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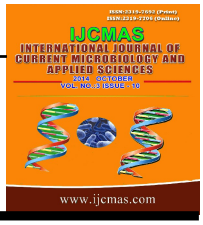

Original Research Article

Isolation and Identification of fungal flora from Mangroves of Pudukkottai District, Tamilnadu, India

S.Banupriya*, G.Kanimozhi and A.Panneerselvam

PG and Research Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur (Dt),Tamilnadu, India

**Corresponding author*

A B S T R A C T

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Mangrove ecosystem, PDA medium, Total fungal colony

Over the past several decades, a rapidly expanding field of research known as biodiversity and ecosystem functioning has begun to quantify how the world's biological diversity, as an independent variable, control ecological processes that are both essential for and fundamental to the functioning of ecosystems. The present study was conducted to know the diversity of fungal flora in the Mangrove ecosystem of Pudukkottai district at the three different stations during two seasons (January-2013-June-2013). The result of the study revealed that the mangrove soil showed 21 different fungal species belonging to 8 genera were isolated using PDA medium with standard manual. The dominant species were *Phoma fimeti*, *Verticillium* sp, *Penicillium roqueforti*, *Aspergillus niger*, *A.candidus*, *A.flavipes* and *Fusarium oxysporum*. The Physico-chemical parameters were analysed and statistically correlated with fungal flora. The results were discussed in detail.

Introduction

Mangrove forests are extremely important coastal resources, which are vital to our socio-economic development. A vast majority of human population lives in coastal area, and most communities depend on local resources for their livelihood. The mangroves are sources of highly valued commercial products and fishery resources and also as sites for developing a burgeoning eco-tourism (Kathiresan and Bingham, 2001). Biological diversity (biodiversity) encompasses the variety of life forms occurring in nature, from a kingdom distinct from plants and animals gradually became accepted only after Whittaker (1969).

Presently the "fungi" as a mega-diverse group spans three kingdoms, most belonging to the fungi (Eumycota), while others are classified in the protozoa and Chromista (Straminipila) (Cavalier-smith 1998, James *et al.*, 2006). The word "fungi", lower case and not in italics, is commonly used as a collective term for organisms traditionally

studied by mycologist from all three kingdoms (Hawksworth, 1991).

Fungi play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as biofertilizers and in many other ways. Fungal biotechnology has become an integral part of the human welfare (Manoharachary *et al.,* 2005). However, the fungi in marine environment are having terrestrial counterpart, which in their active phase may interact and influence the marine fungi and hence, it becomes essential to study the terrestrial fungi associated with the marine habitats (Kohlmeyer and Kohlmeyer., 1979).

The soil microbes decompose the plant and animal residues entering the soil and convert them into soil organic matter, which influences on soil physical, chemical and biological properties and on creating a complimentary medium for biological reactions and life support in the soil.

Microfungi play a focal role in nutrient cycling by regulating soil biological activity. However, the rate at which organic matter is decomposed by the microbes is interrelated into the chemical composition of the substrate as well as environmental conditions (Arunachalam *et al.,* 1997). This study deals with the seasonal variations in soil fungal population in relation to the soil nutrient variability in one of the least studied Mangrove field in South India.

Sample stations (Pudukkottai Dt)

Materials and Methods

Collection of Mangrove rhizosphere and salt pan soil sample (January-2013 to June 2013)

The mangrove rhizosphere soil such as *Avicennia marina, Suaeda monica, Salicornia depressa* and salt pan soil. The rhizosphere soils were collected from mangrove environment of Arasankarai, Muthukuda, and salt pan soil were collected from Ammapattinam, Pudukkottai Dt.

Isolation of microfungi

Rhizosphere soil of mangrove environment and salt pan soil were analysed for the soil fungi with the help of the following three methods.

1. Direct method
2. Dilution method
3. Hyphal isolation method

Dilution plate technique described by (Warcup, 1995) was used for the isolation of fungi from soil sample. 10g of soil from each sample was weighed separately and then dissolved in 100ml of distilled water. The flasks were shaken thoroughly in order to get uniform distribution of the soil. The soil suspensions were diluted in 10 fold increment from 10^{-2} to 10^{-3}. One ml of the diluted sample was plated onto sterilized Potato Dextrose Agar medium supplemented with 1% streptomycin sulphate (1gram of streptomycin sulphate was mixed thoroughly in 100ml of sterilized distilled water). The plates were incubated at room temperature 28˚C for 3-4 days. Three replicates for maintained for each sample. After three to four days of incubation, the colonies growing on Potato Dextrose Agar plates, with different morphology, were counted and purified on medium separately.

Presentation of data

Population of fungi
1g of the soil sample = No. of Colonies × Dilution factor

Pure culture and identification of soil fungi

After the isolation of the microfungi their pure cultures were made by single-spore culture method. A portion of the growing edge of each colony was picked up with the help of a pair of needles and mounted on a clean slide with lactophenol cotton blue. The slide was gently heated over the flame so as to remove air bubbles. The excess stain was wiped off with the help of tissue paper and then the cover slip was sealed with transparent Nail polish/DPX mountant.

The slide was observed under Microscope and Microphotography of the individual fungal species was also taken using Nikon Microphotography Microscope (Japan). Identification of the organisms were made with help of the relevant literature (Thom and Raper 1945., Raper and Thom 1949 and Gillman, 1957).

Soil analysis

(Correlation were analyzed by SPSS package)

The physicochemical analysis of the soil were analysed and statistically correlated with soil fungal flora (Table 2, 3).

Results and Discussion

The correlation between the number of colonies and physico-chemical parameters revealed that pH, Electrical Conductivity, Organic carbon were positively correlated. The Organic matter, Available Phosphorus, Available zinc, Available copper, Available iron, Available Manganese, Sodium, Potassium were negatively correlated. However the relationship in Organic carbon, Available nitrogen, Calcium, Magnesium were statistically significant at 0.05 level.

The correlation between the number of colonies and physico-chemical parameters revealed that pH, Electrical conductivity were positively correlated. However the relationship in Organic matter and Sodium are statistically significant at 0.01 level and Available potassium, Available copper, Available iron, Available Manganese and Calcium were also statistically significant at 0.05 levels.

It is generally accepted that only about 7% of all fungi have so far been discovered and about 93% of them to be discovered. Fungi are neglected organisms and they are not well protected, but like animals and plants, they are endangered by human activities. Although the 1992 convention on Biological Diversity extends protection to all organisms, it is worked in terms of “animals, plants and microorganisms” and fungi do not fit well in to these categories (Minter, 2010).

Threats to fungi throughout the globe are of concern since they are not only beautiful but also play a significant role in human welfare. Three steps were suggested by Moore *et al.,* (2001) for fungal conservation: (1).Conservation of habitats; (2). In situ conservation of non-mycological reserves/ecological niches; and (3).Ex situ conservation especially for saprobic species growing in culture. To help collections of fungal cultures to maintain appropriate standards, the World Federation for Collections (WFCC) has formulated guidelines which outline the necessary requirements (Hawksworth 1991, Smith *et al.,* 2001, Smith 2003).

Table.1 Cultural characteristic on PDA of the isolated genera

S.no	Species	Upper surface			Lower surface	Observation
		Cultural aspects	Density	Color		
1.	*Absidia glauca*	Effuse globose	Medium	Yellow to brown	Idem to upper face	Hyphae, septate with Sporangiospore
2.	*Alternaria alternate*	Effuse globose	Thick	Velvety to cottony, light to dark olivacious, gray-brown	Similar, very dark (brown to black)	Hyphae, septate with conidiophore
3.	*Alternaria tenuis*	Effuse globose	Medium	Brown-green	Olive-green or brownish black	Septate with conidiophore
4.	*Aspergillus alliaceus*	Effuse floccose	High	First white later become black	Idem to upper face except the color (yellow or orange)	Hyphae, septate with conidiophore
5.	*Aspergillus awamori*	Effuse floccose	High	Dull yellow brown	Fading of the textile color	Hyphae, septate with conidiophore
6.	*Aspergillus candidus*	Effuse Globose	High	White or yellowish cream	Uncolored to pale yellow	Hyphae, septate with conidiophore
7.	*Aspergillus chevalieri*	Effuse globose	Medium	Blueish gray in center	Marron in center to orange at margin	Hyphae, septate with conidiophore
8.	*Aspergillus flavipes*	Effuse Floccose	Medium	Sulphur-yellow	Yellow to orange or brown	Hyphae, septate with conidiophore
9.	*Aspergillus flavus*	Effuse floccose	Medium	Conidial head yellow to green	Idem to upper face	Hyphae, septate with conidiophores
10.	*Aspergillus nidulans*	Effuse globose	High	Dark cress-green	Idem to upper face except the color (purplish-red)	Hyphae, septate with conidiophores
11.	*Aspergillus niger*	Effuse globose	High	Blackish brown; phialides	Idem to upper face	Hyphae, septate with conidiophores

12.	*Aspergillus ruber*	Effuse globose	Medium	Ferruginous to morocco red	Shade of dark red brown	Hyphae, septate with conidiophores
13.	*Aspergillus terricola*	Effuse globose	Thick	Yellow-ochre to brown or umber	Idem to upper face	Hyphae, septate with conidiophores
14.	*Aspergillus variecolor*	Effuse globose	Medium	Purple red	Shadow of purple red	Hyphae, septate with conidiophores
15.	*Fusarium oxysporum*	Effuse globose	High	Brownish white to violet	Idem to upper face except the color (carmine yellow)	Oval to reniformchlamydospores
16.	*Penicillium citrinum*	Effuse globose	High	Bluish green to clear green, becoming olive to brownish-olive	Dull blue-green to yellow green	Aerial hyphae with conidiophores
17.	*Penicillium purpurogenum*	Effuse floccose	Thick	Yellow to pinkish shades, finally light gray-green	Deep red to purple	Hyphae, septate with conidiophore
18.	*Penicillium roqueforti*	Effuse globose	Medium	Gray-green to clear green	Colorless or cream to yellowish	Submerged hyphae with conidiophore
19.	*Phoma fimeti*	Effuse powder	Medium	White to light yellow(in oat meal agar medium)	Idem to upper face	Hyphae, septate with conidiophore
20.	*Thamnidium* sp.	Effuse globose	Thick or medium	Initially white and turns gray to yellowish brown in time	Dark brown in color	Sporangiophore bearing a terminal sporangium (*Mucor*like primary sporangium)
21.	*Verticillium* sp.	Effuse floccose	Thick	Blue-green, pale green	Purple to olive	Hyphae, septate with conidiophore

Table.2 Correlation between the number of colonies and the physico-chemical parameters in season I (postmonsoon)

season- postmonsoon

	TNC	pH	EC	OC	OM	AN	AP	APO	AZ	AC	AI	AM	C	M	S	P
TNC	1															
pH	0.153	1														
EC	0.536	0.218	1													
OC	0.506	0.528	0.765*	1												
OM	-0.585	-0.262	-0.234	-0.569	1											
AN	-0.006	-0.722	-0.597	-0.781*	0.071	1										
AP	0.454	0.037	0.032	-0.170	-0.291	0.420	1									
APO	-0.193	0.271	-0.364	-0.426	0.039	0.254	0.725	1								
AZ	0.212	-0.548	0.120	0.134	-0.597	0.371	0.203	-0.174	1							
AC	0.268	-0.545	0.036	-0.216	0.328	0.412	0.288	-0.091	0.121	1						
AI	-0.205	-0.314	-0.674	-0.381	-0.296	0.593	0.319	0.395	0.517	0.152	1					
AM	0.322	-0.598	0.117	-0.404	-0.025	0.681	0.600	0.188	0.430	0.341	0.045	1				
C	0.555	-0.095	0.336	0.088	-0.544	0.319	0.852*	0.421	0.558	0.114	0.197	0.727	1			
M	0.215	-0.347	-0.247	-0.397	-0.284	0.712	0.871*	0.635	0.517	0.353	0.667	0.664	0.785*	1		
S	0.173	0.034	-0.631	-0.442	-0.371	0.642	0.633	0.623	0.133	-0.031	0.681	0.260	0.400	0.705	1	
P	0.126	-0.126	-0.015	0.037	0.449	-0.077	-0.354	-0.480	-0.384	0.653	-0.197	-0.326	-0.577	-0.393	-0.309	1

***. Correlation is significant at the 0.05 level (2-tailed).**

Table.3 Correlation between the number of colonies and the physico-chemical parameters in season II (summer)

SEASON- SUMMER

	TNC	pH	EC	OC	OM	AN	AP	APO	AZ	AC	AI	AM	C	M	S	P
TNC	1															
pH	0.262	1														
EC	0.570	0.190	1													
OC	0.601	-0.387	0.220	1												
OM	0.601	-0.387	0.220	10.000**	1											
AN	0.498	0.214	-0.338	0.244	0.244	1										
AP	-0.057	0.575	0.301	-0.369	-0.369	-0.347	1									
APO	0.574	-0.452	-0.126	0.814*	0.814*	0.624	-0.620	1								
AZ	-0.091	0.572	-0.305	-0.608	-0.608	0.238	0.566	-0.325	1							
AC	0.474	-0.571	0.060	0.601	0.601	0.288	-0.733	0.812*	-0.386	1						
AI	0.418	0.814*	-0.116	-0.177	-0.177	0.702	0.336	0.015	0.677	-0.292	1					
AM	0.405	-0.120	-0.363	0.456	0.456	0.801*	-0.154	0.744	0.220	0.313	0.454	1				
C	-0.429	-0.089	0.063	-0.329	-0.329	-0.440	-0.386	-0.434	-0.472	-0.077	-0.470	-0.804*	1			
M	-0.344	-0.373	-0.189	0.072	0.072	0.056	-0.360	0.027	-0.561	-0.185	-0.301	0.039	0.353	1		
S	-0.545	-0.445	-0.889**	-0.030	-0.030	0.189	-0.627	0.253	-0.073	0.233	-0.218	0.160	0.237	0.248	1	
P	0.181	0.232	0.398	-0.420	-0.420	-0.177	0.530	-0.289	0.602	-0.025	0.173	-0.085	-0.335	-0.632	-0.567	1

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Rani and Panneerselvam, 2010 reported that the diversity and distribution of different organisms in the marine environment are influenced by the physico-chemical properties of both water and the sediments. Point calimere includes many diverse habitats such as sandy and muddy shores and mangroves, which have various physico-chemical features. A total of 59 fungal species were isolated from all four stations. In our study total of 30 soil microfungi were isolated. However, only a few fungal species were observed in different seasons of the year across the sites.

Senthilkumar *et al.* (2009) suggested that 15 soil samples were collected from three different stations along the Muthupet mangroves in Tamilnadu and examined by dilution plating method on PDA medium to access fungal diversity and the population diversity. Of the 22 species recorded the Aspergillus, Penicilliumwas represented as dominant one of each. Incorporate with our study species like Aspergillussp, Penicilliumsp. and Trichoderma sp. were common to all sites. Some fungal species encountered were rare and restricted to particular site. Dominance of the genus *Aspergillus* sp,

Penicillium sp. in the present study sites may be due to their greater rate of spore production, dispersal and partly due to their resistance over extreme environmental conditions (Schimel, 1995).

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