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Research Article

Isolation of fungi from *Suaeda monoica*, Karankadu Mangrove forest of Ramanathapuram Dt.

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Abstract

A total number of 27 endophytic fungi were isolated from young mature and senescent leaves of *Suaeda monoica*. The 11 taxa obtained, *Aspergillus* was dominant community. The senescent leaves 19 species were found to more endophytic diversity than the young 9 species ones. *Aspergillus conicus, Penicillium janthinellum, Phomopsis amygdale* were occurred on young, mature and senescent leaves. Fungi were observed.

Keywords: Isolation of endophytic fungi in *Suaeda monoica*.

Introduction

Fungi are ubiquitous occurring, eukaryotic, heterotrophic organisms. Beside the well – known mushrooms, fungal life is found worldwide in soil samples as well as deep sea vents and arctic ice and often reveals symbiotic traits. Similar to plants, there is a long history of the utilization of fungi by mankind as remedies and in everyday life. Nearly 3000 years ago the mayans used fungi to treat intestinal ailments (Strobel *et al.,* 2004). The discovery of penicillin isolated from *Penicillium notatum* by Sir Alexander Fleming in 1928 which resulted in a breakthrough in the treatment of bacterial infections that fungi became an important source of drugs for the treatment of a variety of diseases.

Endophytes

Endophytes are microbes that colonize living, internal tissues of plants without causing, any immediate overt negative effects (Bacon and White, 2000) As almost all vascular plant species appear to be inhabited by endophytic bacteria or fungi these represent important components of microbial diversity. The relationship between the host plant and its endophytic shows symbiotic characteristics as the endophytic occupant usually obtains nutrients and protection from the host plant and in return profoundly enhances the fitness of the host by producing certain functional metabolites (Tanandzou, 2001)

Fungal endophytes are a polypheletic group of primarily Ascomycetous fungi, whereas Basidiomycetes, Deuteromycetes and Oomycetes are rarely found (Saikkonen *et al* 1998; Arnold, 2007)

Mangroves

The specific regions where mangrove plants grow are termed as “mangrove ecosystem”. Mangrove forests occupy several million hectares of coastal area worldwide and distributed in over 112 countries and territories comprising a total area of about 1,81,000 km^2 in over one fourth of the world’s coastline (Alongi, 2002; spalding *et al*; 1997). According to forest survey of India (FSI.1999). Out of 4,87,100 ha of mangrove wetlands in India, nearly 56.7% (2,75,800ha) is present along the East coast and 23.5% (1,14,700ha) along the west coast and the remaining 19.8% (96,600ha) is found in the Andaman and Nicobar islands. The largest single area of mangroves in the world lies in the Bangladesh part of the Sunderbans, covering an aera of almost 6,00,000 ha

including waterways. There are about 6.9 million hain the indo-pacific region, 3.5 million ha in Africa, 4.1 million ha in the America including the Caribbean. Mangroves also survive in some temperature zones but there is a rapid decrease in the number of species with increasing latitude. (Chapman, 1977, Tomlinson, 1986, Bandranayake, 1998)

Materials and Methods

Sample Collection

The young, nature and senescent leaves of mangrove plants namely S*uaeda monoica* were collected from mangrove environment of Karankadu. The collect leaves were carefully stored in polythene bags and transported to the laboratory for the mycological examination.

Sterilization of plant materials

For isolation of the endophytic, selected mangrove plant leaves were subjected to surface sterilization. They were first washed with running water and then immersed in aqueous ethanol (3:7) for 1 minute, then in 3% aqueous Sodium hypochlorite solution for 4 minutes and, again in aqueous ethanol (0.2:9.8) for 0.5 minute, finally ringing in distilled water three times, with further drying in sterilized paper in a biosafety chamber (Petrini *et al.*, 1982)

Isolation of endophytic fungi

The potato dextrose agar medium (potato - 200g, Dextrose -20g, Agar – 18g, Distilled water – 1000ml and ph-6.5) was used for isolation of endophytic fungal species.

The potato tubers were peeled and weighed for about 200gms. The tubes were chopped in to small pieces with the help of a sterile knife. The chopped potatoes were transferred in to a conical flask containing about 1000ml of distilled water. The contents were boiled for 20 minutes. The supernatant was collected and filtered through muslin cloth and the filtrate was collected. To this filtrate dextrose and agar were added and shaked well to dissolve the ingredients and made up to 1000ml by addition of distilled water. Finally, the medium was autoclaved at 121^0c for 20 mins at 15lbspressure. Streptomycin sulphate (50$\mu g/ml$) was added and mixed well to prevent the bacterial contamination.

From each leaf lamina, six pieces approximately 5mm diameter were excised from the tip middle and base. The leaf bits were placed on to a potato dextrose agar PDA petri plate. The plates were incubated in a dust free cupboard at the room temperature (24+2^0C) for 5-7 days.

Observation

The colonies growing from the leaf fragments on PDA plates with different morphology were observed. The fungal cultures were then transferred, subcultured and the pure cultures were maintained on PDA medium. A portion of the growing edge of the colony was picked up with the help of a pair of needles and mounted on a clean slide with lactophenol cotton blue strain. The slide was gently heated in a spirit lamp so as to facilitate the staining and remove air bubbles. If any the excess stain was removed with the help of tissue paper and then the cover slip was sealed with transparent nail polish. The slide was observed under a compound microscope. Microphotography of the individual fungal species was also taken using Nikon phase contrast microscope (Nikon, Japan)

Identification

Colony colour and morphology were observed besides hyphal structure, spore size, shapes and spore bearing structures. They were compared with the standard works of Manual of Soil Fungi (Gillman, 1957); Higher fungi (Kohlmeyer and Kohlmeyer, 1979) and soil fungi (Domsch *et al.*, 1980)

Results

A total of 27 endophytic fungi were isolated from young, mature and senescent leaves of *Suaeda monoica*. Table (1). Of the 11 taxa obtained, *Aspergillus* was dominant community. The senescent leaves (19 species) were found to more endophytic diversity than the young (9 species) ones. *Aspergillus conicus*, *Penicillium janthinellum*, *Phomopsis amygdale* were occurred on young mature and senescent leaves of one plant three stages of plants.

Table.1 Isolated endophytic fungi from young, mature, and senescent leaves of *Suaeda monoica* Karankadu mangrove forest, Ramanathapuram Dt.

S. No.	Isolated endophytic fungi	Young leaves	Mature leaves	Senescent leaves
1.	*Absida glauca*	+	-	+
2.	*Alternaria alternata*	-	-	+
3.	*Aspergillus awamori*	-	+	-
4.	*Aspergillus conicus*	+	+	+
5.	*Aspergillus flavus*	-	+	+
6.	*Aspergillus fumigatus*	+	+	-
7.	*Aspergillus granulosis*	+	+	-
8.	*Aspergillus humicola*	-	-	+
9.	*Aspergillus niger*	-	-	+
10.	*Aspergillus repens*	-	+	+
11.	*Aspergillus ruber*	+	+	-
12.	*Aspergillus sulphureus*	+	+	+
13.	*Aspergillus terreus*	+	-	+
14.	*Aspergillus ustus*	+	-	+
15.	*Aspergillus variecolor*	+	-	-
16.	*Bipolaris oryzae*	+	-	-
17.	*Curvularia lunata*	-	-	+
18.	*Currularia senegalensis*	-	+	+
19.	*Fusarium moniliforme*	+	-	-
20.	*Fusarium semitectum*	-	+	+
21.	*Helminthosporium oryzae*	-	-	+
22.	*Penicillium chrysogenum*	+	+	-
23.	*Penicillium janthinellum*	+	+	+
24.	*Penicillium japonicum*	+	-	-
25.	*Pestalotiopsis* sp.	-	-	+
26.	*Phomopsis amygdali*	+	+	+
27.	*Verticillim* sp.	+	-	+

Discussion

Diverse endophytic fungi exist within plant aerial tissues, with a global estimate of upto a million undescribed species. These endophytes constitute a rich bio resource for exploration to discover new natural products. Endophytic fungi are reported from plants growing in various environments including tropic, temperate, xerophytes and aquatic. But few studies have investigated the endophytes from mangrove plants. Mangrove a kind of special host plants is a resource of abundant endophytic fungi. More than 200 species of endophytic fungi have been isolated and identified from mangrove trees and have despite the short period of research on the chemistry of mangrove endophytes already been proven to be a well established source for structurally diverse and biologically active secondary metabolites with great potential for antimicrobial antioxidant, anticancer and antitumor activities.(Strobel2003., Li *et.al.*, 2008., Pang *et.al*., 2008 Li *et.al*., 2009)

In the present investigation focused on the endophyte assemblages on the mangrove plant leaves of different age levels. Studies on mangrove endophytic fungi were initiated recently and it has been realized that mangrove plants harbour an extremely diverse endophytic fungal flora. (Chaeprasert *et al.*,2010, Costa *et al.*, 2012)

In the present investigation totally 27 endophytic fungal species belonging to 11 genera were isolated from the young, mature and senescent leaves of *Suaeda monoica* Karankadu mangrove forest Ramanathapuram District. Similarly, fourteen different taxa of endophytic fungi were isolates from *Avicennia marina* by Mehdi and additionally, endophyte assemblage varies with different parts and age of the host plant and with different season.

(Kumaresan and Suryanarayana, 2002). In the present study, diversity of mycoflora showed variations in young mature and senescent leaves. Higher number of endophytes were obtained in senescent leaves followed by mature and young leaves of the study plants.

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