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BIOLOGICAL DIVERSITY OF ENDOPHYTIC FUNGI ISOLATED FROM UNDERGROUND PARTS OF INDIAN MEDICINAL PLANTS

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ABSTRACT

Microorganisms like endophytic fungi generally reside asymptomatically in the tissues of higher plants. The host endophyte relationship is supposed to be complex and different from plant to microbe. Intention of present report is to provide studies on endophytic fungi, particularly on diversity of fungal endophytes from some medicinal plants of India such as *Aloe vera* (L.), *Ocimum sanctum* (L.) and *Curcuma longa* (L.)**.** Diverse fungal taxa isolated majorly *Fusarium* (12%), *Alternaria* (12%), *Ascomycete* (12%), *Mycelia sterilia* (10%), *Eurotium* (28%) and *Oidodendran* (13%) and other unidentified taxa (13%). **Results:** Different fungal taxa was isolated from designated medicinal plants. These are majorly *Fusarium*, *Alternaria*, *Ascomycete*, *Mycelia sterilia*, *Eurotium* and *Oidodendran* and other unidentified taxa identified by molecular sequencing and phylogenetic analysis of fungal endophytes. **Conclusion :** Investigations have been carried on tissues of selected indigenous medicinal plants The present study provides evidence that isolated endophytes are capable to survive inside plants. Results of present study indicate that selected plants and their parts are highly colonized by microbial endophytes, and endophytes are not host specific.

Key words: Endophytic fungi, fungal taxa, diversity, medicinal plants.

INTRODUCTION

Fungi are the second major group after insects and important component of tropical ecosystems throughout world. They are abundant with diverse habitats extending from psychrophilic to thermophilic and curiously play a vital role in every ecosystem. Fungi are better known than other microbes such as, viruses and viroids. There are 72,000 approximetly

named species and new species are being added at rate of almost 1500 each year (Bennett & Faison 1997).During evolution when plants colonized the land effectively, fungi developed type of relationship with them. The group now know as 'endophytes' their existence have been traced in the fossil chronicles, suggesting that endophyte-host association may have evolved from the time of apperance of first higher plants on earth (Rodriguez & Redman 1997, Strobel 2003).

The term "endophytes" includes a group of microorganisms that grow intra or intercelullarly in the tissues of higher plants without causing any symptoms on the plants in which they live, (Tan & Zou 2001, Li et al. 2008) or Fungal endophytes are organisms living in a plant host for at least a part of their life, without causing any apparent disease (Saikkonen et al. 1998). Such mutualistic interaction between endophyte and host plant results in fitness benefits for both partners (Kogel et al. 2006).

The endophyte provide defense and survival environment to their host plant by producing a plethora of substances having potential use in industry, agriculture, and medicine (Strobel and Daisy 2003, Strobel et al. 2004). Approximately 300, 000 plant species growing on the earth are host to one or more endophytes (Strobel 2003), and the presence of bio different endophytes in huge number plays an important role on bionetworks with greatest biodiversity, for example, the tropical and temperate rainforests (Strobel & Daisy 2003).Studies on fungal endophytes over the past 30 years indicate that they occupy a unique environmental position and are also supposed to influence distribution, physiology and biochemistry of plant. Interrogation arises why do plants quay such a burden of endophytes? or Would it is an unnecessary burden on plant metabolism? These questions need to be answered adequately. The purpose of this research is to isolate and study distribution and diversity pattern of endophytic fungi from some Indian medicinal plants.

MATERIALS AND METHODS

Plant selection strategies for isolation of endophytes

It is essential to recognize the methods and rationale used to make available the best opportunities to isolate endophytic microorganisms. Some reasonable hypothesis governing this plant selection strategies are as follows

i. Plants that have ethano botanical history
ii. Medicinally used by indigenous peoples

iii. Plant that are endemic, having an unusual longevity and also occupied a certain ancient land mass.

iv. Plants growing in temperate region having huge biodiversity with prospects of hosting endophytes with enormous biodiversity.

Sampling area and collection of plant materials

Mature, healthy plant materials were collected and identified by expertise from Department of Botany, Moolji Jaitha College Jalgaon, India. Collected plant samples were immediately brought to laboratory and were used within 8 hours for isolation of fungal endophytes. Healthy plants were selected growing in different regions of Jalgaon geographically located at 21.01 0 N75.56 0 E and average elevation about 209 meters (Figure 1.) Endophytic flora were isolated from roots of *Aloe vera* (L.), *Ocimum sanctum* (L.) and rhizomes of *Curcuma longa* (L.) .

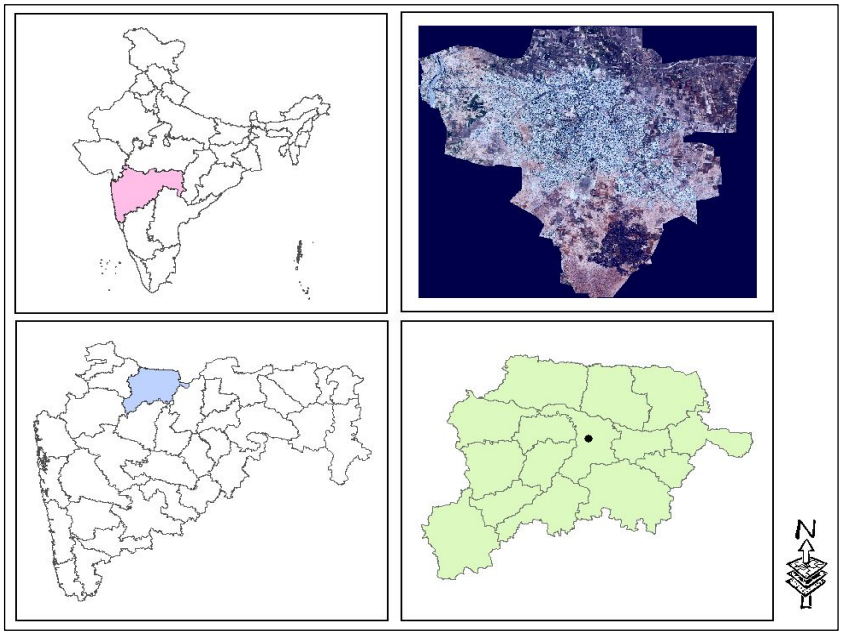


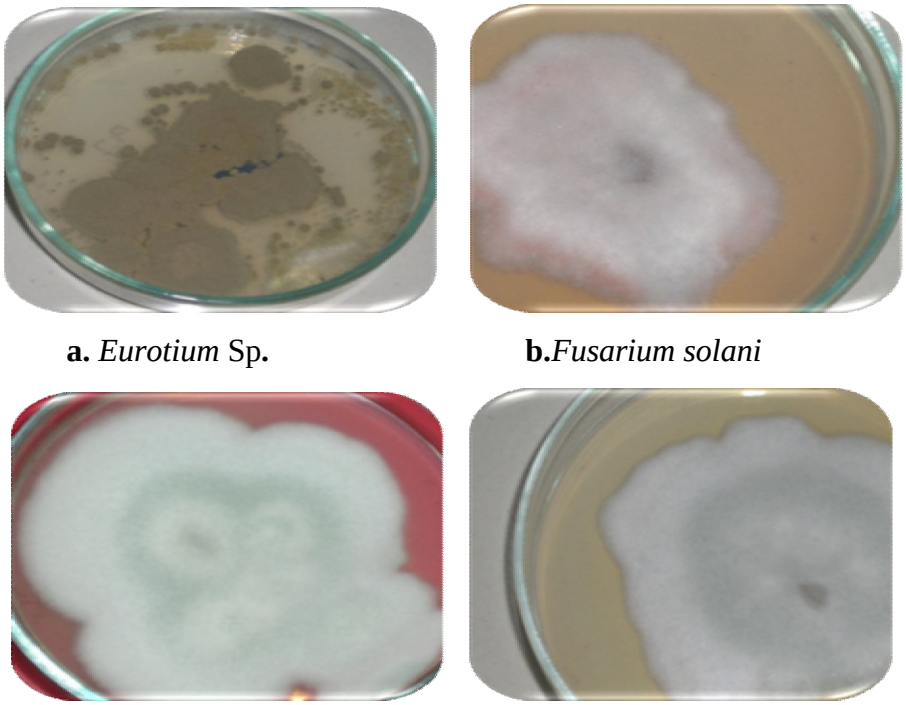
Figure 1.Geographical location of Jalgaon

Processing and disinfecting samples for isolation of endophytic fungi

Sampling different parts of the selected medicinal plants, ten samples were taken from each host plant. These samples were wash in running tap water to remove soil particles and adhered debris, and finally with distilled water. From each sample sub samples were prepared for further isolation of endophytes. Root/rhizome tissues were immersed in 70% ethanol for 1-3 min and 5% aqueous solution of sodium hypochlorite 1 min, 2 min with 70 % ethanol, and 1 min with 0.1% mercury chloride and rinsed with sterile distilled water (Strobel et al. 2004, Castillo et al. 2007). Disinfected samples were selected by aseptic cutting using sterile

knife and inner tissues were excised and rinsed three times with sterile distilled water and were blotted in the laminar air flow. Size of samples range between 1 cm long and 3-4 mm broad. The fungi discussed in this report were isolated by decontaminating the endophytic bacteria. The dissected tissues were placed on potato dextrose agar, with 50mg/l chloramphenicol. Endophytic fungi usually began to produce hypal filaments after 5-6 days of incubation at 30^0C. The hypal tips appeared were carefully transferred to fresh potato dextrose agar plates (Strobel et al. 2004, Kharwar et al. 2008).

RESULTS AND DISCUSSION



a. *Eurotium* Sp**.** **b.***Fusarium solani*

c. *Eurotium amstelodami* **d.** *Alternaria alternate*

Figure 2 . Plate morphology of diverse endophytic fungal flora isolated from different medicinal plants, a. *Eurotium* Sp., b. *Fusarium solani,* c. *Eurotium amstelodami,* d. *Alternaria alternate* .

Photoplates of isolated endophytic fungi grown on cultural media are shown above in Figure 2. further details are presented below.

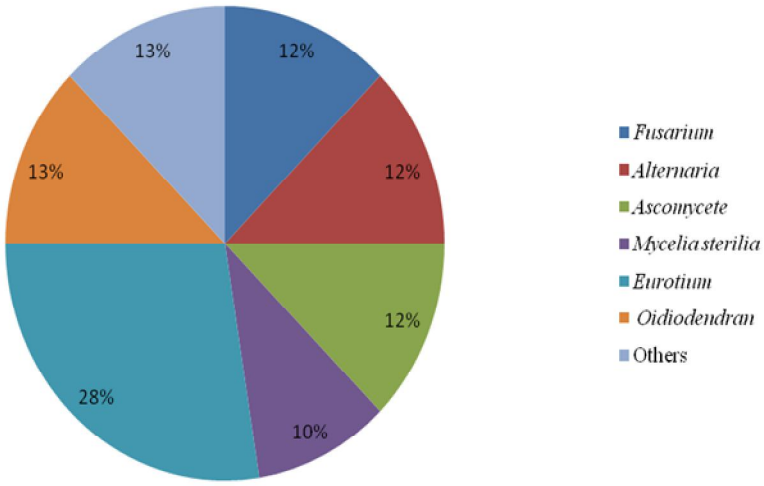


Figure 3. Relative frequency of endophytic fungi isolated from medicinal plants

Diverse fungal taxa isolated from medicinal plants. These belongs majorly to *Fusarium* (12%), *Alternaria* (12%), *Ascomycete* (12%), *Mycelia sterilia* (10%), *Eurotium* (28%) and *Oidodendran* (13%) and other unidentified taxa (13%) (Figure 3.).

Molecular sequencing and phylogenetic analysis of fungal endophytes

Eurotium Sp.

CTCCCATCCGTGTCTATCTGTACCCTGTTGCTTCGGCGTGGCCACGGTTCCGCCGA
AGACTAACATTTGAACACTGTCTGAAGTTTGCAGTCTGAGTTTTTAGTTAAACAA
TAATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG
CGAAACGCGATAATTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGA
ACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGC
TGCCCTCAAGCACGGCTTGTGTGTTGGGCTTCCGTCCCTGGTAACGGGGACGGGC
CCAAAAGGCAGTGGCGGCACCATGTCTGGTCCTCGAGCGTATGGGGCTTTGTCAC
CCGCTCCCGTAGGTCCAGCTGGCAGCTAGCCTCGCAACCAATCTTTTTAACCAGG
TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA

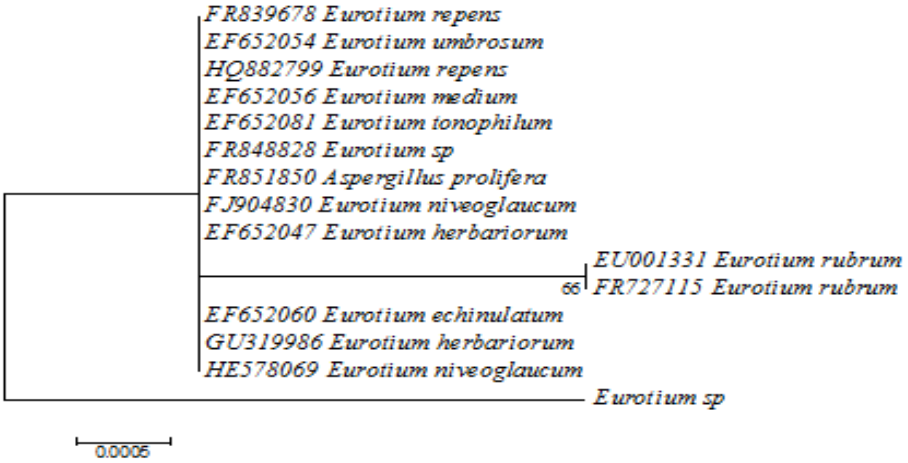


Figure 4.Phylogenetic analysis of endophytic fungi, *Eurotium* Sp

Fusarium solani

TAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGT
TCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGGA
AGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAG
CTTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCC
GTAAAACACCCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCCGCTGA
ACTTAAGCATATCAATAAGCGGAGGAA

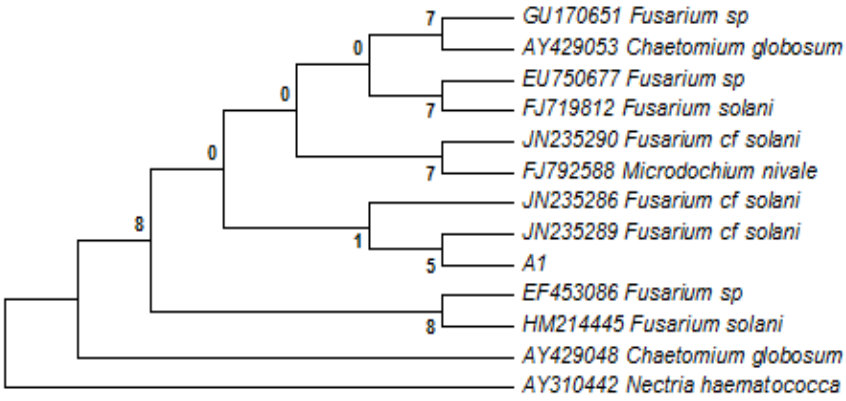


Figure 5.Phylogenetic analysis of endophytic fungi, *Fusarium solani,*

Eurotium amstelodami

GAACGCTGTCTGAAGTTTGCAGTCTGAGTTTTTAGTTAAACAATCGTTAAAACTT
TCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT
AATTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGC
GCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGC
ACGGCTTGTGTGTTGGGCTTCCGTCCCTGGCAACGGGGACGGGCCCAAAAGGCA
GTGGCGGCACCATGTCTGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCCCGT
AGGTCCAGCTGGCAGCTAGCCTCGCAACCAATCTTTTTAACCAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATC

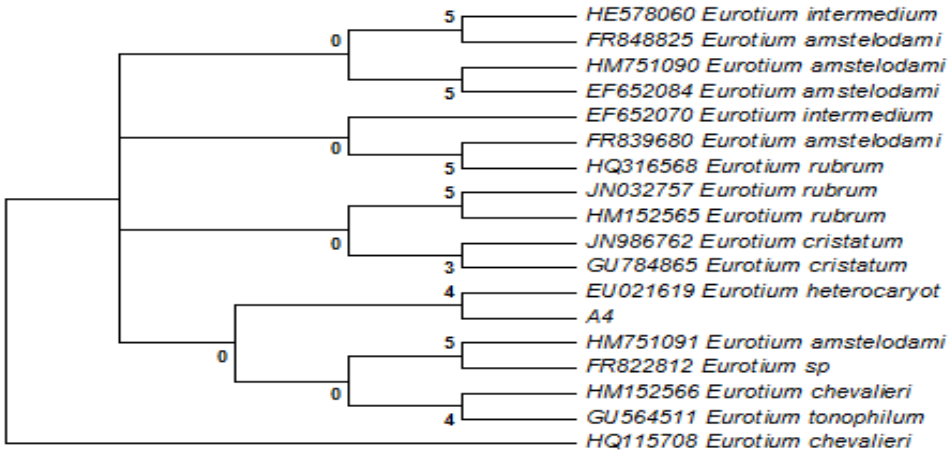


Figure 6. Phylogenetic analysis of endophytic fungi, *Eurotium amstelodami*

Alternaria Alternate

GGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTT
GGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCG
TCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCAT
CGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCT
GTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTA
GCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAG
CGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTT
TTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA
TA

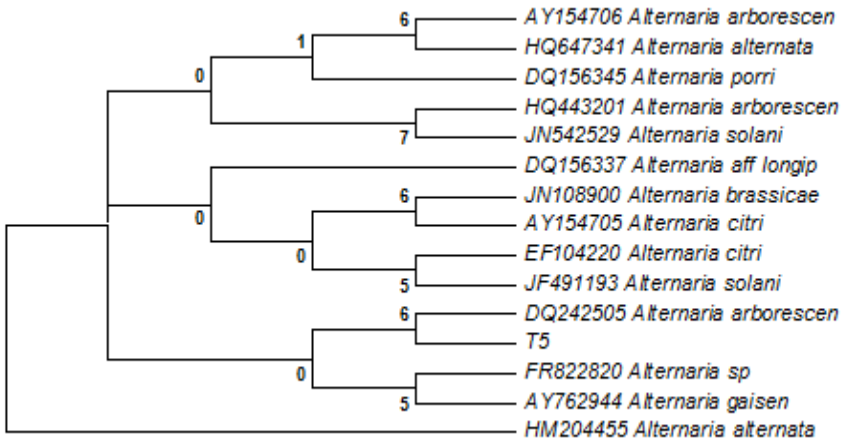


Figure 7. Phylogenetic analysis of endophytic fungi, *Alternaria alternate*

Endophytic fungi were sequence on molecular level by 16S RNA using ITS (for internal transcribed spacer) as shown above. With the help of sequence base pairs phylogenetic analysis were done. Isolate forms divergent linkage from clusters (Figure 4-7. Phylogenetic analysis of endophytic fungi, **a**. *Eurotium* Sp, **b**. *Fusarium solani*, **c**. *Eurotium amstelodami*, **d**. *Alternaria alternate*). Therefore, on the basis of phylogency isolates were identified as *Eurotium* Sp., *Fusarium solani*, *Eurotium amstelodami*, *Alternaria alternate.* For further characterization up to species level, few more genes have to be sequence.

Endophytic fungi are organisms living in host plant for at least a part of their life, without causing any apparent disease (Saikkonen et al. 1998). The traditional view about these microbes was that the interaction is mutualistic, the fungi get nutrients and protection from the host and provide various competition advantages to the host (Carroll 1998) but most of these host-parasite relationships are not well characterized (Arnold 2003). According to Stone et al. (2000) endophytic microbes in relation to their host plant organ belong to diverse classes. Major groups are as follows: 1) Endophytic *Clavicipitaceae*, 2) Fungal endophytes of dicots, 3) Endophytic fungi, 4) Other systemic fungal endophytes, 5) Fungal endophytes of lichens, 6) Endophytic fungi of bryophytes and ferns, 7) Endophytic fungi of tree bark, 8) Fungal endophytes of xylem, 9) Fungal endophytes of roots, 10) Fungal endophytes of galls and cysts, 11) Prokaryotic endophytes of plants (includes endophytic bacteria and actinomycetes). Such ubiquitous endophytes dominated their assemblages of distinct hosts suggesting that certain genera of fungi are well adapted to make an endophytic way of life (Bills et al. 2004).

There has been increasing curiosity in systematics, evolutionary biology, ecology and applied research of fungal endophytes. This type of research based ranges from biological control to bioprospecting based on pioneering work done by different workers worldwide on this cryptic guild of fungi (Arnold 2007, Backman & Sikora 2008). In count, interactions and mutualistic symbiosis among endophytes and host plants had also been studied in detail (Clay & Schardl 2002). Tropical and temperate rainforests reflects most biologically diverse terrestrial ecosystems on earth (Mittermeier et al. 1999). The endophytic fungi from healthy aerial tissues are mostly documented from conifers (Wang et al. 2007).It is approved that endophytic fungal diversity heights in tropical forests where woody angiosperm diversity is also higher (Arnold 2001, Banerjee 2011). When compared to other tropical countries, studies on the diversity of endophytic fungi in India are particularly insufficient though India has rich plant diversity of about 17,527 angiosperm and 67 gymnosperm species (Karthikeyan 2009, Annon 2009).

Indian, studies of endophytic fungi has been focused on tree species, different tissue viz. stem, roots, leaf, cotyledons, seed and petiole were screened to isolate endophytes (Suryanarayanan et al. 2002, 2003; Tejesvi et al. 2005, Kharwar et al. 2008, Jalgaonwala et al. 2011).We authors isolated endophytes from root, stem and leaf of fifteen different hosts plants based on seasonal variations from Jalgaon India. Studies have also highlighted ecological factors reflecting the diversity and relationship between occurrence, distribution of foliar and root endophytes from respective geographical locations (paper communicated).Existence and distribution of foliar endophytes of four dissimilar types of tropical forests found in India, such as dry thorn forest, dry deciduous forest, moist deciduous forest and semi-evergreen forest of Western Ghats of Southern India were studied by Suryanarayanan et al. 2002. They calculated twenty tree species for their endophyte collections and concluded that though the tropical trees were rich with endophytic microbes, overall endophytic diversity of the total plant community was not exceptional. Qualitative and quantitative changes in diversity were found in endophytic mycoflora of Neem from different regions and plant parts Varanasi India (Kharwar et al. 2008). However, most of the studies support the hypothesis that qualitative and quantitative endophytic diversity proliferate with precipitation.

Currently, studies on microbial endophytes from medicinal plants are under attention as these plants tend to produce natural products beneficial for us. A number of medicinal plants have

been screened in different regions of world for endophytes (Strobel & Daisy 2003; Jalgaonwala et al. 2011). Still knowledge on comprehensive diversity of endophytic fungi from medicinal plants is scanty.

Future prospects

From the foregoing discussion, the following areas are recommended to be strengthened in future. - We need systematic investigation for endophytic fungi from special extreme environment such as deep-sea, hypoxic zones (with low oxygen levels) and hydro- thermal vents for enzymes, degradation of xenobiotics, bioremediation, genomic and proteomic studies with novel organisms will hopefully help in basic research on evolutionary biology.

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