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Fungi living in diverse extreme habitats of the marine environment

Seshagiri Raghukumar[1]*, Chandralata Raghukumar[1] and Cathrine Sumathi Manohar[2]
[1]*Myko Tech Private Limited, 313 Vainguinnim Valley, Dona Paula, Goa – 403004, India.*
[2]*CSIR-National Institute of Oceanography, Dona Paula, Goa – 403004, India.*
Corresponding author Email: sraghu865@yahoo.co.in
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ABSTRACT

The marine environment contains several habitats characterized by extreme living conditions. However, extremophilic marine fungi were neither well known, nor often studied. Many studies in recent years have shown that fungi do inhabit such habitats. Fungi are capable of withstanding high salinity conditions, such as those in intertidal mangrove environments and salt pans. Cold water, psychrotolerant fungi have been identified from polar waters. Numerous studies have shown that fungi grow actively in deep-sea sediments, under high hydrostatic conditions. Yeasts predominate deep-sea waters and many have also been shown to be psychrotolerant. Fungi have also been found in shallow water hydrothermal vents. Diversity studies on fungi in these habitats has shown the common presence of terrestrial species. Cryptic species and novel lineages have also been discovered . Extremophilic, or extremotolerant marine fungi could prove to be useful for biotechnological applications.

Keywords: Marine, fungi, extreme, habitats, diversity

INTRODUCTION

That terrestrial microorganisms, particularly bacteria and archaea can tolerate extreme conditions such as pH, temperature is well known. Extremophilic species are also represented by several terrestrial fungi. There has been a growing interest in marine organisms that can tolerate, or even prefer extreme conditions of pH, temperature and hydrostatic pressure. Most studies on extremophilic marine microorganisms have been confined to bacteria. The role of fungi in the marine ecosystem has not been studied sufficiently (Satyanarayana *et al*., 2005; Singh *et al*., 2013). As a result, our knowledge on extremophilic, marine fungi is also rudimentary. This article reviews information available presently on fungi occurring in marine habitats that show extreme conditions in terms of salinity, temperature and hydrostatic pressure. Much less is known about fungi living in these habitats. Nevertheless, research in the last ten years have begun to indicate that diverse fungi inhabit these regions and might probably play a definite role in the biogeochemistry of these ecosystems. Such fungi will also be important tools in biotechnology.

SALINITY

With a sodium chloride concentration of nearly 30 ppt or 3.0 %, corresponding to approximately 0.5 M of sodium, salinity is the most significant characteristic of seawater. There are three possible patterns by which salinity can affect marine microorganisms.

1) Mangrove intertidal sediments are exposed to daily fluctuations in salinity, the salt content increasing drastically during receding tides and when exposed to sunlight. Especially in the upper reaches of the intertidal sediment where exposure to seawater is rare and temperatures are high, salinity may even reach 100 to 160 ppt (Olafsson *et al*., 2000). Organisms living in such soils will face rapid environmental changes and will require corresponding adaptations. Fungi have been isolated from mangrove sediments. Ghizelini *et al*. (2012) point out the present lacuna in our knowledge on mangrove sediment fungi and how this could be an important aspect of mangrove microbial ecology, as well as biotechnology.

2) Solar salterns face steadily increasing salinity, starting from the normal seawater salinity of 34 ppt and culminating in salinities of more than 200 ppt (20 %) salt content. Few organisms, such as the halophilic bacteria and a few cyanobacteria were believed to grow at these extreme salinities (Sorensen *et al*., 2004). However, Zajc *et al*. (2012) provide evidence that many others, such as the black yeast *Hortaea werneckii* and the obligate halophilic basidiomycete *Wallemia ichthyophaga* might be important and ecologically significant inhabitants of salterns. Many other mitosporic fungi also have been isolated from salterns. Thus, several studies by Nazareth *et al*. (2011; 2012) in recent years have shown the occurrence, as well as heavy metal tolerance of fungi isolated from salterns. The physiology of these organisms will throw much light on their survival and growth mechanisms and could be useful for biotechnology applications. It is likely that other organisms survive and grow during increasing salinities, an aspect which has not been studied in detail for fungi. Similar to salterns, the Dead Sea is another important extremophilic, hypersaline marine environment. At least one species, *Gymnascella marismortui* seems to be an endemic fungus of the Dead Sea (Oren and Gunde-Simerman, 2012). Several others belonging particularly to *Eurotium* and *Aspergillus* have been isolated from the Dead Sea waters. Presently there is no evidence that fungi play a role in the ecosystem dynamics of these waters. However, any such study should focus on fungi growing on solid organic particles in this environment, rather than isolating them from the water. Fungal degradation of such organic matter present in the Dead Sea might throw more light on their role in this extreme environment.

3) In the open sea, salinity changes are not drastic enough to affect microbial physiology the way they do in intertidal mangrove sediments or salterns.

An important aspect of salinity is the effect of salt concentration on enzyme activities. Many fungi, particularly yeasts and the stramenopilan fungi, the thraustochytrids grow freely in the water column and may be expected to secrete extracellular enzymes into their surroundings. For example, the stramenopilan fungi produce a variety of extracellular enzymes (Bongiorni, 2012). Fungi in mangrove sediments degrade mangrove leaf detritus by secreting a number of enzymes (Raghukumar *et al*., 1994). However, even sodium concentrations in normal seawater are bound to affect the physiology of microorganisms. While the harmful effects of sodium on the intracellular enzyme machinery and the sodium elimination biochemistry is well known, the effect of the sodium on extracellular enzymes secreted by microorganisms is poorly studied. Chandralata Raghukumar isolated a species of *Ulkenia* from shallow water hydrothermal vents and studied the properties of its extracellular proteases and found that the enzyme was active over a wide range of NaCl concentrations from 0 to 5 %, which is much beyond the average seawater salinity of 3.4 % (Unpublished Data; **Fig. 1**). Such salt tolerant enzymes may be common in thraustochytrids. The same enzyme also showed high activities at pH 10.0 and a temperature of 50 °C (**Fig. 2**).

ANOXIA

Fermentative yeasts and rumen fungi are popular topics while discussing fungi growing in anoxic or suboxic conditions. Such conditions are not rare in the ocean. Eastern Arabian Sea possesses a characteristic, permanent oxygen minimum zone (OMZ) in the open ocean region that contributes to nearly 50% of the total marine oxygen depleted environment. The very high productivity in this region and limited circulation with poorly oxygenated water leads to the development of this world's major mid-water oxygen minimum zone (Naqvi *et al*., 1998). In these regions the dynamic steady state between oxygen supply and consumption is altered and the oxygen concentration is < 0.2 ml L^{-1}. Apart from the mid water pelagic denitrification, seasonal oxygen deficient conditions also occur in greater intensity along the coastal waters of western India. This occurs from 30 to 200 m depth along the shelf during June to December, with the maximum intensity in September and October (Naqvi *et al*., 2000). Studies on the microbial process from this ecosystem has been studied based on chemical (nitrite concentrations, NO and N_2O productions), microbiological (bacterial productivity) and enzymatic parameters such as electron transport system (ETS), nitrate reductase (nar), nitrite reductase (nir) and ammonia oxygenase (amo) of the bacterial population (Shailaja, 2001; Shailaja *et al*., 2006).

Fungi were believed to have negligible role in the marine, oxygen depleted environment (ODE) because they are classically considered to be aerobes and were not known to adapt to alternate respiratory process such as denitrification. However, dissimilatory denitrifcation has been reported in a few terrestrial fungi belonging to *Ascomycota*, *Basidiomycota* and *Zygomycota* (Daiber *et al*., 2005; Hayatsu *et al*., 2008). Studies from the Arabian Sea ODE by direct detection technique has revealed the presence of hyphae in the sediments, confirming its active growth and occasionally fungal spores were also detected. Cultivation dependent studies have shown that fungal colony forming units are not affected by the changing dissolved oxygen levels (Jebaraj and Raghukumar, 2009). However, diversity indices show that the diversity is low in these regions in comparison with oxic coastal and mangrove regions. Fungi isolated were grouped into phylum *Ascomycota* or *Basidiomycota* based on the 18S rDNA sequence analysis and a few novel and new species were also reported from these regions (Jebaraj *et al*., 2010; 2012). Nitrate reduction capacity was also detected in the fungi isolated from the Arabian Sea ODE (Jebaraj and Raghukumar, 2009). These studies have determined the abundance and diversity of fungi from the marine ODE, but further studies are required to understand the actual role of fungi in these regions to the biochemical cycles.

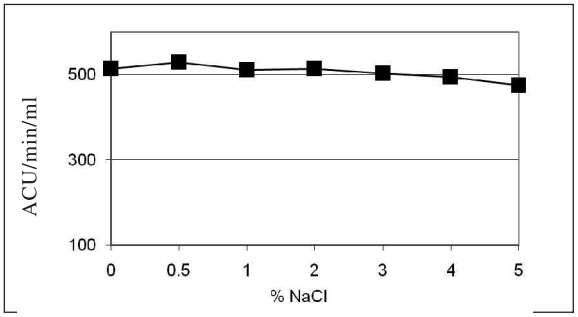

Fig. 1. Azocasein Units (ACU) of protease activity of *Ulkenia* sp. isolated from shallow water hydrothermal vents of Azores, Portugal, at different NaCl concentrations.

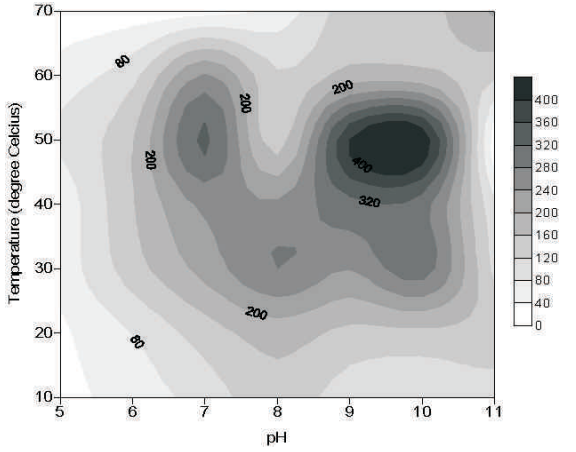

Fig. 2. Protease activity of *Ulkenia* sp. isolated from shallow water hydrothermal vents of Azores, Portugal, at different temperature and pH ranges.

Much less studied are fungi inhabiting the anoxic layers of mangrove sediments. The enormous organic matter input through fallen and decaying leaves and intertidal fauna, accompanied by rapid bacterial respiration results in anoxia even just a few centimeters from the surface of the intertidal mangrove soils. Fungi in these habitats deserve special attention. Some of the common straminipilan fungi inhabiting these regions are the thraustochytrids. These are extremely common in the marine environment. *Aurantiochytrium mangrovei* and *Aurantiochytrium limacinum* are two of the most common thraustochytrids found in these habitats. The latter is known to synthesize polyunsaturated fatty acids using a unique, anaerobic synthesis, instead of the conventional anaerobic unsaturation found in other organisms. Indeed, PUFA synthesis is enhanced during fermentation of these organisms by lowering oxygen levels to below 3 % (Barclay *et al*., 2010). It is likely that such thraustochytrids have other adaptations to cope with hypoxia and anoxia.

PSYCHROPHILES AND PSYCHROTOLERANTS

The Antarctic continent is one of the coldest and harshest environments on earth. Fungi inhabiting various habitats therein have been studied by several authors (Gonçalves *et al*., 2013). Gonçalves *et al*., (2013) isolated several isolates of cold- and salt-tolerant *Penicillium solitum* from a number of Antarctic marine sediments and suggested that this species could be an interesting eukaryote model for the study of structure-function relationships. An excellent example of a psychrophilic fungus is that of the straminipilan fungus *Thraustochytrium antarcticum*, which was described from Ross Sea waters by Bahnweg and Sparrow (1974). This species grew optimally between 2 and 5 °C and had a maximum temperature tolerance of only 10 °C. A similar psychrophile, *Schizochytrium aggregatum* was reported by Ulken (Raghukumar, 2002). Riemann and Schaumann (1993) have reported dense growth of thraustochytrids on the under surface of fast ice in the Weddell Sea of the Antarctic. This indicates that psychrophilic thraustochytrids may be common. Unfortunately few have attempted to isolate these straminipilan fungi from polar waters.

Extremely low temperatures of about 2 °C characterize the deep sea environment. High hydrostatic pressures compound problems for organisms living in the deep sea environment. Psychro- and barotolerant fungi inhabiting deep-sea sediments has been studied in detail by Damare *et al.* (2006a). The production of psychrotolerant protease from a deep-sea fungus has been reported by Damare *et al*. (2006b).

THERMOPHILES AND THERMOTOLERANTS

Interestingly, oceans also contain habitats that are characterized by extremely high temperatures. These are the hydrothermal vents, characterized by extremely high water temperatures. The existence of thermophilic and chemoautotrophic bacteria archaea from hydrothermal vents has been well known for nearly 30 years now (Nakagawa and Takai, 2008). Although the high production and organic nutrient contents of these habitats is favourable for growth of fungi, few studies have addressed fungi in deep-water hydrothermal vents. Raghukumar *et al*. (2008) recorded the presence of culturable filamentous fungi and thraustochytrid stramenopilan fungi from the shallow-water hydrothermal vent. Growth and protease production in one of the thraustochytrid cultures, were not affected by the addition of Fe, Mn and Pb to the culture medium. The presence of such metal resistant thraustochytrids may suggest their adaptation to survive in metal-enriched waters of hydrothermal vents. It is likely, therefore, that interesting and useful, metal-tolerant fungi and thraustochytrids inhabit hydrothermal environments.

OLIGOTROPHIC WATERS

Typical copiotrophic bacteria has the capacity to grow rapidly in the presence of high concentrations of nutrients and to undergo reductive cell division to form resting-stage cells when exposed to nutrient deprivation. This may be referred to as a feast-and-famine response. The unique, oligotrophic, extremely small, ultramicro bacteria, having an average size 0.1 mm are the most abundant in oceanic waters. These grow even at nanomolar concentrations of nutrients and proliferate extremely slowly, the doubling time ranging from 12 h to 5 days. *Sphingomonas* sp. strain RB2256 is one such characteristic bacterial species. RB2256 is well suited for growth in low concentrations of nutrients, it may be expected that it would rarely encounter nutrient levels that would cause starvation, and as a result it may not have the genetic potential to respond to complete nutrient deprivation in the same way as copiotrophic bacteria (Fegatella and Cavicchioli, 2000). Temperature and salinity are not actually stresses for organisms which prefer to grow and multiply optimally under those conditions. It is not clear if the same is true for the extremely low oligotrophic environment. Are there organisms which prefer to grow only when nutrient conditions are extremely low, or is oligotrophy an extreme stress condition limiting the growth and multiplication of organisms other than extremely hardy ones? On the other hand, there are 'feast and famine' kind of situations, especially in the deep-sea, where microorganisms will have to face long periods of nutrient deprivation and sudden inputs of high amounts of nutrient. Such a situation calls for an extremely rapid growth and multiplication when nutrients are available, a capability for prolonged dormancy when nutrients are depleted and an extremely quick revival of dormancy when nutrients again appear. The deep sea is also characterized by elevated hydrostatic pressure, which, therefore, is yet another extreme condition for fungi growing in that environment. These are discussed below.

ELEVATED HYDROSTATIC PRESSURE

The deep-sea is characterized by high hydrostatic pressures and cold temperatures. Hydrostatic pressure

increases by 1 bar at every 10 m depth in the sea. Hydrostatic pressure at 5000 m depth in the sea corresponds to 500 bar or 50 MPa (0.1 MPa= 1 bar pressure). Fungi in the deep-sea have been studied much in recent years. A variety of special techniques are employed in isolating and culturing fungi from such depths, one of them being the use of high pressure culture vessels (Raghukumar *et al*., 2010). Methodologies such as dilution-to-extinction employed in culturing difficult-to-culture bacteria (Giovannoni and Stingl, 2007) and microencapsulation technique involving gel microdroplets (GMD) combined with flow cytometry (Zengler *et al*., 2002) need to be applied to culture fungi from deep-sea sediments. Fungi have been unequivocally demonstrated through direct microscopic detection in calcareous shells and deep-sea sediments, using Calcofluor White, a fluorescent brightner (Raghukumar and Raghukumar, 1998; Damare *et al*., 2006a). A fluorescent *in situ* hybridization (FISH) technique has been used to detect the presence of yeasts in animals collected from deep-sea hydrothermal vents (Burgaud *et al*., 2010). Newer improved techniques need to be tried for direct detection of fungi in environmental samples. Growth, physiology and enzyme production by fungi under simulated deep-sea conditions are discussed at length by Raghukumar *et al*. (2010).

1) Diversity of cultured fungi: Takami (1999) cultured *Penicillium lagena* and *Rhodotorula muciloginosa* from sediments collected at 10,500 m depth of Mariana Trench. Raghukumar *et al*. (2004) cultured *Aspergillus sydowii* from 364 cm depth of a ~ 480 cm long sediment core obtained from 5000 m depth in the Central India Basin. They demonstrated its growth and spore germination at 500 bar under simulated deep-sea conditions in the laboratory. Using culture-based classical taxonomy Damare *et al*. (2006a) reported dominance of *Aspergillus* spp and several unidentified non-sporulating fungi in sediments at ~5000 m depth in the Central Indian Basin (CIB). Singh *et al*. (2010) cultured 16 filamentous fungi and 12 yeasts from CIB. These were identified using ITS and 18S sequences of SSU rDNA. They reported for the first time the occurrence of *Sagenomella* sp., *Exophiala* sp., *Capronia coronata* and *Tilletiopsis* sp. In a subsequent work based on 18S rDNA phylogeny, they identified cultured fungi affiliated to *Trametes versicolor, Cerrena* sp., *Ascotricha lusitanica, Hortaea werneckii* (Singh *et al*. 2012a). Majority of the cultured fungi belonged to *Ascomycota* whereas most of the yeasts isolated belonged to *Basidiomycota* (**Table 1**). A total of 62 filamentous fungi and 32 isolates of yeasts were isolated from animals in deep-sea hydrothermal vents by Burgaud *et al*. (2009; 2010). A new deep-sea ascomycete, *Alisea longicola* was obtained from sunken wood in the Pacific Ocean described by its morphology, 18S and 28S rDNA sequences (Dupont *et al*., 2009). Connell *et al*. (2009) isolated several yeast and yeast-like fungi from cold basalt rock surfaces from an active deep-sea volcano of Samoa. Biddle *et al*. (2005) recovered species of *Cladosporium, Penicillium* and *Acremonium* by direct plating and by enrichment culturing techniques from sediment core collected at 200 m below sea floor (mbsf) from 252 m water depth on the outer shelf edge of the Peru Basin. These results clearly showed that both, truly marine and non-marine fungi are present from deep-sea sediments at different geographical locations by various workers.

Table 1. Diversity of culture-dependent fungi isolated the deep-sea sediments during the cruisesAAS 34, AAS 46, AAS 61, ABP 26 and ABP 38 in the Central Indian Basin (Damare *et al*. 2006a; Singh *et al*., 2010; 2012 a;b).

Identification of isolates	No. of Isolates
Identified by microscopy (Cruise # AAS 34, AAS 46 & AAS #61)	
Aspergillus sp.	31
Non-sporulating mycelial fungi	26
Aspergillus terreus	25
Aspergillus restrictus	22
Cladosporium sp.	14
Penicillium sp.	12
Aspergillus sydowii	5
Unidentified ascomycetes	4
Curvularia sp.	1
Fusarium sp.	1
Aureobasidium sp.	1
Unidentified yeasts	4
Unidentified sporulating fungi	44
Identified by 18S rDNA gene sequencing (Cruise # ABP 26 & ABP 38)	
Penicillium sp.	1
Pezizomycotina sp.	2
Cladosporium sp.	3
Sagenomella sclerotialis	3
Unidentified ascomycetes	3
Tilletiopsis albescens	2
Aspergillus restrictus	1
Acremonium sp.	1
Capronia coronata	1
Rhodotorula cassiicola	2
Rhodotorula mucilagenosa	1
Cryptococcus vishniacii	2
Graphiola cylindrica	1
Sporidiobolu sjohnsonii	4
Rhodosporidium toruloides	1
Coniosporium perforans	1
Nigrospora oryzae	1
Trametes versicolor	1
Chaetomiume latum	1
Aspergillus versicolor	2 (95%)
Ascotricha lusitanica	1
Pleospora herbarum	1
Eurotium herbariorum	1
Cerrena sp.	4
Penicillium griesofulvum	1
Sagenomella sp.	1
Hortaea werneckii	2 (97%)

The data within brackets indicate OTUs showing less than 98 % homology to the closest relative. The rest of the cultures showed more than 97% homology.

2) Culture-independent Diversity: Methods employing amplification of sediment DNA with fungal specific primers to study culture-independent fungal diversity in deep-sea has gained popularity. Le Calvez *et al*., (2009) reported rich fungal diversity with new species in three fungal phyla, namely *Chytridiomycota*, *Ascomycota* and *Basidiomycota* from hydrothermal vent samples. Several of the species identified are unknown. On the other hand, Bass *et al*., (2007) claimed low diversity of filamentous fungi and a high diversity of yeasts in 11 deep-sea water samples taken from different oceans. Fungal sequences from both DNA- and RNA-based clone libraries were reported from 37 mbsf in the Peru Trench (Edgcomb *et al*., 2010). Basidiomycetous fungi were the dominant phylotypes in this site. Lai *et al*., (2007) reported several fungal sequences in methane hydrate-bearing deep-sea sediments. These sequences were not associated with any known fungi or fungal sequences in the public data bases. However, clones phylogenetically homologous to *Phoma, Cylindrocarpon, Hortaea, Cladosporium, Emericella, Aspergillus, Malassezia, Cryptococcus, Lodderomyces, Candida* and *Pichia* spp. were also isolated from these sediments.

Singh *et al*. (2011; 2012a;b) carried out a detailed analysis of culture-independent fungal diversity in the Central Indian Basin using multiple sampling locations and multiple primer sets. Amplification of community DNA isolated from three locations in the CIB using universal and fungal-specific ITS and universal 18S rDNA primer pairs resulted in recovery of 39 fungal operational taxonomic units (OTUs), with 32 distinct fungal taxa (Singh *et al*., 2011). The majority of the recovered sequences belonged to diverse phylotypes of *Ascomycota* and *Basidiomycota* (*Sordariomycetes*, *Dothideomycetes, Microbotryomycetes, Wallemiomycetes, Ustilagomycetes, Saccharomycetes, Eurotiomycetes* and *Tremellomycetes*). Individual primer sets appeared to amplify different fungal taxa occasionally (**Table 2**). Eight new sequences were recovered by using these multiple primers. In another approach, sediment DNA from one single sediment core obtained from ~ 5000 m depth in the CIB, was amplified with four different primer sets (Singh *et al*., 2012a). These were fungal-specific primer pair ITS1F/ITS4, universal 18S rDNA primers NS1/NS2, Euk18S-42F/Euk18S-1492R and Euk18S-555F/Euk18S-1269R. These sequences yielded 8 fungal OTUs with ITS and 19 OTUs with 18S rDNA primer

Table 2: Phylogenetic affiliation of the fungal OTUs obtained with different primer sets at three locations of the CIB (Singh *et al*. 2011).

Closest relative	Phylum	OTUs with fungal-specific ITS primers	OTUs with universal ITS primers	OTUs with universal 18S rDNA primers
Sagenomella sp.	*Ascomycota*	1	1	-
Dothidiomycete sp.	*Ascomycota*	-	1	-
Aspergillus penicillioides	*Ascomycota*	-	1	1
Aspergillus restrictus	*Ascomycota*	-	1 (94%)	2
Hortaea sp.	*Ascomycota*	1(95%)	1(95%)	-
Stenella musicola	*Ascomycota*	1	1	-
Candida sp.	*Ascomycota*	1	1	-
Debaryomyces yamade	*Ascomycota*	-	1	-
Pichiajadinii	*Ascomycota*	1	-	-
Nodulisporium sp.	*Ascomycota*	1	-	-
Aspergillus niger	*Ascomycota*	-	-	1 (96%)
Candida glucosophila	*Ascomycota*	-	-	1
Aspergillus fumigates	*Ascomycota*	-	-	1
Aspergillus sp.	*Ascomycota*	-	-	1
Phoma herbarum	*Ascomycota*	-	-	1
Aspergillus unguis	*Ascomycota*	-	-	1
Ulospora bilgramii	*Ascomycota*	-	-	1
Capnodium coffeae	*Ascomycota*	-	-	1(93%)
Bionectriaceae sp.	*Ascomycota*	-	1	-
Fungal sp.	*Ascomycota*	1	-	-
Uncultured	*Ascomycota*	-	-	3
Candida parapsilosis	*Ascomycota*	-	1	-
Malassezia pachydermatis	*Basidiomycota*	-	1(96%)	-
Trichosporon asahii	*Basidiomycota*	1	1	-
Wallemia sebi	*Basidiomycota*	-	-	1
Rhodosporidium sphaerocarpum	*Basidiomycota*	1	-	-
Fungal sp.	*Basidiomycota*	-	-	1
Uncultured	*Basidiomycota*	-	1 (94%)	1

Fungal specific ITS primer set ITS1F/ITS4; Universal ITS primer set ITS ITS1/ITS4; Universal 18S rDNA primers NS1/NS2.The data within brackets indicate OTUs showing less than 98 % homology to the closest relative. The rest of the OTUs showed more than 97% homology.

sets (**Table 3**). The OTUs belonged to 20 distinct fungal genera of the phyla *Ascomycota* and *Basidiomycota*. Seven sequences appeared to be novel with only 79-97% homology to the known sequences in public database. Amplification of fungal sequences with eukaryotic as well as fungal specific primers indicates dominance of fungi in the sampling site of the CIB.

In another sampling, sediment DNA from two cores from the CIB were amplified using fungal specific ITS1F/ITS4 ad universal 18SrDNA NS1/NS2 primer sets. This resulted in recovering 18 and 28 OTUs with 18S and ITS rRNA gene primer sets, respectively (**Table 4**). Of the total 46 OTUs, 42 OTUs represented distinct fungal species belonging to *Ascomycota* and *Basidiomycota* (Singh *et al*., 2012b). The primer pair NS1/NS2 amplified 10 different phylotypes of *Ascomycota* belonging to the class *Euratiomycetes, Saccharomycetes* and *Dothidiomycetes*. Four basidiomycetous phylotypes amplified belonged to the classes *Agaricostilbomycetes, Agaricomycetes* and *Exobasidiomycetes*. The primer pair ITS1F/ITS4 amplified 16 fungal phylotypes belonging to *Sordariomycetes, Eurotiomycetes, Saccharomycetes* and *Dothidiomycetes*, whereas 12 phylotypes of *Basidiomycota* belonged to seven different classes (**Table 4**).

Some of the fungi such as *Sagenomella, Horteae, Cerrena, Aspergillus*, *Penicillium* spp. which were detected by culture-independent technique were also obtained in culture suggesting that they may be active members of the deep-sea fungal community. However, the NCBI blast searches of these fungi showed different accession numbers for the cultures isolated and environmental sequences. Therefore, the cultured isolates and the environmental sequences belonging to the same genera may not be identical phylotypes.

SUMMARY AND CONCLUSION

To summarize, the diversity of cultured as well as uncultured fungi was found to be comparatively high in the deep-sea sediments of the Central Indian Basin. Most of the fungi recovered were either mesophiles or psychrotolerant and barotolerant. No true barophiles, halophiles or psychrophiles were recovered. Fungal communities detected in deep-sea sediments mostly belonged to phyla *Ascomycota* and *Basidiomycota* using both culture-dependent and culture-independent approaches. No genera belonging to *Chytridiomycota* or *Zygomycota* were recovered in any of the five cruises. Nagano *et al*. (2010) repoted the presence of novel fungal groups, including *Chytridomycota* from deep-sea sediments in the Pacific Ocean. Fungal OTUs belonging to marine sequences in existing database were detected. Occurrence of similar phyla/subclasses in the environmental libraries from different stations of the CIB suggests their abundant nature in such extreme environments. Some of the deep-sea fungi recovered showed similarities with animal parasites (*Malassezia* sp). They may play an important role in impacting host population and diversity. They might be also in symbiotic association with marine animals.

Fungi in the deep-sea, which typifies such an environment have been studied in recent years. A thraustochytrid isolated from salp fecal pellets in the Arabian Sea actively produced proteases under high hydrostatic conditions (Raghukumar, 2002).

A strong note of caution needs to be added here with regard to studies on fungal diversity in the marine environment. While both culture dependant, as well as culture-independent studies have shown novel lineages, suggesting that some interesting fungi might inhabit

Table 3: Phylogenetic affiliations of the fungal OTUs obtained with four different primer sets from one station in the CIB (Singh *et al*. 2012 a; b).

Closest relative	Phylum	*Class*	ITS1F/ITS4	NS1/NS2	18s-42F/Univ149RE	EK555F/EK1269R
Unidentified fungi	*Ascomycota*	*Dothideomycetes*	1	-	-	-
Unidentified fungal clone	*Basidiomycota*	*Wallemiomycetes*	1(79%)	-	-	-
Unidentified fungal clone	*Basidiomycota*	*Exobasidiomycetes*	1	-	1 (79%)	-
Uncultured isolate	*Basidiomycota*	*Wallemiomycetes*	1	-	-	-
Unidentified member of *Aphyllo-phorales*	*Basidiomycota*	*Agaricomycetes*	1	-	1	-
Nectria mauritilicola	*Ascomycota*	*Sordariomycetes*	1	-	1	-
Rhodotorula calyptogenae	*Basidiomycota*	*Cystobasidiomycetes*	1	-	-	-
Trichosporonasahii	*Basidiomycota*	*Tremellomycetes*	1	-	-	-
Uncultured *Malassezia* clone	*Basidiomycota*	*Exobasidiomycetes*	-	1	-	2 (96%)
Uncultured *Aspergillus* clone	*Ascomycota*	*Eurotiomycetes*	-	1	-	1 (97%)
Fungal clone	*Ascomycota*	*Sordariomycetes*	-	-	1	-
Unidentified fungus	*Basidiomycota*	*Wallemiomycetes*	-	-	2	-
Unidentified fungus	*Ascomycota*	*Dothideomycetes*	-	-	1	-
Wallemia sp.	*Basidiomycota*	*Wallemiomycetes*			1 (80%)	-
Rhodotorula sp.	*Basidiomycota*	*Microbotryomycetes*	-	-	1	-
Member of *Dothidio-mycete*	*Ascomycota*	*Dothideomycetes*	-	-	1 (94%)	-
Aspergillus restrictus	*Ascomycota*	*Eurotiomycetes*	-	-	-	1
Wallemia sebi	*Basidiomycota*	*Wallemiomycetes*	-	-	-	1
Candida ortho-psilopsis	*Ascomycota*	*Saccharomycetes*	-	-	-	1
Aspergillus penicillioides	*Ascomycota*	*Eurotiomycetes*	-	-	-	1(91%)

ITS1F/ITS4 fungal specific primer pair, NS1/NS2, Euk18S-42F/Euk18S-1492RE and Euk18S-555F/Euk18S-1269R universal 18S rDNA primer pairs.The data within brackets indicate OTUs showing < 98 % homology to the closest relative. The rest of the OTUs showed > 97% homology.

Table 4. Phylogenetic affiliation of the fungal OTUs obtained with two primer pairs from two sediment cores of the CIB.

Closest relative	Phylum	Class	NS1/NS2	ITS1F/ITS4
Uncultured soil Ascomycete fungus	*Ascomycota*	*Eurotiomycetes*	1 (97%)	-
Uncultured fungus clone	*Ascomycota*	*Eurotiomycetes*	2 (95%)	-
Uncultured *Aspergillus* clone	*Ascomycota*	*Eurotiomycetes*	1	-
Phialosimplex carninus	*Ascomycota*	*Eurotiomycetes*	1 (97%)	-
Saccharomyces sp.	*Ascomycota*	*Saccharomycetes*	1	-
Uncultured marine fungus clone	*Ascomycota*	*Saccharomycetes*	1	-
Uncultured soil Ascomycete fungus	*Ascomycota*	*Sordariomycetes*	1	-
Pycnoporus sp.	*Basidiomycota*	*Agaricomycetes*	1	-
Uncultured *Malassezia* clone	*Basidiomycota*	*Exobasidiomycetes*	1	-
Sterigmatomyces halophilus	*Basidiomycota*	*Agaricostilbomycetes*	1	-
Uncultured soil Ascomycete fungus	*Ascomycota*	*Dothidiomycetes*	1	-
Dothidiomycete sp.	*Ascomycota*	*Dothidiomycetes*	1	-
Cerrena unicolor	*Basidiomycota*	*Agaricomycetes*	-	1
Sterigmatomyces	*Basidiomycota*	*Agaricostilbomycetes*	-	1
Gibberella moniliformis	*Ascomycota*	*Sordariomycetes*	-	2
Schizophyllum commune	*Basidiomycota*	*Agaricomycetes*	-	1
Uncultured fungal clone	*Ascomycota*	*Saccharomycetes*	-	1
Uncultured fungal clone	*Basidiomycota*	*Agaricomycetes*	-	1 (97 %)
Rhodotorula slooffiae	*Basidiomycota*	*Cystobasidiomycetes*	-	1
Rhodotorula sp.	*Basidiomycota*	*Cystobasidiomycetes*	-	1
Uncultured endophytic fungus clone	*Basidiomycota*	*Wallemiomycetes*	-	1
Debaryomyces hansenii	*Ascomycota*	*Saccharomycetes*	-	1
Stenella musicola	*Ascomycota*	*Dothidiomycetes*	-	1
Malassezia slooffiae	*Basidiomycota*	*Exobasidiomycetes*	-	1
Resinicium friabile	*Basidiomycota*	*Agaricomycetes*	-	1 (97 %)
Aspergillus sp.	*Ascomycota*	*Eurotiomycetes*	-	1
Rhodotorula mucilaginosa	*Basidiomycota*	*Microbotryomycetes*	-	1
Malassezia restricta	*Basidiomycota*	*Exobasidiomycetes*	-	1
Aspergillus penicillioides	*Ascomycota*	*Eurotiomycetes*	-	1 (96 %)

NS1/NS2 universal 18S rDNA primer set; ITS1F/ITS4 fungal specific primer set. The data within brackets indicate OTUs showing < 98 % homology to the closest relative. The rest of the OTUs showed > 97 % homology.

marine, extreme environments. Results on the presence of known, terrestrial species could be highly misleading because cultures and DNA could have been obtained from inactive, dormant spores of fungi carried by air or runoff from land and deposited in the sea. Such fungi will not have any role in the biochemical cycles of the ocean. Therefore, it is important to correlate occurrence of particular species of fungi with demonstration of actively growing hyphae of those species to prove that they grow in extreme marine environments, play a role in marine biogeochemical cycles and have the potential to be used in biotechnology.

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