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Diversity of Endophytic Fungi from Salt Tolerant Plants

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Abstract : Endophytes are organisms which may be fungi or bacteria that live inside the tissues of living plants. The following halophyte plants *Spinifex littoreus* (Poaceae), *Salicornia* sp. (Amaranthaceae), *Portulaca portulacastrum* (Aizoaceae) and *Bauhinia* sp. (Fabaceae) were probed for its presence of endophytic fungi which grows in salt marshes, on beaches, and among mangroves, was explored for its presence of fungal endophytes. The plants from different areas in and around Chennai (Besant Nagar, Tiruvanmiyur and Kovalam) were collected and studied for their presence of endophytic fungi. Altogether 600 segments were screened from the Leaf, Stem, Root regions of those plant samples. A total of 24 different species and sterile forms were recorded. Among them 24 belongs to Hyphomycetes and the remaining sterile forms. Jaccard's similarity coefficient showed the species composition of the endophytes recovered from all the 4 plant samples from any two tissues did not overlap by more than average of 1.66%. The *Salicornia* sp. showing maximum number of similarity between Root, followed by stem and leaf.
Key words: Biodiversity, Endophytic fungi, *Spinifex littoreus*, *Salicornia* Sp., *Portulaca portulacastrum*, *Bauhinia* Sp.

Introduction

Endophytes are microorganisms that invade the healthy tissues of living plants without causing any harm to their host [1]. These endophytes protect their host from infectious agents and adverse conditions by secreting bioactive secondary metabolites [2]. Endophytes are now considered as an important component of biodiversity. Endophytes can have many effects on their host such as enhancement of stress, insect and disease [3]. The colonization and propagation of endophytes can be potential biological agents and will play an important role in ecological agriculture.

Many reports showed that in a plant-microbe relationship endophytes contribute substances that posess various types of bioactivity, such as antibacterial and antifungal. The endophytes of medicinal plants [4], Gymnosperms [5] and floral endophytes [6] were already reported. In this study, the endophytes of halophytic plants were explored for the presence of endophytic fungi. The following plant species, i.e. *Spinifex littoreus, Salicornia* sp., *Portulaca portulacasrtum, Bauhinia* sp. were explored for their presence of fungal endophytes. The study was

also conducted to explore the species composition and diversity of such endophytic fungi from different tissues of halophytic plants.

Materials and methods

Sample collection

The halophytic plants samples which were screened for their endophytic assemblages were collected from different areas in and around Chennai (Besant Nagar, Thiruvanmiyur and Kovalam). To acquire endophytes, healthy plant samples were collected using separate, sterile polythene bags from their natural habitat. The samples were brought into the laboratory and processed within 24 hours according to Fisher and Petrini [7]. The plants were collected from different places were mixed together to have random isolation.

The halophyte plants collected for the isolation of endophytic fungi are as follows: *Spinifex littoreus* belonging to family Poaceae is a perennial grass with stolen forming stems, hard, stout, many-noded, rooting and copiously branching at nodes and normally grows to the height of about 1 to 3 feet. *Salicornia* sp. belonging to family Amaranthaceae are small, usually less than 30cm tall, succulent herbs with a jointed horizontal stem and erect lateral branches. The leaves are small and scale-like, and as such, the plant may appear leafless. *Portulaca portulacastrum* belonging to family Aizoaceae is a native, herbaceaous perennial found on sea coasts. The thick, fleshy leaves are borne on succulent, reddish-green stems that branch regularly forming dense stands close to the ground. *Bauhinia* sp. belonging to family Fabaceae is a creeper spread 3–6 m outwards. The lobed leaves usually are 7–9 cm across.

Isolation of Endophytes

The collected samples were first washed thoroughly in running tap water, From the plant samples, 50 segments of different tissues each (Root, Stem & Leaf) were screened for the presence of fungal endophytes. Leaf segments (approx 1 cm) were surface sterilized following a method of [8]. The segments were dipped in 70% ethanol for 5 seconds, immersed and rinsed in sterile water for 10 seconds. Stem and root tissues were cut into 1cm segments and the segments were surface sterilized following the method of [9]. The stem and root segments were dipped in 75% ethanol 60 seconds, immersed in 4% mercurric chloride for 90 seconds and dipped in 75% ethanol for 30 seconds. Ten segments of each plant parts were placed on 20ml PDA (Potato Dextrose Agar) medium amended with streptomycin (150mg) in petridishes (9cm dia) and were sealed using parafilm.

The petri dishes were incubated in a light chamber for a period of four weeks [10]. The light chamber had a bank of three four feet philips day light fluorescent lamps. The segments received 2200 lux of light through the petridishes lid as measured by a lutron (Germany) 1X-10' lux meter. The incubation temperature was 26$\pm$1°C. The petridishes were observed periodically and the fungi which grew out from the tissues were transferred to fresh PDA slants. To prevent the rapidly growing fungi from inhibiting the slow growing species, the former were removed following isolation.

Sporulating isolates were identified down to species level with the help of standard manuals [11-14]. The sterile isolates which could not be assigned to any taxonomic growth were sorted into morphospecies on the basis of colony surface texture, hyphal pigmentation, exudates, and growth rates. Such sterile forms were included as 'Sterile form' for the analysis of the results.

Statistical analysis

Colonisation frequency:

$$Cf = \frac{\text{Total number of individual fungi recorded}}{\text{Total number of segments observed}} \times 100$$

Endophytic infection rates (EIR%) were calculated

$$EIR = \frac{\text{Total number of infected segments}}{\text{Total number of segments screened}} \times 100$$

Jaccard's similarity coefficients were calculated for all possible pairs of host tissues to compare the endophytic assemblages according to the formula:

Similarity coefficient = C/(A+B-C)

Where A and B are the total number of fungal species isolated from any two tissue type and C the number of fungal species found in common.

Results:

Altogether six hundred segments were screened from Leaf, Stem and Root of halophytic plants, *Spinifex littoreus, Salicornia* sp. *Portulaca portulacastrum* and *Bauhinia* sp. A total of 141 isolates were obtained. Among them 1 isolate was sterile morphospecies. The remaining isolates were classified into 24 species which belonged to 10 genera. Among them 3 species belongs to Ascomycotina and 21 species belongs to Mitosporic fungi. Maximum number of isolates were recorded from *Spinifex littoreus* of Root (10) followed by Leaf (8) and Stem (6). The list of species isolated and their colony frequency is reported in (Table- 1).

Table 1. List of species and their Colonization Frequency recorded for the endopytes of halophytic plants.

S.No	Species	Spinifex littoreus			Salicornia sp.			Portulaca portulacastrum			Bauhinia sp.		
		L	S	R	L	S	R	L	S	R	L	S	R
HYPHOMYCETES													
1	Alternaria alternata	2	-	-	-	-	-	-	-	-	-	2	-
2	Aspergillus flavus	-	-	-	2	-	2	-	4	6	2	-	4
3	A.fumigatus	4	4	2	-	2	2	2	-	-	-	-	-
4	A. glaucus	2	-	-	-	-	-	-	-	-	-	-	-
5	A. japonicas	-	-	-	4	-	-	6	-	-	-	-	-
6	A. nidulans	-	-	2	-	2	-	-	-	-	-	-	-
7	A. niger	8	6	-	4	6	8	10	2	6	4	4	8
8	A. ochraceous	-	-	-	-	-	-	-	2	-	-	-	-
9	A. terreus	2	8	6	-	2	2	-	-	6	2	-	2
10	A. tamarii	-	-	-	-	-	-	-	-	-	2	4	-
11	Chaetomium globosum	-	-	6	-	-	-	-	-	-	6	-	-
12	Chaetomium sp.	-	-	-	-	-	2	-	-	-	-	-	-
13	Chrysonilia sitophila	-	-	2	-	-	-	-	-	-	-	-	-
14	Cladosporium sp.	-	-	-	-	-	2	-	-	-	-	-	-
15	Curvularia brachyspora	4	-	2	-	-	-	-	-	-	2	-	-
16	Curvularia lunata	-	-	2	-	-	-	-	-	-	2	-	-
17	Emericella nidulans	-	-	-	-	2	-	-	-	-	-	-	-
18	Fusarium culmorum	-	2	-	-	-	-	-	-	-	2	8	-
19	F. monoliformae	-	-	-	-	-	-	-	-	-	-	-	2
20	F. oxysporum	-	-	2	-	-	-	-	-	-	2	4	2
21	Fusarium sp.	-	-	-	-	-	2	-	-	-	-	-	2
22	Penicillium corylophilum	-	-	-	-	2	-	-	-	-	-	-	-
23	Penicillium sp.	-	-	-	-	-	-	2	4	2	-	-	-
24	Trichoderma sp.	2	-	2	-	-	-	-	-	-	-	-	-
	Sterile forms	10	6	8	-	-	-	-	2	2	8	10	4

L –Leaf **S**-Stem **R**-Root

The endophytic infection rates (EIR%) revealed that EIR were high in *Bauhinia* Sp. Stem (100%) and Leaf (100%). This was followed by stem (82%) and leaf tissues (100%). However, least number of EIR was recorded for *Salicornia* sp. stem (18%) and leaf (36%) tissues and root (60%). The EIR recorded for different tissues of halophytic plants are presented in (Table- 2). Jaccard's Similarity Coefficient showed the species composition of the endophytes recovered from any two tissues did not overlap by more than 1.66% (Table- 3).

Table 2. Endophytic Infection Rate (EIR %)

S.No	Tissue type	Total Number of segments screened	Total Number of segments infected by endophytic fungi	Endophytic Infection Rates(EIR %)
Spinifex littoreus				
1.	Leaf	50	50	100%
2.	Stem	50	41	82%
3.	Root	50	48	96%
***Salicornia* sp.**				
1.	Leaf	50	18	36%
2.	Stem	50	9	18%
3.	Root	50	30	60%
Portulaca portulacastrum				
1.	Leaf	50	33	66%
2.	Stem	50	32	64%
3.	Root	50	16	32%
Bauhinia malabarica				
1.	Leaf	50	50	100%
2.	Stem	50	50	100%
3.	Root	50	40	80%

Table 3: Jaccard Similarity Coefficient for the endophytic fungi isolated from the halophytic plants

	Spinifex littoreus			**Salicornia sp.**			**Portulaca portulacastrum**			**Bauhinia sp.**		
	Leaf	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**
Leaf	-	1.75	0.33	-	1.66	3	-	0.75	1	-	1	0.3
Stem	1.75	-	0.33	1.66	-	2.33	0.75	-	1	1	-	0.66
Root	0.33	0.33	-	3	2.33	-	1	1	-	0.3	0.66	-

Discussion:

Most of the species recorded in this study from halophyte plants also grow as sporulating saprophytic parasites on leaf tissues. It may be that certain endophytes become pathogenic when the host plant is stressed. Evidence suggests that endophytes have evolved directly from plant pathogenic fungi[15]. Endophytes were considered to have common attributes, i.e., (a) they are internal, at least subticular and derive nutrition from the living host tissue; (b) they establish at least a transitory biotrophic nutritional relationship with their host; (c) infected host tissues remain symptom less[16]. Endophytes also constitutes valuable source of secondary metabolites for the discovery of new potential therapeutic drugs [17]. Endophytic fungal investigations in the tropics have recently gained importance, although they have occurred in a wide variety of plants in temperate parts of the world [18].

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References

1. Bacon, C.W. and M.R. Siegel. "Endophytic parasitism of tall fescue". *Journal of production Agriculture* 1988, 1, 45-55.
2. Azevedo, J.L., W. Maccheroni, J.O. Jr. Pereira and Araujo, W.L. 2000. "Endophytic microorganisms: a review on insect control and recentAdvances on tropical plants". *Electroni J. Biotechnol.* 2000, 13.
3. Clay, K. "Fungal endophytes, grasses, and herbivores". In: Microbial mediation of plant-herbivore interactions. ('eds.) P. Barbosa, V.A. Krischik, and C.G. Jones. John Wiley & Sons, New York. 1991.
4. Kannan K. P. "Biodiversity of endophytic mycobiota from selected medicinal plants and some Gymnoperms". Ph.D., Thesis. University of Madras, Chennai, India. 2002.
5. Kannan, K.P., N. K. Udaya Prakash and Muthumary, J. "A comparative account on the endophytic mycoflora of Gymnosperms from Bangalore and Singapore". *Pollution Research.* 2005, 24, 1 – 6.
6. Kannan, K.P., D. Madhankumar, N.K. Udaya Prakash, R. Muthezilan, G. Jamuna, N. Parthasarathy and S. Bhuvaneswari. "Fungal Endophytes: A Preliminary report from marketed flowers", *International Journal of Appied Biology*, 2011, 2, 14-18.
7. Fisher, P.J. and O. Petrini. "Location of fungal endophytes in tissues of Suaeda fruticosa: A preliminary study". *Trans. Br. Mycol. Soc.* 1987, 89, 2, 246-249.
8. Dobranic, J.K., J.A. Johnson, and Q.R. Alikhan. "Isolation of endophytic fungi from eastern larch (Larix larcina) leaves from New Brunswick, Canada". *Can. J. Microbiol.* 1995, 41, 194-198.
9. Fisher, P. J., L.E. Petrini and B.C. Sutton. "A study of fungal endophytes from leaves, stem and roots of *Gynoxis oleifolia*, Muchler (Compositae) from Ecuador". *Nova Hedwigia* 1995, 60, 589-594.
10. Bills, G. F. and J.D. Polishook. "Recovery of endophytic fungi from *Chamaecyparis thyoides*". *Sydowia*. 1992, 44, 1-12.
11. Ellis, K. "Dematiaceous Hyphomycetes". Commonwealth Mycological Institute, Kew, Surrey, England, 1971.
12. Sutton, B. C. "The Coelomycetes". CMI, Kew, Surrey, England, 1980.
13. Onions, A. H. S., Allsopp,D., Eggins, H.O.W. "Smith's Introduction to Industrial Mycology". Edward Arnold Ltd. London. 1981.
14. Udayaprakash, N.K. "Indoor Molds: Isolation and Identification". Color Wings (P) Ltd, Chennai, 2004.
15. Isaac, S. "Fungal Plant Interactions". Chapman and Hall. New York, 1992.
16. Stone, J.K., Viret, O., Petrini, O. and Chapela, I.H. "Histological studies of the host penetration and colonization by endophytic fungi". In: Host wall alterations by parasitic fungi (Eds. O. Petrini and G. B. Oullette). APS Press. USA, 1994.
17. Miller, S.L.1995. "Functional diversity in fungi". *Can. J. Microbiol.* 1995, 73, (Suppl): S50 – S 57.
18. Petrini, O. "Fungal endophytes of tree leaves". In: Andrews JH and Hirano SS (Eds), Microbial Ecology of Leaves, pp. 179-197, Springer-Verlag, New York. 1992.

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