Biodiversity of endophytic fungi associated with *Ficus religiosa* and *F. benghalensis*

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Abstract. Endophytic fungi were isolated from leaf and bark tissues of *Ficus religiosa* and *F. benghalensis* (*Moraceae*) in a tropical forest in southern India. Five hundred leaf and bark segments from each plant species were collected. Endophytic fungi were more numerous from leaf segments than bark. In this study, hyphomycetes were the most dominant group followed by coelomycetes, ascomycetes, zygomycetes, and sterile fungi. Leaf and bark tissues of *F. religiosa* had more endophytic fungi than *F. benghalensis.* Some endophytic fungi were common to both hosts, and few appeared to be host specific.

Key words: bark, endophytic fungi, *Ficus benghalensis, Ficus religiosa*, leaf, *Moraceae,* Tropics

Introduction

Endophytic fungi occur within plant tissues without producing any symptoms or diseases and their presence may confer advantages to the hosts (Carroll 1991; Suryanarayanan *et al.* 1998; Abdessamad *et al.* 2009; Xuan *et al.* 2010; Rajagopal *et al.* 2011). Medicinal plants are reported to harbor endophytes, which in turn may provide protection to their host from infectious agents. Some endophytes produce metabolites useful to pharmaceutical and agricultural industries (Petrini *et al.* 1992; Strobel 2002; Gangadevi & Muthumary 2008). Endophytic fungi have been suggested as major potential sources for new, useful metabolites (Dreyfuss & Chapela 1994). A single endophytic fungal strain may produce multiple bioactive compounds including alkaloids, steroids, terpenoids and peptides (Tan & Zou 2001). Some of the more interesting compounds produced by endophytic fungi include taxol, cryptocin, cryptocandin, jesterone, oocydin, isopestacin, the pseudomycins and ambuic acid (Strobel 2002; Kathiravan & Muthumary 2009). Different types of endophytic fungi were associated with medicinal plants (Rajagopal *et al.* 2011) but there are very few studies regarding endophyte associations of medicinal trees, including *Ficus religiosa* L. and *Ficus benghalensis* L., although such fungi have been reported from other tropical medicinal herbs and trees (Gangadevi *et al.* 2008; Rajagopal *et al.* 2010). The present study examined the assemblages of endophytic fungi of *F. religiosa* and *F. benghalensis* from a tropical forest in southern India.

Materials and methods

Leaf and bark tissues were collected from matured trees (approximately 15 m) growing in a tropical forest (12°41' N, 79°58' E) 50 km south of Chennai in southern India. *Ficus religiosa* and *F. benghalensis* are the dominant forest trees and have religious significance in India. Ten trees from each species were selected for this study and one hundred leaves were collected from lower parts of the crown of each tree about 3 m above the ground. Bark peelings were collected from each tree 3 m above the ground. Samples were transported to the laboratory in closed sterile polythene bags and processed within 24 h of collection (Petrini & Fisher 1988; Suryanarayanan *et al.* 1998). Five hundred segments, approximately 5 × 5 mm² were cut from the

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upper, middle and lower portion of healthy leaves. Similarly 5 × 5 mm² pieces were cut from the bark (Rajagopal 1998). The segments were surface sterilized by dipping in 70% ethanol (Merck, German) (30 s), immersed in 4% sodium hypochlorite (Sigma, St. Louis, MO, USA) (90 s) and rinsed in autoclaved double distilled water for 5 s (Dobranic *et al.* 1995; Rajagopal 1998). Five hundred segments from each tree species were placed onto potato dextrose agar (PDA) Petri plates with 100 mg of chloramphenicol added to inhibit the growth of bacteria. Inoculated plates were incubated at 27 °C under 12 h white fluorescent light with 12 h dark cycles (Bills & Polishook 1992). Plates were observed daily for up to 3–4 weeks. Fungi that grew out from sterile segments were transferred to fresh slants. Sterile isolates that could not be identified using morphological characters were given codes using culture characteristics including growth rate, colony surface texture, colony margin and pigmentation (Bills & Polishook 1994; Rajagopal 2004).

The density of colonization was calculated as the percentage of leaf and bark segments that were colonized by a one or more isolate(s) from the total number of segments of each tissue incubated × 100. Isolation rate (IR) was determined as the number of isolates obtained from tissue segments divided by the total number of segments incubated. IR could measure fungal richness in a given sample of tissue segments and the incidence of multiple infections per tissue segments (Fisher & Petrini 1987). Simpson dominance index and Shannon-Wiener's diversity index were calculated for fungal diversity (Poole 1974; Groth & Roelfs 1987).

Table 1. Endophytic fungi isolated from leaf and bark of *Ficus benghalensis* and *F. religiosa*

S.no.	Endophyte	FR-Leaf	FR-Bark	FB-Leaf	FB-Bark
I.	**Ascomycetes**				
	Chaetomium globosum (1019)	15.0	6.5	5.5	15.2
	Chaetomium indicum (1020)	9.3	15.2	7.3	6.5
	Glomerella sp. (1034)	6.7	8.7	1.8	-
	Thielavia sp. (1055)	-	-	1.0	2.2
II.	**Coelomycetes**				
	Botryodiplodia theobromae (1018)	7.0	8.0	5.5	8.7
	Colletotrichum gloeosporioides (1022)	5.3	6.5	4.6	6.5
	Colletotrichum sp. 2 (1023)	8.0	15.2	5.5	15.2
	Phyllosticta sp. (1052)	2.0	-	1.8	-
III.	**Hyphomycetes**				
	Acremonium murorum (1011)	-	-	0.9	-
	Alternaria alternata (1012)	5.3	4.3	11.0	4.3
	Alternaria tenuissima (1013)	-	-	2.8	-
	Aspergillus niger (1015)	3.8	-	11.0	-
	Bipolaris sp. (1017)	4.0	2.4	-	2.8
	Cladosporium sp. (1021)	4.3	4.0	-	2.1
	Curvularia lunata (1026)	5.3	6.5	9.2	6.5
	Drechslera halodes (1028)	5.3	2.1	5.5	10.8
	Fusarium oxysporum (1031)	8.0	-	4.6	-
	Nigrospora sphaerica (1042)	6.7	-	1.8	2.0
	Penicillium sp.1 (1044)	4.0	2.1	-	4.3
	Penicillium sp.4 (1047)	5.0	2.0	2.8	-
IV.	**Zygomycetes**				
	Mucor sp.1 (1040)	2.7	2.1	1.8	-
V.	**Mycelia Sterilia**				
	VUCC 1 (1035)	4.0	-	-	-
	VUCC 2 (1036)	5.2	-	-	-
	VUCC 3 (1037)	1.6	-	-	-
	VUCC 4 (1038)	-	-	2	-

Abbreviations: FR – *Ficus religiosa,* FB – *Ficus benghalensis,* VUCC – Vels University Culture Collection

Results and discussion

Several medicinal herbs and shrubs have been intensively screened for endophytic fungi, whereas medicinal trees have rarely been studied for the presence of endophytic fungi in the tropics (Li *et al.* 2001; Strobel 2002; Suryanarayanan & Thennarasan 2004; Sowparthani & Rajagopal 2011). In the present study, endophytic fungi colonized both species of *Ficus.* Hyphomycetes were the dominant endophytes, ascomycetes and coelomycetes were equally distributed but zygomycetes and sterile forms were low in frequency. Basidiomycetes were absent and are usually isolated in low numbers in endophyte research (Suryanarayanan *et al.* 1998). *Ficus religiosa* leaves yielded a total of 22 taxa and 109 isolates, and 14 taxa 48 isolates in bark respectively, while *F. benghalensis* leaves yielded 18 taxa and 75 isolates, and 13 taxa 46 isolates in bark respectively. In this study, more endophytic fungi could be isolated from leaf than bark. These results are in agreement with Rajagopal & Suryanarayanan (2000), Devarajan *et al.* (2002), Kumaresan & Suryanarayanan (2002), Gangadevi & Muthumary (2007) who found that the leaves of the tropical hosts had more endophytic fungi than present in bark.

Although 25 different fungal taxa were present in both plants (Table 1), only 13 endophytic fungi showed appreciable densities of colonization (above 5%). *Colletotrichum gloeosporioides, Colletotrichum* sp., *Chaetomium indicum, Chaetomium globosum, Botryodiplodia theobromae, Alternaria alternata, Curvularia lunata* and *Drechslera* sp. occurred in both plants (Table 1). The endophyte assemblages of both hosts were dominated by *Colletotrichum* sp., *Chaetomium indicum, Chaetomium globosum, Botryodiplodia theobromae, Alternaria alternata, Curvularia lunata* and *Drechslera* sp. According to Petrini (1986), Suryanarayanan *et al.* (1998) and Rajagopal (1998), only one or few endophytic taxa dominate a single host species. Although both *F. religiosa* and *F. benghalensis* shared endophyte species, VUCC1, VUCC2 and VUCC3 were unique to *F. religiosa.* Similarly, *Thielavia* sp., *Alternaria tenuissima, Acremonium murorum* and VUCC4 were unique to *F. benghalensis.* Some endophytic fungi, e.g. *Phyllosticta* sp., *Alternaria tenuissima, Acremonium murorum, Fusarium oxysporum, Aspergillus niger,* VUCC1, VUCC2, VUCC3 and VUCC4 were present only in leaf tissues of the two hosts. The differences in species composition and densities of colonization indicated that certain endophytic fungi were host specific.

Petrini (1986) grouped endophytic fungi into xylariaceous, coprophilous, epiphytic and true endophytic forms. In the present investigation, representatives from these categories, excluding the xylariaceous forms, were observed to constitute the endophyte assemblage of the tree species. In addition to these categories of fungi, non-sporulating forms (Mycelia sterilia) accounted for 14.2% and 4.5% of the endophytic fungi population of *F. religiosa* and *F. benghalensis* respectively. Such sterile endophytic fungi continue to frustrate mycologists because of their uncertain taxonomic status. It is likely that many may be host specific species (Bills & Polishook 1992). We found sterile mycelia VUCC1, VUCC2, VUCC3 and VUCC4 that appeared to be host specific (Table 1) and corroborated the conclusion of Bills & Polishook (1992). Kowalski & Kher (1992) and Suryanarayanan & Rajagopal (2000) proposed the term 'phellophytes' to circumscribe endophytes that colonize bark tissues from those endophytes that colonize living tissues. The host range of phellophytes varied. Some of the phellophytes were isolated from both hosts (Table 1). Kowalski & Kher (1992) obtained similar results for some European trees species. Sixteen of the twenty-five endophytic species could also be isolated from leaf tissues of the two hosts screened suggesting that some phellophytes switched to endophytic mode of life.

Apart from environmental factors, there appeared to be another factor that affected endophyte diversity and dominance. The highest Simpson dominance index of 1.8175 in *Ficus religiosa* and 1.7778 in *Ficus benghalensis* (Table 2), did not reveal differences in their endophytic fungal assemblages. These combined values are significantly higher than the individual values, which ranged from 0.8752 to 0.9344 and indicated that rare species in the individual tissue is lower than in the overall endophytic community. The Shannon-Wiener diversity index indicated the uneven distribution of endophytic fungal population in the tissues of both *F. religiosa* and *F. benghalensis. Ficus religiosa* shows more diversity in both leaf and bark tissue (3.024, 2.47) compared to *F. benghalensis* (2.919, 2.439). *Ficus religiosa* shows comparatively higher diversity and dominance in its endophytic fungal population than *F. benghalensis*, although the difference is low in the dominance index and high in the diversity index between the two species. Further research is in progress for extraction and characterization of bioactive compounds from dominant endophytic fungi isolated from both host species.

Table 2. Diversity and Dominance indices of endophytic fungi in *Ficus religiosa* and *F. benghalensis*

Name of the host	Name of the tissue	Total no. of isolates	Total no. of taxa	Simpson index (1-D)	Shannon-Wiener index (Hs)
Ficus religiosa	Leaves	113	21	0.9344	3.024
	Bark	82	14	0.8831	2.47
Ficus benghalensis	Leaves	77	19	0.9026	2.919
	Bark	82	13	0.8752	2.439

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