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Biodiversity of Endophytic Mycoflora from *Lannea coromandelica*

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In recent years endophytic fungi from plants have gained tremendous importance with emerging evidence of their high biotechnological potential for their valuable source of useful metabolites. In the present study an attempt has been made to isolate endophytes associated with leaves and bark of *Lannea coromandelica*(Houtt.) Merr (Anacardiaceae).Five hundred bark and leaf segments were analysed and a total of 586 isolates representing 20 taxa, with a colonization rate of 59.6% were isolated. A total of 586 isolates belonging to 20 taxa comprising 5.70% Ascomycetes, 5.20% Zygomycetes, 18.45% Coelomycetes, 67.78% Hypomycetes, 2.85% sterile mycelia were obtained. Species of, *Aspergillus, Alternaria*,and *Colletotrichum* were isolated as dominant endophytes. Colonization frequency of *Aspergillus flavus*(21.64 %) was highest which was isolated from all the tissues.

Key words: *Lannea coromandelica* ,endophytic fungi , diversity.

The term endophyte (Gr. endon, within; phyton, plant) – was first coined by de Bary[1]. First reports describing these mirobes date back to the turn of the 19th and 20[th]century[2]. Endophyte includes all organisms which colonize the living internal tissues of their hosts without producing symptoms of disease[3].Endophytic fungi represent an important and quantifiable component of fungal diversity, with an estimate of at least 1 million species[4]. The internal tissues of plants harbouring endophytes may well account for a substantial number of new fungi [5,6]. All vascular plants harbour endophytic organisms[7].

Since these fungal endophytes have been recognized as a repository of novel secondary metabolites, which have antibiotic, antimycotic, immunosuppressive, and anticancer activity[8]**.** A recent comprehensive study has indicated that 51% of bioactive substances isolated from endophytic fungi were previously unknown[9]**.** Hence, endophytic fungi provides an enormous opportunity to explore the relatively untapped source of information about ecological and biological activity.

Medicinal plants are known to harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products[7]. Therefore, it is important to explore the endophytic mycoflora in the medicinal plants. In the present study, *Lannea coromandelica*, an important medicinal plant, was investigated for the presence of endophytic community.

Lannea coromandelica which is commonly known as "The Indian Ash Tree" is a deciduous tree which grows up to 14 metres high. It belongs to the family Anacardiaceae. The bark of the tree and leaves are used as traditional medicine to cure sprains, bruises, skin eruptions, heart diseases, dysentery , mouth sores., toothache and diabetes [10]

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MATERIALS AND METHODS

Sample collection

Leaf and bark tissues were collected from mature trees growing in Velachery area (12°.97'N , 80°.22'E) in Chennai, South India.The plant samples were collected for a period of one year.Samples of healthy mature living symptomless leaves were chosen at random from the lower part of the crown and bark peelings were collected from each tree 3m above the ground. The samples were transported to the laboratory in closed, sterile polythene bags and processed within 24 h of collection. Five hundred segments, approximately 5 × 5 mm were cut from healthy leaves and bark[11].

Isolation of endophytic fungi

The leaves were washed thoroughly in running water. The leaf segments (0.5 cm^2) cut from the median and lateral vein portion of the leaf and surface sterilised. Surface sterilization was done in the following modified method of [12].The leaf segments were dipped in 70% ethanol for 5 seconds, followed by 4% sodium hypochlorite for 90 seconds and sterile water for 10 seconds. The excess moisture was blotted in a sterile filter paper.In each petri dish, 4 - 5 segments were placed on Potato Dextrose Agar (PDA) supplemented with antibiotics streptomycin (100 µg/mL concentration), (Sigma, St. Louis, MO, USA) to suppress the growth of bacteria .Afterinoculation the petri dishes were sealed with parafilm and incubated at 27°C ± 1°C for 7 days.Another segment of the same origin without surfacesterilization was cultured as a negative control to check the presence of contaminated microbes on the segment surface. The plates were examined for fungal growth,the fungus grown out from explant were sub cultured in PDA plates. Later the purified endophytic fungi were transferred to PDA slants separately and were kept at 4ºC after being cultured at 28ºC for 7 days.

Identification of endophytes

The endophytic fungi grew out from the leaf and bark were identified on the basis of cultural characteristics like texture, colour, surface, elevation reverse side and margin, and the morphology of fruiting bodies and spores and was identified down to species level using standard monographs [13].

Statistical analysis

The relative frequency (CF%) of colonization of endophytic fungi was calculated using the formula given by Hata[14].

$$CF\% = N_{col}/N_t \times 100 ;$$

Where N_{col} = the number of segments colonized by each fungus, and N_t = the total number of segments.

The Dominance ofendophytes was calculated using the formula as per kumerasan[15].

$$\text{Dominance} = \frac{\text{CF\% of a given endophyte}}{\text{Sum of CF\% of all endophytes}} \times 100$$

RESULTS AND DISCUSSION

Endophytic fungi have been found associated with every plant species investigated so far from tropical and temperate hosts, yet they are poorly investigated because of their cryptic nature[16,17].In the present study a total of 596 isolates of endophytic fungi were recovered from 1000 samples of bark and leaf tissues of *Lannea coromandelica*. Colonization frequency rate of bark and leaf tissue (36.4% and 84.2%) indicate

Table 1. Colonization frequency of endophytic fungi isolated from leaf and bark of *Lannea coromendalica*

Endophytes	Bark	CF %	LEAF	CF%	Total	CF%
Ascomycetes	7	1.4	27	4.6	34	3.4
Coelomycetes	34	6.8	76	15.2	110	11
Hyphomycetes	130	26	274	54.8	404	40.4
Zygomycetes	8	1.6	23	6.2	31	3.1
Mycelia Sterilia	6	1.2	11	3.4	17	1.7

the wide variation between two tissues .The overall fungal composition includes 20 species comprising 5.70% Ascomycetes, 5.20% Zygomycetes, 18.45% Coelomycetes, 67.78% Hypomycetes, 2.85% sterile mycelia were obtained.(Table 1). Among the endophytes, class Hyphomycetes was dominant. Such dominance of Hyphomycetes as endophytes has also been reported from several plants such as *Azadirachta indica* and *Terminalia indica*[18,19], indicating their ubiquity among the plant kingdom. Species of, *Aspergillus, Alternaria, Botryodiplodia, Colletotrichum and Drechslera* were isolated as dominant endophytes (Table 2). The dominant endophytic isolates are also found commonly as the plant pathogens and they might have evolved to endophytic lifestyle due to loss of virulence[20]. Moreover in the present study, there were no bark-specific taxa in the overall isolates which coincides with the earlier work done on *Kigelia pinnata*[21].

In the present study, more endophytic isolates were obtained from leaf tissue(84.2%) than on bark tissue (36.4%) (Table 1).Colonisation of the host tissues by endophytic fungi is dependent on successful penetration of the protective external plant layers, which can be achieved by enzymatic digestion of cuticular and epidermal layers by endophytes. Leaves contain mainly parenchymatous cells that are thin-walled,with chloroplasts and rich in starch, whereas bark tissueis covered with periderm which mainly strengthens the plant than supplying nutrient elements on which the endophyte depends. These differences may be a reflection of tissue recurrence in individual species and mayreflect their capacity for utilizing or surviving within a specific substrate[22,23].

In the present study, more endophytic isolates were obtained during the monsoon season. It also showed higher species richness

Table 2. Endophytic fungi isolated from *Lannea coromendalica*

Name of Endophyte	No of Endophytes			colonization frequency	dominant fungi
	Bark	leaves	Total		
ASCOMYCETES					
Chaetomium globusum	6	15	21	2.1	3.52
Nodulisporium sp	1	8	9	0.9	1.51
Xylaria sp	0	4	4	0.4	0.67
COELOMYCETES					
Botryodiplodia sp	12	23	35	3.5	5.87
Phoma sp	7	13	20	2.0	3.35
Collectrotrichum sp	9	28	37	3.7	6.20
Phomopsis sp	6	12	18	1.8	3.02
HYPOMYCETES					
Nigrospora sp	3	10	13	1.3	2.18
Aspergillus flavus	40	89	129	12.9	21.64
Aspergillus niger	20	48	68	6.8	11.40
Aspergillus clavatus	14	20	34	3.4	5.70
Curvularia lunata	8	19	27	2.7	4.53
Alternaria alternata	23	50	73	7.3	12.24
Drechslera sp	12	22	34	3.4	5.70
Fusarium oxysporum	8	13	21	2.1	3.52
Rhizoclonia sp	2	3	5	0.5	0.83
ZYGOMYCETES					
Rhizopus sp -1	4	13	17	1.7	2.85
Mucor sp -1	4	10	14	1.4	2.34
STERILE FUNGI					
Mycelia sterilia (brown)	2	6	8	0.8	1.34
Mycelia sterilia (haline)	4	5	9	0.9	1.51
Total	185	411	596	59.6	-

than the summer isolates. This could be due to higher precipitation rates which might have favoured spore germination in fungi indicating horizontal transfer of the endophytes in the host tissues. A strong correlation has been observed between the endophyte infection levels and cumulative precipitation [24]. Our results were similar to many instances, where leaves sampled during the wet season harbored more endophytes than those screened during the dry season [25].

Endophyte assemblages are composed of dominant or plural species which are frequently isolated and some rare or singleton species which are isolated only once or very few times, from a given host[26].Usually one or few taxa of endophytic fungi predominate a single host which may be due to its specificity for tissues and host. Other factors such as competition for space,nutrients,humidity and antagonism plays and important role. In the present study *Aspergillus flavus* was found to be the predominant species. The Colonization frequency of *Aspergillus flavus* being (21.64 %) was highest which was isolated from all the tissues.(Table -2)Most taxa, however, were recovered sporadically, this may be due to various environmental factors which were not conducive for their growth or that more competitive endophytes have already achieved a significant colonization of the host tissue.

CONCLUSION

This present study is perhaps the first report on endophytic fungal diversity of the medicinal plant *Lannea coromandelica*. Hence comprehensive efforts are needed to tap the vast potential of the endophytes of medicinally important plants which are the rich source of valuable bioactive compounds. Further work is being carried out on the fermentation of fungal endophytes to obtain bioactive compounds with therapeutic applications.

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