

# ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI FROM *AVICENNIA MARINA* IN MANGROVE ENVIRONMENTS

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## Abstract

The aim of this study was to identify the endophytic fungi of the mangrove plant *Avicennia marina* (Forsk) vierh. Totally 51 species of endophytic fungi were successfully isolated from *Alternaria alternata*, *Aspergillus awamori*, *A. chevalieri*, *A. conicus*, *Aspergillus favipes*, *A. funiculosus*, *A.flavus*, *A.clavatus*, *A.fumigatus*, *Penicillium candidum*, *Penicillium janthinellum*, *Penicillium citrinum*, and *Aspergillus flavus* were screened and identified.

**Keywords:** Isolation and Identification, Endophytic fungi, Mycodiversity.

## Introduction

Endophytic organisms are those that live internally in apparently healthy and asymptomatic hosts. Endophytes appear to be ubiquitous; indeed, no study has yet shown the existence of a plant species without endophytes (Nisa *et al.*,2015). The term “Endophyte” was introduced (De Bary, 1866) and was initially applied to any organism found within a plant that causes asymptomatic infections entirely within plant tissues without any symptoms of disease (Wilson,1995). The endophytic fungus lives in mycelial form in biological association with living plant at least for some time. Therefore, the minimal requirement before a fungus to be termed as an endophyte should be the demonstration of its hyphae in the living tissue (Kaul *et al.*,2012). Endophytic fungi spends the whole or part of its life cycle colonizing inter and/or intra-cellularly inside the healthy tissues of the host plants, typically causing no apparent symptoms of diseases (Petrini, 1991). These are fungal microorganisms which asymptotically inhabit plant tissues and have been isolated from many species of woody plants and grasses (Hyde, 2008;Petrini, 1991).Endophytic fungi are found in all kinds of plants, i.e. trees, grasses, algae and herbaceous plants. Medicinal plants had been used to isolate and characterize directly the bioactive metabolites.

Endophytic fungus is an endosymbiont that live within a plant for least part of life without causing apparent harm. Dreyfuss and Chapela (1994) estimated that there may be at least one million species of endophytic fungi alone. Almost all plant species harbor one or more endophytic fungi (Tan and Zou, 2001). The plants have been extensively investigated for their endophytic biodiversity. The occurrence of endophytic microorganisms in different plant species is extensively studied. The endophytic fungal diversity

of *Avicennia marina* in Muthukuda mangrove forest is not reported so far. The present study forms the first report on the endophyte diversity of *Avicennia marina* plant of Muthukuda mangrove forest.

#### Isolation of fungi from leaf of *Avicennia marina* mangrove of Muthukuda, Pudukkottai District

S. no	Name of the fungi	Young leaves	Mature leaves	Senescent leaves	Fallen leaves
1.	<i>Alternaria alternata</i>	08	04	05	-
2.	<i>Aspergillus awamori</i>	04	03	02	-
3.	<i>A. chevalieri</i>	03	01	01	01
4.	<i>A. conicus</i>	07	02	-	-
5.	<i>A. flavipes</i>	03	02	-	02
6.	<i>A. flavus</i>	09	07	-	03
7.	<i>A. fumigatus</i>	08	05	03	02
8.	<i>A. funiculosus</i>	04	03	01	-
9.	<i>A. granulosis</i>	04	02	-	-
10.	<i>A. humicola</i>	03	01	-	04
11.	<i>A. niger</i>	06	04	05	-
12.	<i>A. ochraceous</i>	04	04	-	-
13.	<i>A. panamensis</i>	03	-	-	-
14.	<i>A. puniceus</i>	05	02	-	-
15.	<i>A. ruber</i>	03	02	-	-
16.	<i>A. rugulosus</i>	-	-	-	-
17.	<i>A. parsus</i>	06	-	03	01
18.	<i>A. speluneus</i>	07	06	-	-
19.	<i>A. sydowii</i>	-	04	-	-
20.	<i>A. tamarii</i>	04	-	-	-
21.	<i>A. terreus</i>	03	02	01	01
22.	<i>Bipolaris oryzae</i>	05	-	02	-
23.	<i>Botrytis cinerea</i>	-	01	-	-
24.	<i>Chaetomium sp.</i>	-	03	04	-
25.	<i>Cladosporium sp</i>	02	-	-	03
26.	<i>Curvularia geniculata</i>	02	-	04	05
27.	<i>C. lunata</i>	-	-	03	02
28.	<i>Fusarium moniliforme</i>	04	05	-	-
29.	<i>F. oxysporium</i>	-	-	04	-
30.	<i>Fusarium sp.</i>	-	03	-	-
31.	<i>Gloeocercospora sorgh</i>	-	02	-	-
32.	<i>Helminthosporium oryzae</i>	02	01	-	-
33.	<i>Humicola sp.</i>	06	04	-	-
34.	<i>Hyalopus sp.</i>	05	-	-	02
35.	<i>Masoniella sp.</i>	-	-	-	01
36.	<i>Mortierella humicola</i>	-	-	04	-
37.	<i>Penicillium chrysogenum</i>	06	07	04	-
38.	<i>P. citrinum</i>	11	08	07	03
39.	<i>P. janthinellum</i>	05	02	01	-
40.	<i>P. purpurescens</i>	08	07	04	-
41.	<i>P. Purpurogenum</i>	06	04	-	-
42.	<i>P. turbatum</i>	-	-	-	03
43.	<i>Rhziopus stolonifer</i>	02	-	-	-
44.	<i>Sclerospora sp.</i>	01	03	02	-

45.	<i>Spicaria divaricata</i>	-	06	-	07
46.	<i>Thamnidium</i> sp.	07	05	-	-
47.	<i>Thielaviopsis basicola</i>	-	-	06	04
48.	<i>Trichoderma harzianum</i>	-	04	-	-
49.	<i>Trichothecium</i> sp.	08	06	-	07
50.	<i>Torulla allii</i>	-	-	06	-
51.	<i>Verticillium</i> sp.	07	04	-	-
Total No.of Species		0181	0131	078	051
Total No.of Colonies		36	35	21	17

## MATERIAL AND METHODS

### Sample Collection

Foliage, mature and senescent and fallen leaves of *Avicennia marina*, were collected from Muthukuda mangrove forest Pudukkottai district.

### Isolation of Endophytic Fungi

Three types of leaves were sampled for the investigation of endophytic fungal communities. Healthy and mature plants were carefully chosen for sampling. The surface sterilization usually initializes with plant material being washed in running tap water and soaked in 0.1% mercuric chloride. The plant material washed is then subjected to dry under airflow (Comcheon *et al.*, 2005). Subsequently a surfactant such as ethanol was employed to rinse the plant material, followed by a sterilizing agent, such as sodium hypochloride (schulz and Boyle, 2005). Leaves were cut into pieces of about 3-4mm x 0.5-1 cm length and thereafter, plated onto the culture medium normally potato dextrose agar (PDA) (Suryanarayanan *et al.*, 2003) supplemented with antibiotic agent Streptomycin to restrain bacterial growth until emergence of fungal colony from the plant segments. Afterward, the plates were incubated at temperatures ranging from 18°C to 28°C for 7 days. Fungal out growth from the plant tissues were sub-cultured on fresh antibiotic- free medium for identification based on morphological examination and conidial characters. Colonization Frequency (CF) was calculated as described by Suryanarayanan *et al.*, (2003).

### 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 AND 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: 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 example is anticancer drug taxol, which had been previously to occur only in the plants (Strobel and Daisy, 2003).

A survey has been conducted for the diversity of endophytic fungi associated with the mangrove plant *Avicennia marina*. A total of 51 fungal endophytes were isolated and cultured in the laboratory. The results are in accordance with Rajendran and Kathiresan (2007) who studied microbial flora associated with *Avicennia marina* and *Rhizophora apiculata*. Most of the species encountered in the study was also observed by other works (Maria *et al.*, 2005; Liv *et al.*, 2007). In the current investigation started that the initial assessment was performed for the antibacterial activity of the isolated endophytic fungi. The fungal diversity were observed and the endophytic fungi like *Alternaria alternate*, *Aspergillus awamori*, *A. chevalieri*, *A. conicus*, *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. funiculosus*, *A. granulosis*, *A. humicola*, *A. niger*, *A. ochraceous*, *A. panamensi*, *A. puniceus*, *A. ruber*, *A. rugulosus*, *A. parsus*, *A. speluneus*, *A. sydowii*, *A. tamari*, *A. terreus*, *Bipolaris oryzae*, *Botrytis cinerea*, *Chaetomium* sp., *Cladosporium* sp., *Curvularia geniculata*, *C. lunata*, *Fusarium moniliforme*, *F. oxysporum*, *Fusarium* sp., *Gleocercospora sorgh*, *Helminthosporium oryzae*, *Humulola* sp., *Hyalopus* sp., *Masoniella* sp., *Mortierella humicola*, *Penicillium chrysogenum*, *P. citrinum*, *P. janthinellum*, *P. purpurrescens*, *P. Purpurogenum*, *P. turbatum*, *Rhizopus stolonifer*, *Sclerospora* sp., *Spicaria divaricata*, *Thamnidium* sp., *Thielaviopsis basicola*, *Trichoderma harzianum*, *Trichothecium* sp., *Torulla allii* and *Verticillium* sp. with four different samples of young leaves(36), Mature leaves(35), Senescent leaves(21) and Fallen leaves(17) were differentiated from the *A. marina* plant leaves.. The methanolic extracts from the culture of endophytic fungi grown aerobically in nutrient agar medium displayed antibacterial activity. Some extracts were effective against all the bacterial strains included in the study. Three different solvents were used for the extraction of antimicrobial metabolites from the culture filtrate of the selected fungi Distilled water extract showed least antibacterial activity while Methanol extraction of fungi, shows higher antibacterial activity than Ethanol extracts. It is accepted widely that the use of organic solvents always provides a higher efficiency in extracting an antimicrobial compounds when compared with water extraction (Rosell and Srivastava, 1987).

## CONCLUSION

The association between fungal endophytes and their host plant with various stages of leaf in the results of possibly unique adaptations including the development of a signalling system. It concluded that the better understanding of the basic biology of endophytes and host symbiosis were performed. It improve the precision of knowledge about the process involved in this unique symbiosis and develop a complete inventory of fungal endophytes with this host were investigated.

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