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Assessment of mycological diversity of marine sediment of south east coast of Tamilnadu, India

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ABSTRACT

Marine fungi are an ecological rather than a taxonomic group and comprise an estimated 1500 species, excluding those that form lichens. They occur in most marine habitats and generally have a pantropical or pantemperate distribution. Their importance lies in their ability to produce pharmaceutically valuable secondary metabolites. They may be important in the degradation of dead animals and animal parts. Marine fungi are important pathogens of plants and animals and also form symbiotic relationships with other organisms. Hence a concerted effort has been made to investigate the diversity along the south east coast of Tamilnadu.

Keywords: Marine fungi, Lichens, Secondary metabolites.

INTRODUCTION

The importance of terrestrial bacteria and fungi as sources of valuable bioactive metabolites has been very well established for more than half a century. As a result, over 120 of the most important medicines in use today (penicillins, cyclosporin A, adriamycine, etc.) are obtained from terrestrial microorganisms at first sight thus, the expectable enormous biodiversity of marine microorganisms might have been the reason for the interest in their study. An additional possible explanation should be that marine microorganisms constituted the ultimate ‘‘inviolated’’ frontier for the search of marine natural products.

Oceans cover 70% of the Earth, and most ocean bottom is covered in sediments ranging from gravel to fine muds; this makes it the largest habitat on our planet in areal coverage. Some sediment is uniform in grain size, some are mixed, some are biological in origin and others are geological. Much of this habitat (- 83%) is greater than 1000 m depth [1], so most marine sediments are located in a cold, lightless, high pressure habitat where food is supplied from distant surface waters. Fungi are cosmopolitan in oceans and estuaries and occur commonly on

decomposing organic matter such as drift and intertidal wood. Initial studies of marine fungi in India were confined to marine sediment and mangrove mud. An extensive survey of marine fungi from the west coast of India, particularly Maharashtra coast, was made by Borse, [2] and Raghukumar, [3].

Fungi produce a vast range of secondary metabolites [4]. Some of these are high value products with pharmaceutical applications such as penicillins [5]. More specifically marine fungi are also believed to be prolific resources of natural products [6 – 9]. However, their potential has not yet been fully investigated. Unlike the terrestrial fungi, which were initially exploited for drug discovery, marine fungi have attracted great attention as considerable resources only since the late 1980s (9). Furthermore, it was reported that the corresponding chemistry of marine fungi was structurally diverse and related to terrestrial fungi [10].

Marine fungi comprise of an estimated 1500 species excluding those that from lichens [11]. This number is low compared to the number of described and estimated terrestrial fungi (over 2,50,000) [12]. So far, less than 500 filamentous higher marine fungi have been described and only 79 are associated with algae as parasites or symbionts, and 18 with animal hosts [13]. A number of interesting compounds, such as cytoglobosins [14] and halovirs [15], had been isolated from marine fungi. Hence, we made an attempt to assess the mycopopulation of south east coast of Tamilnadu.

MATERIALS AND METHODS

Study Area

The sampling spots are located on 10.20°N 79.24°E on the coast of the Bay of Bengal, South east coast of Tamilnadu. The sampling spots included in the present study are Pudhupattinam, Adirampattinam, Mallipattinam and Rajamatam. Marine ecosystem is one of the richest and most productive areas of organic detritus and form the base of the food chain. Marine fungi play an important role in nutrient regeneration cycles as decomposers of dead and decaying organic matter in the estuaries. Although mangroves are the dominant features of Indian coastline and provide niches and habitats for many marine organisms. Nevertheless very little is known about the fungi associated with them till recently.

Isolation of fungi

The samples were collected during April 2008 – May 2009. The marine sediment samples were collected in sterile polythene bags and sterilized 50% seawater was added to each bag in order to maintain moisture condition. The bags were tied with a string and incubated at room temperature for 7 days. The collected sediment samples were used for fungal population analysis on Rose Bengal Agar (HiMedia, Mumbai, India) medium supplemented with chloramphenicol 100 mg/l and Malt Extract Agar medium (HiMedia, Mumbai, India) supplemented with 0.1 g streptomycin and 10,000 units of penicillin/100 ml to inhibit bacterial growth (Raghukumar et al., (16, 17). An inoculum of 0.5 ml was inoculated by spread plate method from the undiluted samples (50% w/v). All the sample aliquots were analyzed in duplicate and incubated for one week to 15 days at 27°C temperature or as soon as the colonies appeared prior to spore formation to avoid over estimation due to autoinoculation (Raghukumar et al., [16]. The colonies formed on the plates were then counted and expressed as CFU/g dry sediment weight. All the colonies were frequently picked up, subcultured and maintained in slants for further studies.

Identification

Identification was achieved by taxonomic processes such as direct comparison of specimens and by the use of keys, descriptions and illustrations. The microscopic examinations of cultures were done by preparing the semi-permanent slides with Lactophenol Cotton Blue stain. Then the slides were observed under microscope (400X) and identified with the help of keys given by Barghoorn and Linder (1944), [18], Johnson and Sparrow (1961), [19], Barnett and Hunter (1972), [20], Anisworth et al. (1973a,b) [21, 22], Kohlmeyer and Kohlmeyer (1979, 1991) [12, 23] and following the taxonomic arrangement proposed in the 6th edition of Ainsworth and Bisby's Dictionary of the Fungi (Anisworth, 1971) [24].

RESULTS

The results of quantitative analyses for filamentous fungi are shown in Table I. List of fungi recorded from individual sampling stations were given in Table – II, III, IV and V. Diverse filamentous fungi were recovered from the sediment samples of Adirampattinam coastal environs along the East of India. Of 63 fungi (Table – I) belonging to 37 genera comprising 53% Ascomycota, 2% Mucoromycotina, 2% Anamorphic fungi, 1% Hyphomycetes and 5% Mitosporic fungi. The present study reveals the Adirampattinam coastal environs shows maximum number of isolates when compared to other sampling spots Fig – I and Table - VI.

DISCUSSION

Population density of filamentous marine fungi from the south east coast of Bay of Bengal in the present study ranged from 6.224 to 12.432 CFU/g. However, these estimates were lower than that observed by Sinclair and Ghiorse [25] from the deep subsurface sediments, Upadhyay et al., [26] from the tropical sand belt of Kanyakumari, D'souza et al. [27] and Araujo et al., [28] from the west coast of India. For a fungus, sea water poses three problems. First, it is a medium of a relatively low water potential; second, it contains relatively high concentrations of ions, being potentially capable of exerting toxic effects on cell processes and third, it has an alkaline pH (Jennings, 1986). As a result, filamentous fungi are dominated by facultative forms than the true forms since most of the filamentous marine fungi are host specific and uncultivable in a common medium (Hyde et al., 1987). There are also reports on the prevalence of these facultative forms (which are of terrestrial origin) in the marine environment, even in the deep sea (Raghukumar et al., 2004) [17], raising the question ''Do they function in the sea?''. Of course, their habit is not suitable for the planktonic mode of life and they are not capable of producing spores in sea water due to mycostatic effect of fresh sea water (Kohlmeyer and Kohlmeyer, 1979) [12]. However, these facultative fungi in the marine environment may occur in the form of fragmented mycelia associated with detritus (Nadimuthu, 1998) [29].

Distribution of filamentous fungi in the present study has shown a higher diversity. Of the 63 isolates belonged to 37 genera comprising 53% Ascomycota, 2% Mucoromycotina, 2% Anamorphic fungi, 1% Hyphomycetes and 5% Mitosporic fungi However, the present study matches the findings of several investigators who found Ascomycetes fungi as the major contributor to the filamentous higher marine fungi (Kohlmeyer, 1980 [30]; Kohlmeyer and Kohlmeyer, 1979 [12], 1986 [31], 1991 [23]; Jones et al., 1988 [32]; Hyde and Jones, 1988 [11]; Sridhar and Prasannarai, 2001) [33].

Christophersen et al. (1999) [34] remarked that the marine derived fungi so far investigated do not include Aspergillus and Penicillium as ubiquitous genera. But, Sponga et al. (1999) [35] reported several fungal genera from marine environment and supported the present findings. In

addition, the genus Penicillium was reported to be the prevailing strain in the marine environment followed by Aspergillus and Cladosporium (Salvo et al., 2005) [36]. Further, Damare et al. (2006) [37] reported the presence of Aspergillus, Penicillium, Cladosporium, and Fusarium from the deep sea sediment samples. They described that most of the marine fungal genera isolated were usually considered to be soil inhabitants and might have resulted either from dormant spores or actively growing mycelia, which is also evident in the present study. Generic composition of mycoflora in the present study indicated Aspergillus as the species rich genus (14 species) followed by Penicillium (6 species). This is an indication for the wealth of fungal biodiversity in the south east coast of Bay of Bengal. Each species of filamentous fungi had its own contribution to the total mycopopulation. In general, facultative marine fungi are the major contributors and among them, Aspergillus and Penicillium are noteworthy (Nadimuthu, 1998) [29].

Table – I: Total number of fungal isolates recorded from different sampling stations

BIODIVERSITY OF MARINE FUNGI			
S. NO	**NAME OF THE ISOLATE**	**TAXONOMIC CLASSIFICATION**	**FAMILY**
1.	*Penicillium funiculosum*	Ascomycota	Trichocomaceae
2.	*Acremonium sp*	Ascomycota	Hypocreaceae
3.	*Penicillium lutetium*	Ascomycota	Trichocomaceae
4.	*Fusarium oxysporum*	Ascomycota	Nectriaceae
5.	*Aspergillus awamori*	Ascomycota	Trichocomaceae
6.	*Aspergillus sulphureus*	Ascomycota	Trichocomaceae
7.	*Aspergillus flavus*	Ascomycota	Trichocomaceae
8.	*Aspergillus sydowii*	Ascomycota	Trichocomaceae
9.	*Aspergillus niger*	Ascomycota	Trichocomaceae
10.	*Rhizopus nigricans*	Mucoromycotina	Mucoraceae
11.	*Geotrichum candidum*	Ascomycota	Endomycetaceae
12.	*Penicillium granulatum*	Ascomycota	Trichocomaceae
13.	*Aspergillus nidulans*	Ascomycota	Trichocomaceae
14.	*Penicillium expansum*	Ascomycota	Trichocomaceae
15.	*Aspergillus fumigatus*	Ascomycota	Trichocomaceae
16.	*Absidia glauca*	Mucoromycotina	Mucoraceae
17.	*Massarina japonica*	Ascomycota	Massarinaceae
18.	*Alternaria tenuis*	Ascomycota	Pleosporaceae
19.	*Aspergillus terreus*	Ascomycota	Trichocomaceae
20.	*Acrophilophora fusipspra*	Ascomycota	Mitosporic Ascomycota
21.	*Trichocladium acrasporum*	Anamorphic fungi	Mitosporic Ascomycota
22.	*Aspergillus glaucus*	Ascomycota	Trichocomaceae
23.	*Aspergillus granulosus*	Ascomycota	Trichocomaceae
24.	*Aspergillus ustus*	Ascomycota	Trichocomaceae
25.	*Aspergillus versicolor*	Ascomycota	Trichocomaceae
26.	*Aspergillus clavatus*	Ascomycota	Trichocomaceae
27.	*Aureobasidium pullulans*	Ascomycota	Dothioraceae
28.	*Alternaria geophila*	Ascomycota	Pleosporaceae
29.	*Chaetomium spp*	Ascomycota	Chaetomiaceae
30.	*Chrysosporium spp*	Hyphomycetes	Mitosporic Onygenales
31.	*Cladosporium spp*	Ascomycota	Davidiellaceae
32.	*Gliocladium spp.*	Ascomycota	Mitosporic Hypocreales
33.	*Verticillium spp.*	Ascomycota	Plectosphaerellaceae
34.	*Cunninghamella spp.*	Ascomycota	Cunninghamellaceae
35.	*Curvularia spp*	Ascomycota	Pleosporaceae
36.	*Fusarium proliferatum*	Ascomycota	Nectriaceae
37.	*Verticillium serrae*	Ascomycota	Plectosphaerellaceae
38.	*Aspergillus Eryzae*	Ascomycota	Trichocomaceae

39.	*Phoma glomerata*	Ascomycota	Incertae sedis
40.	*Penicillium notatum*	Ascomycota	Trichocomaceae
41.	*Penicillium chrysogenum*	Ascomycota	Trichocomaceae
42.	*Trichoderma spp*	Ascomycota	Hypocreaceae
43.	*Alternaria alternata*	Ascomycota	Pleosporaceae
44.	*Cladosporium brevicompactum*	Ascomycota	Davidiellaceae
45.	*Varicosporina ramulosa*	Anamorphic fungi	Halosphaeriaceae
46.	*Clavatospora bulbosa*	Mitosporic fungi	Halosphaeriaceae
47.	*Ascochyta sp*	Mitosporic fungi	Mitosporic Pleosporales
48.	*Cumulospora marina*	Mitosporic fungi	Sordariomycetes incertae sedis
49.	*Dendryphiella salina*	Mitosporic fungi	Pleosporaceae
50.	*Periconia prolifica*	Mitosporic fungi	Halosphaeriaceae
51.	*Verruculina enalia*	Ascomycota	Didymosphaeriaceae
52.	*Salsuginea ramicola*	Ascomycota	Pleosporales incertae sedis
53.	*Savoryella paucispora*	Ascomycota	Hypocreaceae
54.	*Savoryella lignicola*	Ascomycota	Hypocreaceae
55.	*Pleospora pelagica*	Ascomycota	Pleosporaceae
56.	*Marinosphaera mangrovei*	Ascomycota	Phyllachoraceae
57.	*Lignincola tropica*	Ascomycota	Halosphaeriaceae
58.	*Leptosphaeria australiensis*	Ascomycota	Phaeosphaeriaceae
59.	*Halosarpheia ratnagiriensis*	Ascomycota	Halosphaeriaceae
60.	*Aniptodera chesapeakensis*	Ascomycota	Halosphaeriaceae
61.	*Aigialus grandis*	Ascomycota	Massariaceae
62.	*Massarina bipolaris*	Ascomycota	Massarinaceae
63.	*Halorosellinia oceanicum*	Ascomycota	Xylariaceae

Table – II: Total number of colonies (CFU/g) and Percentage contribution of fungi from PUDHUPATTINAM (SS1)

S.No	Name of the organism	Total number of colonies	% of Contribution
1	*Ascochyta sp*	4	1.659
2	*Aniptodera chesapeakensis*	2	0.829
3	*Aigialus grandis*	7	2.904
4	*Acremonium sp*	18	7.468
5	*Aspergillus awamori*	3	1.244
6	*Aspergillus flavus*	24	9.958
7	*Aspergillus nidulans*	7	2.904
8	*Acrophilophora fusipspra*	4	1.659
9	*Aureobasidium pullulans*	7	2.904
10	*Chaetomium sp*	10	4.149
11	*Chrysosporium sp*	15	6.224
12	*Cladosporium sp*	10	4.149
13	*Curvularia sp*	10	4.149
14	*Cunninghamella sp*	11	4.564
15	*Geotrichum candidum*	22	9.128
16	*Halorosellinia oceanicum*	1	0.414
17	*Lignincola tropica*	2	0.829
18	*Leptosphaeria australiensis*	3	1.244
19	*Massarina bipolaris*	6	2.489
20	*Penicillium luteum*	25	10.373
21	*Penicillium granulatum*	20	8.298
22	*Penicillium expansum*	8	3.319
23	*Penicillium notatum*	8	3.319
24	*Penicillium chrysogenum*	9	3.734
25	*Salsuginea ramicola*	2	0.829
26	*Savoryella paucispora*	1	0.414
27	*Savoryella lignicola*	2	0.829
		241	

Table – III: Total number of colonies (CFU/g) and Percentage contribution of fungi from ADIRAMPATTINAM (SS2)

S. No	Name of the organism	Total number of colonies	% of Contribution
1.	*Acremonium sp*	18	5.625
2.	*Aspergillus nidulans*	13	4.062
3.	*Aspergillus terreus*	15	4.687
4.	*Aspergillus fumigatus*	17	5.312
5.	*Aspergillus awamori*	5	1.562
6.	*Aspergillus sulphureus*	12	3.75
7.	*Aspergillus flavus*	19	5.937
8.	*Aspergillus sydowii*	9	2.812
9.	*Aspergillus niger*	6	1.875
10.	*Absidia glauca*	9	2.812
11.	*Alternaria tenuis*	3	0.937
12.	*Alternaria geophila*	5	1.562
13.	*Alternaria alternate*	2	0.625
14.	*Clavatospora bulbosa*	3	0.937
15.	*Cumulospora marina*	7	2.187
16.	*Cladosporium sp*	13	4.062
17.	*Cunninghamella sp*	5	1.562
18.	*Dendryphiella salina*	1	0.312
19.	*Geotrichum candidum*	19	5.937
20.	*Massarina japonica*	7	2.187
21.	*Massarina bipolaris*	7	2.187
22.	*Penicillium chrysogenum*	19	5.937
23.	*Penicillium notatum*	20	6.25
24.	*Penicillium luteum*	19	5.937
25.	*Penicillium granulatum*	20	6.25
26.	*Penicillium expansum*	14	4.375
27.	*Rhizopus nigricans*	5	1.562
28.	*Salsuginea ramicola*	1	0.312
29.	*Savoryella lignicola*	2	0.625
30.	*Trichocladium acrasporum*	7	2.187
31.	Trichoderma sp	7	2.187
32.	*Verticillium sp*	4	1.25
33.	*Verticillium serrae*	4	1.25
34.	*Varicosporina ramulosa*	1	0.312
35.	*Verrculina enalia*	2	0.625
		320	

Table – IV: Total number of colonies (CFU/g) and Percentage contribution of fungi from MALLIPATTINAM (SS3)

S.No	Name of the organism	Total number of colonies	% of Contribution
1.	*Acrophilophora fusipspra*	4	1.568
2.	*Acremonium sp*	23	9.019
3.	*Absidia glauca*	12	4.705
4.	*Aspergillus glaucus*	8	3.313
5.	*Aspergillus granulosus*	3	1.176
6.	*Aspergillus ustus*	5	1.960
7.	*Aspergillus versicolor*	1	0.392
8.	*Aspergillus clavatus*	2	0.784
9.	*Aspergillus awamori*	2	0.784
10.	*Aspergillus sulphureus*	3	1.176
11.	*Aspergillus flavus*	21	8.235
12.	*Aspergillus sydowii*	8	3.313
13.	*Aspergillus niger*	2	0.784
14.	*Aspergillus oryzae*	1	0.392
15.	*Chaetomium sp*	6	2.352

16.	*Clavatospora bulbosa*	5	1.960
17.	*Cladosporium sp*	9	3.529
18.	*Curvularia sp*	9	3.529
19.	*Geotrichum candidum*	21	8.235
20.	*Phoma glomerata*	3	1.176
21.	*Penicillium notatum*	20	7.843
22.	*Penicillium chrysogenum*	19	7.450
23.	*Penicillium luteum*	18	7.058
24.	*Penicillium granulatum*	22	8.627
-25.	*Penicillium expansum*	8	3.313
26.	*Salsuginea ramicola*	3	1.176
27.	*Savoryella paucispora*	4	1.568
28.	*Savoryella lignicola*	4	1.568
29.	*Pleospora pelagica*	9	3.529
		255	

Table – V: Total number of colonies (CFU/g) and Percentage contribution of fungi from RAJAMATAM (SS4)

S.No	Name of the organism	Total number of colonies	% of Contribution
1.	*Acremonium sp*	17	9.189
2.	*Acrophilophora fusipspra*	1	0.540
3.	*Aspergillus flavus*	23	12.432
4.	*Aspergillus niger*	5	2.702
5.	*Aspergillus nidulans*	2	1.081
6.	*Aureobasidium pullulans*	4	2.162
7.	*Aniptodera chesapeakensis*	1	0.540
8.	*Aigialus grandis*	1	0.540
9.	*Chrysosporium sp*	3	1.621
10.	*Cladosporium sp*	1	0.540
11.	*Cumulospora marina*	1	0.540
12.	*Dendryphiella salina*	1	0.540
13.	*Geotrichum candidum*	18	9.729
14.	*Halosarpheia ratnagiriensis*	3	1.621
15.	*Massarina japonica*	2	1.081
16.	*Marinosphera mangrovei*	1	0.540
17.	*Phoma glomerata*	2	1.081
18.	*Penicillium chrysogenum*	18	9.729
19.	*Penicillium granulatum*	17	9.189
20.	*Penicillium notatum*	19	10.270
21.	*Penicillium expansum*	8	4.324
22.	*Penicillium luteum*	19	10.270
23.	*Periconia prolific*	2	1.081
24.	*Pleospora pelagic*	1	0.540
25.	*Verticillium sp*	7	3.783
26.	*Verticillium serrae*	5	2.702
27.	*Varicosporina ramulosa*	1	0.540
28.	*Verruculina enalia*	1	0.540
29.	*Halorosellinia oceanicum*	1	0.540
		185	

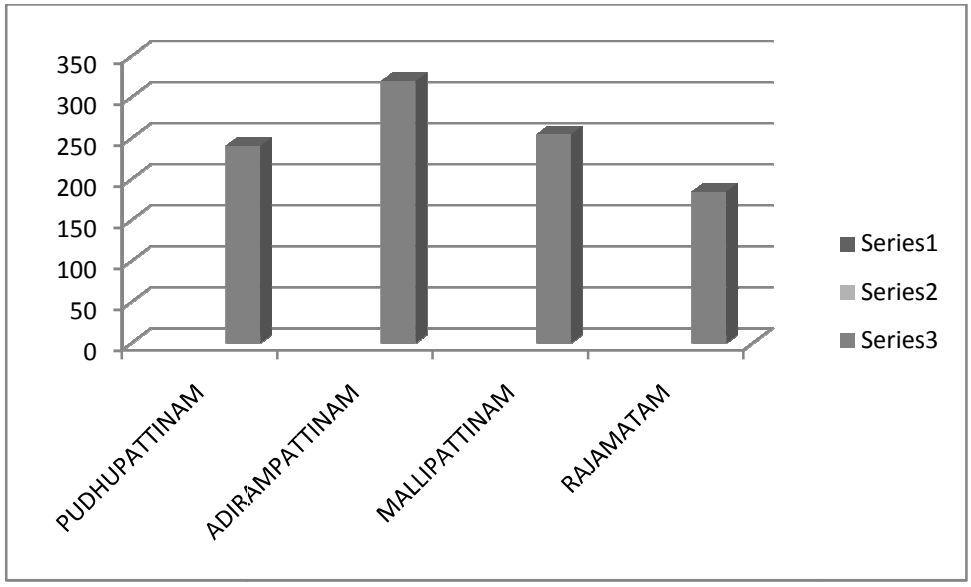

Fig – I: Histogram showing the highest number of colonies recorded spot

Table – VI: List of sample collection spots and total number of colonies recorded

S. No	Name of the sampling station	Total number of colonies recorded
1	SS1	241
2	SS2	320
3	SS3	255
4	SS4	185

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