

A Review on Deep-sea Fungi: Occurrence, Diversity and Adaptations

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Abstract

The few studies that have been carried out on deep-sea fungi in recent years either by culturing or molecular signatures, have provided evidence about their presence. A majority of culture-dependent and culture-independent fungi recovered showed homology to species reported in the terrestrial environment indicating their possible arrival in deep sea either with wind or terrestrial runoffs. However, presence of novel fungal sequences with less than 97 % similarity to previously identified fungal sequences in public databases has also been reported. Several filamentous fungi and yeasts recovered from deep-sea sediments of the Central Indian Basin from a depth of ~5000 m showed growth under hydrostatic pressures of 20-40 MPa and 5°C. Some of these fungi showed unusual morphology during the initial culturing and also when grown at 20 MPa/5°C. Direct detection of fungal hyphae in deep-sea sediments is a daunting task as they are present in low abundance. We demonstrated the presence of fungal hyphae in deep-sea sediments by direct staining of the sediments with Calcofluor, a fluorescent optical brightener. This review presents data on the occurrence, diversity and adaptations of fungi to various deep-sea habitats, with a special emphasis on their possible ecological role under these extreme environmental conditions.

Key words: adaptations; culturing; deep-sea; fungi; hydrostatic pressure.

Introduction

Conditions, like low temperature, elevated hydrostatic pressure, and low nutrient availability, combine to make the deep sea an extreme environment. Most of the deep-sea bottom is stable, cold and dark, therefore it was presumed that most of the life forms may be present in a state of suspended animation in this largest refrigerator of the world. [ZoBell and Johnson \(1949\)](#) first coined the term barophile and [ZoBell and Morita \(1957\)](#) obtained the first evidence of piezophilic growth in mixed microbial cultures recovered from the deep sea. [Yayanos \(1979\)](#) reported the first isolate of pressure-adapted bacteria. Subsequently, many psychrophilic piezophiles with various optimal growth pressures have been isolated and characterized physiologically and genetically ([see Kato 1999](#)). Marine sediments overlay two-thirds of the earth's surface and harbor diverse and abundant communities ([Snelgrove et al. 1997](#)). It was estimated earlier that of the 3.8×10^{30} prokaryotes calculated to be in the unconsolidated subsurface sediments, 97 % or 3.7×10^{30} occur at depths shallower than 600 m. The estimated number of prokaryotes for deeper sediments is only 0.13×10^{30} cells ([Whitman et al. 1998](#)). This value was uncertain because it was based on extrapolation, but it still represents considerable microbial biomass. One would then wonder about their function, metabolism and biogeochemical role at these depths.

[Barghoorn and Linder \(1944\)](#) demonstrated the presence of an indigenous marine mycota, showing growth and reproduction on submerged wood after defined periods of time. Subsequently, filamentous fungi have been recovered from a variety of materials in the sea. They appear to be associated with decomposing algal and plant tissues including intertidal and benthic algae, sea grasses and mangroves as well as a wide variety of cellulosic materials from land, such as driftwood, and leaves ([see Raghukumar 2008](#)). Calcium carbonate deposits are often and apparently actively reworked by filaments of boring fungi ([Golubic et al. 2005](#)), which are more widespread and abundant than endolithic algae. The presence of fungi in oceanic waters and deep-sea have been sporadically reported in the past ([see Raghukumar and Damare 2008](#)). This review intends to present the state-of-art on fungi in the deep sea with an emphasis on techniques of isolation, direct detection, diversity, adaptations and their ecological role.

Techniques for collection of sediment and water samples

The prerequisite for studying fungi in deep oceans is collection of samples from such depths. Many sampling devices were designed and used for collection of water and sediments. A survey of the literature reveals that several bacteriological water samplers have been described introducing new

principles or modifications, but they all have limitations which restrict their usefulness. The majority of the samplers which have been described are suitable only for collecting surface samples or samples from shallow depths. Niskin bottles are used universally for collecting water samples in a sterile container at any oceanic depth (Fig. 1) while sediments are collected by a variety of corers. The first pressure-retaining sampler for collecting water sample from deep sea was successfully tested and used by Jannasch et al. (1973). A deep-sea workstation was developed under the Deep-Star program at JAMSTEC, Japan, for retrieving and culturing deep-sea organisms under simulated deep-sea conditions. This system also contains a pressure-retaining sediment sampler, which is able to obtain a sediment sample while maintaining ambient pressure and low temperature after sampling at the deep-sea bottom (Yanagibayashi et al. 1999). The most common samplers to collect deep-sea sediments are the box corers, multiple corer or long gravity corers. Sampling with box corers is efficient where the topography of the ocean floor is more or less flat. Raghukumar et al. (2001) used USNEL box corer (50 x 50 x 50 cm) during the cruises to the Central Indian Basin (CIB) to collect sediment samples (Fig. 2). Sub-cores were collected from the center of the box corer with alcohol-sterilized PVC cylinders of 5 cm diameter. Subsections of 2 cm down to 10 cm depth and thereafter every 5 cm were extruded from these sediment cores of approximately 30 to 40 cm length directly into sterile plastic bags to avoid any aerial contaminants. The bags were closed with rubber bands and carried to the working area in the microbiology laboratory on board. Long gravity cores up to 5 m in length were also used for collecting sediment samples (Raghukumar et al. 2004). Precautions to maintain aseptic conditions during sampling are of importance. Samplers need to be sterilized by washing thoroughly with hot water and surface sterilized with steam and alcohol. More recently, samples are collected with titanium syringes or sediment samplers attached to robotic arms on deep-sea submersibles in hydrothermal vent sites (Le Calvez et al. 2009, Burgaud et al. 2009, 2010).

Techniques for culturing deep-sea fungi

The best possible way of isolating deep sea fungi is to do isolation on board ship, immediately after the sample is retrieved. This way one maximizes the chances of getting more culturable fungi which sometimes may not remain viable till transported to laboratory on land for isolations. Strict procedures to monitor aerial contamination are to be followed during culturing on board. The working area is alcohol-sterilized each time before handling the sediment cores. The culturing is always done in a closed low-temperature room. In order to check aerial contaminations during culturing on board the research vessel, nutrient plates are to be exposed in the sampling place and in the culturing place like laminar hood and laboratory during each sampling. The culturing or sediment

enrichment is to be strictly carried out in the laminar flow hood in front of gas or spirit burners. [Damare \(2007\)](#) and [Singh et al. \(2010\)](#) isolated filamentous fungi and yeasts from deep-sea sediments and water column respectively using such stringent procedures on board the research vessels. For isolating yeasts from water column, a membrane filtration technique was used ([unpublished results](#)). In this, 100 ml water samples were filtered through sterile cellulose ester membranes with a pore size of 0.45 μm (Millipore, USA). These membranes were then placed on solid mycological media plates, supplemented with antibiotics to suppress bacterial growth. The agar plates were incubated at a desired temperature (10°C) till visible colonies appeared on the membrane. [Le Calvez et al. \(2009\)](#) and [Burgaud et al. \(2009\)](#) isolated fungi associated with hydrothermal vent animals by crushing them in sterile sea water and plating on to several different mycological media.

Apart from the conventional dilution plating method, a modified particle plating method along with pressure incubation methods were also used for obtaining culturable fungi ([Damare et al. 2006a](#), [Damare 2007](#), [Singh et al. 2010](#)). In the dilution plating method, approx. 0.1 g of sediment was suspended in sterile sea water, vortexed for 1 min and 100 μl aliquots were spread-plated. In a particle plating technique ([Bills and Polishook 1994](#)), approximately 1 g of sediment slurry was sieved successively through a mesh size of 200 and 100 μm screens. The particles that passed through 200 μm mesh but were retained on the 100 μm mesh were spread-plated. For both the above techniques, the plates were incubated at 5°C at 0.1 MPa pressure (1 bar pressure) for 15-20 days. In the pressure incubation method, approximately 0.5 g of sediments were placed in sterile plastic pouches (of 4x4 cm size) containing 2 ml of sterile sea water malt extract broth (MEB) and the open ends of the pouches sealed with an electrical sealing machine (Quickseal, Sevana, India). The bags were placed in a deep-sea culture vessel (Tsurumi & Seiki Co., Japan) filled with sterile water and pressurized. The pressure vessels were immediately placed at 5°C and incubated for 30 days. At the end of this incubation period, 100 μl of the sediment was spread-plated on nutrient media prepared in sea water and the plates incubated at 0.1 MPa and 30°C for recovery of fungi, till fungal colonies appeared (within 8-10 days). The percentages of culturable fungi obtained by incubation of deep-sea sediments under elevated hydrostatic pressure were higher than those obtained by dilution plating in the three cruises to the Central Indian Basin ([Table 1](#)).

Bacteriologists have used several techniques to culture the uncultured bacteria. One such method, called dilution- to- extinction, has yielded difficult to culture bacteria like SAR11 ([Giovanni and Stingl 2007](#)). In this technique, the sediment is diluted to give a final concentration to achieve 1-5 bacterial cells ml^{-1} and plated in a modified very low nutrient medium prepared in ambient sea

water. [Zengler et al. \(2002\)](#) reported a microencapsulation technique for culturing the uncultured bacteria from environmental samples. It involved encapsulation of cells in environmental samples in gel microdroplets (GMD). The gel microdroplets were incubated in amended sea water medium for several weeks resulting in encapsulated microcolonies. These gel microdroplets were sorted out by flow cytometry, based on their distinct light-scattering signature. This property enabled discrimination between unencapsulated single cells, empty or singly occupied GMDs and GMDs containing a microcolony. The GMDs were further used for massive cultivation under various low nutrient conditions. This technique helped in culturing previously uncultured phylotypes. Such culturing techniques for culturing deep-sea fungi needs to be developed to tap this immense reservoir of fungal diversity.

Detection and diversity of fungi in deep sea

Presence of fungi in shells collected from deep-sea waters of 4610 m depth ([Höhnk 1969](#)) was the first report on deep-sea fungi. This was followed by isolation of fungi from water samples collected from subtropical Atlantic Ocean, from surface to 4500 m depth using sterile van Dorn bags or Niskin samplers ([Roth et al. 1964](#)). Deep-sea fungi were obtained by directly submerging wooden panels at 1615-5315 m depth ([Kohlmeyer 1977](#)). These fungi were however not cultured. Four of these fungi were found growing on wooden panels and one on chitin of a hydrozoan. Mycelial fungi growing inside shells of mollusks at 4830 m depth in the Atlantic were documented by [Poulicek et al. \(1986\)](#). Several filamentous fungi were isolated from surface sterilized calcareous fragments collected from 300-860 m depth in the Bay of Bengal ([Raghukumar et al. 1992](#)). These fungi were isolated using 1/5 diluted malt extract medium prepared with seawater. It was observed that “tests for the tolerance of high pressures and low temperatures can indicate whether the isolated fungal species are indigenous deep-sea forms or aliens from other habitats” ([Kohlmeyer and Kohlmeyer 1979](#)). In accordance, it was demonstrated that conidia of *Aspergillus ustus* (Bainier) Thom & Church and *Graphium* sp. isolated from the calcareous sediments germinated at 10 and 20 MPa pressure in Czapek-Dox medium and on shells suspended in seawater ([Raghukumar and Raghukumar 1998](#)).

Recovering culturable fungi from deep-sea sediments and other such extreme environments is always fraught with an apprehension of contaminants. Therefore, only detection by direct examination of sediments provides proof of their existence. Direct microscopic detection of fungi in deep-sea sediments has seldom been attempted. Detection of fungal filaments in formalin-preserved calcareous fragments obtained from 965 m depth in the Arabian Sea confirmed that they were actively growing in these shells. These calcareous fragments were treated with EDTA to dissolve the

calcium carbonate and subsequently stained with the fluorescent brightener Calcofluor to visualize fungal filaments under an epifluorescence microscope (Raghukumar and Raghukumar 1998). Isolation and direct detection of fungi was reported in deep-sea sediments collected from the Chagos Trench in the Indian Ocean at a depth of 5900 m (Raghukumar et al. 2004). Fungal hyphae could not be detected in sediments by bright field microscopy but were visible by epifluorescence microscopy after staining the sediments with Calcofluor White (Raghukumar et al. 2004). Damare et al. (2006a) detected fungal mycelia in deep-sea sediments by using polyclonal antibodies. Polyclonal antibodies were produced against *Aspergillus terreus* Thom, the most commonly isolated fungus from these sediments. Presence of *A. terreus* in the sediment was confirmed with immunofluorescence probe. These studies indicated that the presence of fungi in deep-sea sediments might have gone undetected by conventional microscopy.

Very recently, Burgaud et al. (2010) used a fluorescent in situ hybridization (FISH) technique for detecting the presence of yeasts in animals collected from deep-sea hydrothermal vents. Although yeasts were obtained from some of the vent fauna, no FISH signal was observed in them. The FISH which involves singly labeled rRNA-targeted oligonucleotide probe suffers from a problem of low signal intensity of the targeted microbes. Therefore, the absence of FISH signal does not rule out the presence of isolated yeasts in these vent fauna. These authors however detected FISH signal in concentrated sea water indicating that yeast population is quite low in deep-sea vents. To detect fungal presence in deep-sea animals and sediments, one could use improved techniques such as CARD-FISH (Amann and Fuchs 2008) or DOPE-FISH (Stoecker et al. 2010). In catalyzed reporter deposition (CARD)-FISH, horseradish peroxidase-labeled oligonucleotide probe is used resulting in several fold higher sensitivity than standard FISH. Further improvement of this technique was to label oligonucleotide probes with multiple fluorescent dyes to increase the FISH signal intensity. The double-labelling-of-oligonucleotide-probes (DOPE)-FISH increases the signal intensity of standard FISH probes by at least a factor of 2 without causing specificity problems. Such advanced techniques are being targeted by our group to detect fungi in the CIB sediments and anoxic sediments of oxygen minimum zone (OMZ) in the Arabian Sea, off Goa, India.

Culturable fungi, *Penicillium lagena* (Delitsch) Stolk & Samson and *Rhodotorula mucilaginosa* (A. Jørgensen) F. C. Harrison, were retrieved from a depth of 10,500 m sediment samples from the Mariana Trench in the Pacific Ocean (Takami 1999). Using classical morphology-based taxonomy, Damare et al. (2006a) reported *Aspergillus* sp. as the most dominant form followed by unidentified non-sporulating cultures from the Central Indian Basin (CIB). Using ITS and 18S sequences of SSU rDNA to identify culturable fungi, Singh et al. (2010) reported 16 filamentous

fungi and 12 yeast species from CIB. Using this approach these authors reported for the first time occurrence of *Sagenomella* sp., *Exophiala* sp., *Capronia coronata* Samuels and *Tilletiopsis* sp. from deep-sea sediments (Table 2). These authors observed that most of the culturable filamentous fungi belonged to ascomycetes whereas most of the yeast isolates belonged to basidiomycetes. Burgaud et al. (2009) isolated 62 filamentous fungi mostly belonging to ascomycetes associated with animals from various deep-sea hydrothermal vent sites. Isolation of fungi associated with deep-sea dwelling macrofauna and zooplankton should be targeted in future to describe their diversity and ecological role. A new genus of deep-sea ascomycetes, *Alisea longicola* J. Dupont & E. B. G. Jones with one new species was described by analyses of 18S and 28S rDNA sequences and morphological characters (Dupont et al. 2009). This was isolated from sunken wood obtained from Pacific Ocean off Vanuatu Islands. Connell et al. (2009) isolated eight yeasts and yeast-like fungal species from cold hydrothermal environment and basalt rock surface from an active deep-sea volcano, Vailulu'u Seamount, Samoa. Recently Burgaud et al. (2010) obtained 32 isolates of yeasts belonging to Ascomycota (n=11) and Basidiomycota (n=21) phyla associated with deep-sea fauna from hydrothermal vents. In terms of species richness, ascomycetous yeasts belonged to seven different clusters, whereas basidiomycetous yeasts belonged to five.

Occurrence and diversity of microbial eukaryotes from a deep-sea in the aphotic zone at 250-3000 m depth in the Antarctic polar front (Lopez-Garcia et al. 2001), Guaymas Basin hydrothermal vent (Edgecomb et al. 2002), hydrothermal sediment at the Mid-Atlantic Ridge and Lost City hydrothermal field (Lopez-Garcia et al. 2003, 2007) and hypersaline anoxic deep-sea basin of L'Atalante (Alexander et al. 2009) have been reported using PCR amplification of SSU rRNA genes and sequence analyses. As these reports showed fungi as one of the eukaryotic groups in these sediments, methods employing amplification of sediment DNA with fungal specific primers to study culture-independent diversity started gaining popularity. Le Calvez et al. (2009) investigated culture-independent fungal diversity by analyzing the small-subunit rRNA gene sequences amplified by PCR using DNA extracts from hydrothermal vent samples. They reported unsuspected diversity with new species in three fungal phyla. Many of the species identified are unknown, even at higher taxonomic levels in the Chytridiomycota, Ascomycota and Basidiomycota. On the contrary, Bass et al. (2007) reported very low diversity of filamentous fungi with only 18 fungal 18S-types from 11 deep-sea sediment samples. These reports were based on direct amplification of small-subunit ribosomal RNA genes from water. Thus, it can further be concluded that combining culturing with phylogenetic analysis will give us a better picture of the diversity of fungi in the deep sea environment. Further, diversity of fungi in deep-sea sediments reported by culture-independent methods (Lai et al. 2007,

Takishita et al. 2007, Le Calvez et al. 2009, Nagano et al. 2010, Biddle et al. 2008, Edgecomb et al. 2010) emphasizes the challenge that culture-dependent methods must overcome.

A majority of the fungi reported to date from the deep-sea are terrestrial-like fungi. However, Alker et al. (2001) and Zuccaro et al. (2004) have isolated “so called terrestrial fungi” from marine habitats, that may have evolved into marine forms. We need to understand if they are physiologically different from their terrestrial counterparts in their nutritional requirements and enzyme activities. No true piezophilic fungi have been reported so far. Probably they are to be found associated with deep-sea dwelling marine fauna. This is possible only when we have instruments to retrieve deep-sea samples without depressurization. Some of the novel clones or operational taxonomic units (OTUs) described by several research groups (Lopez-Garcia et al. 2001, Gadanho and Sampaio, 2005, Lai et al. 2007, Le Calvez et al. 2009, Nagano et al. 2010, Singh et al. unpublished) from various deep-sea locations may turn out to be true piezophiles. We also need to devise newer culturing methods and media for obtaining yet-uncultured forms in culture. The association of many eukaryotic phylotypes with parasitic organisms may have significant impact on host population and diversity in the deep-sea environment (Brown et al. 2009).

Sampling of deep marine seafloor sediments by the Ocean Drilling Program (ODP) has enabled examination of microbial abundance and diversity in this extreme environment (Parkes et al. 2005). Biddle et al. (2005) reported recovery of ascomycetous fungi belonging to the genera *Cladosporium*, *Penicillium* and *Acremonium* spp. by direct plating as well as enrichment culturing technique from sediment core collected at 200 mbsf (meter below sea floor) from 252 m water depth on the outer shelf edge of the Peru margin. These cultured fungi were identified by ITS sequencing. From the deep-sea sediment cores down to 37 m below the seafloor of the Peru Margin and Peru Trench Edgecomb et al. (2010) have recovered fungal sequences from both DNA- and RNA-based clone libraries. Basidiomycetous fungi were the most consistent phylotypes recovered from these sites. Working with RNA-based clone libraries, these authors could also identify the active members of the community. Besides, presence of fungi has been reported from some more extreme environments. The basidiomycetous yeast *Cryptococcus curvatus* (Diddens & Lodder) Golubev was found to be the dominant fungal phylotype in oxygen-depleted sediments from deep-sea methane seeps (Takashita et al. 2006, 2007). The seeping fluids in such areas contain methane and hydrogen sulfide. Fungal sequences have been recovered from gas-hydrate bearing sediments (Cao 2010). Gas hydrates are ice-like minerals that have crystallized under low temperature, elevated hydrostatic pressure and methane concentrations. In gas-hydrates, methane is the dominant hydrocarbon in the gas mixture held in water molecules. Lai et al. (2007) reported several fungal sequences in methane

hydrate-bearing deep-sea sediments that were not associated to any known fungi or fungal sequences in the public database. Besides, clones phylogenetically similar to *Phoma*, *Cylindrocarpon*, *Hortaea*, *Cladosporium*, *Emericella*, *Aspergillus*, *Malassezia*, *Cryptococcus*, *Lodderomyces*, *Candida* and *Pichia* were also recovered.

Physiological characterization of fungi isolated from the deep-sea hydrothermal vents has shown the presence of true halophiles and those that were halotolerant (Burgaud et al. 2009, 2010, Le Calvez et al. 2009). On the other hand, most of the recovered fungi from the CIB sediments were mesophiles or psychrotolerant, but no true halophiles or psychrophiles were reported (Damare et al. 2006a, Singh et al. 2010). Several of the fungi isolated showed similarity with fungi reported as animal parasites (e.g. Burgaud et al. 2009, Le Calvez et al. 2009, Nagano et al. 2010) and thus these may play an important role as facultative parasites of deep-sea animals and impact host population and diversity in the deep-sea environment (Moreira and Lopez-Garcia 2003, Brown et al. 2009). It is also likely that some of these fungi are in symbiotic association with deep-sea fauna or have a stimulating effect on host defense response. A *Cryptococcus* sp. associated with the healthy coral *Pocillopora damicornis* Linnaeus elicited a cryoprotective response from the soft tissue of the coral. The fungus was demonstrated to extend the survival of coral cells by 2 days, selectively maintaining skeletogenic cell types (Domart-Coulon et al. 2004). Nagano et al. (2010) reported a special group designated as the DSF-Group 1, containing at least 14 OTUs, some of which are reported from oxygen-depleted deep-sea environments like methane cold seep, anoxic bacterial mats and below the sea floor, but not from shallow –sea or surface waters (Bass et al. 2007, Takashita et al. 2007). It is likely that they may be anaerobic or facultative anaerobic fungi. Several of the yeasts isolated by Connell et al. (2009) produced siderophores, a class of molecules used to assimilate and utilize Fe (III) from the environment and one isolate was capable of oxidizing Mn(II). These results indicate that fungi may play an active role in biomineralization process in the deep-sea. Presence of methylotrophic yeasts in methane seeps (Lai et al. 2007) could play a significant role in global methane and carbon cycles and may find application in diagnostic processes.

Growth of deep-sea isolated fungi under simulated deep-sea conditions

The basic requirement for these studies is an instrument, which would be able to simulate the conditions existing in the deep-sea. Different types of instruments have been used for this, but the majority of the work was carried out with bacteria (Jannasch and Wirsen 1977, Helmke 1979, Bernhardt et al. 1987). Lorenz and Molitoris (1992) produced a system, modified from equipment developed for bacteria, to cultivate fungi, especially yeasts, under simulated deep sea condition.

ZoBell and Johnson (1949) and Yamasato et al. (1974) showed that yeasts isolated from terrestrial and marine environments grew at elevated hydrostatic pressure, with an upper limit of approximately 50 MPa. However, they grew the cultures in closed glass tubes, and only the oxygen dissolved in the medium was available for the growth of the cells. This was also true for other methods used for bacteria, such as cultivation in thick-walled silicone tubing (Groß et al. 1994) and in plastic syringes (Alongi 1990). Helmke (1979) demonstrated that such cultivation techniques did not produce sufficient biomass for growth assessment of actinomycetes. To overcome this limitation, Helmke cultured actinomycete cells in polypropylene bags in a fluorocarbon-filled pressure vessel, as fluorocarbon has high oxygen solubility. Berger and Tam (1970) cultured aerobic bacteria in plastic bags but used water, which had low oxygen solubility, and due to lack of exchange of oxygen, the system became anaerobic very rapidly. In all the growth-related studies we used oxygen-permeable polypropylene bags resulting in a good biomass yield in terms of dry weight of filamentous fungi, as well as yeasts (Damare et al. 2006a, Singh et al. 2010).

Under simulated laboratory conditions, we compared the growth and production of biomass by deep-sea filamentous fungi and yeasts (measured as mg dry weight in 20 ml culture medium). Growth measurements in yeasts were carried out by biomass estimation and not by measuring the optical density because yeast cells tended to stick to the surface of culture pouches and scrapping them off with medium resulted in dilution of the turbidity. Most of the fungi were piezotolerant, showing growth under elevated pressure. A greater number of the filamentous fungi and yeasts showed higher biomass production at 0.1 MPa and 30°C, than under elevated hydrostatic pressure (20 MPa) at 5 and 30°C (Table 3). Six filamentous fungi produced biomass in the range of 10-15 mg dry weight in 20 ml culture medium at 20 MPa/30°C and 6 fungi produced biomass in the range of 25-30 mg in 20 ml culture medium at 20 MPa/ 5°C (Table 3). Five yeasts produced biomass in the range of 2-4 mg dry weight in 20 ml culture medium at 20 MPa/ 30°C as well as at 20 MPa/5°C (Table 3). These cultures showed better growth in media prepared in seawater than in distilled water. However, none of the cultures showed an absolute requirement for seawater for growth (Damare et al. 2006a). In one experiment, out of the 32 filamentous fungi compared for biomass production in media prepared in seawater and distilled water, an equal number showed biomass maximas in seawater and distilled water (Singh et al. 2010). However, biomass production was higher in seawater than in distilled water. Similar results were obtained with deep-sea yeasts (Singh et al. 2010). Methods to accurately measure the biomass of yeasts and filamentous fungi continuously to determine specific growth rates under simulated deep-sea conditions using bioreactors, as has been used with bacteria (Miller et al. 1988) needs to be developed for fungi. Terrestrial fungi also showed

growth and biomass production under hydrostatic pressures of 20 MPa at 5° and at 30°C (Damare et al. 2006a). Daniel et al. (2006) opined that most, if not all, organisms can live under a large range of hydrostatic pressures. Surface organisms can survive and grow up to a pressure of 20 MPa (equivalent to 2 km depth in oceans). These authors are of the opinion that as life originated in the deep-sea environment, tolerance to elevated hydrostatic pressure is a physical/chemical parameter found in all organisms. It needs to be verified, however if not all, at least a majority of terrestrial fungi can survive under deep-sea conditions.

Response of spores and mycelia to low temperature and elevated hydrostatic pressure

Germination of fungal spores in the deep sea may face several obstacles like the mycostatic effect of seawater (Kirk 1980), low temperature, elevated hydrostatic pressure and low nutrient conditions. A defining characteristic of spores is their ability to develop into a new individual without fusion with another reproductive cell. The first step in this is spore germination, which can be defined as the sequence of events that converts the resting/dormant spore into a rapidly growing germ tube from which the mycelium is produced by elongation, septum formation and branching. A spore is defined as having germinated if the germ tube is at least as long as the width of the spore. The effect of extreme conditions on the germination of conidia of the deep-sea *Aspergillus* isolates, and their viability, was compared (Damare et al. 2008). Spores of three deep-sea and terrestrial *Aspergillus* isolates and a deep-sea *Cladosporium* isolate germinated much better in sediment extracts of different dilutions at 20 MPa pressure at 30°C but not at 5°C (Table 4). However, percentage germination decreased gradually with increasing pressure. The spores failed to germinate at a low temperature of 5°C, even at 0.1 MPa pressure during an incubation period of 20 days. Although all of these fungi could produce biomass at elevated hydrostatic pressure and low temperature when initiated with mycelial inoculum, spores did not germinate under these conditions (Damare 2007). Thus it appears that low temperature and not elevated hydrostatic pressure is a limiting factor for spore germination and further biomass production in the deep-sea. A temperature shock of 15 min at 50°C could break the dormancy of spores in three *Aspergillus* species resulting in their germination at 0.1 MPa at 5°C but not at 20 MPa/5°C (Table 5) indicating that this shock was not effective enough against the combined negative effects of low temperature and elevated pressure. It can further be concluded from these results that fungal mycelial fragments which appear to be metabolically more active are more tolerant to elevated hydrostatic pressure and low temperature than the dormant fungal spores. Thus in conclusion, mycelial fragments have better chances of propagation under deep-sea conditions than do spores. As observed by Ivanova and Marfenina

(2001), we suspect that the critical size of mycelial fragments may also influence the survival of fungal species in stressful environments.

Pressure effects on yeasts

The term piezophysiology is used to describe the effects of high pressure on growth, viability and cellular responses in living cells (Abe 2004). *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, a piezotolerant yeast is used as a model organism to study the effects of hydrostatic pressures. Growth and cellular activity in *S. cerevisiae* is unaffected at pressures lower than 20-30 MPa. Higher pressures induce various stress response through pressure-inducible genes and proteins. Piezotolerance depends on the duration of high pressure application. Yeast cells in stationary phase are more resistant to pressure than actively growing cells (Abe 2004, Fernandes 2005). Hydrostatic pressure of 200 MPa for 30 min induced several morphological changes in wild-type and trehalose-6-phosphate synthase (*tsp1*) mutant cells of *S. cerevisiae* (Fernandes et al. 2001). Such mutant cells are unable to accumulate trehalose, a disaccharide, a well-known cell membrane protectant against adverse environmental conditions. When the cells were subjected to preheat treatment at 40°C for 60 min before subjecting to the pressure treatment, both the types of cells (wild and mutant) acquired resistance to the pressure treatment. Such induced barotolerance in *S. cerevisiae* by heat shock was earlier reported by Iwahashi et al. (1991).

Effect of lethal pressures on yeast cells were studied by several groups (Iwahashi et al. 1991, 2003, Fernandes et al. 2004). Iwahashi et al. (2003) studied DNA microarrays of *S. cerevisiae* and analysed expression levels of ~6000 genes. The genome-wide expression profiles suggested that high pressure (180 MPa at 4°C for 2 min) caused damage to cellular organelles and it was similar to the damage caused by detergents, oils, freezing and thawing. Fernandes et al. (2004) studied gene expression patterns in response to hydrostatic pressure of 200 MPa for 30 min in *S. cerevisiae* also by whole genome microarray hybridization. Among the 6200 genes analysed by these authors, ~5 % were affected by hydrostatic pressure treatment, 131 were >2-fold induced while 143 were <2-fold down-regulated. The majority of the up-regulated genes were involved in stress defense and carbohydrate metabolism while, a large number of down-regulated genes were in the categories of cell cycle progression and protein synthesis. It was noted that the effects of pressures that cause growth inhibition in *S. cerevisiae* are different from those caused by lethal pressures (Abe 2004). Accordingly, Iwahashi et al. (2005) studied genome-wide mRNA expression profiles of *S. cerevisiae* grown under 30 MPa pressure, which was shown to cause growth inhibition. They reported up-

regulation of genes involved in membrane metabolism and the response were essentially different from the response to pressures that cause cellular death.

Pressure effects on filamentous fungi

Microbial activity under elevated pressure, compared with that at 0.1 MPa is inhibited. All aspects of activity, including growth, respiration, and specific biochemical processes, appear to be affected at elevated pressures (Abe and Horikoshi 1995, Fernandes et al. 2004, Daniel et al. 2006, Abe 2007). Pressure-inducible genes, which aid in pressure acclimatization, have been proposed to exist in marine bacteria, which experience large vertical changes in the water column (Bartlett 1991). In the case of bacteria, the pressure effects on gene expression, membranes, membrane proteins, DNA structure and function, cell division, protein and enzyme functions have been studied in detail, while in the case of fungi the studies have remained restricted only up to their detection in the deep-sea sediments and ability to grow under elevated pressure and produce extracellular enzymes active under elevated hydrostatic pressure (see Raghukumar and Damare 2008).

Exposure of both prokaryotic and eukaryotic cells and/or tissues to a variety of physiological stresses results in the rapid synthesis of a specific class of proteins. This phenomenon is known as 'stress response' and the newly formed transient proteins are termed as 'stress proteins'. These proteins play a significant role in cells and tissues in manifestation of adaptation and may serve as a defense mechanism against a variety of stress conditions (Lindquist and Craig 1988). As the adaptation of other kinds of stress or shock condition is often mediated by the production of such specific stress proteins (e.g. heat shock, cold shock or antifreeze proteins), a suitable approach to high-pressure response would be to look for alterations in the protein patterns of microorganisms grown at different hydrostatic pressures, ranging from atmospheric pressure to the limit of viability.

Some of the fungi showed abnormal morphology immediately after isolation from deep-sea sediments of the CIB (Damare et al. 2006a). These showed extremely long conidiophores with vesicles being covered by long hyphae, instead of phialides of metulae or conidia, as is typical of the genus *Aspergillus*. Most of these abnormal features disappeared after repeated (~4-6 times) subculturing. Several fungi isolated from deep-sea sediments were initially non-sporulating, but formed spores after repeated (~ 6-8 times) subculturing at 0.1 MPa pressure. Several of these fungi showed distinct swellings when grown under elevated hydrostatic pressure. The type of nutrients used for culturing also seemed to affect the morphology. When deep-sea fungi were grown in malt extract seawater broth, the fungal hyphae showed several swellings, while the hyphae grew perfectly

normal in sediment extract medium (Damare and Raghukumar 2008). Two of the deep sea fungi showed microcyclic conidiation when grown at 10 MPa (Raghukumar and Raghukumar 1998). In this, immediate conidiation was seen following conidial germination without the growth of vegetative mycelium. Microcyclic conidiation is reported to occur under nutrient-limiting conditions and might help the fungi to complete their life cycles in a shorter time. This might be occurring due to arrest of apical growth followed by lateral differentiation of conidium-producing cells.

Possible role in the deep-sea environment

1. Aggregate formation and carbon contribution

Bacteria and fungi are major players in transformation of organic matter in terrestrial soil. In contrast to land, however, most studies on deep-sea sediments have focused exclusively on bacteria and have demonstrated their intense metabolic activities therein (Turley and Dixon 2002). The fungi and their role in the deep-sea sediments have remained neglected mainly due to the fact that they are not easily seen / observed. This is because most of the times, fungi remain embedded in aggregates and hence go unnoticed (Damare and Raghukumar 2008). Increased sighting of fungi after treating the deep-sea sediments with chelating agent, like EDTA, was reported by these authors. EDTA is a chelating agent and effective in solubilising the polysaccharides as well (Liu et al. 2002). It was further suggested that fungi in deep-sea sediments may be involved in humic aggregate formation by processes very similar to those in terrestrial sediments. Reducing sugars and amino acids, formed, as by-products of microbial metabolism in terrestrial sediments are known to undergo nonenzymatic polymerization and form brown-coloured products, constituting humus (Tisdall and Oades 1982). The humic material combines with soil particles to form microaggregates. Fungal hyphae further act as binding agents to form macroaggregates by trapping fine particles into the microaggregates (Kandeler et al. 1999). Fungi or bacteria can thus remain protected in certain particle size classes (Suberkropp and Meyers 1996). Cations such as Si^{4+} , Fe^{3+} , Al^{3+} and Ca^{2+} form bridges between particles in terrestrial microaggregates (Bronick and Lal 2005).

Although fungi are generally believed to be more effective at aggregate stabilization than other soil microflora, several studies have concluded that their primary contribution to aggregation is through hyphal entanglement of soil particles (Molope et al. 1987). Damare and Raghukumar (2008) demonstrated formation of microaggregates by fungi grown in sediment extract medium under 20 MPa pressure. The microaggregates stained positively for humic substances indicating that the fungal activities are responsible for the formation of humus possibly in deep-sea sediments also as

described previously for terrestrial environments (Stevenson 1994). The fungi, thus present, concealed inside these microaggregates, contribute to the nutrient cycling in the deep-sea habitat, by acting upon them from inside the aggregates. Aggregation protects the soil organic matter which is an important, biotically regulated mechanism for accumulation and maintenance of organic matter in soil (Beare et al. 1994). This is particularly a helpful feature in natural environment as it prevents the extracellular enzymes from diffusing away from the cells secreting it. Thus, these humic-enzyme complexes have an important role to play in overall nutrient dynamics of the sediments (Burns 1978). Fungi are known to produce a large amount of exopolysaccharides (Selbmann et al. 2003), which in turn may enrich the aggregates. Hyphal sheaths often have an adhesive role in the attachment of fungal mycelium to surfaces and particle entrapment (Hyde et al. 1986).

Fungal biomass constitutes a large portion of the potentially mineralizable organic matter associated with forest and grassland soils (Chiu et al. 2006). Damare and Raghukumar (2008) estimated fungal biomass in deep-sea sediments based on fungal biovolume. By measuring microscopically the hyphal lengths and diameter of fungi in Calcofluor-stained sediments and assuming it to be a cylinder, fungal biovolume was calculated using the formula – $\Pi r^2 \times \text{hyphal length}$. The biovolume was converted to carbon assuming an equivalent of 1 pg of C for every μm^3 (Van veen and Paul 1979). Based on these values, the fungal organic carbon contribution in the deep sea sediments of Central Indian Basin was reported to be 2.3 to 6.3 $\mu\text{g g}^{-1}$ of dry sediment. The bacterial carbon contribution for the same site was reported in the range of 1 to 4 mg g^{-1} of dry sediment (Raghukumar et al. 2001). Fungal carbon contribution in the coastal waters off Goa ranged from 10-150 pg g^{-1} sediment (Jebraj and Raghukumar 2009). In contrast, fungal carbon contribution in soil from grassland and forests ranged from 453-3375 $\mu\text{g g}^{-1}$ of dry sediment (Chiu et al. 2006). The results obtained by us could be much less than the actual contribution, as fungal biomass estimated by hyphal length may often underestimate the actual value when compared to other biochemical methods like phospholipids (Balsler et al. 2005), hexosamine (Gessner and Newell 2001) or ergosterol (Mille-Lindblom et al. 2004) content. Melanin and chitin, the polymers of fungal cell wall are not easily degradable, whereas phospholipids of bacterial cell walls are easily degraded. Thus fungus-mediated C storage is expected to be more persistent in contrast to the bacterially sequestered one (Bailey et al. 2002). A comparative quantitative study using ^{14}C -labeled sugars and amino acids with pure cultures of deep-sea bacteria and fungi for growth, uptake kinetics and carbon sequestration under simulated deep-sea conditions could help us in understanding their individual role to a certain extent.

2. Extracellular enzymes

Several enzymes are known to be involved in the cycling of nutrients in the deep-sea and can be used as potential indicators of nutrient cycling processes. In aquatic ecosystems like that of the deep sea, alkaline phosphatase activity (APA) plays an important role in the regeneration of inorganic phosphate through its catalysis of organic esters to inorganic P (Chrost 1991). Organic P content in the CIB measured during three cruises varied from 0.007- 0.11 % and sediment alkaline phosphatase activity was in the range of 0.06 to 7.8 U which is equivalent to μmol of phosphate phosphorus released $\text{h}^{-1} \text{g}^{-1}$ dry sediment (Raghukumar et al. 2006). Damare (2007) tried to differentiate the contribution of fungi and bacteria towards the APA in deep-sea sediments of the CIB in simulated deep-sea conditions in laboratory microcosm experiments (Table 6). The plain sediment without any additional nutrients showed very low APA. On addition of detritus, the APA increased 20-fold, indicating acute increase in microbial activity, typical of a 'feast and famine' deep-sea habitat, where the nutrient supply is mostly in the form of pulse, meaning sometimes available in plenty and sometimes not available for days together. APA was higher in the presence of antibacterial antibiotic, indicating a major share of fungi towards the APA (Table 6). This indicates that fungi might play an important role in degradation of detritic material as reported in the terrestrial and mangrove environments (Newell 1996). In contrast, addition of autoclaved *Artemia* larvae and cysts had no effect on the APA. A protease enzyme might play a direct role in degradation of such animal detritus. The deep-sea fungal isolate *A. terreus* (#A4634), in the presence of antibacterial antibiotics, showed a very high APA at both the pressures of incubation as compared to APA shown by deep-sea bacteria in the presence of a fungicide in the same microcosm experiment (Table 6). These results indicate that fungi could be playing an equally important role in the P cycle in the deep-sea.

Deep-sea microorganisms growing under extreme conditions are a good source for screening of industrially useful enzymes with novel properties (Synnes 2007, Dang et al. 2009). Protease is one of the several extracellular enzymes being produced by the fungi playing a major role in their nutritional requirements. Damare et al. (2006b) demonstrated that 11 % of the total fungi (n=221) isolated from CIB deep-sea sediments showed low temperature-active protease production. These fungi when grown under elevated pressure synthesized extracellular protease, but in very low quantity compared with that produced under 0.1 MPa. As enzyme production is governed by growth, reduced growth at 5°C and elevated pressure appeared to be responsible for low levels of enzyme. One of the fungi isolated from the deep-sea produced 1639 Azocasein units ml^{-1} of alkaline protease with optimum activity at pH 9.5, showing 45 and 25 % of its activity at 20 and 15°C, respectively. It

was active in the presence of several commercial detergents and 0.5 M NaCl, equivalent to 29 PSU (Practical Salinity Unit) of seawater salinity. These features are desirable for commercial application as a detergent additive for cold wash (Raghukumar et al. 2009). The proteases from deep-sea fungi could be a potential source in the search for detergent enzymes.

Fungal polygalacturonases (PGase) are useful enzymes for clarification of fruit juices in the food industry. Two novel endopolygalacturonases active at 0-10°C were purified from the culture supernatant of a deep-sea yeast (strain N6) isolated from the Japan Trench at a depth of 4500-6500 m (Miura et al. 2001, Abe et al. 2006). The hydrolytic activity of N6-PGases remained almost unchanged up to a hydrostatic pressure of 100 MPa at 24°C (Abe et al. 2006). Interestingly this strain of *Cryptococcus* was tolerant to CuSO₄ up to a concentration of 50 mM and showed high activity of superoxide dismutase, an enzyme responsible for scavenging superoxide radicals (Abe et al. 2001). High pressure can shift the temperature requirement for a given chemical reaction towards lower temperature (Daniel et al. 2006). Therefore, it could be possible for a reaction requiring a thermophilic enzyme to be carried out effectively with a mesophilic enzyme at elevated hydrostatic pressure.

Conclusion

Deep-sea sediments appear to be one of the niches to look for hidden fungal diversity and therefore a systematic survey of world oceans for culture-dependent and culture-independent fungi is recommended. Newer methods and media to isolate and culture them need to be devised. Their role in macroaggregate formation is important in biogeochemical cycling of nutrients and carbon sequestration in the deep-sea. They could be a source for novel secondary metabolites and enzymes with novel properties (Pettit 2010).

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Table 1. Number of fungi isolated from deep sea sediments by different methods during 3 cruises in the Central Indian Basin (CIB).

	Particle plating	Dilution plating	Pressure incubation*	Direct incubation in MEB** at 5°C
Total number of sediment samples used	532	260	263	153
Number of fungi recovered***	101	50	94	3
% recovery	19	19	36	2

* Incubated at 30 MPa pressure

** Malt extract seawater broth. Fungi were isolated as described in detail by [Damare et al. \(2006a\)](#) and [Singh et al. \(2010\)](#).

*** Number of distinct morphological types obtained during the three cruises in the CIB.

Table 2. Diversity of culturable fungi isolated from the deep sea sediments during three cruises AAS 46, AAS 61 and ABP 26 in the CIB (Damare et al. 2006a, Singh et al. 2010).

Identification of isolate	No. of isolates
<i>Aspergillus</i> sp.	31
Non-sporulating mycelial fungi	26
<i>Aspergillus terreus</i>	25
<i>Aspergillus restrictus</i> G. Sm.	22
<i>Cladosporium</i> sp.	14
<i>Penicillium</i> sp.	12
<i>Aspergillus sydowii</i> (Bainier & Sartory) Thom & Church	5
Unidentified Ascomycetes	4
<i>Sagenomella</i> sp.	3
<i>Tilletiopsis</i> sp.	2
<i>Acremonium</i> sp.	2
<i>Exophiala</i> sp.	2
<i>Capronia coronata</i>	1
<i>Fusarium</i> sp	1
<i>Sporidiobolus johnsonii</i> Nyland	4
<i>Rhodotorula</i> sp.	3
<i>Cryptococcus</i> sp.	2
<i>Rhodosporidium toruloides</i> Banno	1
<i>Graphiola cylindrica</i> Kobayasi	1
<i>Coniosporium perforans</i> Sterf.	1
<i>Aureobasidium</i> sp.	1

Table 3. Biomass produced by filamentous fungi and yeasts under varying hydrostatic pressures and temperatures.

Biomass range (mg dry weight in 20 ml culture medium)	No. of filamentous fungi *			
	0.1 MPa / 30°C	0.1 MPa / 5°C	20 MPa / 30°C	20 MPa / 5°C
5-10	0	0	4	0
10-15	0	3	6	0
15-20	1	5	5	1
20-25	5	5	1	4
25-30	2	2	0	6
30-35	1	1	0	4
35-40	1	0	0	1
40-45	4	0	0	0
45-50	1	0	0	0
50-55	1	0	0	0

Biomass range (mg dry weight in 20 ml culture medium)	No. of yeast cultures*			
	0.1 MPa / 30°C	0.1 MPa / 5°C	20 MPa / 30°C	20 MPa / 5°C
0-2	0	1	6	5
2-4	0	5	5	5
4-6	1	3	0	2
6-8	2	2	1	0
8-10	5	1	0	0
10-12	2	0	0	0
12-14	2	0	0	0

*Number of filamentous fungi and yeast showing the biomass production in a particular range under specific growth condition. Culturable fungi and yeasts were isolated during cruise # ABP26 (Singh et al. 2010). Biomass as dry weight for mycelial fungi was determined after 20 days incubation in malt extract broth under different pressure and temperature conditions. Biomass as dry weight for yeasts was determined after 20 days incubation in yeast extract, peptone, dextrose broth under various pressure and temperature conditions as described in detail by Singh et al. (2010).

Table 4. Effect of sediment extract dilutions and elevated hydrostatic pressures on spore germination of deep-sea isolated and terrestrial fungi.

Concentrations of sediment extract**	% germination of spores							
	<i>Aspergillus terreus</i> (deep-sea isolate)		<i>Aspergillus ustus</i> (deep-sea isolate)		<i>Cladosporium sp.</i> (deep-sea isolate)		<i>Aspergillus terreus</i> (terrestrial isolate)	
	0.1 MPa / 30°C	20 MPa / 30°C	0.1 MPa / 30°C	20 MPa / 30°C	0.1 MPa / 30°C	20 MPa / 30°C	0.1 MPa / 30°C	20 MPa / 30°C
1 (undiluted)	100	36	100	23	100	19	100	28
1 : 10	100	31	100	26	114	20	90	25
1 : 20	93	30	100	19	78	21	82	21
1 : 40	77	35	100	24	77	17	77	20
1 : 60	73	27	96	20	73	10	79	13
1 : 80	65	22	88	10	70	10	67	7
1 : 100	51	12	63	10	60	9	52	9
Hydrostatic pressure***	% germination of spores at varying hydrostatic pressures at 30°C *							
0.1 MPa	94		100		92		90	
10 MPa	10		29		22		21	
20 MPa	9		25		20		19	
30 MPa	5		16		12		5	
50 MPa	3		8		5		6	

* No germination observed at 5°C for all the pressures. **Data taken from [Damare and Raghukumar \(2008\)](#); *** Data taken from [Damare et al. \(2006a\)](#).

Table 5. Effect of varying hydrostatic pressures and temperatures on spore germination.

Incubation conditions	% Spores Germinated								
	<i>Aspergillus terreus</i> (deep- sea isolate)			<i>Aspergillus ustus</i> (deep-sea isolate)			<i>Aspergillus terreus</i> (MTCC479, terrestrial isolate)		
	A	B	C	A	B	C	A	B	C
20 MPa /30°C	14 ± 4	12 ± 3	16 ± 4	6 ± 2	9 ± 2	8 ± 3	13 ± 3	16 ± 4	18 ± 4
20 MPa/5°C	0	0	0	0	0	0	0	0	0
0.1 MPa/30°C	100	100	100	100	100	100	100	100	100
0.1 MPa/5°C	0	0	100	0	D	100	0	0	100

A - malt extract broth (MEB); B - MEB with 20% sucrose; C - spores given a heat shock of 50°C for 15 min and then incubated in MEB with 20 % Sucrose; D – doubtful germination. Data taken from [Damare and Raghukumar \(2008\)](#).

Table 6. A comparison of bacterial and fungal contribution to alkaline phosphatase activity (APA) under simulated deep-sea conditions in the presence and absence of additional nutrients .

Experimental condition	Release of MUF ($\mu\text{g l}^{-1} \text{h}^{-1}$)**	
	20 MPa / 5°C	50 MPa / 5°C
1 Plain sediment	5.6 \pm 0.3	4.6 \pm 0.9
2 Sediment + antibacterial antibiotic	4.5 \pm 1.2	4.9 \pm 0.1
3 Sediment + Fungicide	1.5 \pm 0.9	0.2 \pm 0.1
4 Sediment + detritus	101.2 \pm 25.4	98.6 \pm 21.1
5 Sediment + detritus + antibacterial antibiotic	90.0 \pm 8.4	97.0 \pm 15.9
6 Sediment + detritus + Fungicide	73.5 \pm 34.9	70.1 \pm 34.9
7 Sediment + artemia larvae	5.0 \pm 1.6	2.6 \pm 0.4
8 Sediment + artemia larvae + antibacterial antibiotic	3.9 \pm 0.6	2.0 \pm 0.7
9 Sediment + artemia larvae + Fungicide	3.4 \pm 1.5	2.6 \pm 1.2
10 Sediment + detritus + Deep-sea fungus* + antibacterial antibiotic	108.0 \pm 0	93.9 \pm 9.3
11 Sediment + detritus + Deep-sea bacteria + Fungicide	84.9 \pm 16.6	67.4 \pm 0.6

**Aspergillus terreus*. **APA activity was estimated by measuring the released fluorescent compound 4-methylumbelliferone (MUF) from MUF- phosphate at 340 nm excitation and 450 nm emission. The pH of the reaction mixture was 8.3 (Hoppe 1983). Ampicillin (0.1 g in 100 ml of the medium) and penicillin (4000 U in 100 ml of the medium) were used as the antibacterial agents. BavistinTM at 0.5 % final concentration was used as an antifungal compound. The concentrations of the antibiotic and the fungicide used in the study were standardized by growing bacteria and fungi in the presence of varying concentrations of both the inhibitors. The second lowest concentration not showing cross inhibition (fungicide not inhibiting bacteria and vice versa) were used in the study. To stimulate sporadic enrichment of the deep-sea sediments, phytodetritus and dead *Artemia* larvae were used for spiking the sediments. Detritus was collected from shallow waters of Dona Paula Bay (Goa, India) with 200 μm plankton net. It was a mixture of phyto- and zooplankton (Damare 2007).

Legends to the figures

Figure 1 Niskin sampler is being lowered into water column for collecting water samples. Several such samplers can be attached on the winch wire for collecting water samples from different depths.

Figure 2 Box corer is being lowered for collecting sediment samples.



Fig. 1

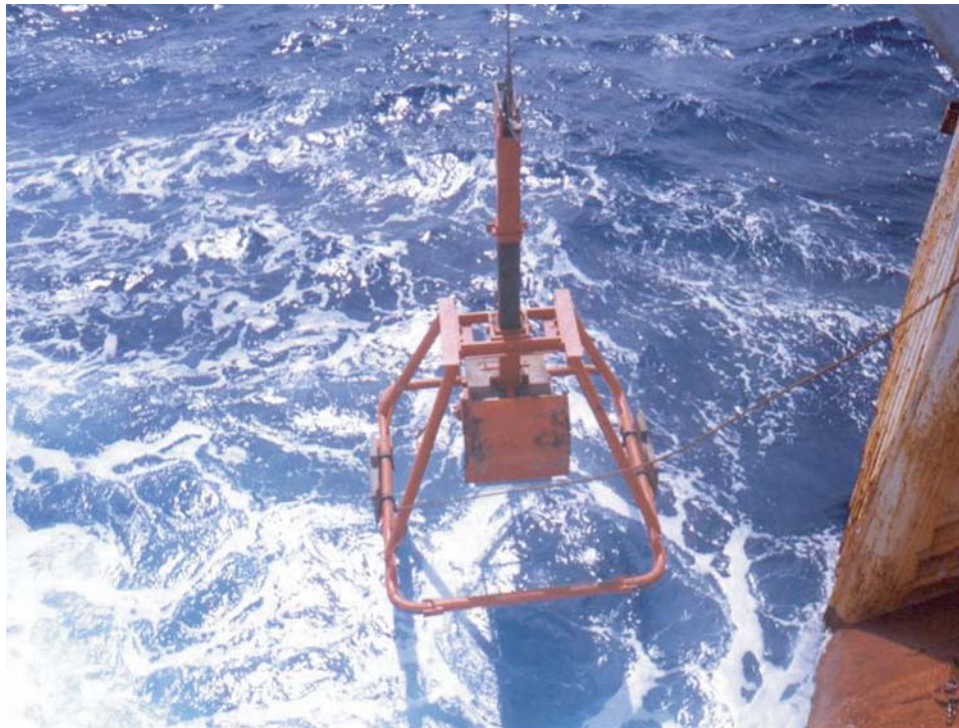


Fig. 2