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# Isolation and Identification of Endophytic Fungi from *Salicornia brachiata* in Manakkattai, Muthupet Mangroves



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## ABSTRACT

*Salicornia* genus is succulent halophytic plant (salt tolerant) which belonging to the family Amaranthaceae that grows in salt marshes, on beaches and among mangroves. The aim of the research is to isolate and identify the fungal endophytes from the leaves of *Salicornia brachiata*. Totally ten isolates of fungal endophytes were isolated from the leaf of *Salicornia brachiata*. The endophytic fungi such as *Absidia sp*, *Aspergillus candidus*, *A. fumigatus*, *A. flavus*, *A. granulosis*, *A. janus*, *A. nidulan*, *A. ochraceous*, *A. sydowi*, *A. terreus*, *Fusarium sp*, *F. oxysporum*, *F. moniliforme*, *Penicillium sp*, *P. citrinum*, *P. janthinellum*, *Sporotrichum olivaceum*.



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## INTRODUCTION

Mangrove forests are fascinating and complex ecosystems (Feller *et al.*, 2010). Mangrove plants are salt-tolerant plants which act as primary producers in the estuarine food chain and they produce novel metabolites unique to the environment with various important economic and environmental functions (Bandarnayake 2002). Most mangrove plants do not consider the fact that plants in natural ecosystems have symbiotic associations with fungi. *Salicornia* species are native to North America. Common names for the genus include glasswort, pickleweed and marsh samphire. These common names are also used for some species, not in *Salicornia*. The main European species is often eaten, called marsh samphire in Britain and the main North American species is occasionally sold in grocery stores or appears on restaurant menus, usually as sea beans or samphire greens.

The *Salicornia* species are small, usually less than 30 cm tall, succulent herbs with a jointed horizontal main stem and erect lateral branches. The leaves are small scale-like, the plant may appear leafless. Many species are green but their foliage turns red in autumn. The hermaphrodite flowers are wind pollinated and the fruit is small and succulent contains a single seed. The ashes of glasswort and saltwort plants and of kept were long used as a source of soda ash (mainly sodium carbonate) for glassmaking and soap making. The introduction of the LeBlanc process for industrial production of soda ash superseded the use of plant sources in the first half of the 19<sup>th</sup> century. Endophytic fungi that live inside the tissues of living plants are under-explored group of microorganisms. Recently, they have been received considerable attention after they were found to protect their host against insect, pests, pathogens and even domestic herbivores. Almost all the plant species harbour one or more endophytic microorganisms. Endophytes are hidden within healthy host plants are a poorly investigated group of microorganisms but they represented an abundant and dependable source of novel bioactive compounds with huge potential for exploitation in a wide variety of medical, agricultural and industrial areas (Tan and Zou 2001).

These fungi are important to the structure, function and health of plant communities and may be responsible for the adaptation of plants to environmental stresses (Clay and Holah 1999). In addition, mutualistic symbiosis with mycorrhizal and endophytic fungi can confer salt tolerance

to plants and decrease yield losses in cultivated crops grown in saline soils (Baltruschat *et al.* 2008).

Endophytic fungi have been recognized as possible sources of bioactive secondary metabolites (Schulz *et al.*, 2002, Strobel *et al.*, 2003). There is a need to search new ecological niches for potential sources of natural bioactive agents for different pharmaceutical, agriculture and industrial applications.

## MATERIALS AND METHODS

### Collection of plant samples

The leaves of *Salicornia brachiata* were collected from the Muthupet mangrove Thiruvarur district. Healthy and mature plants were carefully chosen for sampling. The plants randomly collected and brought to the laboratory in sterile bags for further investigation.

### Isolation of endophytic fungi

Asymptomatic healthy leaf materials were thoroughly washed in running tap water, then surface sterilized by a modified method of Raviraja (2005). The selected leaf segments were immersed in 95% ethanol for 30sec, 45% sodium hypochlorite solution for 15sec and 95% ethanol for 30sec followed by rinsing with sterile distilled water three times for 10sec and allowed to surface dry under sterile conditions. After drying, each leaf segment was cut into approximately 0.5cm squares and placed on Petri Plates containing potato dextrose agar medium (PDA). The Streptomycin sulphate (100mg/L) was added to prevent the growth of bacteria. Then it was monitored every day for growth of endophytic fungal colonies. Fungi growing out from the samples were subsequently transferred to fresh PDA plates.

$$\text{Colonization frequency of endophytes} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments analysed}} \times 100$$

### Identification of endophytic fungi

The identification of fungi was done using cultural and microscopic characteristics such as shape, colour, pattern arrangement of mycelium, conidial arrangement, types of spores by using

standard manuals such as A manual of Penicillia by Kenneth (1949). The genus *Aspergillus* by Kenneth Raper (1965), Fungi in Agricultural Soils by Domsch and Gams (1972) and A manual of soil fungi by Joseph C. Gilman (1957). All the isolated fungi were identified up to genus level on the basis of detailed culture and microscopic study and by consulting relevant literature. The pure culture of isolated fungal strains was maintained in PDA slants at 28°C.

### **Lacto phenol cotton blue mounting**

A loopful culture was picked up with the help of a sterile inoculation loop and semi-permanent slides were prepared using lacto phenol cotton blue. The slides were gently heated in a spirit lamp so as to release the air bubbles if any present inside the cover glass. The excess stain was removed by using tissue paper and the cover glass was sealed with white nail polish.

### **RESULTS**

This resulted in the association of endophytic fungi with *Salicornia brachiata*, was selected and its leaves are only taken for isolation of endophytic fungi. Each isolate were sub-cultured into a PDA agar plates to remove the adherent plant metabolite from the mycelia and stored at 4°C for further studies.

In present study, seventeen endophytic fungi have been isolated from the *Salicornia brachiata* leaves such as *Absidia sp.*, *Aspergillus candidus*, *A. fumigatus*, *A. flavus*, *A. granulosis*, *A. janus*, *A. nidulans*, *A. ochraceous*, *A. sydowi*, *A. terreus*, *Fusarium sp.*, *F. oxysporum*, *F. moniliforme*, *Penicillium sp.*, *P. citrinum*, *P. janthinellum*, *Sporotrichum olivaceum*.

Name of the fungi isolated from leaves sample

S.NO	Name of The Fungi
1	<i>Absidia sp</i>
2	<i>Aspergillus candidus</i>
3	<i>A. fumigatus</i>
4	<i>A. flavus</i>
5	<i>A. granulose</i>
6	<i>A.janus</i>
7	<i>A. nidulans</i>
8	<i>A. ochraceous</i>
9	<i>A. sydowi</i>
10	<i>A. terreus</i>
11	<i>Fusarium sp</i>
12	<i>F. oxysporum</i>
13	<i>F. moniliforme</i>
14	<i>Penicillium sp</i>
15	<i>P. citrinum</i>
16	<i>P. janthinellum</i>
17	<i>Sporotrichum olivaceum</i>

Fungi class and family

S.No	Isolated Fungi	Macroscopic View	Microscopic View	Taxonomy
1	<i>Absidia sp</i>	Gray-green color when young stage then becoming clear yellow brown	Sporangium puriform measuring 40-50 $\mu$ in diameter $\times$ 44-60 $\mu$ in length	Class: Phycomycetes Order: Mucorales Family: Mucoraceae
2	<i>Aspergillus candidus</i>	Green	Conidiophores arise separately from the substratum 400-700 $\mu$ chains of conidia few phialides upto 10-15 $\times$ 3-5 $\mu$	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae

3	<i>A. fumigatus</i>	Green to dark green	Conidiophores short, usually densely crowded submerged hyphae or as branches from aerial hyphae septate or non septate, gradually enlarged conidia	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
4	<i>A. flavus</i>	Green	Conidiophores arise separately from the substratum 400-700 $\mu$ chains of conidia few phialides upto 10-15 $\times$ 3-5 $\mu$	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
5	<i>A. granulose</i>	Pale blue-green Reverse dull yellow and brown	Conidial heads few clustered in small groups mostly at margin, hemispherical to radiate, 75-125 $\mu$ in diameter	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
6	<i>A. janus</i>	Crowed reverse dull yellow to light brown dark green	Conidial heads loose in texture, with radiating and divergent chains of conidia, commonly 150-200 $\mu$ in diameter	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
7	<i>A. nidulans</i>	Dark cress-green	Hyphae, septate with ascospore	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
8	<i>A. ochraceous</i>	Shades, orange to vinaceous purple	Conidiospores and conidial heads with little arial mucelium conidiophores variable in length conidia globose 3.5-3 $\mu$ or 3.5-4 $\mu$ m	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae

9	<i>A. sydowi</i>	Blue green with the bluish effect prominent velvety Reverse orange to red	Conidiophores mostly arise from submerged hypha upto 500×4-8μ colourless smooth thick walled heads radiate or globose vesicles 12-20μ in diameter.	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
10	<i>A. terreus</i>	Pinkish velvety Reverse pale or bright yellow	Conidiophores to 150μ long × 5-8μ, more or less flexuous with walls smooth, septate with apex enlarged to form a vesicle commonly 12-18μ in diameter.	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
11	<i>Fusarium sp</i>	Brownish, white to violet	Conidial layer cushion shaped or somewhat extended without a spindle or sickle shaped, many celled with indistinct cross walls stroma	Class: Deuteromycetes Order: Moniliales Family: Tuberculariaceae
12	<i>F. oxysporum</i>	Brownish white to violet	Sclerotical hard bodies, and 0.5-3mm or 3-6mm in thickness more or less wrinkled, under moist conditions usually covered by fascicled medium high aerial mycelium.	Class: Deuteromycetes Order: Moniliales Family: Tuberculariaceae
13	<i>F. moniliforme</i>	Rosy white	Conidia, fusiform curved oval to riniform chlamydospores	Class: Deuteromycetes Order: Moniliales Family: Tuberculariaceae
14	<i>Penicillium sp</i>	Bluish green gray green	Surface growth networks of hyphae and ropes of hyphae	Class: Deuteromycetes

		bright green	conidiophores 30-40×2μ. Phialides	Order: Moniliales Family: Moniliaceae
15	<i>P. citrinum</i>	Bluish green to clear green reverse yellow	Conidiophores arise separately from submerged hyphae or from mycelium on the surface, usually upto 150μ in length (rarely 300μ) green, slightly granular.	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
16	<i>P. janthinellum</i>	Bluish-green, gray-green	Conidiophores 30-40 X 2μ arising from creeping hyphae or ropes of hyphae, with all smooth penicillus a single one-sided	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae
17	<i>Sporotrichum olivaceum</i>	White olive-gray	Conidiophores alternate, with secondary two to three phialides at the bear single spores at their tips. Conidia, olive, globose, to slightly ellipetic, 2-4μ in diameter.	Class: Deuteromycetes Order: Moniliales Family: Moniliaceae

## DISCUSSION

The endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and may produce beneficial substance for host (Weber, 1981 and Shiomi *et al.*, 2006). Endophytic organisms have received considerable attention after they were found to protect their host against insect pests, pathogens and even domestic herbivorous (Weber, 1981). Fungi have been widely investigated as a source of bioactive compounds an excellent responsibility for anticancer drug taxol, which had been previously to occur only in the plants (Strobel and Daisy, 2003).



In the present investigation, seventeen endophytic fungi were isolated from the leaves of *Salicornia brachiata*. The endophytic fungi such as *Absidia sp*, *Aspergillus candidus*, *A. fumigatus*, *A. flavus*, *A. granulosi*, *A. janus*, *A. nidulans*, *A. ochraceous*, *A. sydowi*, *A. terreus*, *Fusarium sp*, *F. oxysporum*, *F. moniliforme*, *Penicillium sp*, *P. citrinum*, *P. janthinellum*, *Sporotrichum olivaceum*.

## CONCLUSION

In the present study, totally seventeen endophytic fungi were isolated from *Salicornia brachiata* in Manakkattai, Muthupet mangroves focusing on the investigation of endophytic fungal diversity. The endophytes isolated during this study offer an excellent platform for the discovery of natural product-transforming fungus novel drugs.

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