiversity. In such respect, endophytic fungi became an effective solution because one can isolate the compound of plant/host origin without destroying the plant population. After the discovery of taxol (billion dollar drug) from the endophytic fungus *Taxomyces andreanae* (Stierle et al. 1993), it proved itself as a novel source of taxol production without loss of the *Taxus* plant. After this discovery, the endophytic research came to light and microbes have been considered as a novel and alternative source for new biologically active compounds and/or compounds of host origin such as taxol (Stierle et al. 1993), vincristin (Tung et al. 2002), camptothecin (Shweta et al. 2010), piperin (Verma et al. 2011), azadirachtin (Kusari et al. 2012), etc. Today, credits go to endophytic microbes for producing a number of new and effective bioactive natural compounds that can be used in agriculture, medicine and industry. In addition, more than 100 anticancer compounds have been (57 % novel and 43 % known) isolated only from endophytic fungi (Kharwar et al. 2011). In this chapter, we have focused mainly on the diversity of endophytic fungi of eight medicinal (*Azadirachta indica, Agele marmelos, Catharanthus roseus, Eucalyptus citriodora, Nyctanthes arbor-tristis, Adenocalymma alliaceum, Tinospora cordifolia, Cinnamomum camphora*) plants of Uttar Pradesh, India, with their antimicrobial potential.

3.2 Transmission of Endophytic Fungi

Transmission describes the spreading of microbes within and among host population. Endophytic fungi have two transmission modes, vertical and horizontal. Vertical transmission occurs when fungi travel from host to their offspring via host tissues such as host seeds and vegetative propagules. Systemically infected endophytic fungi have vertical transmission mode that differs from horizontal transmission where fungus travels by its sexual or asexual spores.

3.3 Ecology and Biodiversity of Endophytic Fungi

Endophytic fungi are important, hidden, highly diverse, less exploited and highly potential component of the environment. Almost all plant species studied to date for endophytic diversity were found to act as a reservoir for potential of microbes to be used to resolve the problems of mankind. The endophytes were observed in all green biota ranging from algae (Yang et al. 2006), bryophytes (Chambers et al. 1999), pteridophytes (Schmid and Oberwinkler 1995), gymnosperms (Huang and Wang 2011) and to angiosperms (Mishra et al. 2012a), including underground root to all aerial parts of host (Kharwar et al. 2008). Endophytic fungi isolated from water-stressed deserts (Bashyal et al. 2005), cold-stressed arctic (Fisher et al. 1995), Antarctic ocean (Rosa et al. 2009; 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) and mangrove swamps (Maria et al. 2005). Fungal endophytes were isolated from either all or specific organs of selected hosts showing the impacts of environmental variables on their colonization frequency (CF), diversity and antimicrobial activity (Hyde and Soytong 2008; Mishra et al. 2012a; Verma et al. 2011; Verma et al. 2013). Our earth harbours almost 300,000 higher plants species, and each species represents either one or plethora of endophytic community which is well proved by the previous reports of higher plants fungal endophytes (Strobel 2002). Out of these plants that exist on the earth, only a few dozen, have been studied related to their endophytic biology, and every plant studied has an endophytic community. Including fungal endophytes, the ratio of fungal to plant species will reach up to 33:1 from 6:1 (Hawksworth and Rossman 1987).

3.3.1 Endomyco Diversity in *Adenocalymma alliaceum* Miers

A. alliaceum, is commonly known as garlic creeper or *lahsun lata* plant. It is a member of the family Bignoniaceae, a highly medicinal, evergreen tropical shrubby vine plant that is native to the Amazon rainforest. In the absence of garlic, its leaf can be used as a substitute for cooking purposes. Every part of the plant is well used by the indigenous people of the Amazon as folk medicine for curing various disorders. Despite having several compounds, it is considered analgesic, anti-inflammatory, depurative, purgative and widely used against arthritis, rheumatism, body aches, muscle pain, cholesterol and injuries. Its leaves are also used to cure flu, pneumonia, cough, fever and headache. Kharwar et al. (2011) reported the isolation of total 149 fungal endophytic isolates belonging to 17 fungal taxa from 270 segments of leaf, stem and petiole (90 segments of each tissue). Collectively, among the total isolates recovered, hyphomycetes were more frequent (74.47%) followed by mycelia sterilia (10.07%), ascomycetes (8.05%) and coelomycetes (4.03%) (Table 3.1, Fig. 3.1). Among all tissues studied, leaves showed greater colonization of endomycobiota (72.22%) compared to stem (67.78%) and petiole (25.54%). *A. alternata* (6.30%), *A. niger* (5.93%), *Stenella agalis* (5.20%), *Fusarium oxysporum* (5.18%), *C. lunata* (4.18%) and *Fusarium roseum* (4.07%) were recovered as the dominant genera. However, *Penicillium* sp. and *Rhizoctonia* sp. were the least frequent with equal CF of 1.85%. Out of 17 taxa, *Penicillium* sp., *C. globosum* and *Rhizoctonia* sp. were only restricted to stem tissue, and as per authors this may be because of displacement of their spores from root and substrate specificity supported by stem.

Table 3.1 Endophytic fungal diversity among eight different medicinal plants

Host plants	*A. alliaceum*	*A. marmelos*	*A. indica*	*C. roseus*	*C. camphora*	*E. citriodora*	*N. arbortristis*	*T. cordifolia*	Total	
Total segments plottedt	270	550	600	300	105	600	800	7200	10425	
Endophytic fungi	17	32	44	19	26	32	34	29		Total CF
Acremonium acutatum			34						34	0.329
Acromonium sp.			15				40	6	61	0.585
Alternaria alternata	17	29	22	16		25	59	78	246	2.35
Alternaria chlamydospora			12						12	0.115
Alternaria cinerariae					10				10	0.095
Alternaria dennsii			19						19	0.182
Alternaria longipes			19	4					23	0.220
Alternaria sp.	5					6			11	0.105
Arthrinium sp.					1				1	0.009
Arthrobotrys sp.					6				6	0.057
Aspergillus flavus			18					52	70	0.671
Aspergillus fumigatus	8	14	2	10	7	19	20		80	0.767
Aspergillus niger	16	29	38	10	17	8	25	61	204	1.956
Aspergillus oryzae			7						7	0.067
Aspergillus sydowii								36	36	0.345
Aspergillus terreus				6		10		28	44	0.422
Aspergillus tubingensis								20	20	0.191
Aureobasidium pullulans						22	24		46	0.441
Aureobasidium sp.		63							63	0.604
Basidiobotrys sp.						8			8	0.076
Bipolaris sp.				12					12	0.115
Botryosphaeria rhodina								38	38	0.364
Botrytis cinerea						21			21	0.201
Botrytis sp.								16	16	0.153
Cercinella mucoroides			1						1	0.009
Chaetomium crispatum			2						2	0.019
Chaetomium globosum	6	37	9	3		20	25	93	193	1.85
Chaetomium sp.	6				3				9	0.086
Chaetophoma sp.					1				1	0.009
Chloridium virescenc			1	9					10	0.095
Cladosporiella sp.						7			7	0.067
Cladosporium acaciicola			5						5	0.047
Cladosporium apicale								16	16	0.153
Cladosporium cladosporioides		53	39	16		65	94	87	354	3.39
Cladosporium sp.							13		13	0.124
Cladosporium tennuissimum					8				8	0.076
Colletotrichum crassipes								27	27	0.258
Colletotrichum dematium		12					33	77	122	1.17
Colletotrichum gloeosporioides		18				10			28	0.268
Colletotrichum linicola								58	58	0.556
Colletotrichum sp.			3	6					9	0.086
Corynespora sp.		1					13		14	0.134
Curvularia catanulata			3						3	0.028
Curvularia fallax							3		3	0.028

Table 3.1 (continued)

Host plants	*A. alliaceum*	*A. marmelos*	*A. indica*	*C. roseus*	*C. camphora*	*E. citriodora*	*N. arbortristis*	*T. cordifolia*	Total	
Total segments plottedt	270	550	600	300	105	600	800	7200	10425	
Endophytic fungi	17	32	44	19	26	32	34	29		Total CF
Curvularia intermedia								31	31	0.297
Curvularia lunata	13	32	21	5	1	39	56	56	223	2.13
Curvularia oryzae							17		17	0.163
Diatrype sp.							14		14	0.134
Drechslera sp.			1	11	4				16	0.153
Drechslera ellisii		12					25		37	0.354
Drechslera graminea								25	25	0.239
Drechslera rostrata			2			15			17	0.163
Emericella nidulans								31	31	0.297
Emericella sp.		3							3	0.028
Fusarium chlaydosporum			12						12	0.115
Fusarium moniliformae			4	3					7	0.067
Fusarium oxysporum	14	4	21			8	8	35	90	0.863
Fusarium roseum	11	8		15					34	0.326
Fusarium soloni			3						3	0.028
Fusarium sp.			9						9	0.086
Gliomastix sp.			1		7				8	0.076
Guignardia sp.								6	6	0.057
Helicosporum sp.							4		4	0.038
Humicola grisea			2		6	7	19		34	0.326
Humicola sp.		14		9		20		23	66	0.633
Macrophoma sp.							16		16	0.153
Melanconium sp.						20	37		57	0.546
Monilia sp.								10	10	0.095
Morphospecies 1					6				6	0.057
Morphospecies 2					5				5	0.0479
Morphospecies 3					7				7	0.067
Morphospecies 4					7				7	0.067
Mycelia sterilia		2	18						20	0.191
Mycelia sterilia	4								4	0.038
Mycelia sterilia	6								6	0.057
Mycelia sterilia	5								5	0.047
Mycelia sterilia		9							9	0.086
Mycelia sterilia		3							3	0.028
Mycelia sterilia		1							1	0.009
Mycelia sterilia		10							10	0.095
Mycelia sterilia						14			14	0.134
Mycelia sterilia						8			8	0.076
Mycelia sterilia						7			7	0.067
Mycelia sterilia							7		7	0.067
Mycelia sterilia							4		4	0.038
Mycelia sterilia							35		35	0.335
Mycelia sterilia							12		12	0.115
Mycelia sterilia							7		7	0.067
Nigrospora oryzae		13	18	6	6	12	39	70	164	1.57

Table 3.1 (continued)

Host plants	*A. alliaceum*	*A. marmelos*	*A. indica*	*C. roseus*	*C. camphora*	*E. citriodora*	*N. arbor-tristis*	*T. cordifolia*	Total	
Total segments plottedt	270	550	600	300	105	600	800	7200	10425	
Endophytic fungi	17	32	44	19	26	32	34	29		Total CF
Oidiodendron clamydosporum						13			13	0.124
Penicillium citrinum				16					16	0.153
Penicillium cristata			7			10			17	0.163
Penicillium crysogenum				11					11	0.105
Penicillium sp.	5	2	1		8		9		25	0.239
Penicillium sp. 1								87	87	0.834
Penicillium sp. 2								58	58	0.556
Periconia sp.			1		5				6	0.057
Periconia tirupatiensis						12			12	0.115
Pestalotia macrotricha		28	2						30	0.287
Pestalotiopsis sp.			34		10				44	0.422
Pestelotia sp.						9			9	0.086
Phacidium sp.					2				2	0.019
Phaeotrichoconis sp.							7		7	0.067
Phoma eupyrena			18						18	0.172
Phoma glomerata		25				58	31		114	1.09
Phoma herbarum		20							20	0.191
Phoma sp.		15							15	0.143
Phomopsis helianthi							50		50	0.479
Phomopsis oblonga			27						27	0.258
Phomopsis sp.	6	28			4	24	19		81	0.776
Phyllosticta minima			2						2	0.019
Phyllosticta nobilis					11				11	0.105
Pseudofusicoccum violaceum								8	8	0.076
Rhizoctonia sp.	5	16					22		43	0.412
Scytalidium sp.			2				7		9	0.086
Stachybotrys					4		5		9	0.086
Stenella agalis	14	4							18	0.172
Stenella sp.			2						2	0.019
Trichoderma harzianum					11				11	0.105
Trichoderma sp.	8								8	0.076
Trichoderma viride		1	27			23		8	59	0.565
Ulocladium chlamydosporum			7						7	0.067
Mycelia sterilia/unidentified		4							4	0.038
Mycelia sterilia/unidentified				15	5				20	0.191
Mycelia sterilia/unidentified 1						11			11	0.105
Mycelia sterilia/unidentified 2						7			7	0.067
Mycelia sterilia/unidentified 5						14			14	0.134
Veronaea musae								10	10	0.095
Verticillium albo-atrum			2						2	0.019
Verticillium sp.									1	0.009
Verticillium tenuissimum			2						2	0.019
Total isolates	149	511	495	183	162	552	799	1151	4002	38.38

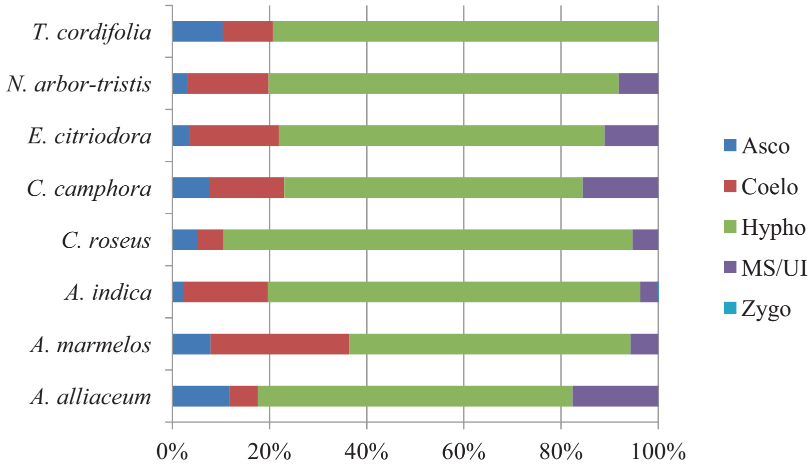

Fig. 3.1 Per cent recovery of different classes of endophytic fungi

3.3.2 Endomyco Diversity in *Aegle marmelos*

A. marmelos is an Indian plant having medicinal and religious importance as well. The plant is used in Indian system of ayurvedic medicine against variety of diseases including diarrhoea, dysentery and dyspeptic symptoms. Green leaves of the plant are used for lowering blood sugar level. The plant was also reported to possess antifungal and antibacterial properties. Gond et al. (2007, 2011) isolated total of 511 endophytic fungal isolates representing 32 endophytic fungal taxa from 550 segments of bark, leaf and root. In the study, bark was found to harbour greater number of endophytic fungi followed by leaf and root. Among total taxa recovered, the *Aureobasidium* sp. (11.45 % CF) was found to be the highly dominated taxon. Among different endophytic classes, hyphomycetes showed maximum colonization 57.92 % followed by 28.57 % coelomycetes, 7.82 % ascomycetes and 5.67 % mycelia sterilia (Table 3.1, Fig. 3.1).

3.3.3 Endomyco Diversity in *Azadirachta indica*

A. indica is native to India and one of the most effective and popular medicinal plant, commonly known as neem, belongs to family Meliaceae. Different parts or extracts of the plant are used as antibacterial, antiretroviral, antiarthritic, anti-inflammatory and antiulcer. Over 400 bioactive compounds from neem plant and 32 from its endophytes have been reported so far. Verma et al. (2007) isolated 495 endophytic fungal isolates from 600 segments of leaf, stem and bark, root and fruit of neem collected from Varanasi region. The total endophytic fungal isolates recovered belonged to 44 fungal species including mycelia sterilia. In whole of the study, hyphomycetes dominated with 76.56 % followed by 17.37 % coelomycetes, 3.63 % mycelia sterilia, 2.22 % ascomycetes and interestingly only a single isolate (0.02 %) of zygomycete (Table 3.1, Fig. 3.1). However, genera like *Cladosporium, Aspergillus, Acremonium, Pestalotiopsis, Phomopsis, Curvularia and Trichoderma* were observed as dominant fungi. Among 495 isolates, 223 isolates were recovered from 200 segments of leaf, bark and stem while 272 isolates were isolated from 400 segments of root and fruit (Verma et al. 2007, 2011; Verma 2009).

3.3.4 Endomyco Diversity of *Catharanthus roseus*

C. roseus is commonly known as Madagascar periwinkle or sadabahar belonging to family Apocynaceae. A number of anticancer vinca alkaloids such as vincristine, vindesine, vinorelbine, vinblastin and vinflunine have been isolated from the plant. It has also been used as a folk remedy to cure diabetes and high blood pressure. Kharwar et al. (2008) reported the isolation of 183 fungal endophytic isolates under 19 fungal species from 300 segments of stem, leaf and root of Varanasi region. Hyphomycetes showed maximum recovery (86.88 %) followed by mycelia sterilia or unidentified groups (8.19 %), 3.27 % coelomycetes and least by ascomycetes 1.63 % (Table 3.1, Fig. 3.1). The CF was found higher in root sample followed by leaf and stem. Root tissues were heavily colonized by genera such as *Alternaria, Cladosporium* and *Aspergillus*. Leaf tissues showed a greater diversity of endophytes and *Drechslera, Curvularia, Bipolaris, Alternaria* and *Aspergillus* spp. were the dominant fungi.

3.3.5 Endomyco Diversity in *Cinnamomum camphora*

C. camphora is commonly known as camphor or *kapoor* plant. The plant belongs to the family Lauraceae and is native to Taiwan, southern Japan, Southeast China and Indochina. The oil of camphor is used as an anti-inflammatory, antiseptic, a cardiac, carminative, diuretic, febrifuge, an insecticide, a laxative, rubefacient, stimulant and vulnerary agent. Kharwar et al. (2012) claimed the isolation of 162 endophytic fungal isolates belonging to 26 species from more than 100 segments of leaf, stem and petiole. Among isolates recovered, hyphomycetes ranked first with 62.96 % isolation frequency (IF) followed by coelomycetes 16.66 %, mycelia sterilia 15.43 % and least from ascomycetes 4.93 % (Table 3.1, Fig. 3.1). Among all the segments studied, leaf harbour maximum (40.44 %) endomyco isolates followed by stem (29.04 %) and petiole (30.24 %). Among all the species observed, *A. niger* (10.49 %) was found to be most dominated followed by *Phyllosticta nobilis* and *Trichoderma harzianum* with equal IF of 6.79 % while *Arthrinium* sp. and *C. lunata* were recorded as rare isolates with IF value of 0.61 %.

3.3.6 Endomyco Diversity of *Eucalyptus citriodora*

Basically *E. citriodora* is a long tree and native to Australia, but it is frequently grown in the northeastern states of India. The bluish-green leaves of the plant contain fragrant volatile oil that have antiseptic, expectorant, antibacterial, anti-inflammatory, deodorant, diuretic and antispasmodic properties. Commonly used and a very important essential oil, it is known as eucalyptol, isolated from the leaves and used as an anti-cough syrup, for aromatherapy, dentistry, and to treat bronchitis, sinusitis, chronic rhinitis and asthma, etc. (Gond et al. 2010; Gond 2011). A total of 552 fungal endophytic isolates belonging to 32 fungal species from 600 segments of leaf and stem at Varanasi and Sonbhadra regions were isolated. Hyphomycetes was found to be the highly dominated group (67.02 %) followed by coelomycetes (18.29 %), mycelia sterilia or unidentified taxa (11.05 %) while ascomycetes represented the least IF (3.62 %; Table 3.1, Fig. 3.1). *Cladosporum cladosporioides* with an IF of 11.77 % was the most dominant taxon followed by *P. glomerata* at 10.50 %.

3.3.7 Endomyco Diversity of *Nyctanthes arbor-tristis*

N. arbor-tristis is a well-known medicinal plant native to the Indian subcontinent and grows abundantly in all parts of the country. It is commonly known as Harsinghar, Parijata, or night jasmine and belongs to the family Oleaceae. The flowers and leaves of *N. arbor-tristis* are well known for their interesting antibacterial, antifungal, antileishmanial and cytotoxic activity. Gond 2011 described the endomyco diversity of leaf and stem of *N. arbor-tristis* collected from Varanasi and Sonbhadra regions. From 800 segments (400 segments for each tissue) of leaf and stem, the author reported the isolation of 799 endophytic isolates. In this study, the recovery of hyphomycetes was found maximum with 72.09 % followed by coelomycetes 16.64 %, mycelia sterilia 8.13 %

and least from ascomycetes 3.12 % (Table 3.1, Fig. 3.1). A total of 34 endophytic fungal species were observed from both tissues collectively. Among the total 34 species recorded, 32 were isolated from the leaves while only 19 species from the stem. *C. cladosporioides* (11.63 %), *A. alternata* (7.38 %), *Phomopsis helianthi* (6.25 %) were observed as dominated taxa. *C. cladosporioides, C. lunata, C. dematium, Drechslera ellisii, Acremonium* sp., *N. oryzae, Phomopsis* sp. and *Rhizoctonia* sp. were isolated as common species for both tissues; *Aspergillus fumigates, A. niger, Helicosporium* sp. *Scytilidium* sp. and *Stachybotrys* sp. were only isolated from the leaf segments while isolate NAH3 only reported from stem segments; however, these results are a fine example of tissue specificity of endophytic fungi. Gond 2011 concluded that leaves harbour a higher number and high diversity of endophytic fungi in comparison to the stem, and this may be due to the large surface area of leaves exposed to the outer environment and the presence of stomata providing passage to the entry of fungal mycelia.

3.3.8 Endomyco Diversity in *Tinospora cordifolia* Miers

T. cordifolia is a widely used medicinal plant in the Indian Ayurvedic system of medicine. It is commonly known as Guduchi, Gurch, Giloe or Amrita, having a large, glabrous, deciduous, shade-loving climbing shrub belonging to family Menispermiaceae. A number of chemical constituents such as alkaloids, diterpenoids, lactones, phenolics, glycosides, aliphatic compounds and steroids have been isolated from *T. cordifolia.* It is used as an anti-inflammatory, antiperiodic, antifever, antidyspepsia, antiarthritic, anti-allergic and antidiabetic agent. The plant is also used to cure scorpion stings, and its watery extract used in febrifuge which is called 'Indian quinine' (Chopra et al. 1982; Singh and Panda 2005). The plant contains a polyclonal B cell mitogen with antioxidant activity which can be used as an immunomodulator (Venna et al. 2002). Mishra et al. (2012a) isolated 1,151 endophytic fungal isolates representing 29 taxa from 7,200 segments of leaf, stem, petiole and root (1800 segments of each tissue) collected at three locations of Varanasi district in three different seasons (winter, summer and monsoon). The IF of hyphomycetes (74.80 %) was found greater followed by coelomycetes(14.07 %) and ascomycetes (11.12 %; Table 3.1, Fig. 3.1). Leaf tissues harbour maximum endophytes (29.38 % of the isolates), followed by stem (18.16 %), petiole (10.11 %) and root segments (6.27 %). The leaf segments harbour greater species (29) followed by stem (26), petiole (23) and root (18). CF was maximal during monsoon (23.23 %) followed by winter (15.35 %) and minimal during summer (8.85 %). Among the isolates, *Penicillium* spp. were dominant (12.62 % of all isolates), followed by *Colletotrichum* spp. (11.75 %), *Cladosporium* spp. (8.93 %), *C. globosum* (8.06 %), *Curvularia* spp. (7.55 %) and *A. alternata* (6.75 %). *Trichoderma viride, Monilia* sp., *Acremonium* sp. and *Guignardia* sp. were rare (0.69 %, 0.86 %, 0.52 % and 0.52 %). The paper suggested that some endophytes are season specific for example *Colletotrichum linicola* occurred almost exclusively in winter and *F. oxysporum* only in winter and summer but never during monsoon while *C. lunata* was found only in winter and during monsoon but never in summer. It was concluded that the effect of season and tissue type on CF and species diversity was much more pronounced than the effect of the location.

3.4 Biopotential of Endophytic Fungi

Microbes have played an important role in the discovery of novel and effective drugs. More than 22,000 secondary metabolites from natural sources are reported with various bioactive properties, but not more than 200 compounds could reach the market which certainly is a discouraging figure (Bérdy 2005). Due to the rising resistance ability in pathogens against existing antibiotics and ingress of newer diseases in society, there is an urgent need to discover the novel and potent antimicrobials. For this, one should go with a novel alternative source. This is the reason that endophytic fungi are getting attention from the scientific community for their ability to produce novel natural metabolites. As the literature suggests, the microbes residing in special niches may be able to produce novel and potent compounds as well. After the discovery of taxol from an endophytic fungi, *T. andreanae* isolated from the Pacific yew (Stierle et al. 1993), the endophytic research came to light as an alternative source and till today over 100 (57 % novel and 43 % known) anticancer compounds have been isolated and characterized from endophytic fungi (Kharwar et al. 2011a). Not only anticancer but a number of effective and potential antibacterial and antifungal compounds were also isolated from endophytic fungi against a range of Gram +ve and Gram −ve strains. Some of them are colletotric acid isolated from *Colletotrichum gloesporioides,* an endophytic fungus of *Artimisia mongolica* active against *Bacillus subtilis, Staphylococcus aureus* and *Sarcina leutea* (Zou et al. 2000); Javanacin isolated from endophytic fungus *Chloridium* sp. resident of *A. indica* showed strong antibacterial property against *Bacillus* sp., *Escherichia coli, Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. The compound was also active against several fungal pathogens (Kharwar et al. 2009). Cryptocandin isolated from endophytic *Cryptosporiopsis quercina* showed avtivity 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). Excluding other diseases, malaria alone is responsible to kill about 1 million people throughout the world every year. Endophytic fungi produce several antimalarial compounds such as phomoxanthones A and B from an endophyte *Phomopsis* sp., which are known to display antimalarial activity against *Plasmodium falciparum* K1 (Isaka et al. 2001). Pestacin and isopestacin, obtained from endophytic *Pestalotiopsis microspora* from the interior of *Terminalia morobensis,* displayed an antioxidant activity (Strobel et al. 2002). Cytonic acids A and B are novel protease inhibitors, isolated from *Cytonaema* sp., an endophyte of *Quercus* sp. against human cytomegalovirus (hCMV) (Guo et al. 2000). L-783 and 281 are 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). Subglutinol A and B are immunosuppressive, noncytotoxic diterpene pyrones isolated and characterized from an endophytic fungus *Fusarium subglutinans* of *Tripterygium wilfordii* (Lee et al. 1995). Nodulisporic acid A is a potential insecticide obtained from an endophytic fungus *Nodulisporium* sp. of *Bontia daphnoides* (Ondeyka et al. 1997). 3-Hydroxypropionic acid was isolated from *Phomopsis phaseoli* endophytically present in *Betula pendula and Betula pubescens* showed selective nematicidal activity against the plant-parasitic nematode *Meloidogyne incognita* (Schwarz et al. 2004). In addition to endophytic fungal diversity of eight plants, this chapter also covers the biopotential of the endophytic diversity. Literatures reveal that of the total endophytic community reported to have bioactive potential, 35 % belong to medicinal plants, followed by crops at 29 %, and the rest is equally divided between plants with special niches and other plants, each at 18 % (Selim et al. 2012).

3.4.1 Biopotential of Endophytic Fungi of *Adenocalymma alliaceum*

Out of 17 endophytic taxa, only 12 taxa were tested for their antibacterial activity against five human bacterial pathogens. Among 12 endophytic taxa, nine were found to be active against at least one bacterial pathogen. *A. alternata, C. globosum, C. lunata and Penicillium* sp. were active against 4 of 5 tested pathogens. *Salmonella enteritidis* (IMS/GN3) was found to be the most susceptible pathogen (Kharwar et al. 2011b).

3.4.2 Biopotential of Endophytic Fungi of *Aegle marmelos*

3.4.2.1 *Antibacterial Activity*

Seventeen endophytic fungi isolated from *A. marmelos* were tested for antibacterial activity against human pathogenic bacteria. Fifteen (88.23 %) endophytic fungi showed antibacterial activity against one or more pathogenic bacteria. Out of 17 endophytic fungi, four were active against five bacteria (*Shigella flexnii, Shigella boydii, S. enteritidis, Salmonella paratyphi* and *P. aeruginosa*). *Phoma herbarum* had exhibited an impressive antibacterial activity against seven of eight bacteria tested. The extract of *P. herbarum* showed strongest activity (inhibition zone 23 mm) against *S. boydii*. Among the endophytes of *A. marmelos, P. herbarum* gave least minimum inhibitory concentration (MIC; 40 µg/ml) against *S. flexnii* and *S. boydii*. *S. boydii* was found to be most susceptible followed by *P. aeruginosa* towards the extract of endophytic fungi. Fifteen endophytic fungal extracts were active against *S. boydii* and 13 against *P. aeruginosa* (Gond 2011).

3.4.2.2 *Antifungal Activity*

Seventeen endophytic fungi of *A. marmelos* were also tested against eight pathogenic fungi by dual culture assay. Out of 17 endophytic fungi, 10 were found to be active against one or more fungal pathogens. *P. herbarum* was most active that inhibited growth of five out of eight fungal pathogens. It inhibited 54.47 % growth of *C. lunata*. *Pestalotia macrotricha* was most active against *C. cladosporioides* showing 47.03 % growth inhibition, while *C. globosum* showed 24.03, 26.90, 27.07 and 39.13 % growth inhibition against *C. cladosporioides, F. oxysporum, Fusarium udum* and *C. lunata,* respectively. *Colletotrichum dematium* showed activity against *C. cladosporioides, F. oxysporum, F. udum* and *C. lunata* with 29.03, 32.7, 33.73 and 43.00 % inhibition, respectively. The endophytic *Phomopsis* sp. showed inhibitory activity against *A. alternata* (40.73 %), *C. cladosporioides* (30.03 %) and *C. lunata* (38.89 %). The pathogenic *C. lunata* was found most susceptible whereas *Microsporium gypseum* was resistant against all endophytic fungi tested (Gond 2011).

3.4.2.3 *Antimarial Activity*

P. herbarum (*A. marmelos*) was assessed for antimalarial activity against 3D7 strain of *P. falciparum*. The extract of *P. herbarum* gave only 55 % Schizont maturation inhibition of 3D7 strain of *P. falciparum* at the concentration of 50 µg/ml (Gond 2011).

3.4.2.4 *Antioxidant Activity*

The free radical-scavenging activity of fungal extract was carried out by using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The IC_{50} of *P. herbarum* isolated from *A. marmelos* was 125.63 µg/ml (Gond 2011), which could further be studied for detail and precise activity.

3.4.2.5 Extracellular Enzyme Production

Out of 32 endophytic fungi, only *A. alternata, C. globosum, P. herbarum, C.olletotrichum dematium, T. viride* of *A. marmelos* were tested for extracellular production of amylase, xylanase and phosphate solubilization. All five endophytic fungi were found to produce amylase, while only *P. herbarum, C. dematium* secreted xylanase whereas no fungi were observed for solubilizing the phosphate in solid media (Gond 2011).

3.4.3 Biopotential of Endophytic Fungi of *A. indica*

Among endophytic isolates of *A. indica,* six endophytic fungi (*Alternaria* sp., *Colletotrichum* sp., *Chloridium* sp., *Nigrospora* sp., *Pestalotiopsis* sp., *Scytalidim* sp.) were evaluated for their anti-dermatophyte activity. Among the six endophytic taxa, ethyl-acetate-extracted *Pestalotiopsis* metabolite was found more effective against dermatophytes at MIC 80 µg/ml while acetone-extracted *Scytalidium* sp. exhibited least activity with 400 µg/ml. Javanicin, a napthaqunone isolated from *Chloridum* sp., an endophytic fungus resident of neem tree root (Kharwar et al. 2009), showed antibacterial as well as antifungal activity. Among all tested pathogens, *P. fluorescens* and *P. aerugenosa* were observed more sensitive at MIC 2 µg/ml followed by *Cercospora arachidicola* at 5 µg/ml. At the rate of 10 µg/ml, the compound inhibited the growth of *Rhizoctonia solani* and *Verticillium dahalae,* and *F. oxysporum* at 20 µg/ml, whereas the suppression of *Bacillus* sp., *E. coli* and *C. albicans* were observed at 40 µg/ml (Kharwar et al. 2009). The isolation of azadirachtin was previously only known from *A. indica* but Kusari et al. (2012) described the isolation and characterization of azadirachtin A and B from *Eupenicillium parvum* isolated from *A. indica*.

3.4.4 Biopotential of Endophytic Fungi of *C. roseus*

The endophytic fungi isolated from *C. roseus* collected in China are known to produce vinka alkaloids. Endophytic *Alternaria* sp. and *F. oxysporum* isolated from the phloem of *C. roseus* were able to produce vinblastine and vincristine. These alkaloids have anticancer property (Guo et al. 1998; Zhang et al. 2000).

3.4.5 Biopotential of Endophytic Fungi of *Cinnamomum camphora*

Five out of 26 endophytic taxa were tested against 11 fungal (five human and five phytopathogens) and single bacterial pathogens. *Pestalotiopsis* sp. showed significant inhibition against *Phytophthora cryptogea* (57.7 %), *Pythium aphanidermatum* (54.5 %), *Microsporum nanum* (51.4 %), *T. rubrum* (49.7 %), *Microsporum gypseum* (48.5 %) and *P. fluorescence* (47.1 %), while *Phomopsis* sp. showed significant inhibition only to *P. aphanidermatum* (50.6 %) (Kharwar et al. 2012).

3.4.6 Biopotential of Endophytic Fungi of *Eucalyptus citriodora*

3.4.6.1 *Antibacterial Activity*

Thirteen (72.22 %) out of 18 endophytic fungi were found active against one or more human bacterial pathogens. *C. globosum, Rhizoctonia* sp., *P. glomerata* and *T. viride* were found to be active against four bacteria. *Pestalotia* sp. was most active against *S. flexnii* and *S. boydii* with an inhibition zone of 16.33 mm and 16.00 mm, respectively. *Periconia* sp. showed the activity only against *S. enteritidis*. *S. paratyphi* showed most susceptibility against *Rhizoctonia* sp. with 10 mm diameter of inhibition zone. The extract of seven endophytic fungal species inhibited *P. aeruginosa.* An unidentified species ECB2 (mycelia sterilia) gave maximum inhibition to *P. aeruginosa* with 12 mm diameter. *Citrobacter freundii* was only inhibited by extract of *Pestalotia* sp. *P. vulgaris* was inhibited by *P. glomerata and T. viride.* However, *Morganella morganii* was resistant against all the fungal extracts (Kharwar et al. 2010).

3.4.6.2 *Antifungal Activity*

Out of 18 endophytic fungi, eight were found active against at least one phytopathogenic fungus. *Phomopsis* sp. was the most active taxon against *C. lunata* followed by an unidentified fungus ECB1 and with 48.88 and 47.1 % radial growth inhibition, respectively. *C. globosum* also inhibited 32.87 % growth of *T. rubrum*. *Phomopsis* sp. and ECB1 inhibited growth of four pathogenic fungi out of the eight tested. Pathogenic *A. alternata* was inhibited only by endophytic *F. oxysporum* (34.57 %) while *F. udum* was inhibited (30.7 %) only by *Phomopsis* sp. (Kharwar et al. 2010).

3.4.6.3 Extracellular Enzyme Production

Eight endophytic fungi of *E. citriodora* were tested for amylase, xylanase and phosphate solubilization activity. Except *C. globosum,* all seven were found to produce amylase. Among them, *Periconia* sp. gave maximum zone of amylase production on solid agar medium. Only *Colletotrichum gloeosporioides* and *Aspergillus terreus* were observed to produce xylanase. Like *A. marmelos,* none of the endophytic fungus of *E. citriodora* had exhibited phosphate-solubilization activity (Gond et al. 2012).

3.4.7 Biopotential of Endophytic Fungi of *Nyctanthes arbor-tristis*

3.4.7.1 Antibacterial Activity

Sixteen endophytic fungi isolated from *N. arbor-tristis* were tested for antibacterial activity by disc diffusion assay against eight clinical isolates of human pathogenic bacteria (*S. flexnii, S. boydii, S. enteritidis, S. paratyphi, P. aeruginosa, C. freundii, M. morganii* and *P. vulgaris*). Among them, 12 (75 %) were found active at a rate of 5 mg/disc. *C. dematium* and *Chaetomiun globosum* exhibited antibacterial activity against five pathogens with inhibition ranged from 6.00 to 14.00 mm while *Nigrospora* sp. inhibited the growth of four bacterial pathogens, i.e. *S. paratyphi* (22.00 mm), *S. flexnii* (15.00 mm), *S. boydii* (18.00 mm) and *P. aeruginosa* (15.66 mm). In a study, *C. freundii, M. morganii* and *Proteus vulgaris* were found resistant against all the fungal extracts. *S. boydii* was found most susceptible and was inhibited by ten endophytic fungal extracts (Gond et al. 2012).

3.4.7.2 Antifungal Activity

Nine out of 16 endophytic fungi exhibited antifungal activity. *C. dematium* displayed the inhibitory activity against five phytopathogens, however its maximum activity was pronounced against *C. lunata* producing 55.87 % radial growth inhibition in dual culture. *Acremonium* sp. and *N. oryzae* inhibited the growth of three of eight pathogenic fungi. *C. cladosporioides* was found to be the most susceptibile species that was inhibited by *Aspergillus fumigatus,* and *F. oxysporum* with 39.66 %, 39.57 % while 31.60 % by *Dreschlera rostrata*. The growth of *F. oxysporum* was restricted by a single unidentified fungus MS/NAB2 up to 38.47 %.

3.4.7.3 Antimalarial Activity

N. oryzae isolated from *N. arbor-tristis* showed 100 % Schizont maturation inhibition of a malarial parasite 3D7 strain of *P. falciparum* at the concentration of 50 µg/ml (Gond 2011).

3.4.7.4 Antioxidant Activity

The free radical-scavenging activity of fungal extract of *N. oryzae* was carried out by using DPPH . The IC_{50} for *N. oryzae* was found at 265.53 µg/ml (Gond 2011), which was quite higher than *P. herbarum* isolated from *A. marmelos*.

3.4.7.5 Extracellular Enzyme Production

Nine endophytic fungi of *N. arbor-tristis* were tested for amylase, xylanase and phosphate-solubilization activity. Only *N. oryzae, Helicosporium* sp., *Diatrype* sp., *Macrophoma* sp. were found to produce amylase. Except *N. oryzae,* none were observed to produce xylanase. Unlike *A. marmelos* and *E. citriodora,* three endophytic fungi of *N. arbor-tristis*, i.e. *P. glomerata, Scytilidium* sp. and *Diatrype* sp. were able to solubilize phosphate (Gond 2011).

3.4.8 Biopotential of Endophytic Fungi of *Tinospora cordifolia*

Twenty nine endophytic taxa were tested for their antibacterial activity against eight human bacterial pathogens. More than 50 % (15 out of 29) of the endophytic taxa exhibited antimicrobial activity. *Botryosphaeria rhodina* (JQ031157) and *C. globosum* showed activity against all bacterial human pathogens tested, with the former showing higher activity than the latter. *B. rhodina* (JQ031157) exhibited strongest activity against *C. freundii* (IMS/GN5) producing an inhibition zone of 45.66 ± 0.33 mm whereas lowest against *M. morganii* (IMS/GN6) with an inhibition zone of 12.83 ± 0.16 mm at the rate of 5 mg/ml. *C. linicola, A. alternata, C. cladosporioides, N. oryzae* and *Pseudofusicoccum violaceum* (JQ031159) were active against a single pathogen. *S. flexnii* IMS/GN1 was observed to be the most susceptible bacterial pathogen, inhibited by 11 endophytic taxa followed by *E. coli* ATCC 25922, inhibited by six endophytic taxa, *S. paratyphi* and *P. vulgaris* inhibited by five endophytic taxa whereas *S. enteritidis* IMS/GN3, *P. aeruginosa* ATCC 27853, *C. freundii* IMS/GN5, *M. morganii* IMS/GN6 were found to be the least susceptible and were only inhibited by three endophytic taxa (Mishra et al. 2012a).

3.5 Future Prospective

Endophytic fungi are relatively less studied, unexploited and hidden microbes of the microbial community. All the plants studied to date for their endophytic fungi were found to harbour either at least a single or plethora of fungi. 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, and this study raises the question, Where are the rest of the fungi? Are they in form of endophytes or somewhere else? These are some of the basic questions regarding the diversity of endophytic fungi that require more endophytic research which may help in the isolation and characterization of new fungal species and/or bioactive compounds. Since a considerable number of novel fungal genera and species have been reported from this relatively hidden (inside healthy plant tissues) source that may be a good repertoire for filling the gap between reported and estimated fungal diversity. Literatures suggest that endophytes enhance 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 (Mishra et al. 2012b). Today a major problem in the front of scientists is the development of resistance in pathogenic microbes (bacteria, fungi and other microbes, malarial parasite, viruses, etc.), pests and weeds that have become a serious trouble for humans, animals and agriculture. To overcome this problem, there is an urgent need for a novel and alternative source for drug discovery. Endophytic fungi can serve as a good alternative because a number of antibacterial, antidiabetic, antifungal, antimalarial, antioxidant, antiviral and other bioactive compounds exhibited promising activity isolated and characterized from this source. The endophytic fungi isolated from the above described eight medicinal plants are under the process of isolation and characterization of antibacterial, antifungal, antioxidant and antimalarial compounds. Suryanarayanan and his colleagues found endophytic fungi as a prolific source for production of extracellular amlyases, cellulases, chitinases, chitosonases, laccases, lipases, pectinases and proteases (Suryanarayanan et al. 2012).

The isolation of several fungi and their isolates (Alternaria, Phomopsis, Chaetomium, Chollelotrichum, Fusarium, etc) from these medicinal plants indicate that some endophytic fungi may be probable candidates to produce some cytotoxic compounds. Another interesting aspect of fungal endophytes is to produce antimicrobial volatile organic compounds (VOCs) reported from mitosporic xylariales fungus *Muscodor albus* and *Muscodor vitigenus* isolated from *Cinamomum zeylanicum* (Strobel et al. 2001). Recently, some hydrocarbon derivatives as major constituents of diesel fuel (Mycodiesel) were reported for the first time from a fungal endophyte *Gliocladium roseum,* NRRL 50072 (Strobel et al. 2008); however, some genuine technical questions on mycodiesel production were raised (Stadler and Schulz 2009). Nevertheless, the successful trial of running a Honda (100 cc) motorbike using eucalyptol, a better and safe alternative of gasoline received from a fungal endophyte of *Eucalyptus* sp. (Tomsheck et al. 2010) furthered our understanding towards other interesting aspects.

Very recently, some interesting works have been published in order to enhance the production of cryptic and known bioactive compounds through epigenetic modulations, and these works may point the way in future that can reduce the stigma of reduced yield of fungal endophytes in successive generations (Sun et al. 2012; Hassan et al. 2012). A huge diversity of endophytic fungi isolated from these plants and significant antimicrobial and biochemical activity of crude extracts provide us potential fungal endophyte pools to isolate pure and novel bioactive compounds. In future, epigenetic modulation may play a very crucial role in isolating the cryptic secondary metabolites which are not either known, or it may enhance multifold production of known compounds from fungal source which may serve the need of society.

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