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ORIGINAL PAPER

Endophytic fungi of wild legume *Sesbania bispinosa* in coastal sand dunes and mangroves of the Southwest coast of India

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Abstract Evaluation of 450 surface sterilized tissue segments of a seasonal wild legume, *Sesbania bispinosa* (Jacq.), of coastal sand dunes and mangroves of southwest India yielded 546 isolates comprising 39 endophytic fungi with six dominant taxa (*Aspergillus flavus*, *Aspergillus niger*, *Cladosporium tenuissimum*, *Fusarium moniliforme*, *Penicillium chrysogenum* and morpho sp. 1). A consortium of saprophytic, pathogenic and toxigenic fungi exists as endophytes in *S. bispinosa*. Number of segments colonized, number of isolates obtained, species richness and diversity were higher in *S. bispinosa* in mangroves compared to coastal sand dunes. Seeds yielded more fungal isolates, but species richness and diversity were low. In spite of low fungal colonization in root segments, the diversity was high. Up to 30–40 % endophytic fungi of *S. bispinosa* differed between coastal sand dunes and mangroves revealing partial host- and habitat-specificity. As *S. bispinosa* is extensively used as green manure and forage in southwest India, further studies especially on the bioactive compounds of its endophytic fungi might broaden its range of uses. In addition to conventional morphological techniques, molecular tools would provide precise insight on the endophytic fungi of coastal sand dunes and mangroves.

Keywords Legumes · *Sesbania bispinosa* · Endophytic fungi · Coastal sand dunes · Mangroves

Introduction

The Indian subcontinent consists of 10 biogeographic zones in 25 provinces encompassing more than 400 biomes (Mehta 2000). Coastal habitats have been considered one of the major biogeographic zones supporting a wide range of flora, fauna and microbes. The coastal sand dunes along the southwest coast of India harbor a variety of wild legumes, which are nutritionally and agriculturally valuable (Rao and Meher-Homji 1985; Rao and Sherieff 2002). About 25 species of legumes are known from the mid- and hind-dunes of coastal sand dunes of this region (Bhagya and Sridhar 2009). Similar to coastal sand dunes, mangroves of the Indian subcontinent also support a wide variety of legumes (Rao and Suresh 2001; Kathiresan and Rajendran 2005). The Nethravathi mangroves in southwest India are endowed with several mangrove associates along with wild legumes of economic importance (Rao and Suresh 2001). They consist of seasonal to perennial herbs, shrubs, under-shrubs, climbers and woody plants. About 14 wild legumes have been identified in the Nethravathi mangrove with dominance of *Canavalia cathartica, Derris triflorum, Sesbania bispinosa* and *S. speciosa* (Anita 2010). Many wild legumes fix nitrogen symbiotically with rhizobia in coastal sand dunes as well as mangroves (Arun and Sridhar 2004). They are common in different tidal ranges of coastal sand dunes or mangroves and extend to

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agricultural fields where they improve soil fertility and provide green manure and fodder for livestock, sources of food and traditional medicine. *S. bispinosa* in southwest India is self-dispersed, widely distributed in coastal sand dunes and mangroves and extends to agricultural fields (e.g. paddy, sugarcane and vegetable) and plantations (e.g. coconut and areca) (Bhat 2003).We compared the assemblage and diversity of endophytic fungi in five tissues selected from five different organs (leaf, stem, root, tender pods and seeds) of *S. bispinosa* grown on the coastal sand dunes and mangroves of southwest India. The major objectives of this study were to determine whether the endophytic fungi of *S. bispinosa* is host- or habitat-specific and to compare our results with earlier studies.

Materials and methods

Plant species and processing

Five mature plants of *S. bispinosa* (Jacq.) W.F. Wight growing about 50 m apart in coastal sand dunes (hind-dunes) of Someshwara (12°47′N, 74°52′E) and Nethravathi mangroves (12°50′N, 74°51′E) were chosen during post-monsoon season (November–December 2010). Plants were uprooted, transferred to the laboratory and processed within 4–5 h of collection. Four tissue pieces (leaf, stem, root and tender pod) from each plant were excised into nine segments each of one cm length ($4 \times 5 \times 9 = 180$ segments) and nine dry seeds per plant ($9 \times 5 = 45$ seeds) were randomly chosen. To remove the extraneous matter, tissue segments and seeds were rinsed in distilled water and blotted. They were surface sterilized according to Taylor et al. (1999) with a slight modification (sequence of 95 % ethanol, 1 min; 6 % sodium hypochlorite, 5 min; 95 % ethanol, 0.5 min; followed by three rinses in sterile distilled water).

Fungal identification

Surface sterilized tissue segments and seeds were placed on 1.5 % potato dextrose agar (PDA) medium (HiMedia Laboratories Pvt. Ltd., Mumbai, India) amended with antibiotic (tetracycline, 250 mg L^{-1}; Sigma Chemical Co., St. Louis, Missouri). The plates were incubated (27 ± 2 °C) up to 4 weeks at 12 h light and dark regimes. Tissues and seeds were periodically screened for growth of mycelia or development of colonies on the medium or on the tissue or seeds. Actively advancing mycelial portions were transferred aseptically to fresh antibiotic-free PDA medium. Fungi were identified based on colony characteristics and spore morphology using standard monographs and taxonomic keys (Ellis 1971, 1976, Carmichael et al. 1980; Ellis and Ellis 1987; Cai et al. 2006; Bhat 2010).

Data analysis

The percent colonization frequency (C_F, %), total colonization frequency (T_F, %) and relative abundance (R_A, %) of each fungus in different tissues/seeds were calculated:

$$C_F = [(N)/(T)] \times 100 \quad (1)$$

$$T_F = [(N_1)/(T_1)] \times 100 \quad (2)$$

$$R_A = [(C_F)/(T_F)] \times 100 \quad (3)$$

where N is the number of segments of each tissue colonized, N_1 the number of segments of all tissue colonized, T the total segments of each tissue screened, and T_1 is the total segments all tissue screened. Diversity (D', Simpson; H', Shannon) (Magurran 1988) and evenness (J', Pielou's) (Pielou 1975) of fungal taxa on each tissue and all tissues were calculated:

$$D' = 1 \Big/ \sum (pi)^2 \quad (4)$$

$$H' = -\sum (p_i \ln p_i) \quad (5)$$

$$J' = (H'/H'_{max}) \quad (6)$$

where p_i is the proportions of individual that fungal taxon i contributes to the total number of individuals of all fungal taxa; H'_{max} is the maximum value of diversity for the number of fungal taxa present. To compare the richness of fungal taxa based on the number of isolations and number of segments assessed, the expected number of species [$E_{(t)}$] was calculated using rarefaction indices by Ludwig and Reynolds (1988). $E_{(t)}$ in a random sample of n isolations taken from a total population of N isolations was estimated:

$$E_{(t)} = \sum_{i=1}^{s} \left\{ 1 - \left[\binom{N - n_i}{n} \Big/ \binom{N}{n} \right] \right\} \quad (7)$$

where n_i is the number of fungal isolations of the ith fungal taxon. Percent Sørensen's similarity coefficient (C_s, %) was calculated pair-wise among the different tissues based on the presence or absence of each fungal taxon (Chao et al. 2005):

$$C_s = [(2c)/(a+b)] \times 100 \quad (8)$$

where a is total number of fungal taxa in tissue 1, b the total number of fungal taxa in tissue 2, and c is number of fungal taxa common to tissues 1 and 2.

Results

The incubated plates were periodically screened for growth of fungi from the edges of the segments embedded on the medium under low-power microscope. No symptoms of projection of mycelia or fruiting of fungi were seen up to

1 week of incubation. This indicated the efficiency of surface sterilization in destruction of surface mycelia and spores, which resulted in growth of any fungi present in the interior of the tissues. Although the number of sampled host plants and the number of assessed segments of different organs from each host were low for assessment of the diversity of endophytic fungi, the sample sizes provided reasonable results for comparison of endophytic fungi between habitats and between tissue segments of host plant organs.

Coastal sand dune plants

Out of a total of 225 segments examined, 88 % yielded 227 isolates (Table 1). The number of segments colonized as well as the number of isolates were highest in seeds (44 and 69) followed by leaves (43 and 62). However, the number of core-group taxa was highest in roots (4 vs. 3 in other tissues), but the expected number of species out of 30 random segments was lowest (21 in roots vs. 25–27 in other tissues) (Fig. 1). Simpson and Shannon diversities and Pielou's evenness were higher in root than in other tissues (Simpson: 0.889 vs. 0.825–0.850; Shannon: 3.359 vs. 2.757–3.238; Pielou's evenness, 0.937 vs. 0.809–0.875). A total of 26 taxa were recovered with a maximum of 16 taxa in leaves. The total frequency of occurrence of *P. chrysogenum* was highest (20.2 %) followed by morpho sp. 1 (19.1 %) and they belonged to core-group taxa in all tissues. *A. flavus, A. niger, C. tenuissimum* and *F. moniliforme* were also the core-group taxa in at least one of the tissues. A total of 10 taxa was confined to one of the tissues assessed (exclusive species) with a maximum of four taxa each in leaves and roots (leaf: *Aspergillus sydowii*, *A. tamarii, Paecilomyces* sp. and *Rhizopus* sp.; root: *Codinaea assamica, Colletotrichum dematium, Fusarium solani* and *Phialophora* sp.; pod: *Penicllium citrinum* and *Pestalotiopsis neglecta*) (Table 2). Fungal similarity among the tissues ranged from 29 % (root vs. pod) to 80 % (stem vs. seed) (Table 3). Morpho sp. 1 did not sporulate on the PDA and it also failed to sporulate on the malt extract agar (MEA).

Mangrove plants

In mangrove plants, out of 225 segments, 91.6 % yielded 319 isolates (Table 1). All tested seeds yielded endophytic fungi and the number of isolates (130 vs. 35–70 %) as well as core-group taxa were higher in seeds than in other tissues (5 vs. 2–3). The expected number of species was higher in pods than in other tissues (29 vs. 23–25) (Fig. 1). Simpson diversity was higher in roots than in other tissues (0.897 vs. 0.775–0.884), while Shannon diversity was higher in leaf segments (3.652 vs. 2.453–3.564 in other tissues). Pielou's evenness was higher in roots than in other tissues (0.912 vs. 0.816–0.876). A total of 31 taxa were recovered with a maximum of 18 taxa in leaves. The total frequency of occurrence of *P. chrysogenum* was highest

Table 1 Fungal assemblage, species richness and diversity of endophytic fungi of *S. bispinosa* of coastal sand dunes and mangroves in the Southwest coast of India

Items	Assemblage				Species richness			Diversity		Pielou's evenness
	SA	SC	FI	FS	Total taxa	Core-group taxa	$E_{(t30)}$	Simpson	Shannon	
Coastal sand dunes										
Leaf	45	43	62	1.4	16	3	26	0.850	3.238	0.809
Stem	45	39	45	1.0	10	3	27	0.839	2.908	0.875
Root	45	37	51	1.1	12	4	21	0.889	3.359	0.937
Pod	45	35	40	0.9	09	3	26	0.825	2.757	0.870
Seed	45	44	69	1.5	10	3	25	0.827	2.848	0.857
All tissues	225	198	227	1.0	26	6	29	0.889	3.749	0.798
Mangroves										
Leaf	45	44	70	1.6	18	3	25	0.884	3.652	0.876
Stem	45	38	37	0.8	12	3	25	0.828	2.926	0.816
Root	45	36	47	1.0	15	3	23	0.897	3.564	0.912
Pod	45	43	35	0.8	07	2	29	0.775	2.453	0.874
Seed	45	45	130	2.9	14	5	25	0.859	3.118	0.819
All tissues	225	206	319	1.4	31	6	28	0.906	3.974	0.802

SA number of segments assessed, *SC* number of segments colonized, *FI* total fungal isolations, *FS* number of fungal isolates per segment, $E_{(t30)}$ expected number of fungal taxa out of 30 random isolations

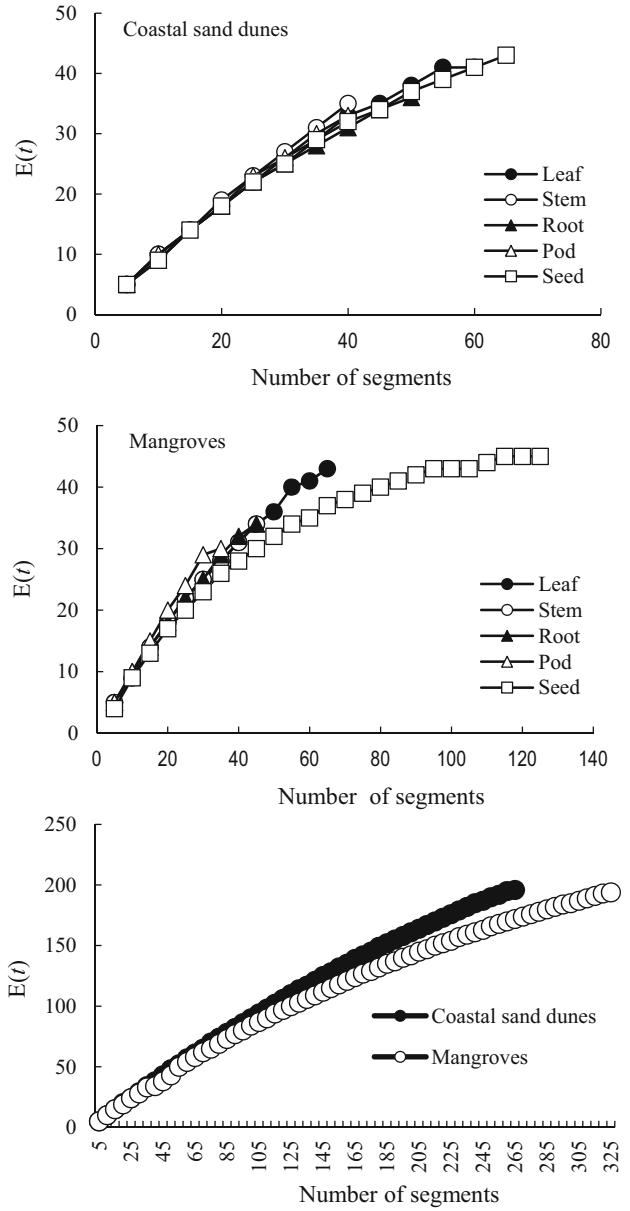

Fig. 1 Rarefaction curves of endophytic fungi in different tissues of *S. bispinosa* in coastal sand dunes and mangroves with rarefaction curves of endophytic fungi in coastal sand dunes and mangroves irrespective of tissues (number of segments vs. expected number of species, $E_{(t)}$)

(22.4 %), which was a core-group taxon in all tissues. Besides *P. chrysogenum*, five other fungal taxa (*A. flavus, A. niger, C. tenuissimum*, *Fusarium moniliforme* and morpho sp. 1) belonged to the core-group at least in one of the tissues (Table 4). A total of 12 taxa were confined to one type of tissue with a maximum of four taxa each in leaves and roots (leaf: *Chaetomium globosum, Curvularia prasadii, P. citrinum* and *Trichoderma harzianum*; stem: *Eurotium chevalieri*; root: *C. assamica, Rhizopus* sp., *Scytalidium lignicola* and *Trichoderma hamatum*; pod: *Aspergillus ochraceus*; seed: *Aspergillus oryzae* and *Cladosporium psoraleae*). The fungal similarity among tissues was 27 % (root vs. pod) with a maximum between leaf and stem (67 %) (Table 3).

Comparison of habitats

In both habitats, more seeds were colonized by endophytic fungi than were other tissues (44–45 vs. 36–44) and seeds yielded more fungal isolates (69–130 vs. 35–70 in other tissues) (Table 1). However, the total number of taxa in seeds was lower than in leaves (10–14 vs. 16–18). In both habitats, species richness was highest in leaves followed by roots, seeds, stem and pods. The exclusive species were more numerous and more consistent in all tissues of mangroves compared to the coastal sand dunes. For the five tissues examined, mangrove habitat showed higher expected number of species than coastal sand dunes (Fig. 1). However, the overall expected number of species out of 265 segments on random assessment was higher in coastal sand dunes than in mangroves (196 vs. 172 taxa). Simpson and Shannon diversities were higher in mangroves than in coastal sand dunes (Simpson: 0.906 vs. 0.889; Shannon: 3.974 vs. 3.749). Overall, 18 fungal taxa were common to coastal sand dunes and mangroves (Tables 2, 4). *P. chrysogenum* was the most frequent and was a core-group taxon in both habitats. The top six taxa of the core-group that were common to both habitats were: *A. flavus, A. niger, C. tenuissimum, F. moniliforme, P. chrysogenum* and morpho sp. 1.The exclusive species of tissues in coastal sand dunes and mangroves were not common except for *C. assamica* in roots (Tables 2, 4). Sørensen's similarity showed least similarity between roots and pods in both habitats (27-29 %), while stem vs. pods (53 %) and roots vs. seeds (55 %) showed almost equal similarity between the habitats (Table 3).

Discussion

In the coastal sand dunes of southwest India, Beena et al. (2000) surveyed endophytic fungi of root segments of three dominant plant species (*Ipomoea pes-caprae, Launaea sarmentosa* and *Polycarpea corymbosa*) employing plating and damp-incubation techniques. Seena and Sridhar (2004) evaluated endophytic fungi in three age groups (seeds, seedlings and mature plants) and five tissue classes (root, stem, leaf, seed coat and cotyledon) of two coastal sand dune wild legumes (*Canavalia cathartica* and *Canavalia maritima*) of southwest India. Foliar endophytic fungi of many mangrove plant species like *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica, Kandelia candel,*

Table 2 Colonization frequency (C_F, %) of endophytic fungi in five tissues of *S. bispinosa* in coastal sand dunes (out of 45 segments of each tissue)

Species	C_F (%)					T_F (%)	R_A (%)
	Leaf	Stem	Root	Pod	Seed		
**Penicillium chrysogenum* Thom	35.6	17.8	11.1	22.2	14.4	20.2	19.6
*Morpho sp. 1	22.2	22.2	22.2	17.8	11.1	19.1	18.5
**Aspergillus niger* Tiegh.	28.9	24.4	17.8	–	2.2	14.7	14.3
**Fusarium moniliforme* J. Sheld.	8.9	6.7	11.1	8.9	6.7	8.5	8.3
**Cladosporium tenuissimum* Cooke	2.2	6.7	–	–	23.3	6.4	6.2
**Aspergillus flavus* Link	–	2.2	–	20	3.3	5.1	5.0
Yeast (white)	2.2	–	6.7	–	5.6	2.9	2.8
**Fusarium oxysporum* E.F. Sm. & Swi	–	6.7	–	–	6.7	2.7	2.6
Alternaria longipes (Ellis &Everh.) E.W. Mason	4.4	–	4.4	–	2.2	2.2	2.1
**Drechslera halodes* (Drechsler) Subram. & B.L. Jain	4.4	–	6.7	–	–	2.2	2.1
*Yeast (pink)	6.7	–	–	4.4	–	2.2	2.1
**Chaetomium globosum* Kunze	–	6.7		2.2	–	1.8	1.8
***Codinaea assamica* (Agnihothr.) S. Hughes & W.B. Kendr.**	**–**	**–**	**8.9**	**–**	**–**	**1.8**	**1.8**
***Colletotrichum dematium* (Pers.) Grove**	**–**	**–**	**8.9**	**–**	**–**	**1.8**	**1.8**
***Fusarium solani* (Mart.) Sacc.**	**–**	**–**	**8.9**	**–**	**–**	**1.8**	**1.8**
**Nigrospora* sp.	4.4	4.4	–	–	–	1.8	1.8
**Eurotium chevalieri* L. Mangin	4.4	–	–	2.2	–	1.3	1.3
**Mucor plumbeus* Bonord.	4.4	–	2.2	–	–	1.3	1.3
***Pestalotiopsis neglecta* (Thüm.) Steyaert**	**–**	**–**	**–**	**6.7**	**–**	**1.3**	**1.3**
***Penicillium citrinum* Thom**	**–**	**–**	**–**	**4.4**	**–**	**0.9**	**0.9**
***Phialophora* sp.**	**–**	**–**	**4.4**	**–**	**–**	**0.9**	**0.9**
**Curvularia clavata* B.L. Jain	–	2.2	–	–	1.1	0.7	0.7
***Aspergillus sydowii* (Bainier & Sartory) Thom & Church**	**2.2**	**–**	**–**	**–**	**–**	**0.4**	**0.4**
***Aspergillus tamarii* Kita**	**2.2**	**–**	**–**	**–**	**–**	**0.4**	**0.4**
***Paecilomyces* sp.**	**2.2**	**–**	**–**	**–**	**–**	**0.4**	**0.4**
***Rhizopus* sp.**	**2.2**	**–**	**–**	**–**	**–**	**0.4**	**0.4**

T_F (%) is percent total percent frequency of occurrence out of 225 tissue segments; R_A (%) is percent relative abundance; * is Common to mangroves (see Table 4); Species and data in boldface are exclusive species

Table 3 Sørensen's similarity coefficient (C_s, %) of endophytic fungi in tissues of *S. bispinosa* in coastal sand dunes and mangroves

Items	Stem	Root	Pod	Seed
Coastal sand dunes				
Leaf	46	57	32	54
	Stem	36	53	80
		Root	29	55
			Pod	42
Mangroves				
Leaf	67	36	40	44
	Stem	30	53	46
		Root	27	55
			Pod	48

Rhizophora apiculata, Rhizophora mucronata, *Sonneratia caseolaris* and *Suaeda maritima* were studied in southeast India (Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000; Kumaresan and Suryanarayanan 2001, 2002). Endophytic fungi of bark, woody tissues and leaves of *K. candel* were studied at Maipo Marshes nature reserve in Hong Kong (Pang et al. 2008). Segments of plant organs including stem, pods, seeds, roots and rhizomes of mangrove species have been studied for endophytic fungi (Ananda and Sridhar 2002; Maria and Sridhar 2003; Anita and Sridhar 2009; Anita et al. 2009). Based on the above literature and the present study, the following sub-sections compare endophytic fungi of plant species in different maritime habitats.

Dominant endophytes

In our study, the core-group fungi (*A. flavus, A. niger, C. tenuissimum, F. moniliforme*, *P. chrysogenum* and morpho sp. 1) seem to render major defense in *S. bispinosa* of coastal sand dunes as well as mangroves. Except for *C. tenuissimum* and *F. moniliforme*, the others were top core-group taxa in *S. bispinosa* in southwest India mangroves (Anita et al. 2009). Similarly, the mangrove landrace *C. cathartica* also supported *A. flavus*, *A. niger*, *P. chrysogenum* and morpho sp. 1 as core-group taxa (Anita and Sridhar 2009). However, *C. cathartica*of coastal sand dunes showed single species dominance by *C. globosum* (Seena and Sridhar 2004). In the current study, *C. globosum* was restricted to stem and pods of *S. bispinosa* in coastal sand dunes, but was confined to leaves in mangroves. In coastal sand dunes non-legumes (*L. sarmentosa* and *P. corymbosa*), *C. globosum* was a dominant core-group taxon (Beena et al. 2000). Occurrence of *C. tenuissimum*, *P. chrysogenum* and morpho sp. 1 as common core-group taxa in seeds *S. bispinosa* indicates the possibility of their vertical transmission.

The present study showed multiple species dominance in all tissues of *S. bispinosa*. Such dominance is common in many mangrove species (e.g. *Acanthus ilicifolius, Acrostichum aureum, A. officinalis, Lumnitzera recemosa, R. mucronata, Sesbania bispinosa* and *S. caseolaris*) (Kumaresan and Suryanarayanan 2001; Ananda and Sridhar

Table 4 Colonization frequency (C_F, %) of endophytic fungi in five tissues of *S. bispinosa* in mangroves (out of 45 segments of each tissue)

Species	C_F (%)					T_F (%)	R_A (%)
	Leaf	Stem	Root	Pod	Seed		
**Penicillium chrysogenum* Thom	42.2	22.2	11.1	17.8	18.9	22.4	18.8
**Fusarium moniliforme* J. Sheld.	13.3	8.9	22.2	8.9	21.1	14.9	12.5
**Cladosporium tenuissimum* Cooke	17.8	26.7	–	–	25.6	14.0	11.8
**Aspergillus flavus* Link	4.4	2.2	–	28.9	32.2	13.5	11.3
**Aspergillus niger* Tiegh.	6.7	31.1	–	6.7	8.9	10.7	9.0
*Morpho sp. 1	8.9	–	11.1	–	16.7	7.3	6.1
Dactylospora haliotrepha (Kohlm. & E. Kohlm.) Hafellner	8.9	4.4	8.9	–	–	4.4	3.7
Alternaria alternata (Fr.) Keissl.	–	6.7	6.7		2.2	3.1	2.6
**Mucor plumbeus* Bonord.	4.4	4.4	–	4.4	–	2.6	2.2
**Drechslera halodes* (Drechsler) Subram. & B.L. Jain	6.7	–	4.4	–	–	2.2	1.9
Penicillium italicum Wehmer	2.2	–	8.9	–	–	2.2	1.9
Pestalotiopsis sp.	–	–	2.2	6.7	2.2	2.2	1.9
Alternaria triticina Prasada & Prabhu	6.7	2.2	–	–	–	1.8	1.5
***Penicillium citrinum* Thom**	**8.9**	**–**	**–**	**–**	**–**	**1.8**	**1.5**
***Cladosporium psoraleae* M.B. Ellis**	**–**	**–**	**–**	**–**	**7.8**	**1.6**	**1.3**
Curvularia lunata (Wakker) Boedijn	–	–	6.7	–	1.1	1.6	1.3
***Chaetomium globosum* Kunze**	**6.7**	**–**	**–**	**–**	**–**	**1.3**	**1.1**
**Curvularia clavata* B.L. Jain	4.4	2.2	–	–	–	1.3	1.1
**Fusarium oxysporum* E.F. Sm. & Swi	4.4	–	–	–	2.2	1.3	1.1
***Scytalidium lignicola* Pesante**	**–**	**–**	**6.7**	**–**	**–**	**1.3**	**1.1**
***Trichoderma hamatum* (Bonord.) Bainier**	**–**	**–**	**6.7**	**–**	**–**	**1.3**	**1.1**
***Aspergillus ochraceous* G. Wilh.**	**–**	**–**	**–**	**4.4**	**–**	**0.9**	**0.8**
**Aspergillus tamari* Kita	2.2	2.2	–	–	–	0.9	0.8
***Curvulariaprasadii* R.L. Mathur & B.L. Mathur**	**4.4**	**–**	**–**	**–**	**–**	**0.9**	**0.8**
***Eurotiumc hevalieri* L. Mangin**	**–**	**4.4**	**–**	**–**	**–**	**0.9**	**0.8**
**Nigrospora* sp.	–	–	2.2		2.2	0.9	0.8
*Yeast (pink)	–	–	2.2	–	1.1	0.7	0.6
***Aspergillus oryzae* (Ahlb.) E. Cohn**	**–**	**–**	**–**	**–**	**2.2**	**0.4**	**0.3**
***Codinaea assamica* (Agnihothr.) S. Hughes & W.B. Kendr.**	**–**	**–**	**2.2**	**–**	**–**	**0.4**	**0.3**
***Rhizopus* sp.**	**–**	**–**	**2.2**	**–**	**–**	**0.4**	**0.3**
***Trichoderma harzianum* Rifai**	**2.2**	**–**	**–**	**–**	**–**	**0.4**	**0.3**

T_F (%) is percent total percent frequency of occurrence out of 225 tissue segments; R_A (%) is percent relative abundance; * is Common to coastal sand dunes (see Table 2); Species and data in bold face are exclusive species

2002; Maria and Sridhar 2003; Anita et al. 2009). However, single species dominance was also seen in foliar tissues of several mangrove species (e.g. *Avicennia marina, B. cylindrica, R. apiculata, R. mucronata* and *S. maritima*) (Suryanaraynan et al. 1998; Suryanarayanan and Kumaresan 2000; Kumaresan and Suryanarayanan 2001). Similar to mangroves, *S. bispinosa*in the present study showed maximum diversity of endophytic fungi in roots (with the exception of Shannon diversity) (Anita et al. 2009).The onset of senescence in host plants may facilitate the endophytic core-group taxa to dominate as saprophytes to decompose the host tissues. However, to prove this notion, additional experiments are necessary to isolate such fungi in dead host tissues.

Terrestrial and marine fungi

Decomposing coastal sand dune and mangrove litter are reported to support more ascomycetes than mitosporic fungi (Kohlmeyer and Volkmann-Kohlmeyer 1991). That the tissues of *S. bispinosa* in our study had a majority of terrestrial mitosporic fungi corroborates earlier studies on foliar and root endophytes (Suryanarayanan et al. 1998; Beena et al. 2000; Kumaresan and Suryanarayanan 2001, 2002; Ananda and Sridhar 2002; Maria and Sridhar 2003; Seena and Sridhar 2004). Almost all endophytic fungi recovered in the current study were typical terrestrial taxa (with the exception of *Dactylospora haliotrepha* in leaves, stem and roots in mangroves). The typical mangrove fungus *D. haliotrepha* was also dominant in the sterilized roots of *S. bispinosa* in mangroves (Anita et al. 2009). *Kallichroma tethys* was recorded from the unsterilized stem, leaves and pods of *S. bispinosa*, which was core-group taxon in leaves (Anita et al. 2009). Besides *D. haliotrepha* and *K. tethys*, another marine fungus *Cumulospora marina* was endophytic in roots of *A. ilicifolius* (Maria and Sridhar 2003). Legumes like *C. cathartica* and *C. maritima* also supported the marine fungus *Halosarpheia* sp. in coastal sand dunes (Seena and Sridhar 2004). Up to 13 % of endophytes were marine fungi (*Monodictys pelagica*, *Periconia prolifica*, *Verruculina enalia* and *Zalerion maritimum*) in roots of coastal sand dune plants (*Ipomoea pes-caprae, L. sarmentosa* and *Polycarpaea corymbosa*) (Beena et al. 2000). However, no mycorrhizal fungi were recorded in association with roots of *Sesbania* in either habitat. The saline condition prevailing in the studied habitats might have hindered their colonization or they did not sporulate in the culture medium and were therefore unidentifiable without molecular characterization.

Saprophytic, pathogenic and toxigenic fungi

Several endophytic fungi are known as saprotrophs or opportunistic pathogens (e.g. *C. globosum* and *Paecilomyces varioti*) (Ananda and Sridhar 2002; Seena and Sridhar 2004; Arnold et al. 2007; Naik et al. 2007; Hyde and Soytong 2008; Vega et al. 2008). *A. flavus, A. niger, Fusarium oxysporum* and *P. chrysogenum* were the top core-group taxa in sterilized as well as unsterilized tissue segments of *S. bispinosa* in mangroves (Anita et al. 2009). Except for *F. oxysporum*, the others were dominant endophytes in our study, indicating their role as saprophytes on plant senescence. The endophytic fungal assemblage of *S. bispinosa* in our study could be divided into three groups, viz. saprophytes, pathogens and toxigens (saprophytes: *Aspergillus niger*, *A. oryzae, A. tamarii*, *C. globosum*, *C. assamica*, *E. chevalieri*, *Mucor plumbeus*, *Penicillium chrysogenum*, *P. citrinum*, *P. italicum* and *T. hamatum*; pathogens: *Alternaria alternata, A. longipes, A. triticina*, *Colletotrichum dematium*, *Curvularia clavata, C. lunata*, *C. prasadii, Drechslera halodes*, *F. moniliforme*, *F. oxysporum* and *F. solani*; toxigens: *A. flavus*, *A. ochraceus* and *T. harzianum*).

Azevedo et al. (2000) reviewed the literature on endophytic entomopathogenic fungi. Webber (1981) showed that elm trees are protected against beetles (*Physocnemum brevilineum*) by an entomopathogenic endophytic fungus *Phomopsis oblonga*. Widely distributed endophytic entomopathogenic fungi include *Beauveria* spp., *Paecilomyces* pp. and *Verticillium lecanii* (Petrini 1981; Bills and Polishook 1991; Ananda and Sridhar 2002; Vega et al. 2008). These fungi are known to protect host plants from insect herbivory and also to increase host plant biomass (Waller et al. 2005; Tejesviet al. 2007). Among these fungi, *Paecilomyces* sp. was recovered in our study exclusively from the leaf tissues of *S. bispinosa* in coastal sand dunes.

Endophytic fungi are the major source of novel bioactive compounds of medicinal value and biological control (Strobel 2003; Hyde and Soytong 2008; Jones et al. 2008; Suryanarayanan et al. 2010). They are known to produce 2-3 fold higher herbicidal metabolites compared to phytopathogenic and soil fungi (Schulz et al. 1999). Many endophytic fungi recorded in our study are potential producers of bioactive metabolites. For example, *Chaetomium* produces chaetocyclinones, chaetoglobosins, chaetomin, chaetoquadrins, chaetospiron, flavipin, orsellides and oxaspirodion (Sekita et al. 1976; Chitwood 2002; Löesgen et al. 2007; Suryanarayanan et al. 2010). In addition, some endophytic fungi also produce compounds that mimic plant hormones (Hyde and Soytong 2008).

Conclusions

Surface sterilized tissue segments of *S. bispinosa* of coastal sand dunes and mangroves of southwest India yielded 39 endophytic fungi with dominance by six taxa (*A. flavus*, *A. niger*, *C. tenuissimum*, *F. moniliforme*, *P. chrysogenum* and

morpho sp. 1). A consortium of identified and unidentified saprophytic, pathogenic and toxigenic fungi existed as endophytes in *S. bispinosa* in coastal sand dunes and mangroves. Besides conventional fungal identification, molecular approaches are necessary to follow the endophytic nature of fungi more precisely. Seeds yielded more fungal isolates than species, revealing limited vertical transmission of their endophytic fungi. Only one typical mangrove fungus (*Dactylospora haliotrepha*) was associated with leaves, stem and roots of mangrove *S. bispinosa*. Up to 30–40 % the endophytic fungal composition of coastal sand dune *S. bispinosa* differed from that of mangrove *S. bispinosa* and other coastal sand dune plant species, indicating their partial host- and habitat-specificity. *S. bispinosa* being is an important green manure and forage legume of southwest India, further insight on its endophytic fungi and production of bioactive metabolites will be beneficial in future.

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