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Diversity of fungi at various depths of marine water

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Fungi are native inhabitants of water and some species behave as opportunistic pathogens in man. Fungi, all above all filamentous fungi can occur almost everywhere, even in marine water. The knowledge about the occurrence and diversity of fungi in water has increased considerably from a low knowledge base. The aim of this study was to gain an overview of the spectrum of filamentous fungi in marine water. Stratified samples of marine water were collected from Arabian Sea near Dona Paula beach, Goa under as sterile conditions as possible from six different depths which varied from 5 to 200 centimeters in depth. Attempts were made to recover fungi from these different water samples collected. Fungi were isolated by using membrane filtration and plating method with subsequent cultivation on agar plates. The different taxa of fungi were identified using routine techniques as well as molecular methods. Fungi were analyzed in all the water samples examined. This paper aims at the isolation and identification of fungi from marine water. The isolation of fungi from different depths of water suggests that there are a wide variety of fungi in marine water and the most prevailing genera were found to be *Aspergillus sp.*, *Cladosporium cladosporioides* and *Thysanophora sp*. This study showed that marine water can also be a reservoir for wide diversity of fungi.

Key words: Fungi, Arabian Sea, marine water, different depths.

Fungi are native inhabitants of soil and water. They are ubiquitous and no geographical area or any group of people is spared by these organisms. Filamentous fungi are usually found wherever organic matter occurs being mostly parasitic and occasionally pathogenic (Arvanbitidou *et al*., 1999).Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments (Arvanbitidou *et al*., 1999).

The number of fungi described worldwide is estimated at around 70,000 but their total number may be as high as 1.5 million species. However the share of marine fungi is mearly 1000 to 1500 only. Marine fungi comprises of saprobic forms present in the open ocean water (pelagic) and in bottom (benthic) zones. Most of the fungi found in marine habitats are microscopic. Higher marine fungi constitute Ascomycotina, Basidiomycotina and Deuteromycotina.

Fungi are a diverse group of organisms belonging to the kingdom *Eumycota*. This kingdom comprises five phyla, namely Ascomycotina, Bascidiomycotina, Zygomycotina, Chytridiomycota, and Glomeromycota (Kirk *et al*., 2001; Schuβler *et al*., 2001). As a practical approach to classification, fungi have been divided into groups such as the filamentous fungi. Some fungi are primarily adapted to aquatic environments,

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and will, therefore naturally be found in water. Fungi have been reported from all types of water, from raw water to treated water, and from heavily polluted water to distilled or ultra-pure water. Fungi have been also reported from bottled drinking water (Cabral & Fernandez 2002; Fujikawa *et al*., 1997; Ribeiro *et al*., 2006).

Fungi have the ability to grow attached to a substrate, forming part of microbial biofilms on pipe surfaces, debris, or sediments. They are likely to become established where there are cracks, pitting or dead ends (Mara & Horan 2006; Paterson & Lima 2005)

Materials and Methods:

a) Collection of Water Sample:

Water samples from different depths were collected from Arabian Sea near Dona Paula beach, Goa with sterile screw-capped bottles. The water was collected from six different depths of the site using sterile screw-capped bottles. During the collection, the bottle was dipped with its cap on, a few below the water surface according to the desired depths, the cap was removed with the other hand and water rushed inside the bottle until it was filled, and the bottle was recapped while it was still in the water. Due to the distance between the site of collection and the laboratory, the water samples were stored in the refrigerator until analysis.

b) Sample Processing and Isolation Method:

A critical point with respect to the study of fungi in water is how the analyses are performed. Water samples were taken and analyzed for fungal growth. The fungi were isolated using two different methods: membrane filtration and plating method. For the membrane filtration, 100 ml of water sample was filtered through membrane filters with a diameter of 47 mm and a pore size of 0.45 µm. The filters were placed in the center of agar plates after filtration. For the plating method, 500 µl of samples were plated on agar plates with a glass spreader. Two different plates were used: Malt extract agar and Sabouraud glucose agar plates, both supplemented with 40 mg/l gentamycin and 100 mg/l Chloramphenicol to inhibit bacterial growth. The agar plates were incubated t 22^{o}C for 3 and 7 days. After 7 days of growth, the numbers of the colony forming units (CFU) per 100 ml of samples were assessed and the different taxa of the cultivated fungi were subcultured on new agar plates at 22^{o}C for up to 10 days. The cultivated fungi were identified using routine microscopy techniques.

Whenever macro- and micro morphology failed to show unambiguous results, PCR of the gene coding for the ribosomal internal transcribed spacers (ITS) with the enclosed 5.8S ribosomal DNA and subsequent sequencing was performed. DNA from fungi was isolated. Thereafter, the ITS region was amplified by PCR using the primer set. Sequencing of the amplified ITS region was accomplished according to the Sanger-Coulson method (or chain termination method using single-stranded DNA) with subsequent analysis of the sequenced products using the Genetic analyzer ABI PRISM 3130. The ITS sequences were then compared with entries in genomic databanks using the Internet free-ware from European Bioinformatics Institute(EMBL) found under http://www.ebi.ac.uk/fasta33/nucleotide.html to identify the specific fungi.

Results and Discussion:

Water samples were collected from six different depths of marine water. The depths selected for water sample collection were 05, 30, 60, 90 and 200 cm. After incubation at 22^{o}C for 3-7 days, the numbers of CFU of the samples were examined. Fungi were isolated from water samples examined. Altogether a total of 44 different taxa of fungi were found and isolated in all samples collected from different depths of water. 20 isolates were isolated at 05 cm depth, 12

isolates at 30 cm, 11 isolates at 60 cm and 09 and 06 isolates at 90 and 200 cm each. The spectrum of isolated fungi along with their number and their site of collection (depth) are listed in table 1.

Table 1: Spectrum of Fungi at different depths

Depth 5cm(20)	Depth 30cm(12)	Depth 60cm(11)	Depth 90cm(09)	Depth 200cm(06)
Acremonium obclavatum	*Thysanophora penicillioides*	*Raciborskiomyces longisetosum*	*Neocosmospora vasinfecta*	*Alternaria raphani*
Stanjemonium onchroroseum	*Raciborskiomyces longisetosum*	*Stanjemonium onchroroseum*	*Illosporium carneum*	*Graphium silanum*
Thysanophora penicillioides	*Guignardia mangiferae*	*Penicillium chrysogenum*	*Penicillium expansum*	*Thysanophora canadensis*
volutela cillate	*Aspergillus ustus*	*Penicillium expansum*	*Pseudotrichia aurata*	*Penicillium herquei*
Monographella nivalis	*Tritirachium sp.*	*Aspergillus ustus*	*Cladosporium cladosporioides*	*Cladosporium cladosporioides*
Fusarium oxysporum	*Penicillium expansum*	*Stanjemonium grisellum*	*Engyodontium album*	*Aspergillus ustus*
Aspergillus ustus	*Verticillium psalliotae*	*Thysanophora penicillioides*	*Raciborskiomyces longisetosum*	
Lewia infectoria	*Bulgaria inquinans*	*Cladosporium cladosporioides*	*Verticillium psalliotae*	
Gibberella avencea	*Illosporium carneum*	*Penicillium herquei*	*Aspergillus ustus*	
Penicillium herquei	*Penicillium herquei*	*Eupenicillium crusceum*		
Neocosmospora vasinfecta	*Cladosporium cladosporioides*	*Tritirachium sp.*		
Cladosporium cladosporioides	*Engyodontium album*			
Raciborskiomyces longisetosum				
Pleospora rudis				
Phacidium coniferarum				
Cephalosporium lanosoniveum				
Meliolina syndowiana				
Nectria haenatococca				
pseudotrichia aurata				
Alternaria brassicola				

Table: 2 Frequency of isolation of fungi in marine water

Fungi genus	Number of similar isolates	Frequency (%)	Similar isolates in different depths
Engyodontium album	2	5.405	30,90
Illosporium carneum	2	5.405	30,90
Pseudotrichia aurata	2	5.405	05,90
Neocosmospora vasinfecta	2	5.405	05,90
Stanjemonium onchroroseum	2	5.405	05,60
Tritirachium sp.	2	5.405	30,60
Verticillium psalliotae	2	5.405	30,90
Raciborskiomyces longisetosum	4	10.810	05,30,60,90
Penicillium herquei	4	10.810	05,30,60,200
Aspergillus ustus	5	13.513	05,30,60,90,200
Cladosporium cladosporioides	5	13.513	05,30,60,90,200
Thysanophora penicillioides	5	13.513	05,30,60,90,200
Total	**37**	**(100)**	

The fungi were isolated from water samples examined. The prevailing isolates were *Penicillium sp., Thysanophora penicillioides* and *Raciborskiomyces sp.* The frequency of the similar isolates at various depths have been tabulated in table: 2

The study was initiated to isolate and identify the presence of different fungi in the marine water in order to obtain information on the possible ubiquitous nature of fungi species recovered from the Sea. Some *Aspergillus* species cause serious disease in humans and animals. *Aspergillus flavus* produces aflatoxin which is both a toxin and a carcinogen, and which can contaminate foods such as nuts (Machida and Gomi, 2010). *Cladosporium cladosporioides* is a fungal plant pathogen that affects wheat. It is the source of the series of chemical compounds known as calphostins. *Thysanophora* species comprise a very small proportion of the fungal biota. This genus is related to Penicillium, Phialocephala, and Gliocladium. No information is available regarding health effects or toxicity.

Penicillium species have been frequently recovered from water in the various studies performed. Several of the species in both genus Penicillium and Aspergillus are known to produce mycotoxins in other substrates, such as food and beverages (Moreau 1979; Pitt & Hocking 1999). The implication of fungi such as e.g. Aspergillus, Penicillium and Cladosporium with allergy, asthma and other respiratory problems have been widely investigated with respect to indoor environments (Denning et al., 2006; Straus 2004).

Conclusions:

This paper explores the study of fungi at various depths of marine water which has demonstrated that fungi are relatively common in water distribution systems. Fungi are everywhere with world-wide occurrence but little attention has been given to their presence in aquatic environment. The presence of fungi and others represent a potential risk to water borne/Skin diseases to the local population who usually visit and swim in the sea. In conclusion, the presence and survival of wide diversity of fungi in marine water showed that fungi can occur ubiquitously and there is a need to keep and practice good hygienic conditions.

Fungi can be a potential risk for many waterborne infections but the critical concentration for people is not known so far. In future, preventive measures may become more important than ever, and the occurrence of fungi in water bodies may require extended regulations. Therefore further research is needed to investigate that may be appropriate and regular mycological investigations should be done.

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