

## Diversity and adaptations of deep-sea microorganisms

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**Abstract:** The discovery of near fresh closed lunch box found after 11 months in the wreck of the submersible Alvin in the year 1969 opened up a Pandora's box of microbial diversity in the deep-sea. Discovery that near-freezing temperatures, elevated hydrostatic pressures and slow decaying process at depth aided preservation opened up a whole new area of research in biology and chemistry of deep-sea waters. This unique and challenging extreme environment is home to native baro- and psychrophiles whereas some are aliens who have acquired a resident status. The presence of Proteobacteria, a gamma subgroup of *Archaea* cohabiting with *Pseudomonas* species and giant single-celled organisms belonging to the group Xenophyophores were some of the interesting discoveries. Microorganisms have been isolated from high-pressure environment of Lake Baikal in Siberia and Lake Vostok located 3-4 km beneath ice sheet in the East Antarctica.

The unique environment of deep sea, methods of collecting deep-sea sediment and water samples, isolation and culturing of microorganisms under *in situ* pressure and temperature conditions are described here. The effect of elevated hydrostatic pressure on barotolerant forms and adaptation mechanisms in barophiles are discussed. Cold-tolerant enzymes, natural products of potential use in human health management and environmental bioremediation using solvent-tolerant microorganisms are some of the potential biotechnological applications of these deep-sea microbes.

**Keywords:** deep-sea microorganisms, barophilic, barotolerant, bacteria, fungi

## **Introduction**

The deep-sea is a challenging extreme environment characterized by high hydrostatic pressures, low temperatures, near to total darkness and “feast or famine” nutrient regimes (Morita, 1982). The average depth of oceans is 4000 m and the hydrostatic pressure increases by 1 bar (0.1 MPa) for every 10 m depth. The deepest recorded part of the ocean at 11,033 m lies in the Mariana Trench in the Pacific Ocean. Depths exceeding 1000 m are considered deep sea and accordingly, 88% of the oceanic environment comes under this zone. There is a constant rain of minute organic particles (marine snow) consisting of faecal matter as well as dead phyto- and zooplankton from surface to deeper waters (Herring, 2002). Carcasses of nektonic organisms, ranging from fishes to dead whales, provide large food source for deep-sea scavengers (Witte, 1999). Contrary to popular belief, the bottom waters in most of the seas are well oxygenated due to the flow of nutrient and oxygen-rich deep and bottom waters by the South Equatorial Currents and the Pacific through-flow (Lalli and Parsons, 1997). The oxygen content of surface sea waters is 5-6 ml L<sup>-1</sup> and in bottom waters is 4-5 ml L<sup>-1</sup> and thus O<sub>2</sub> is rarely limiting. Moreover, solubility of oxygen increases in cold bottom waters and increasing hydrostatic pressures (Lalli and Parsons, 1997). The temperatures in deep seas are around 2-5°C, salinity on an average is 34-35 parts per thousand and the pH is around 8.2 (Lalli and Parsons, 1997).

## **Collection and culturing of deep-sea microorganisms**

Deep-sea microorganisms that grow only under elevated hydrostatic pressure are called barophilic (also called piezophilic) and barotolerant ones grow at 1 atmospheric as well as at elevated hydrostatic pressure. These organisms are either psychrophilic (cold-loving) or psychrotolerant (cold-tolerant), thermophilic and thermotolerant around hydrothermal vents. In this review the focus is on the cold –tolerant piezophiles. Isolation of such microbes is mostly done from water samples collected from different depths in the sea using special water samplers such as Niskin, Nansen bottles, Rossett sampler (Fig. 1) or Zobell sampler (Fig. 2) and sediment sampling is done using different kinds of grabs, multiple corer (Fig. 3) and box corers (Fig. 4, Karl and Dore, 2001). The cultures isolated from water or sediment samples can be tested for their barotolerance or

barosensitivity by culturing them under varying hydrostatic pressures in a special culturing device mimicking deep-sea pressure conditions (Fig. 5).

Different groups of workers have used special devices to obtain deep-sea samples that retain the *in situ* pressure. Jannasch et. al. (1973) developed a deep-sea pressure retaining sampler for collecting water samples for culturing barophilic bacteria (Fig. 6). We have designed a water sampler to collect water samples up to 1200 m depth while retaining *in situ* pressure (Fig. 7). A patent has been filed for the design (Naik and Raghukumar, 1999). The group working on extremophiles at Japan Marine Science and Technology Centre (JAMSTEC) has developed a sophisticated system for retrieving, isolating and culturing deep-sea microorganisms while maintaining their deep sea ambient environmental conditions (Hamamoto and Horikoshi, 1993). Kato et al, (1996) used small corer to collect deep-sea sediment samples by the manned submersible Shinkai 6500. The samples thus obtained were diluted with marine broth and pressurized to desired pressure in a pressure vessel and incubated in a refrigerator at 2-4°C on board the cruise ship. We have used box corer to obtain deep-sea sediments. Subcores of sediments were collected from the centre of the box corer using alcohol-sterilized PVC cylinders of 5 cm diameter. Subsections of 2 cm were extruded from these sediment cores directly in to sterile plastic bags to avoid any aerial contaminants. The sediments were diluted with malt extract broth and pressurized to desired pressure in a pressure vessel and incubated in a refrigerator at 4-5°C on board the research vessel. Fungi were isolated from these sediment slurries after 20-25 days. Subsequently, a test for barophily or barotolerance was conducted by comparing the growth of these fungi under 50-500 bar hydrostatic pressure (Damare et al., communicated).

Helmke (1979) used deep-sea pressure vessel for culturing bacteria from Antarctic waters collected using Niskin water samplers. In order to assess growth of these isolates under elevated pressures, bacterial inoculum was kept in special air permeable bags, sealed and suspended in distilled water (also called hydraulic fluid) in a stainless steel cylinder to which hydraulic pressure is applied. Long time incubation of samples where only distilled water is used as hydraulic fluid does not produce sufficient biomass because of oxygen depletion in water. This problem was overcome by using fluorocarbon-filled pressure vessel (Helmke, 1979). Fluorocarbon liquids are

recommended as oxygen reservoirs for culturing microorganisms in such pressure vessels. Fumiyoshi and Horikoshi (2001) recommend addition of oxygenated fluorinert (FC-72) directly to the culture medium for culturing aerobic microorganisms. Increase in CO<sub>2</sub> concentration and formation of carbonic acids in closed systems was overcome by culturing deep-sea yeasts in gas permeable plastic bags for cultivation under high pressure and a fluorocarbon liquid (FC-77) as hydraulic fluid (Lorenz and Molitoris, 1997). Raghukumar et al. (1992) germinated conidia of the fungus *Aspergillus restrictus* isolated from 860 m depth in dilute malt extract broth and agar discs under hydrostatic pressures of up to 300 bar (30 MPa) in modified plastic syringes suspended in distilled water in a pressure vessel. As these experiments involved observing the germination of conidia, they were completed within 3-7 days and therefore the effect of decreasing oxygen levels in the hydraulic fluid was not felt. Jannasch et al. (1996) developed a pressurized chemostat for culturing marine barophilic bacteria under pressures of up to 700 bar (corresponding to 7000 m depth) using modified high-pressure chromatography equipment. To simulate low nutrient conditions in the deep-sea, Yayanos (2001) incorporated silica gel instead of agar with nutrient deficient medium for culturing barophilic bacteria.

## **Diversity of deep-sea microorganisms**

### ***Prokaryotic diversity***

Organisms growing under hydrostatic pressures of 5-100 MPa, low and high temperatures 2°-100°C, low and high organic nutrients (when dead carcass of large organisms are made available on sea floor) expected to be present in deep-sea conditions. Zobell and Morita (1957) were the first to report presence of barophiles in the deep sea. Deep-sea bacteria have been isolated even from depths of 10,500 m and cultured at > 100 MPa at 2°C and >40 MPa above 100° C (Yayanos et. al. 1981). Barophilic bacteria were isolated from several locations, and identified by 16S rRNA sequences (Kato et al.,1997, Li et al.,1998). Most of the barophilic and barotolerant bacteria belong to the Proteobacteria  $\gamma$  subgroup (Kato et al. 1996). Sediment samples were obtained from the world's deepest seafloor at 11,033 m in the Mariana Trench using unmanned submersible KAIKO belonging to JAMSTEC. DNAs extracted directly from these sediments were

amplified by PCR and the products were sequenced. The results showed coexistence of barophilic Archaea with *Pseudomonas* species in the Mariana Trench (Kato et al. 1997). In addition, filamentous fungi and actinomycetes, were isolated at 1 bar pressure (0.1 MPa) with almost the same frequency as that of facultative psychrophilic bacteria. Besides, several alkalophilic, thermophilic and non-extremophiles were isolated at 0.1 MPa from these sediments (Takami et al. 1997).

Pradeep-Ram et al. (2001) isolated an chemolithotrophic nitrifying bacterium from sediments at 5000 m depth in the Central Indian Basin that grew at 1 bar pressure. This bacterial isolate exhibited dual property of oxidizing ammonia to nitrite and nitrite to nitrate under 1 bar pressure at 28°C as well as under 400 bar pressure at 5°C. Nitrification is generally carried out by known nitrifiers which either oxidize ammonia to nitrite (Phase 1) or nitrite to nitrate (Phase 2). However, the deep-sea bacterial isolate from the Central Indian Basin performed both the phases of nitrification. Kato et al, (1998) isolated several organic solvent-tolerant bacteria from deep-sea sediments. Some of these can grow in the presence of toluene or benzene at concentrations greater than 10-50% (v/v). These authors further report that the frequency of such organic solvent-tolerant bacteria in deep-sea sediments is much more than in terrestrial source. Two extremely barophilic bacteria DB21MT-2 and DB21MT-5 isolated from sediments of the Mariana Trench at ~11,000 m were reported to contain (PUFA) polyunsaturated fatty acids (Fang et al., 2000). This is a unique feature because it has been believed that bacteria are unable to produce PUFA (Tornabene, 1985). Thus, deep-sea is a source of several such novel microbes. Physiology and barotolerance of some of these bacteria has been studied in great details (Table 1).

Culturable actinomycetes ranging in numbers from  $3.4 \times 10^2$  to  $1.6 \times 10^4$  CFU g<sup>-1</sup> deep-sea sediments have been isolated from Pacific Ocean but strangely 16S rDNA clone libraries failed to reveal the presence of markers of the actinomycetes taxa isolated or any actinomycete signatures (see Bull et al., 2000). Thus it appears that culturing and sequence-based techniques need to be rigorously tested with several validation experiments from different geographical sites in the deep sea.

### ***Eukaryotic diversity***

Kohlmeyer and Kohlmeyer (1979) observed fungal colonization of wooden panels suspended at deep-sea between 500 and 3000 m depth. However, these fungi were not cultured. Several culturable fungi such as *Cladosporium* spp., *Alternaria* spp., *Aspergillus sydowii*, *Nigrospora* spp., and *Penicillium* spp. from deep-sea water samples up to 4450 m have been reported from different geographical locations (Roth et al., 1964). Several filamentous fungi were isolated from deep-sea calcareous sediments using a dilute malt extract broth prepared with seawater (Raghukumar et al, 1992). We demonstrated germination of conidia in some of these fungi under 100 and 300 bar pressure (equivalent to 10 to 30 MPa, corresponding to 1000 and 3000 m depth) indicating their barotolerance. Most of these are common terrestrial fungi and it is possible that some of these fungi have acquired barotolerance (Raghukumar et al, 1992). Further, two more filamentous fungi *Aspergillus ustus* and *Graphium* sp were isolated from the deep-sea calcareous sediments of the Arabian Sea and the Bay of Bengal and these grew and produced extracellular protease under hydrostatic pressures of 100 bars (Raghukumar and Raghukumar, 1998). Lorenz and Molitoris (1997) successfully cultivated a few marine yeasts under 20 to 40 MPa pressure. Takami et al. (1997) have mentioned about the presence of fungi in sediments of the deepest trench, the Mariana Trench but no further details are given about kinds and number of fungi obtained.

Recently, a filamentous fungus *Aspergillus sydowii* was isolated at a depth of 370 cm depth of a 470 cm long sediment core sample taken from 5000 m depth in the Central Indian Basin (Raghukumar et al., 2004). The sediment from which *Aspergillus sydowii* was isolated was dated to be 420,000 years old. Besides *Aspergillus sydowii*, some non-sporulating fungi were also isolated from these sediments. The spores of *A. sydowii* germinated at 500 bar pressure. We have also observed spores of mycelial fungi and hyphae in the deep-sea sediments of the Central Indian Ocean (unpublished) after staining the sediments with optical brightener Calcofluor (Fig. 8). Interestingly enough there were viable bacteria at depths of 400 cm of the sediment core (Raghukumar et al., 2004).

Besides fungi, heterotrophic, unicellular protist the thraustochytrids belonging to the kingdom Straminipila are also found in deep-sea waters (Raghukumar et al., 2001). A thraustochytrid protist *Schizochytrium* sp isolated from faecal pellets of the tunicate *Pegea confoederata* was cultured on pine pollen and Artemia larval baits at 100 bar pressure (Raghukumar and Raghukumar, 1999).

Deep-sea protozoan species *Bodo* from a North Atlantic sediment sample showed growth up to 45 MPa but showed no growth at atmospheric pressure. Strangely, this flagellate could be isolated without retaining the *in situ* pressure but required high pressure for its growth (Turley and Carstens, 1991).

### **Adaptations in deep-sea microorganisms**

#### ***Adaptations in prokaryotes***

The extremophiles are native to their respective environment and therefore, should one look for any adaptation strategies in the form of genes or proteins *per se* in these? They may be novel from the point of mesophilic organisms and that is not unexpected. Therefore, most of the studies regarding understanding of pressure effects have come from moderately barophilic or barotolerant microorganisms. The effect of pressure on cell membrane, protein and gene expression are studied in detail in some of these microorganisms.

Cold temperatures and high pressures decrease membrane fluidity and affect a number of membrane-associated processes including ion and nutrient flux and DNA replication (Bartlett, 1992). A barotolerant strain of *Alteromonas* isolated from 4033 m in the Izu-Ogasawara Trench, Japan showed an increase in the proportion of unsaturated fatty acid composition in membrane fraction with increased pressure during its growth indicating its possible involvement in maintaining the fluidity of the cell membrane (Kamimura et al., 1993). The membrane phospholipids of two barophilic bacteria DB21MT-2 and DB21MT-5, isolated from sediments from Mariana Trench at 11,000 m showed wide distribution of polyunsaturated fatty acids, suggesting that adaptation of the barophiles to low temperature and high hydrostatic pressure influenced the synthesis of phospholipids containing polyunsaturated fatty acids (Fang et al., 2000).

Hydrostatic pressure also influences the abundance of cell surface proteins in bacteria adapted to low or high pressures. The moderately barophilic deep-sea bacterium *Photobacterium* SS9 was reported to regulate the abundance of several outer membrane proteins (omp) in response to hydrostatic pressure (Chi and Bartlett, 1993). One outer membrane protein *ompH* was induced 10-100-fold in SS9 cells grown at 28 MPa. These authors suggested that *ompH* probably acts as a non-specific porin protein involving in uptake of substrates larger than 400 daltons. Mutants that lacked *ompH* gene were however not pressure-sensitive suggesting that *ompH* is required for high pressure growth under other physiological conditions present in the deep-sea such as low nutrient conditions or it may be one of the multiple proteins facilitating growth at high pressure (Chi and Bartlett, 1993).

Barotolerant, barophilic and mesophilic bacteria showed elevated levels of several pressure-inducible proteins (PIPs) when analysed by two-dimensional polyacrylamide gel electrophoresis (2-D-PAGE). Many of the identified PIPs were either cold-shock, heat shock or ribosomal proteins (Bartlett et al., 1995). High or low temperatures, high pressure are shown to destabilize tertiary and quaternary structure of proteins (Craig and Gross, 1991). Thus, pressure induced increases in the proportion of partially unfolded proteins or dissociated protein subunits could induce heat-shock proteins. High pressure could also turn on both heat and cold shock proteins (hsps and csps) because of its inhibitory effects on ribosome assembly or functions (Gross et al., 1993).

A promoter, activated at high pressure from a barophilic strain of *Photobacterium* sp. was cloned in *Escheichia coli*. Analysis of transcription showed that the pressure-regulated operon was expressed under elevated pressure and the largest amount of transcript was observed at 70 MPa (Horikoshi, 1998). These highly conserved pressure-regulated operons have been found in many deep-sea adapted bacteria (Bartlett et al., 1995). A moderately barophilic strain of *Shewanella* sp that grows at 30 MPa and 8°C and also at atmospheric pressure (0.1MPa) and 8°C possessing a pressure-regulated operon (containing open reading frames ORF 1 and ORF 2) has been cloned and sequenced. The strain appears to produce different DNA-binding factors under different pressures (Nakasone et al., 1998).

Besides elevated hydrostatic pressures, the deep-sea organisms are also adapted to low temperatures. Synthesis of polysaccharides is greater in cold-adapted microorganisms than in mesophiles. Some psychrotrophs showed differential utilization of substrates at different temperatures (Margesin and Schinner, 1994).

Solubility of oxygen increases at low temperatures and thus its availability also increases and therefore, psychrophilic and psychrotolerant microorganisms are more favourably affected by aeration than mesophiles (Margesin and Schinner, 1994).

High lipid content in membranes of psychrotrophs and psychrophiles facilitates membrane transport of solutes. They also contain more unsaturated fatty acids when grown at low temperatures than mesophiles. An increase in unsaturated fatty acids leads to a decrease in lipid melting point and allows to maintain the lipid in a liquid and mobile state and helps to regulate membrane fluidity (Margesin and Schinner, 1994).

Cold-adapted enzymes have high specific activity and low thermostability (Gerday et al., 2000). These authors further explain that thermostability is derived from high rigidity of the molecule which retards the interaction between substrate and enzyme, leading to a weak specific activity. By contrast flexibility or plasticity of a molecular structure at low temperature enables interactions at a low energy cost, which explains the high specific activity of cold-adapted enzymes.

### ***Pressure adaptations in eukaryotes***

Yeasts have been used as model organisms for studying pressure-adaptations in eukaryotic heterotrophs. It has been hypothesized that chemical reactions that are accompanied by an increase in volume, are inhibited by increasing pressure. Abe and Horikoshi (1998) demonstrated that cytoplasm and vacuole in *Saccharomyces cerevisiae* were acidified under hydrostatic pressure and this was similar to production of carbon dioxide during glycolysis. They suggested that maintenance of the cytoplasm at neutral pH might be one of the mechanisms required for piezoadaptation.

Abe and Horikoshi (2000) showed that inhibition of growth in *S. cerevisiae* under high pressure is due to impairment of uptake of tryptophan which in turn is regulated by high-affinity tryptophan permease Tat2. Addition of excess tryptophan or overexpression

of Tat2 protein enable *S. cerevisiae* cells to grow at 25 MPa suggesting that uptake of tryptophan in yeast cells is one of the key processes for survival under hydrostatic pressure. Iwahashi et al, (1993) generated 10 mutant strains of this yeast by random mutagenesis. Further studies with one of the mutant strain CWG8, the intracellular trehalose concentrations were shown to correlate with pressure tolerance (Fujii et al., 1996). Under the culture conditions employed by these authors, the difference in barotolerance between exponential-phase cells and early stationary-phase cells appeared to be mainly dependent on the difference in cellular trehalose content. Further, the levels of hsp's were not associated with barotolerance in CWG8. Fernandes et al, (1997) demonstrated that mutant of *S. cerevisiae* with deleted trehalose-6-phosphate synthase gene (*tps11*) was unable to accumulate trehalose and was more sensitive to hydrostatic pressure than the wild-type cells.

Yeast cells show increased viability under hydrostatic pressure after heat-shock treatment and it has been proposed that the effect of hydrostatic pressure on yeast is analogous to high temperatures and that a heat-shock pre-treatment is able to induce barotolerance (Komatsu et al., 1991; Iwahashi et al., 1991). However, Fernandes et al, (1997) demonstrated that pressure pre-treatment of *tps11* mutant cells of *S. cerevisiae* to a hydrostatic pressure of 50 MPa did not induce barotolerance.

In prokaryotic and eukaryotic microorganisms response to cold shock is manifested with the synthesis of cold shock proteins (csps). *Trichosporon pullulans*, a psychrotrophic yeast when subjected to a temperature decrease from 21°C to 5°C, produced 26 csps within 12 h (Julseth and Inniss, 1990).

Morphological variations were seen in fungi grown under elevated hydrostatic pressure. The filamentous fungus *Graphium* sp isolated from deep-sea calcareous sediment showed microconidiation, where a conidium gives rise to conidia directly without intervening hyphal growth. *Aspergillus ustus* when grown under elevated pressure of 100 bar, the resting cells called hülle cells showed germination (Raghukumar and Raghukumar, 1998). The fungal hyphae showed thick swellings and beaded-structures (Figs. 9a-c) when grown under elevated pressure (Lorenz, 1993; Raghukumar and Raghukumar, 1998). Lorenz (1993) observed multiple germ tube formation in yeasts

*Rhodospiridium sphaerocarpum* when subjected to 450 bar pressure. A deep-sea isolate of yeast when grown under 200 bar pressure showed filamentous growth (Figs. 9d-e).

Most of these deep-sea fungi showed better growth and germination of conidia at 30°C/200 bar than at 5°C/200 bar pressure. When mycelium was used as the initial inoculum source, terrestrial fungi and fungi isolated from shallow depths also showed growth under elevated pressure. These results further explain the presence of terrestrial fungi in the deep-sea. However, spores of deep-sea fungi and not that of terrestrial species germinated under elevated pressure (Damare et al., communicated). Thus, pressure appears to have different effects on mycelium and spores of fungi. Bartlett (2002) reported that bacterial and archaeal piezophiles in culture are closely related to shallow-water microbes which are not piezophilic. Thus it appears that “high-pressure selection has not required the evolution of dramatically different lineages of life”.

The deep-sea adapted terrestrial fungi showed higher protease production at 100 bar pressure when incubated at 30° C than at 5° C (Fig. 10). Biomass production by these fungi at 5°C and 100 bar is lower than at 30°C/100 bar (Raghukumar and Raghukumar, 1998). Thus, it appears that lower temperature further retards the growth and metabolism in filamentous fungi.

### **Biotechnological applications**

There is a growing interest in extremophilic organisms because “curiosity driven research” is slowly, forcibly or fashionably replaced by “value-added product research”. Extreme environments certainly harbour novel and unusual organisms that are good tools for basic and applied research. Applications of cold- adapted enzymes such as proteases, lipases, cellulases and  $\alpha$ -amylases as additives in detergents are enormous. A reduction in energy consumption and longer life for clothes are the obvious advantages in cold wash. Cold-adapted enzymes are extremely useful in food industry. Proteases for meat tenderization, pectinases for fruit juice extraction, cheese manufacturing are some of the examples where cold adapted enzymes are useful. Bioremediation of hydrocarbons, heavy metals and biopolymers in contaminated soils and waste-waters using cold-adapted microorganisms is an alternative to physicochemical methods in temperate countries (Gerday et al., 2000).

High pressure has been used in place of high temperatures for food processing and the pressure-sterilization of food materials for improved preservation of flavour and color. Short-term treatment at elevated pressure kills or inactivates microorganisms (see Fumiyoshi and Horikoshi, 2001).

The osmolarity of intracellular medium of cells varies in different organisms (Fumiyoshi and Horikoshi, 2001). Proteins in piezotolerant microorganisms that can grow at both atmospheric pressure and elevated hydrostatic pressures undergo different hydration effects under each condition. Such microorganisms might produce certain restriction endonucleases or DNA-binding proteins that are adapted to function either at atmospheric pressure or high pressure under certain conditions of osmolarity (Fumiyoshi and Horikoshi, 2001). Such piezophiles might be of interest to explore for novel restriction endonucleases or other DNA-binding proteins. Low temperature or high temperature piezophiles have received most of the attention. However, piezophiles that grow at 25-37° C (called as mesophilic-piezophiles) will also be worth studying (Fumiyoshi and Horikoshi, 2001). Organic solvent-tolerant bacteria isolated from deep-sea may be useful in degradation of hydrocarbon and other xenobiotics. Some of the deep-sea fungi isolated by us produce substantial amounts of low-temperature-tolerant alkaline protease (Damare et al., communicated).

## **Conclusion**

Deep sea environment offers potential for development of new techniques for retrieval of sediment and water samples while retaining *in situ* conditions and newer methods and skills for isolation of microorganisms. This might yield new microorganisms having novel biomolecules. Deep-sea adapted microorganisms (barotolerant) are better suited for manipulation and maintenance and thus they should also receive equal importance. High-pressure regulated system for gene expression is found not only in deep-sea adapted bacteria, but also in bacteria growing at atmospheric pressure, such as *E.coli* (Sato et al., 1995). There is a possibility that high pressure gene expression system is conserved in life adapted to atmospheric pressure. This might also indicate that life initiated in the deep-sea environment. Therefore, distribution and occurrence of pressure tolerant gene

expression system in one atmosphere-adapted organisms needs to be looked into various bacteria, fungi and actinomycetes to understand its role and importance.

The use of small subunit ribosomal RNA (SSU rRNA) sequences to amplify environmental samples directly has revealed a whole lot of new prokaryotic lineages at phylum and division levels (Dojka et al., 2000). Using the same tools a vast number of heterotrophic eukaryotes in the size range of prokaryotes (1-2  $\mu\text{m}$ ) have been discovered at 3000 m depth in oceanic waters. These included ciliates, fungi and thraustochytrids besides lineages not closely related to any known organisms (Lopez- Garcia et al., 2001). Culturing these huge uncultured hidden microbial diversity in deep-sea waters is going to be a challenging task.

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Table 1. Barophilic and barotolerant microorganisms reported from various regions of the world oceans.

<b>Organism</b>	<b>Growth conditions</b>	<b>Source of isolation</b>	<b>Reference</b>
<b>Bacteria</b>			
<i>Colwellia</i> MT41	103 MPa, 8° C	Amphipod from Pacific Ocean, 10,476 m depth	Yayanos et al. 1981
Strain MT199	90 MPa, 13° C	Pacific Ocean, 10,476 m depth	Yayanos et al. 1981
<i>Shewanella</i> SC2A	14 MPa, 20° C	Pacific Ocean, 1957-10,476 m depth	Yayanos et al. 1982
Strain PT64	90 MPa, 9° C	''''	Yayanos, 1986
<i>Colwellia hadaliensis</i> BNL1	85 MPa, 10° C	Amphipod, Pacific Ocean, 10,476 m depth	Deming et al., 1988
<i>Moritella japonica</i> DSK1	50 MPa, 15° C	Japan Trench, 6269 m depth	Kato et al., 1995
<i>Shewanella violacea</i> DSS12	30 MPa, 10° C	''''	Kato et al., 1995
<i>Shewanella benthica</i> DB6101	50 MPa, 10° C	''''	Kato et al., 1995
<i>Shewanella benthica</i> DB5501	60 MPa, 15° C	''''	Kato et al., 1995
<i>Shewanella benthica</i> DB6705	60 MPa, 15° C	''''	Kato et al., 1995
<i>Shewanella benthica</i> DB6906	60 MPa, 15° C	''''	Kato et al., 1995
<i>Shewanella benthica</i> DB172R	60 MPa, 10° C	Izu-Bonin Trench, 6499 m depth	Kato et al., 1996
<i>Shewanella benthica</i> DB172F	70 MPa, 10° C	''''	Kato et al., 1996
<i>Desulfovibrio profundus</i> 500-1 <sup>T</sup>	15 MPa, 25° C	Japan Sea, 500 m depth	Bale et al., 1997
Strain CNPT3	52 MPa, 8° C	Pacific Ocean	DeLong et al., 1997
<i>Moritella</i> PE36	41 MPa, 15° C	''''	DeLong et al., 1997
<i>Shewanella benthica</i> strain FIA	41 MPa, 8° C	''''	DeLong et al., 1997
<i>Shewanella benthica</i> strain PT48	62 MPa, 8° C	''''	DeLong et al., 1997
<i>Shewanella benthica</i> strain PT99	62 MPa, 8° C	''''	DeLong et al., 1997
<i>Photobacterium profundum</i> Strain DSJ 4	10 MPa, 10° C oC	Ryukyu Trench, Japan, 5,110 m depth	Nogi et al. 1998
<i>Moritella yayanosi</i> DBT1MT-5	80 MPa, 10° C	Mariana Trench, 10,898 m depth	Kato et al., 1998
<i>Shewanella benthica</i> DBT1MT-2	70, 10° C	''''	Kato et al., 1998

<b>Archaea</b>			
<i>Methanococcus thermolithotrophicus</i>	50 MPa, 65 ° C	Hydrothermal vent	Bernhardt et al., 1988
<i>Methanococcus jannaschii</i>	75 MPa, 86 ° C	Hydrothermal vent	Miller et al., 1988
<i>Pyrococcus abyssii</i>	20 MPa, 95 ° C	Hydrothermal vent, Fiji Basin, 2000 m depth	Marteinsson et al., 1997
<i>Thermococcus barophilus</i>	40 MPa, 85 ° C	Hydrothermal vent, Snakepit on Mid-Atlantic Ridge, 3550 m depth	Marteinsson et al., 1999
<b>Barotolerant fungi</b>			
<i>Aspergillus restrictus</i>	30 MPa 30oC	Calcareous sediment, Bay of Bengal	Raghukumar et al., 1992
<i>Aspergillus ustus, Graphium sp</i>	10 MPa, 10° & 30°C	Calcareous animal shells from 860 m in the Arabian Sea and 965 m in the Bay of Bengal respectively	Raghukumar and Raghukumar, 1998
<i>Aspergillus sydowii</i>	50 MPa, 30° & 5°C	Deep-sea sediment core of 470 cm length at ~5000 m depth	Raghukumar et al., 2004
<b>Thraustochytrids</b>			
<i>Schizochytrium sp</i>	10 MPa	Faecal pellets of salps in the Arabian Sea	Raghukumar and Raghukumar, 1999

## Legends to the Figures

Fig. 1. A Rosett sampler fitted on to a CTD frame with several bottles for collecting oceanic water samples from different depths.

Fig. 2. ZoBell sampler for collecting water sample in pre-sterilized bottle for microbiological studies.

Fig. 3. Multiple corer for collecting deep-sea sediment cores.

Fig. 4. Box corer for collecting deep-sea sediments in large volume.

Fig. 5. Culture vessel and a pressurizing unit for culturing bacteria and fungi under hydrostatic pressures.

Fig. 6. A water sampler for collecting water samples with *in situ* pressure and a transfer unit (Jannasch et al., 1973).

Fig. 7. A water sampler for collecting water samples up to depths of 1200 m with *in situ* pressure (Naik and Raghukumar, 1999, patent filed).

Fig. 8. Direct detection of fungal hypha in deep-sea sediment after staining with the optical brightner Calcofluor. The sample is viewed under an epifluorescence microscope where the fungal hyphae show bright blue fluorescence.

Fig. 9. Growth of a fungus (a- c) and a yeast (d, e) under 100 bar pressure.

Fig. 10. Protease production in two fungi, *Aspergillus ustus* and *Graphium* sp under various conditions of pressure and temperature. Values are mean of 3 replicates at 5% confidence level (Raghukumar and Raghukumar, 1998).



Fig. 1

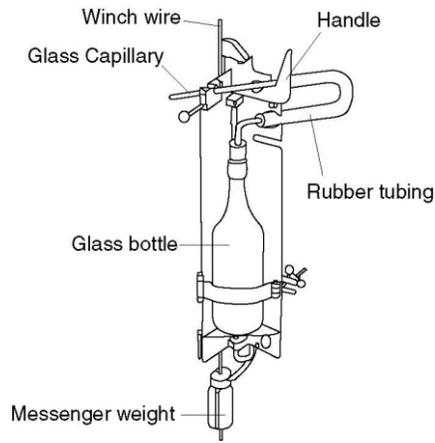


Fig. 2

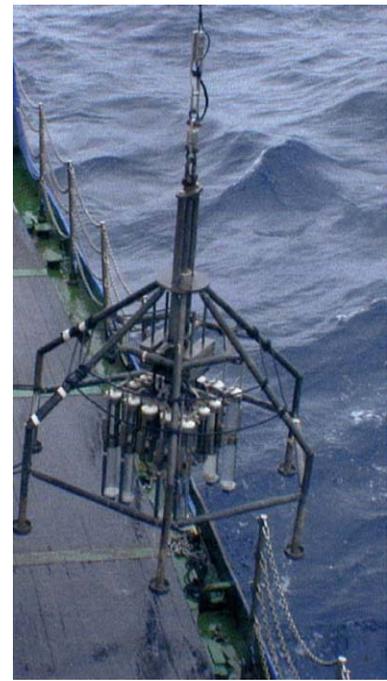


Fig. 3



Fig. 4



Fig. 5

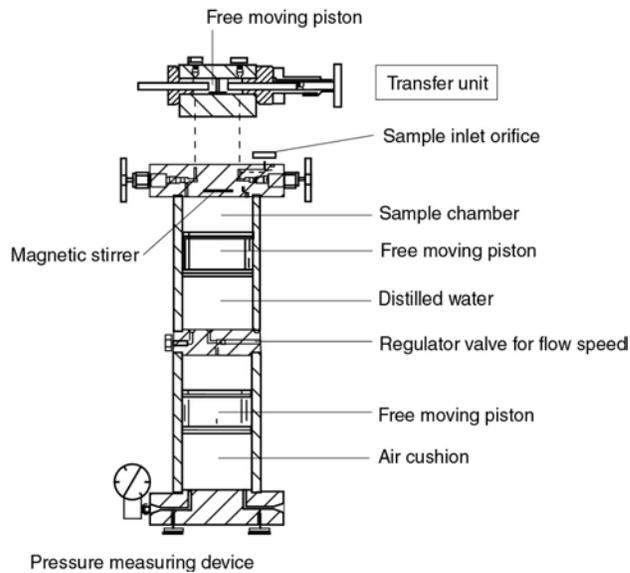


Fig. 6



Fig. 7

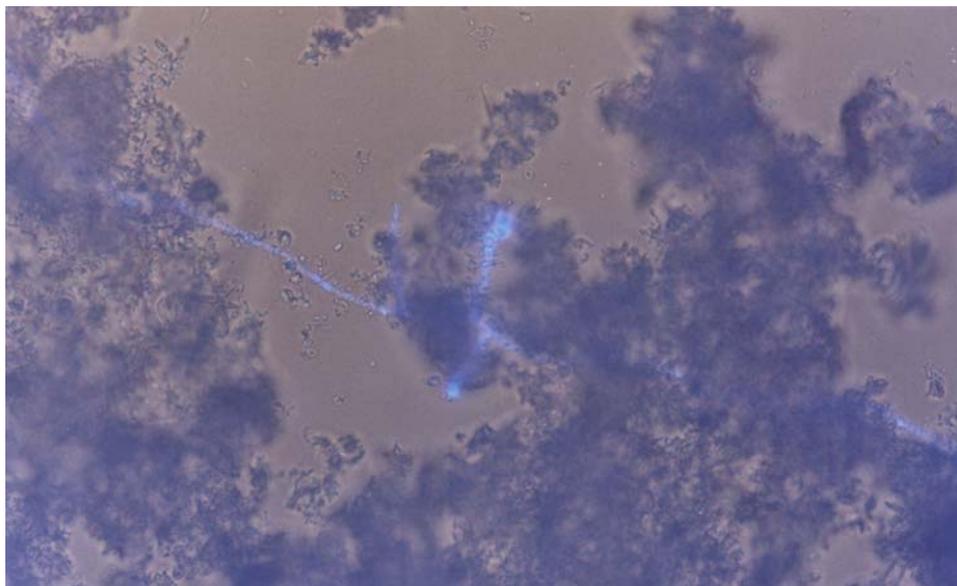


Fig. 8

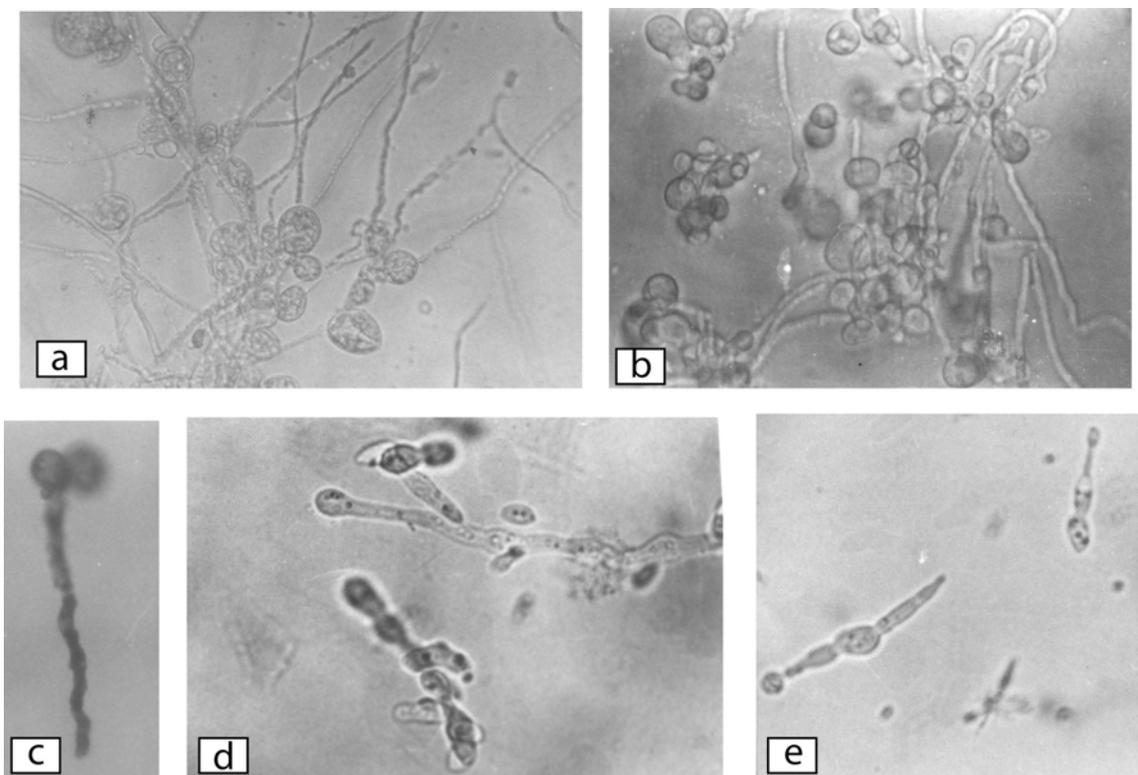


Fig. 9

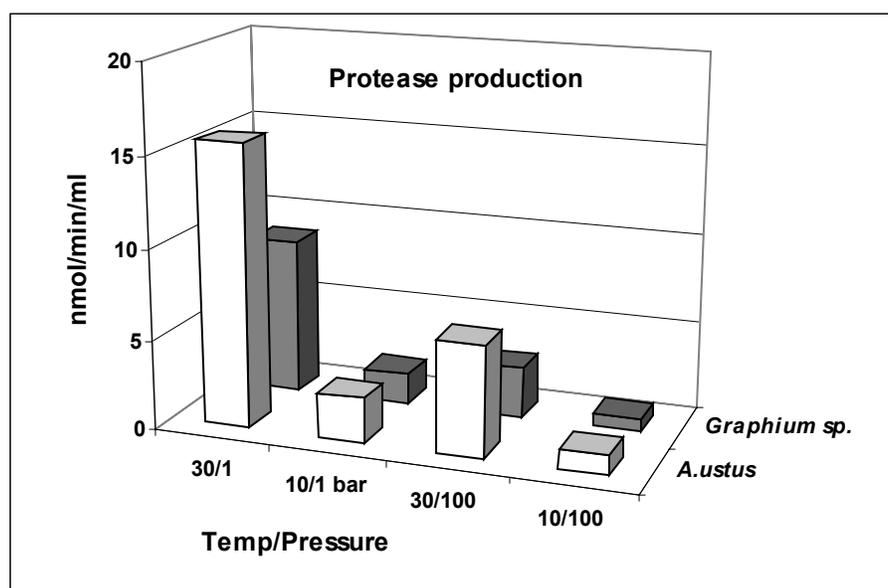


Fig. 10.