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RESEARCH ARTICLE

DIVERSITY OF ENDOPHYTIC AND RHIZOSPHERE SOIL FUNGI OF *SUAEDA MONICA* IN MARAVAKADU MANGROVE FOREST

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ABSTRACT

Muthupet range now consists of six Reserved Forests, viz, Muthpet RF, Thuravikadu RF, Vadakadu RF, Maravakadu RF, Thamarankottai RF, and Palanjur RF. Maravakadu RF (area: 1490.12 ha.): Maravakadu RF was created as per G.O.Ms.No.721, forests and Fisheries, Dept., 6th July 1979 with an area of 1356.07 ha. The Maravakadu RF Extn. Bit-I was created as per Govt.Ir. no.56675/FR-III/86-9, dt 20.8.87 with an area of 134.05. ha. The Maravakadu RF is located in Pattukottai taluk of Thanjavur district. The term "endophytes" includes a suite of microorganisms that grow intra-and/or intercellularly in the tissues of higher plants without causing over symptoms on the plants in which they live, and have proven to be rich sources of bioactive natural products (Tan and Zou, 2001). *Suaeda monica* leaves and rhizosphere soil of the plant was collected during four seasons, premonsoon, monsoon, post monsoon and summer. Sterilized and fragmented leaves were maintained in petridishes with Potato Dextrose Agar medium (PDA) at 28^0 C until the isolation of the fungi. Soil fungi was isolated using Warcup, 1950 method. Thirty species representing 11 genera, 3 morphotypes determined as mycelia sterilia. Maximum number of fungi isolated from the fresh leaves of *Suaeda monica*. *Phoma* sp, *Aspergillus* sp and *Penicillium* sp were most frequently isolated. 9 species recorded from soil and endophytic fungi. *Choanephora* present only in rhizosphere soil. Maximum number of fungi isolated from summer season, minimum number of fungi isolated from monsoon season. Majority of the fungi belongs to Ascomycota and Deuteromycota.

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INTRODUCTION

Muthupet mangrove forest is located at the southern end of the Cauvery delta, covering an area of approximately 13,500 ha of which only 4% is occupied by well-grown mangroves. The rivers Paminiyar, Koraiyar, Kilaithankiyar, Marakkakoraiyar and other tributaries of the river Cauvery flow through Muthupet and adjacent villages. At the tail end, they form a lagoon before meeting the sea. Muthupet range now consists of six Reserved Forests, viz, Muthpet RF, Thuraikadu RF, Vadakadu RF, Maravakadu RF, Thamarankottai RF, and Palanjur RF. Maravakadu RF (area: 1490.12 ha.). More than 20,000 bioactive metabolites are of microbial origin. Soil genera such as *Acremonium*, *Aspergillus*, *Fusarium* and *Penicillium* have shown ability to synthesis a diverse range of bioactive compounds. More than 30% of isolated metabolites from fungi are from *Aspergillus* and *Penicillium* (Berdy 2005). Entophytic fungi were reported to produce novel bioactive metabolites such as antimicrobial, anticancer, and antiviral agents. The discovery of taxol producing fungi increased the importance of endophytes and shifted natural products research to endophytic fungi.

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MATERIALS AND METHODS

Sample Collection

Soil sample was collected from Maravakkadu mangrove forest Pattukottai taluk of Thanjavur district during four seasons namely premonsoon, monsoon, post monsoon and summer.

Isolation of Fungi From Soil –Serial Dilution Agar Plating Method

In serial dilution agar plate method, a known amount of material is suspended or agitated in a known volume of sterial water blank to make a microbial suspension. Serial dilutions 10^{-2}, 10^{-3} to 10^{-7} are made by pipetting measured volumes (usually 1ml or 10 ml) into additional dilution blanks. Finally 1ml aliquot of various dilutions are added to sterile petridishes (triplicate for each dilutions) to which are added 15ml of the sterile, cool PDA medium, supplemented with Sterptopenicillin,10µg/ml. The dilutions 10^{-2} to 10^{-5} are

selected for enumeration of fungi. Upon solidification, the plates are incubated in an inverted position for 3 -7 days at 25^0C.

Isolation of Fungi by Warcup (1950) Method

A single method cannot be used to count all the different types of fungi present, a sample is processed by a variety of techniques. Direct soil plate or warcup soil plate method was developed by warcup in 1950 and is superior to dilution plate method in that is easier to use, less time consuming, requires less glasswares and provides more quantitative information. Added 0.005 to 0.15 g air dried soil each in 5 sterile petriplates with the help of a sterilized cooled loop or transfer needle. Added 15 to 20ml of melted, cooled (45^0c) medium supplemented with streptopenicillin and rose Bengal, to each soil inoculated petriplate. Dispense soil particles throughout the medium by gentle rotation of the petridishes. Allow the plates to solidify. Incubated the plates at 25^0c in an inverted position for 5 days.

Fungal Endophytes

Sample Collection

Fresh, senescent and matured leaves of *Suaeda monica*, was collected from Maravakkadu mangrove forest Pattukottai taluk of Thanjavur district during four seasons namely premonsoon, monsoon, post monsoon and summer.

Isolation of Endophytic Fungi

Three types of leaves were sampled for the investigation of endophytic fungal communities. Healthy and mature plants were carefully choosen 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 and/or Tween 80 was employed to rinse the plant material, followed by a sterilizing agent, such as sodium hypochlorite (Schulz and Boyle, 2005). The effectiveness of surface sterilization was checked by making an imprint of the treated portion on agar media (Schulz *et al.*, 1998). 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 18^0C to 30^0C for 21 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.

Lactophenol Cotton blue mounting

A loopful culture was picked up with the help of a sterile inoculation loop and semi permenent slides were prepared using lactophenol cotton blue. The slides was 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

Isolation of Fungi from Soil

The soil sample was collected during four seasons namely premonsoon, monsoon, post monsoon and summer. 24 species were recorded in premonsoon, 15 species recorded in monsoon, 12 species recorded in post monsoon and 28 species recorded in summer season. (Table 1). *Phoma* sp, *Aspergillus* sp and *Penicillium* sp were most frequently isolated. (Table 2). *Choanephora* present only in rhizosphere soil. Maximum number of fungi isolated from summer season, minimum number of fungi isolated from monsoon season. Majority of the fungi belongs to Ascomycota and Deuteromycota.

Table 1. Isolation of fungi from soil during four seasons

SEASON			
PREMONSOON	SUMMER	POSTMONSOON	MONSOON
24 Isolates	28 Isolates	12 Isolates	15 Isolates

Table 2. Name of the Fungi Isolated from Soil

S.NO	NAME OF THE FUNGI
1	*Aspergillus alliaceus*
2	*A. conicus*
3	*A. niger*
4	*A. terreus*
5	*A. phoenicis*
6	*A. ochraceus*
7	*A. ruber*
8	*A. versicolor*
9	*Candida albicans*
10	*Choanephora* sp.
11	*Curvularia subulata*
12	*Fusarium oxysporum*
13	*Trichoderma viride*
14	*Penicillium janthinellum*
15	*P. levitum*
16	*P. janthinellum*
17	*Phoma chrysanthemicola*
18	*P. hedoricola*
19	*Phoma* sp
20	*Mucor* sp
21	*Rhizopus oryzae*
22	*Saccharomyces* sp
23	*Verticillium* sp

Isolation of Endophytic Fungi

20 fungal species isolated from fresh leaves, 11 fungal species isolated from senescent leaves and 8 fungal species isolated from mature leaves (Table 3). Despite many attempts using different culture media, 2 isolates did not sporulate being classified as Mycelia sterilia and distinguished based on the colour (White or brown) and morphological aspect of the colonies. From those, one from fresh leaves of *Suaeda monica* and one from decomposed leaves of *Suaeda monica.* A total 13 fungi belonging to the species were identified (Table-4).

Table 3. Endophytic fungi isolated from leaves of *Suaeda monica*

S.NO	FRESH LEAVES	SENESCENT LEAVES	MATURE LEAVES
1.	20 Isolates	11 Isolates	8 Isolates

Table 4. Name of the endophytic fungi isolated from leaves of *Suaeda monica*

S.NO	NAME OF THE FUNGI
1	*Aspergillus alliaceus*
2	*A. flavus*
3	*A. niger*
4	*A. ochraceus*
5	*Penicillium levitum*
6	*P. janthinellum*
7	*Phoma chrysanthemicola*
8	*P. hedoricola*
9	*Phoma* sp
10	Vegetative mycelium 1
11	Vegetative mycelium 2
12	Vegetative mycelium 3
13	*Candida albicans*

In this present study *Aspergillus alliaceus, A. niger, A. ochraceus, Penicillium levitum, P. janthinellum, Phoma chrysanthemicoloa, Phoma hedoricola, Phoma and Candida albicans* were recorded both soil and endophytic fungi.

DISCUSSION

Every plant on earth is known to harbour at least one endophytic microbe. Endophytic are one of the most unexplored and diverse group of organisms having symbiotic associations with higher life forms and produce beneficial substances for host (Weber, 1981). However only a few plants have been studied for their endophyte biodiversity and their potential to produce bioactive compounds. Endophytes, are now considered as an outstanding source of bioactive natural products, because they occupy unique biological niches as they grow in so many unusual environments (Strobel and Daisy, 2003). 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). Endophytic organisms have received considerable attention after they were found to protect their host against insect pests, pathogens and even domestic herbivorous (Weber, 1981).

In the present study 23 species were isolated from Rhizosphere soil of *Suaeda monica* in Maravakkadu mangrove forest. *Phoma* sp, *Aspergillus* sp and *Penicillium* sp were most frequently isolated. In the present study 13 species isolated as a endophytes from fresh, senescent and matured leaves of *Suaeda monica* collected from Maravakkadu mangrove forest. Two isolates did not sporulate. The occurrence of sterile mycelia as endophytes demand the use of molecular techniques for classification and induction of sporulation is suggested by means of incubation under near UV or low temperature (Billis, 1996). This study shows a common fungi for both rhizospere soil and endophytic fungi for the same plant of *Suaeda monica*. *Aspergillus alliaceus, A. niger, A. ochraceus, Penicillium levitum, P. janthinellum, Phoma chrysanthemicoloa, P. hedoricola, Phoma and Candida albicans* were recorded both soil and endophytic fungi. Only a few plant species have been investigated for their endophytic fungal population (Strobel and Daisy, 2003). Endophytes are now considered as an outstanding source of bioactive natural products, because they occupy unique biological niches as they grow in so many unusual environments (Strobel and Daisy, 2003). Therefore any information and/or research on endophytic fungi such as in this study is value.

Conclusion

Endophytic fungi are a potential source of natural products for the search for new and biologically active compounds. They produce secondary metabolite with potential for antimicrobial and anticancer property. Endophtic fungi might be useful for the development of newer and potent antioxidants. Many endophytic fungi having the antiviral and anti malarial properties. Hence the present study pave the way for the detection of potential drugs from endophytic fungi.

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