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Isolation and Identification of Marine Fungi from East Coastal Region of Chennai

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Abstract

The present study was confined to the Marine ecosystem in Chennai, Tamil Nadu. Water, sediment were collected to isolate the fungi. All the collected samples were plated, incubated and the fungal colonies were identified. A total of 25 fungal species were isolated and enumerated by plating technique. Among them Hyphomycetes, *Aspergillus* was the common genus.

Keywords: Marine ecosystem, Fungi, Aspergillus sp.

Introduction

The oceans cover more than 70% of the earth's surface and contain about 80% of the world slant and animal tissues. Oceans have plenty of structurally unique metabolites and other resources in the living and dead forms. About 10,000 metabolites have been isolated from different marine organisms. Among them 37% has been isolated from sponges, 21% from coelenterates, 18% from microorganisms, 9% from algae, 6% from echinoderms, 5% from tunicates, 2% from molluscs and 1% from bryozoans (Maloy Kumar *et al*., 2007).

Fungi are found ubiquitously in the environment. They execute a wide range of important ecological functions especially ones associated with decomposition of organic substrates in both terrestrial as well as marine environments, and occasionally in extreme conditions. The magnitude of fungal biodiversity was estimated to be 1.5 million species. However, some estimation exceeded this number to 5.1. Among the estimated 1.5 million, only 5% - 10% has been formally described. Fungi that live in the sea are defined as obligate marine or facultative. Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat and are permanently or intermittently sub-merged in water. Whereas, facultative marine fungi are those that normally occupy freshwater habitats or terrestrial surroundings but are able to grow (and possibly to sporulate) in the marine environment. Currently there are 444 known recognized species of obligate and facultative marine fungi. *Ascomycota* represented by 360 species whereas *Basidiomycota* are represented by 10 species and 74 species of mitosporic fungi. Facultative marine fungi can be transferred to seawater by wind, rain or runoff soil. Some facultative marine fungi were able to evolve adaptation to marine environment and eventually became obligate marine.

Many reasons have posed the need to examine the extent of marine biodiversity of fungi. For example, the emergence marine life diseases to corals, sponges, sea fan corals and fish. Some marine fungi are re-ported as sources of enzymes used in bioremediation. New biosynthetic products have been revealed in marine-derived fungi. Moreover, marine fungi can be exploited for monitoring marine environment pollution. Marine microorganisms have become an important source of pharmacologically active metabolites. More specifically, fungi from the marine environment have shown great potential as suggested by the diversity of secondary metabolites. The intent of this work is to summarize the new marine-derived fungal metabolites. Fungi in the marine environment and taxonomic considerations of marine-derived fungi are intended to be introductory. In addition, most fungi will be referred to as "marine-derived" since the marine ecosystem is comprised of fungi that are obligate fungi and facultative species. A more detailed discussion about marine fungi and obligate marine species has been presented by Kohlmeyer and Kohlmeyer, (1979). Briefly, obligate marine fungi grow and sporulate in a marine environment while facultative marine fungi are from terrestrial or freshwater habitats, but may also grow in the marine environment. Over 75% of the identified fungi belong to the monophyletic Ascomycota which is the most important and diverse phylum.

Marine fungi form an ecological, and not a taxonomic group. Among these, the obligate marine fungi grow and sporulate exclusively in sea water, and their spores are capable of germinating in sea water. On the other hand, facultative marine fungi are those from fresh water or a terrestrial milieu that have undergone physiological adaptations that allow them to grow and possibly also sporulate in the marine environment. About 800 species of obligate marine Fungi belonging to the Fungi have been reported so far. Marine fungi are most common in decomposing wood and plant detritus in coastal waters are also common in calcareous animal shells, Algae and corals. They have been isolated from deep-sea sediments.

Materials and Methods

Site description and sample collection

Soil samples were collected in sterile petri plates from three different sea shore locations of Chennai region, India. Soil was collected from 4cm deep from each of these stations using a sterile spatula. The soil sample was collected in sterile plastic bags from 1-2 cm below the pneumatophores, it was then brought to the laboratories and stored at 4^{o}C till further processing.

Isolation of fungi

Suspensions of all the five soil samples were prepared using physiological saline. Spread plate technique on Potato Dextrose Agar plates was used for the isolation of microorganisms (Dubey and Maheshwari, 2005).

The incubated plates were observed for the development of fungi from 3rd day onwards. The number of colonies in each plate was counted and compared with control. The data obtained were used for calculating the frequency of occurrence. In addition to this, cultural characters of the colonies [color and structure] were also observed and fungi were enumerated. All the isolated fungal cultures were subcultured in test tubes containing agar medium.

Identification by Lactophenol cotton blue method

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

The semi permanent slides of the isolated fungi were prepared using Lactophenol Cotton Blue Staining method (Dring, 1976) and sealed with DPX mountant. The fungal species were photographed using photo micrographic instrument (Nikon AFX II Microscope fitted with Nikon FX-35 camera, Tokyo, Japan).

The identification of fungal taxa was based on illustrated Genera of imperfect fungi (Barnett, 1965), Hyphomycetes (Subramanian, 1971), Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes (Ellis, 1971, 1976), Micro fungi on land plants (Ellis and Ellis, 1985) Micro fungi on Miscellaneous substrate (Ellis and Ellis, 1988), Gilman, J.C. (1957).

Results

During the study period, a total of 25 fungal species were enumerated by plating techniques.

In this study, 21 species of fungi were recovered from sediment samples whereas water samples yielded 15 species.

Table 1: Total number of fungi isolated from marine samples

Zygomycota
Mucor sp.
Rhizopus oryzae
Ascomycotina
Saccharomyces sp.
Deuteromycotina
A. carbonarius
A. flavus
A. fumigatus
A. luchuensis
A. nidulans
A. niger
A. ochraceus
A. sacchari
A. terreus
Aspergillus sp.
P. funiculosum
P. janthinellum
P. purpurrescens
Penicillium sp.
Cladosporium apicale
C. uredinicola
Curvularia lunata
Drechslera avenacea
Exosporium sp
Periconia sp.
Fusarium moniliforme
F. semitectum

Description of isolated fungi

Mucor sp.

Mycelium widespread in and on the substratum, but without rhizoids or especial membraned stolons, richly branched. Sporangiospores springing dingly from the mycelium but usually forming a thick turf, erect. Spores spherical or ellipsoid with thin smooth membrane, colourless or cloured.

Rhizopus oryzae Went and Gerlings

Differs from *R. nigricans* by the smaller spores (7-9 µm long) and the rich number of mycelial gemmae.

Saccharomyces sp.

Cream colour
Literature: Fell (1967)
Marine origin: Marine water

Aspergillus carbonarius (Bain.) Thom

Colonies broadly spreading, more or less zonate with vegetative mycelium white or somewhat yellowish in submerged area Scelrotia produced on the surface of the substratum in old cultures. Conidiophores hyaline below, yellow to yellowish brown toward the tip. Heads globose, upto 500 µm in diam. Conidia smooth to begin with later becoming rough, 5.5-10.5 µm in diam.

Aspergillus flavus Link

The colonies widely spreading with floccosity limited to scantly growth of a few aerial hyphae in older areas. Conidial areas ranging in color from sea – foam yellow, lime green to mignonette - green. Reverse in uncolored or yellow. Conidiophores arise separately from the substratum. Conidia pyriform to almost globose, colorless to yellow green, smooth or rough. Sclerotia at first white, then brown. Cleistothecia not found.

Aspergillus fumigatus Fresenius

The colonies strictly velvety, in others with varying amounts of tufted aerial mycelium up to felt floccose forms, green to dark green, becoming almost black in age spreading. Reverse in yellow or colourless. Conidiophores are short, densely crowded. Conidia dark green in mass, globose.

Aspergillus luchuensis Inui

This form differs from *A. niger* in showing a single series of phialides. Conidia finely roughened. Conidiophores smooth. Phialides in one series.

Aspergillus nidulans (Eidam.) Winter

The colonies spreading broadly, dark – green from abundant conidial heads during the first two weeks. Cleistothecia developing from the center of the colony outward. Reverse in purplish – red, becoming dark in age. Conidial heads short columnar, sinuous with smooth walls in shades of cinnamon brown and terminal vesicles. Phialides in two series. Conidia globose, rugulose, green in mass

Aspergillus niger van Tieghem

The colonies rapidly growing submerged mycelium, yellow color in the hyphae. Reverse usually without color. Conidiophores mostly arise, smooth, septate or non septate. Conidial heads fuscous, blackish – brown, purple – brown, carbonous black, vesicles globose or radiate heads. Phialides in two series. Sclerotia - globose, super ficial but not common.

Aspergillus ochraceus Wilhelm

The colonies ocharaceous shades, consisting of conidiophores and conidial heads with little aerial mycelium. Conidiophores length, rough, yellow, bearing large, radiate conidial heads. Vesicles globose, phialides in to series. Conidia globose to elliptical, smooth or delicately spinulose, yellow, orange to vinaceous or purple. Sclerotia commonly present.

Aspergillus sacchari Chaudhuri & Sachar

The colonies yellow, Reverse colorless in the beginning later yellow, surface somewhat floccose, scletotia begin to form in a weeks time they are white. Conidial heads are globose or elliptical bodies. Conidiophores with smooth and colorless walls. Long non- septate, vesicles globose. Phialides in two series. Conidia colorless, smooth, globose, heads radiate some are columnar with age.

Aspergillus terreus Thom

The colonies with tints of pinkish, cinnamon deeper brown shades in age. Reverse in pale or bright yellow to fairly deep browns. Conidiophores more or less flexuous, smooth, septate or non-septate and phialides usually in two series. Heads becoming solid columnar masses. Conidia elliptical to globose, smooth in long parallal, adherent chains. Cheistothecia not found.

Aspergillus sp.

Conidial heads more or less abundant radiate to somewhat columnar, typically in somewhat of green. Conidiophores smooth-walled. Philadies borne directly on the versicles. Conidia elliptical to subglobose.

***Penicillium funiculosum* Thom**

Colonies deep green, broadly spreading, surface closely floccose; reverse and medium red or purple to almost black. Conidiophores arise laterally from aerial hyphae. Conidia elliptical, green, smooth.

***Penicillium janthinellum* Biourge**

The colonies gelatin bluish green gray – green or bright green ropes of hyphae, yellow to ocharaceus reverse in odor weak, conidiophores arising from creeping hyphae or ropes of hypae, all walls smooth. Phialides in pairs or threes, apparently the Eupenicillium type. Conida globose.

***Penicillium purpurrescens* Sopp**

The colonies thick, mass of mycelium, folded or wrinkled, white, conidia areas as spots or marginal areas. Reverse at first reddish then turn purple and finally black. Hyphae commonly fine. Conidiophores moderately coarse, septate, veriticil of phialides, flask shape. Conidia globose, echunulate. Cleistothecia and Sclerotia not found.

***Penicillium* sp.**

Colonies yellowish – white. Reverse orange-yellow. Conidiophores slender. Conidia oblong, subglobose, 4 × 5 - 5 µm.

***Cladosporium apicale* Berk**

Colonies hypophyllous grey to black. Conidiophores solitary or more often in fascicles arising from dart stomata. Conidia sub spherical, limoniform, ellipsoidal, fusiform or cylindrical, 0-3 septate, pale olive or olivaceous brown, smooth, 3 – 20 × 2.5 – 6 µm.

***Cladosporium uredinicola* Speg**

Colonies effuse, olivaceous, velvety. Conidiophores straight or flexuous, occasionally branched, septate usually Ramo – conidia 25 – 30 µm long. Conidia spherical, fusiform, ellipsoidal or oblong, very pale olive, smooth or minutely verruculose, 0-3 septate, 3-5µ diam or 7.25 × 3-6 µm.

***Curvubria lunata* (Wakkar.) Boedijn**

Colonies spreading, sub floccose, dark olive – gray, reverse bluish – black; hyphae septate, branched olive – brown, 2-3 µm, unbranched. Conidiophores erect, 40-80 × 2-3 µm in diam. Conidia borne in a whorl at the tip of the conidiophores, 3- septate, curved, brown.12-14 × 6-8 µm.

***Drechslera avenacea* (Curt *ex* Cooke.) Shoemaker**

Mycelium thin-walled at first hyaline, later darker, 5-7 µm in diam. Conidiophores appearing singly or in groups, stout, cylindrical, multiseptate, fuliginous 150-200 µm long, 8-12 µm wide. Conidia olivaceous to yellow-brown, cylindrical to slightly swollen in the middle, rounded at the ends, 3-8 septate, sometimes 0-12-septate.

***Exosporium* sp.**

Colonies effuse, dark brown, velvetty. Conidiophores arisng from stromata, flexuous, septate. Conidia ovoid to obclavate, pale brwon, smooth.

***Periconia* sp.**

Condiophores macronematous, occasionally micronematous. Conidia globose, subglobose or rarely ellipsoidal, oblong, one celled, rarely smooth, light to dark brown.

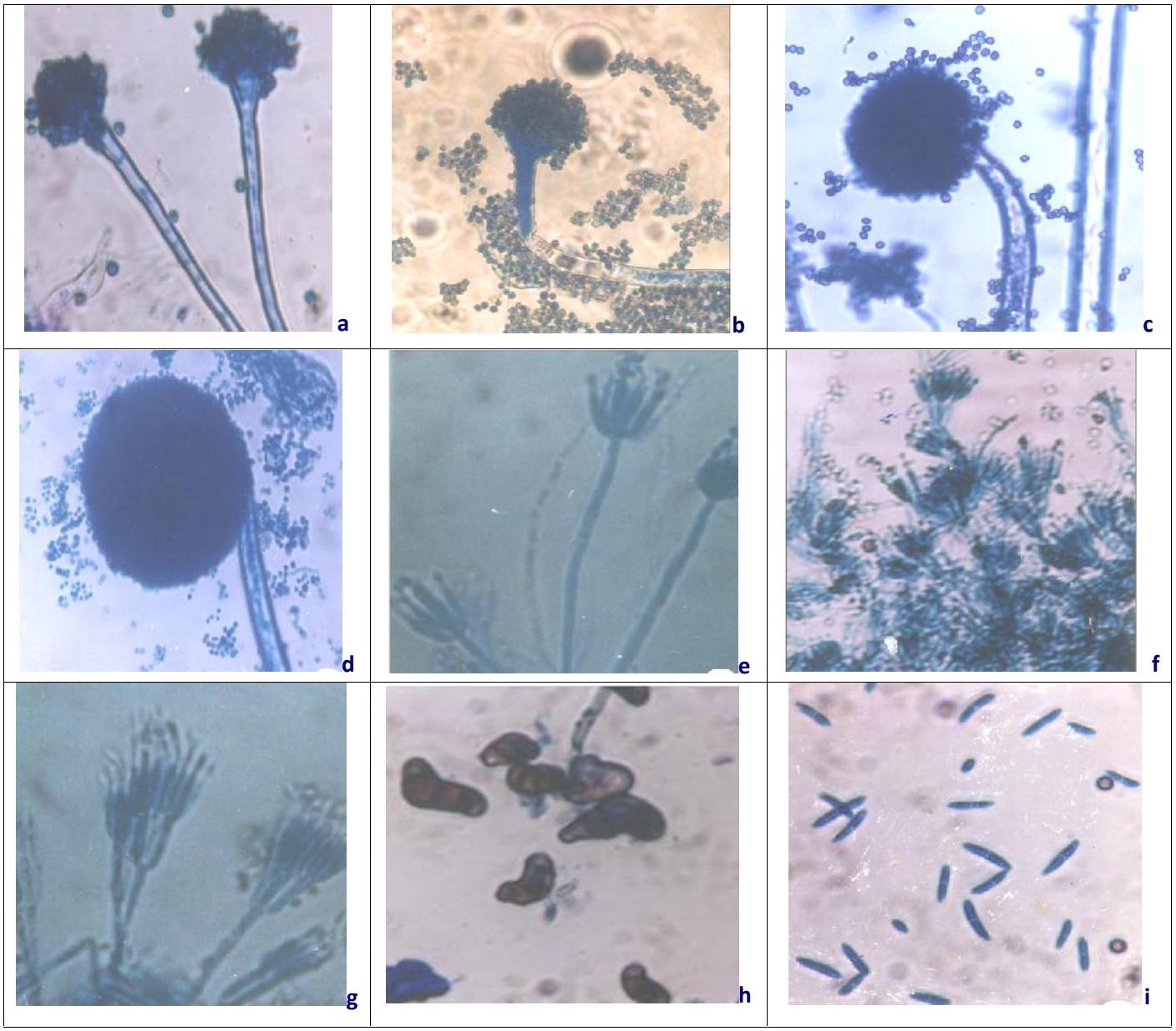
***Fusarium moniliforme* Sheldon**

Microconidia produced in chains and remaining connected, or held in false heads, later becoming scattered over the bright yellow to rosy white aerial mycelium as a transparent shining powder, 1-3 celled, spindle, egg shaped. Macro conidia delicate, awl shaped, tapering at both ends, cinnamon brown or pale, 3-5 septate, seldom 6-7 septate.

***Fusarium semitectum* Berkeley & Ravenel**

Aerial mycelium white – incarnate or isabellin. The lower part of the stoma plectenchymatic, bright brown (in some isolation, violetcarmine). Chlamydosphores intercalary. Conidia scattered on aerial mycelium, spindle, lance or sickle shaped, straight or slightly curved, not pedicellate but often with an attachment wart on the base. Smaller conidia non- two septate, larger three septate, seldom four or five septate, occasionally six or seven septate.

Plate 1: Microphotographic view of isolated fungi



a- *Aspergillus* sp. B- *Aspergillus flavus* c- *Aspergillus niger*, d- *Aspergillus carbonarius* e- *Penicillium purpurrescens* f- *Penicillium janthinellum* g *Penicillium* sp. h – *Curvubria lunata* i - *Fusarium semitectum*

Discussion

In the present study, a total of 25 species were isolated and identified from the water and soil samples. Sivakumar et al., (2006) reported that the distribution of fungi in Muthupettai mangroves along the East coast of Tamil Nadu, India was studied in terms of species diversity, seasonal variation, and frequency of occurrence in five sampling stations at two different seasons and a total of 118species of fungi were isolated, of which maximum 94 species from sediment samples followed by water with 83 species. Among the fungal isolates *Aspergillus* was the common genus followed by *Penicillium*, *Curvualria* and *Alternaria*. Similarly in the present study also *Aspergillus* was the predominant genus.

Considerable effort has been spent investigating the frequency of occurrence of manglicolous fungi (Borse, 1988). Borse (2002) reported that the distribution and substratum range of 166 species 13 Labyrinthulomycota, 4 Chytridiomycota, 20 Oomycota, 1 excluded sp., 120 Ascomycota, 3 Basidiomycota and 23 mitosporic fungi) of marine fungi recorded so far from India on animal substratum, driftwood, intertidal wood, algae, mangroves, sea grasses, salt marsh plants and as propagules in the sea foams samples. In this current investigation among the 25 isolated fungal species 2, 1 and 22 species belong to the phylum Zygomycota, Ascomycotina and Deuteromycotina respectively.

Marine fungi like *Leptosphaeria* species, *Mycosphaerella* species and *Cirrenalia macrocephala* were for the first time recorded only by direct microscopic observation of the pneumatopores of *Avicenia officinalis* from Indian mangrove habitat by Garg (1982). In the present study *Mucor* sp, *Rhizopus oryzae, Saccharomyces* sp, *Aspergillus carbonarius, A.flavus, A. fumigatus, A.luchunensis, A. nidulans, A. niger, A. ochraceus, A. sacchari, A. terreus, Aspergillus sp, Penicillium funiculosum, P.janthinellum, P.purpurrescens, Penicillium sp, Cladosporium apicale, C. uredinicola, Curvularia lunata, Drechslera avenacea, Exosporium* sp, *Periconia* sp., *Fusarium moniliforme, F. semitectum* were isolated from marine soil and water.

A number of publications document the collections of marine fungi made in Thailand, starting with Kohlmeyer and including studies by Hyde (1989). Endophytic fungi of mangrove leaves, stem and roots consists of more terrestrial than marine fungi (Kumaresan and Suryanarayanan, 2001).

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