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Fungal Diversity in the Sediments of Point Calimere, East Coast of India

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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. Soil samples were collected from four stations Viz. Old light house, New light house, Chola light house and Muniyappan lake. A total of 59 species were isolated from all the four stations. Among them 59 species belonged to 20 genera, 6 species belonged to 4 genera were of Phycomycetes, 5 species belonged to 3 genera were of Ascomycetes and 47 species belonged to 13 genera were of Deuteromycetes.

Key words: Ascomycetes, Deuteromycetes, Diversity, Fungi, Mangroves.

Microbial diversity has received particular attention in environmental studies since 1960s, but its functional significance in ecological processes is still a subject of debate and analysis[1]. The ecological importance of fungal diversity in marine ecosystems has been often underestimated, or completely ignored, even though fungi represent a wide range of nutritional groups such as saprobes, pathogens, and symbionts, that form an integral part of the coastal ecosystems[2,3].

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Diverse fungal isolates have been isolated from Point Calimere. Marine fungi are still one of the most understudied ecological groups. There remains some debate as to what constitute a "marine" fungal species. Nevertheless, it is generally accepted that the obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat; while facultative marine fungi are those from freshwater or terrestrial milieus which are able to grow (and possibly sporulate) in the marine environment[4,5].

Because of their ability to sporulate on the seawater medium, most of the isolates in this study likely belong to 'marine fungi'. Nevertheless, many terrestrial fungi have been reported to grow well on media with salt concentrations equal to that of marine

environments[6,7]. In addition, natural seawater medium might not be equivalent to the *in vivo* marine environment. Consequently, it remains to be confirmed whether these isolates are truly obligate or facultative marine fungi. Therefore the present study aims to understand the diversity of fungi on soil samples.

MATERIAL AND METHODS

Study area and Sampling

Point Calimere is in the East Coast of India, situated in Vedaranyam taluk and Nagapattinam district of Tamilnadu (Lat.10°18'; Long.79°51'E). Soil samples were collected from four locations comprising 3 seashores (Old light house, New light house, Chola light house) and one mangrove (Muniyappan lake). 5 samples were collected randomly from the each station. One kg of soil was also collected separately from each station for the analysis of soil physico-chemical properties.

Physico-chemical characteristics of soil

pH

Soil samples were individually mixed with distilled water (1:2 w/v) and the supernatant was examined using pH meter (Elico, India) for the determination of pH.

Electrical conductivity

Soil samples were individually mixed in distilled water (1:2 w/v) and the electrical conductivity was determined by using electrical conductivity meter (Systronics, India, Model 631E).

Nutrients

Macro-nutrients such as available nitrogen, phosphorus, potassium and micronutrients such as Zn, Cu, Mn and Fe were analyzed by the methods described by Barnes[8] and Muthuvel and Udayasoorian[9].

Isolation of fungi from soil

Dilution plate technique described by Warcup[10] was used for the isolation of fungi from soil samples. 10 g of soil from each sample was weighed separately and then dissolved in 100 ml of distilled water. The flasks were shaken thoroughly in order to get uniform distribution of the soil. The soil suspensions were diluted in ten fold increment from 10^{-2} to 10^{-4}. One ml of the diluted sample was plated onto sterilized 50 per cent SWCMA medium(Sea Water Corn Meal Agar Medium - Corn meal powder 20 g, dextrose 20 g, peptone 20 g, agar 20 g) supplemented with 1% streptopenicillin (One gram of streptopenicillin was mixed thoroughly in 100 ml of sterilized distilled water). The plates were incubated at room temperature (28 ± 2°C) for 7 days. Three replicates were maintained for each sample.

After seven days of incubation, the colonies growing on 50% SWCMA plates, with different morphology, were counted and purified on SWCMA medium separately. 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 (Hi-media). 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 microphotographs of the individual fungal species were also taken using Nikon microphotograph microscope (Japan). The organisms were identified with the help of standard manuals such as A Manuals of soil fungi[11]; Hyphomycetes[12]; A Manual of Penicillia[13]; Manual of Aspergilli[14]; Damatiaceous Hyphomycetes and More Damatiaceous Hyphomycetes[15,16].

Presentation of data

The percentage contribution was calculated as follows

$$\% \text{ contribution} \frac{\text{Total number of colonies of individual species}}{\text{Total number of colonies of all the species}} \times 100$$

Simple correlation analysis was performed between the physico-chemical characteristics of the soil and fungal population density.

RESULTS

Physico-chemical characteristics of soil

Soil samples collected from Point Calimere revealed that the pH level was in the range of 7.5 to 8.3, EC 3.5 to 7.5, nitrogen 44.8 to 65.8 kg/acre, phosphorus 13.0 to 18.3 kg/acre, potassium 245 to 350 kg/acre. The micronutrients of soil revealed that the ferrous content was in

the range of 5.7 to 7.65 ppm, manganese 2.75 to 3.56 ppm, zinc 0.30 to 0.54 ppm and copper 0.27 to 0.44 ppm (Fig.1).

Totally 59 species of fungi belonged to 20 genera were isolated from soil samples. Among them, 6 species belonged to 4 genera were phycomycetes, 5 species belonged to 3 genera were Ascomycetes and 47 species belonged to 13 genera were Deuteromycetes

Fungal population density varied from station to station. Maximum population density (1198 CFU/g) was recorded in Old light house and minimum population density (537 CFU/g) was recorded in New light house. Relatively high diversity of 42 species was recorded in the soil samples collected from old light house followed by 34 species in the samples of Muniyappan lake and 24 species from the soil samples of Chola

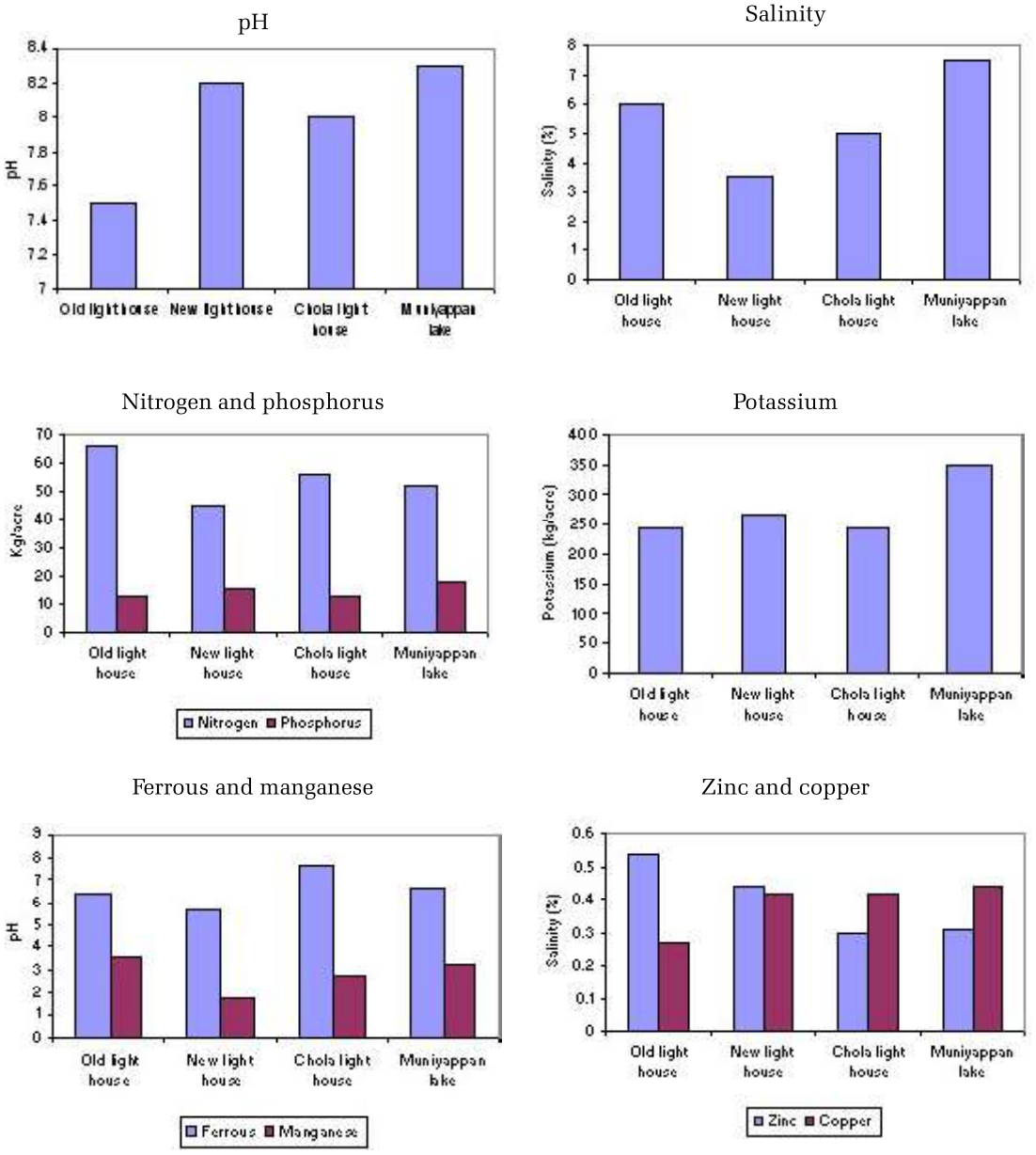

Fig. 1. Physico-chemical properties of soil samples collected from Muniyappan Lake

Table 1. Species of fungi isolated from the soil samples of Point Calimere

S. No.	Fungal species	Old light house	New light house	Chola light house	Muni-yappan lake	Total count	No. of occur rence	% freq-uency	Freq-uency class
Phycomycetes									
1.	*Absidia* sp.	41	-	62	-	103	2	50	O
2.	Blakeslea sp.	12	-	-	15	27	2	50	O
3.	*Rhizopus nigricans*	-	-	19	51	70	2	50	O
4.	*Rhizopus* sp.	25	-	-	33	58	2	50	O
5.	*Syncephalastrum racemosum*	26	-	-	41	67	2	50	O
6.	*Syncephalastrum* sp.	-	32	-	45	77	2	50	O
Ascomycetes									
7.	*Chaetomium globosum*	32	-	-	-	32	1	25	R
8.	*Chaetomium* sp.	48	55	-	-	10	2	50	O
9.	*Emericellopsis* sp.	11	-	-	-	103	1	25	R
10.	*Thielavia terricola*	12	-	-	13	25	2	50	O
11.	*Thielavia* sp.	19	-	14	-	33	2	50	O
Deuteromycetes									
12.	*Alternaria alternata*	22	14	-	-	36	2	50	O
13.	*Alternaria* sp.	-	-	32	12	44	2	50	O
14.	*Aspergillus candidus*	-	-	18	-	18	1	25	R
15.	*A. carbonareus*	-	-	14	-	14	1	25	R
16.	*A. clavatus*	12	-	-	-	12	1	25	R
17.	*A. chevalieri*	45	21	-	27	93	3	75	F
18.	*A. flaviceps*	18	-	-	20	38	2	50	O
19.	*A. flavus*	45	-	36	22	103	3	75	F
20.	*A. fumigatus*	32	21	-	43	96	3	75	F
21.	*A. granuloses*	-	-	32	21	53	2	50	O
22.	*A. humicola*	42	-	-	31	73	2	50	O
23.	*A. koningi*	21	-	-	8	29	2	50	O
24.	*A. luchuensis*	-	-	24	-	24	1	25	R
25.	*A. nidulans*	54	-	36	-	90	2	50	O
26.	*A. niger*	12	36	52	10	110	4	100	C
27.	*A. ochraceous*	5	17	15	28	65	4	100	C
28.	*A. oryzae*	56	28	41	-	125	3	75	F
29.	*A. sulphureus*	22	52	56	-	130	3	75	F
30.	*A. sydowi*	21	42	-	-	63	2	50	O
31.	*A. terreus*	41	-	-	12	53	2	50	O
32.	*A. terricola*	19	6	-	21	46	3	75	F
33.	*Bipolaris* sp.	12	-	-	41	53	2	50	O
34.	*Cephalosporium* sp.	-	-	-	26	26	1	25	R
35.	*Cladosporium lignicola*	5	-	-	28	33	2	50	O
36.	*Cladosporium* sp.	28	-	41	-	69	2	50	O
37.	*Curvularia lunata*	-	32	19	56	107	3	75	F
38.	*C. indica*	16	12	-	-	28	2	50	O
39.	*C. pallescens*	-	12	-	24	36	2	50	O
40.	*Curvularia* sp.	41	-	-	46	87	2	50	O
41.	*Drechslera indica*	56	-	72	15	143	3	75	F
42.	*Drechslera* sp.	41	-	28-	-	69	2	50	O
43.	*Fusarium moniliforme*	-	-	-	22	22	1	25	R
44.	*F. oxysporum*	28	22	-	31	81	3	75	F
45.	*F. semitectum*	12	-	14	-	26	2	50	F

Table 1. Cont.....

46.	*Fusarium* sp.	41	-	-	26	67	2	50	O
47.	*Humicola* sp.	-	-	32	-	32	1	25	R
48.	*Nigrospora* sp.	24	-	-	32	56	2	50	O
49.	*Penicillium chrysogenum*	30	-	-	25	55	2	50	O
50.	*P. citrinum*	-	36	42	-	78	2	50	O
51.	*P. funiculosum*	-	28	-	-	28	1	25	R
52.	*P. janthinellum*	43	-	52	-	95	2	50	O
53.	*Penicillium* sp.	-	32	-	26	58	2	50	O
54.	*Trichoderma harzianum*	32	-	16	8	56	3	75	F
55.	*T. koningi*	14	6	-	28	48	3	75	F
56.	*T. viride*	-	-	42	-	42	1	25	R
57.	*Trichoderma* sp.	28	12	-	-	40	2	50	O
58.	*Verticillium tenerum*	54	21	-	-	75	2	50	F
59.	White sterile mycelium	-	-	-	15	15	1	25	R
	Total species	42	21	24	34				
	Total density (x10^{-2} CFU/g)	1198	537	809	902	3451			

C – Common (76 – 100%); F – Frequent (51-75%); O – Occasion (26-50%); R – Rare (0-25%)

light house. Minimum number of 21 species was recorded in the soil samples collected from New light house.

The genus *Aspergillus* constituted more number of species[19], *Penicillium* 5 species, *Curvularia, Fusarium* and *Trichoderma* (4 species each) and *Syncephalastrum, Chaetomium, Thielavia, Alternaria* and *Cladosporium* (2 species each). All other genera were represented by one species each.

The percentage contribution of *Aspergillus* was 35.78% followed by *Penicillium* 9.09%, *Curvularia* 7.47%, *Fusarium* 5.67% and *Trichoderma* 5.38%. *Aspergillus niger* and *A. ochraceous* (100%) were the common fungi recorded in Point Calimere. *A. chevalieri*, *A. flavus, A. fumigatus, A. oryzae, A. sulphureus, A. terricola, Curvularia lunata, Drechslera indica, Fusarium oxysporum, Trichoderma harzianum, T. koningi* (75%) were recorded frequently *Rhizopus nigricans, Chaetomium globosum,Emericellopsis* sp., *A. clavatus, A. candidus, A. carbonareus, A. luchuensis, Cephalosporium* sp., *Fusarium moniliforme, P. funiculosum, T. viride* were rare. The remaining genera were recorded occasionally (Table 1).

DISCUSSION

Fungi are the ubiquitous eukaryotic micro organisms found in almost all habitat and are important as antagonists, allergents, decomposers, pathogens, organisms of industrial importance, etc., Since the marine environment is distinct in several respects, it is expected to harbour unique groups of micro organisms, especially fungi.

In the present study, the species composition of the Point Calimere soil mycoflora revealed the presence of 59 species belonged to 20 genera. Among them, 6 species were belonged to Phycomycetes, 5 species were belonged to Ascomycetes, and the remaining 47 species were belonged to Deuteromycetes. Among the fungi isolated, *Aspergillus, Penicillium, Trichoderma, Fusarium* and *Curvularia* were the dominant genera. Dominance of Aspergilli and Penicillia are universal feature in various marine habitats as facultative marine organisms has been reported by Prabhu *et al*.[17] and Prabhakaran *et al*.[18] from both East and West Coast of India in different environs. The occurrence of terrestrial fungi namely *Arthrinium* sp., *Aspergillus* sp. and *Penicillium* sp. has been reported as dominant ones during monsoon and compete with marine fungi in the colonization of the leaves has been reported by Ananda and Sridhar[19]. Garrett[20] classified the soil fungi into different ecological groups. *Aspergillus* and *Penicillium* are in the group of saprophytic sugar fungi. Saito[21] investigated the mycoflora of salt marsh and reported that *Penicillium* sp. and *Trichoderma*

vignorum were the common forms in the mud. Isolation of *Aspergillus* species, particularly the members of *A. glaucus* group in greater number and frequency, which was due to the high nutrient level in the swamps. These species prefer a medium with high osmotic concentration and therefore, compete more easily with other forms in the mangrove swamps.

Nicot[22] recorded the dominance of Aspergilli and Penicillia has also been reported in the coastal soils of France. Roth *et al*.[23], Rai and Chowdhery[24] reported that the species of Aspergilli were dominated over Mucorales and Penicillia in the mud of mangrove swamps. Swart[25] studied the mycoflora of the soil collected from mangrove swamps of Inhaca Island and found that these swamps were rich in simple carbohydrates and nitrogen and reported the dominance of the species belonged to *Aspergillus* and *Penicillium*. This indicates their preference for simple organic compounds and their competitive advantage over other fungi for the readily available nutrients.

Terrestrial species of fungi have often been reported as dominant forms in different coastal, marine and mangrove soils. These soils were largely represented by terrestrial soil mycoflora belonged to Deuteromycetes, except for a few Zygomycetes and Basidiomycetes, which were rare or absent[25-29]. In the present study also species of fungi belonged to terrestrial forms were isolated.

Among the four different stations studied in Point Calimere, the percentage frequency of fungi belonged to Phycomycetes, Ascomycetes, and Deuteromycetes were 6.59, 4.28, 86.54 per cent (Old light house), 5.00, 5.27, 89.06 per cent (New light house) 6.95, 5.30, 86.99 per cent (Chola light house) 9.34, 5.52, 84.22 per cent (Muniyappan lake) respectively. Species of Basidiomycetes was absent and members of Ascomycetes were poorly represented as they formed only 8.33 per cent of total fungi (Fig.7). Absence of suitable cellulosic substrates in the soil probably render the soil unsuitable for the colonization of Ascomycetes[30].

Salinity might be one of the factors causing a reduction in Phycomycete population. Swart[31] has given a similar explanation for the absence of Phycomycetes, in the soil of some

Table 2. Correlation co-efficient analysis between physico-chemical properties of soil and total fungal species

	pH	EC	Nitrogen	Phosphorus	Potassium	Ferrous	Manganese	Zinc	Copper	TFS
pH	1									
Salinity	-0.222	1								
Nitrogen	-0.40017	0.8156**	1							
Phosphorus	0.20241	0.125182	0.330735	1						
Potassium	-0.36333	0.8982**	0.64074*	0.06914	1					
Ferrous	0.27461	-0.17171	-0.23263	-0.16115	-0.1134	1				
Manganese	-0.01228	0.50543*	0.55459*	0.28191	0.39549	0.6137*	1			
Zinc	-0.14583	-0.00902	-0.0649	-0.6709*	-0.02619	0.18785	0.0765	1		
Copper	0.304431	-0.54339	-0.5867*	0.324666	-0.42169	-0.2453	-0.6072*	-0.7246*	1	
TFS	-0.01465	0.458991	0.55029*	0.233626	0.11793	-0.1174	0.51621*	0.231827	-0.520*	1

** $p < 0.01$% significant; * $p < 0.05$% significant

mangrove swamps of Inhaca Island. He has also demonstrated that the seawater inhibited *Absidia* sp., a Phycomycete. Poor number of Phycomycetes in the mangrove habitats has been reported by many workers[25,31,32,30]. Thus the present investigation also concludes that Phycomycetes, Ascomycetes and Basidiomycetes are poorly represented, when compared to Deuteromycetes. It is partly because of the presence of high level of salinity, pH, temperature and fluctuation of the nutrient in the marine environment and also the nature of the media used, which did not permit the development of all groups of fungi.

When organic matter or other similar substrate is added to the soil, it becomes subjected to series of waves of colonization by fungi present in soil, and the nature of available energy materials largely determine the activity of fungi. Temperature, moisture, CO_2, oxygen, soil pore and soil durability of fungal mycelium, interaction between soil fauna and soil reaction are the main factors, which influence the growth and activity of fungi in the soil[33].

In spite of the fact that fungi have wide distribution they show variation in their population dynamics. In the present study, it was found that there was significant correlation between environmental characters, physico-chemical properties of soil and total fungal population. It revealed that there was significant positive correlation between N and total fungal species (TFS) ($r = 0.550$; $p < 0.05$); manganese and TFS ($r = 0.516$; $p < 0.05$). It has been reported that the pH, salinity, temperature and nutrients contents of the soils correlated with fungal population[33,34]. The carbon and nitrogen are necessary for the growth of microorganisms. Besides energy sources, the environmental factors such as pH and salinity influence the growth and activity of microorganisms[34,35]. Hence it could be concluded that inspite of the fact that the fungi are ubiquitous, their population dynamics are often influenced by the available nutrients and the physico-chemical condition of the ecosystem.

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