

Phylogenetic diversity of culturable fungi from the deep-sea sediments of the Central Indian Basin and their growth characteristics

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Abstract

Very few studies have addressed the diversity of culturable fungi from deep-sea sediments. We are reporting here the diversity of culturable fungi in deep-sea sediments of the Central Indian Basin obtained from a depth of ~5000 m. A total of 16 filamentous fungi and 12 yeasts isolated from 20 sediment cores of ~35 cm length were identified by 18S and ITS sequencing of SSU rDNA. Most of the filamentous fungi were Ascomycota, while two were Basidiomycota. Microscopic identification of sporulating cultures mostly matched either with 18S or ITS sequences but seldom with both the sequences. Phylogenetic analysis of ascomycetes using 18S sequence data grouped them into 7 clusters belonging to *Aspergillus*, *Sagenomella* sp, *Exophiala* sp, *Capronia* sp., *Cladosporium*, *Acremonium* sp. and *Tritirachium* sp. ITS sequence data grouped isolates into 6 clusters belonging to *Aspergillus* sp, uncultured member of Hypocreaceae, *Exophiala* sp, uncultured soil fungus, Hypocreales and *Trichothecium* sp. The two basidiomycete isolates were a *Tilletiopsis* sp. evident from 18S as well as ITS sequence data. In contrast, most of the yeast isolates belonged to Basidiomycota and only one isolate belonged to the phylum Ascomycota. Sequences of 18S as well as ITS gave matching identification of most of the yeasts. Filamentous fungi as well as the yeasts grew at 200 bar/5°C indicating their adaptations to deep-sea conditions. This is the first report on isolation of *Sagenomella*, *Exophiala*, *Capronia* and *Tilletiopsis* spp from deep-sea sediments. This study reports on the presence of terrestrial fungi as a component of culturable fungi in deep-sea sediments.

Key words: Deep-sea sediments, fungi, 18S, ITS sequences, hydrostatic pressure, Central Indian Basin

Introduction

Although only about 1% of the photosynthetically produced organic carbon reaches the deep-sea floor, the deep sea is considered the largest sink for organic carbon on the earth. A substantial part of the surface carbon dioxide from the euphotic zone is sequestered into the deep sea by biological-mediated processes. Carbon reaching the deep sea is both organic as well as inorganic, the former being contributed by dead plankton, faecal material and other organic waste, the latter being largely calcium carbonate (Raven and Falkowski, 1999). Bacteria and fungi play an important role in decomposition of such organic matter, under elevated hydrostatic pressure and low temperatures of deep-sea habitat. These heterotrophs use organic carbon to build their biomass and respiration, releasing a part of the organic carbon as dissolved CO₂.

Several studies have shown high bacterial diversity and population, both cultured and yet-uncultivated, in relatively low organic carbon-containing deep-sea sediments and water column with its extreme environmental conditions (Takami *et al.*, 1997; Li *et al.*, 1999; Eilers *et al.*, 2000). Diversity of culturable actinomycetes from tropical Pacific Ocean sediments up to a depth of 570 m was reported recently (Jensen *et al.*, 2005). The presence of fungi was reported from deep-sea water columns (Roth *et al.*, 1964), calcareous sediments (Raghukumar and Raghukumar, 1998), sediments from Mariana Trench at 11,500 m depth (Takami *et al.*, 1997), Chagos Trench at a depth of 5500 m (Raghukumar *et al.*, 2004) and the Central Indian Basin at ~5000 m depth (Damare *et al.*, 2006). These developments clearly illustrate the increasing attention being paid to fungal abundance and diversity in deep-sea environments. In all of these reports culturable fungi have been identified by classical microscopic methods. As several of the cultured fungi did not show sporulating structures, these were grouped under non-sporulating fungi (Raghukumar *et al.*, 1992; 2004; Damare *et al.*, 2006). Identification of culturable filamentous and unicellular fungi from deep-sea sediments using molecular methods has not been reported, although ample evidence of fungal signatures in the form of 18S-rDNA genes have been reported from deep-sea sediments (Bass *et al.*, 2007; López-García *et al.*, 2007). The aim of the present study was to isolate culturable fungi using different culturing techniques and culture media from different sections of deep-sea sediment cores obtained at a depth of ~5500 m from the Central India Basin. Further, we aimed to identify them by classical microscopic and molecular methods. Small subunit (SSU) rDNA region was selected for identification by amplification and sequencing since it has been reported to be conserved in nature (Edgecomb *et al.*, 2002). Amplification and sequencing was done for 18S and ITS regions using universal fungal primers (Smit *et al.*, 1999) to identify the cultures

up to species level. The deep-sea fungi were further tested for their growth and spore germination under simulated deep-sea conditions of elevated hydrostatic pressure, low temperature and varying salinities.

Materials and Methods

Collection of Sediment samples

Sediment samples were collected from depths of 4000 to 5700 m in the Central Indian Basin (10-16.5°S and 72-77°E) on board the Russian research vessel Akademik Boris Petrov (Cruise # ABP26) in December 2006 (Table 1). A total of 20 sediment cores were collected with an USNEL-type box corer of 50 cm³ size. Sediments thus collected were mostly undisturbed and compact. Moderate brown sediments dominated the core tops followed by yellow-brown clayish sediments. Intercalations and mottling were seen in most of the sediment cores. Majority The majority of the sediment cores were siliceous in texture. One core (BC-37) was fully calcareous in composition. Sub-cores of sediments were collected from a box corer using an alcohol-sterilized PVC cylinder of 5 cm inner diameter. Subsections of 2 cm down to a depth of 10 cm and thereafter every 5 cm length up to 35-40 cm depth were cut from the above sediment and directly introduced into sterile plastic bags to avoid any aerial contamination. The bags were closed and carried to the microbiology laboratory on board for further processing.

Isolation of fungi

An aliquot of sediment from the central part of the subsection was removed with a sterile spatula and placed in sterile vials for isolation (Raghukumar *et al.*, 2004). Four different media were used for isolation of fungi and they were 1) Malt Extract Agar (MEA), 2) Czapek Dox Agar (CDA), 3) Corn Meal Agar (CMA) and 4) Malt Extract Broth (MEB) (Damare *et al.*, 2006). The above media were used at 1/5 strength to simulate low nutrient conditions of deep-sea sediments. They were prepared in seawater and supplemented with Penicillin (40,000 units in 100 mL medium) and streptomycin (0.1 g in 100 mL medium) to inhibit bacterial growth. Isolation of fungi from sediments was done by the following methods: 1) Particle plating method of Bills and Polishook (1994); 2) Dilution plating 3) Pressure Incubation and 4) Enrichment method (Damare *et al.*, 2006). The plates were incubated at 5°C for 30 days. Air-borne fungi contaminating the culture plates was monitored by exposing various media plates for 10 min on the deck of the research vessel where the cores were received, the microbiology laboratory on board the research vessel and the inoculation hood. This was repeated during every sampling station.

After isolation, fungal cultures were maintained on MEA slants at 5°C. The isolates were originally numbered as NIOCC (National Institute of Culture Collection). The sporulating fungi were deposited in a recognized microbial culture repository, Microbial Type Culture Collection (MTCC) at Chandigarh, India, under the accession number MTCC.

Isolation of DNA from the cultures

The fungi were grown in MEB for 4-5 days for DNA isolation. Yeasts were grown in yeast extract peptone and dextrose (YPD) medium and shaken at 170 rpm for 3-4 days. Mycelia and cells were harvested, lyophilized and crushed in a mortar and pestle to fine powder. Isolation of DNA was carried out following the modified form of standard procedure (Stoeck and Epstein, 2003). The above samples were incubated at 65°C for two hours in a high salt extraction buffer for fungal DNA (100 mM Tris-HCl buffer with 8 pH containing 100 mM Na₂EDTA, 100 mM NaPO₄, 1.5 M NaCl, 1% cetyltrimethylammonium bromide, 20% Sodium Dodecyl Sulphate, Proteinase K (100 µg mL⁻¹ final concentration]) A low salt extraction buffer containing 10 mM Tris-HCl with pH 8, 10 mM Na₂EDTA pH 8, 0.5 M NaCl, 1% cetyltrimethylammonium bromide, 20% Sodium Dodecyl Sulphate and Proteinase K (100 µg mL⁻¹ final concentration) was used for isolating yeast DNA.

PCR amplification of 18S and ITS regions of rDNA

Partial region of SSU rDNA was PCR amplified by using universal fungal primers, NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTTCCGTCAATTCCTTTAAG-3') of ~1100 bp. Full length of ITS region of ~600 bp was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990). The conditions for PCR included an initial hot start incubation (5 min at 94°C) followed by 34 cycles (denaturation at 94° C for 30 sec, annealing at 55° C for 30 sec and extension at 72° C for 1 min) and a final extension at 72° C for 5 min.

Sequencing and phylogenetic analyses

Fresh PCR products were purified by using gel extraction kit (Sigma, Genosys, USA) and sequenced at National Centre for Cell Sciences, Pune, India, using the Big Dye Terminator cycle sequencing kit (V3.1, Applied Biosystems, USA) according to the manufacturer's protocol and analyzed in a DNA Analyzer (3730 DNA Analyzer, Applied Biosystems, USA). Sequence data were edited using Chromas Pro version 1.34. For tentative identification, fungal 18S and ITS rDNA sequences were

compared with NCBI (National Centre for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>) database. Fungal rDNA -18S and ITS sequences in this study and the matched sequences from GenBank were edited and aligned using CLUSTAL-X, version 1.81 (Thompson *et al.*, 1997). The aligned sequences were imported into DAMBE 4.5.47 (Xia and Xie, 2001). Neighbor-joining (NJ) trees were created using pairwise genetic distances using MEGA 3.1 (Sudhir kumar *et al.*, 2008). The quality of the branching patterns for NJ was assessed by bootstrap resampling of the data sets with 1,000 replications.

Growth of mycelial fungi and yeasts under simulated deep-sea conditions

For raising mycelial inoculum, cultures of 16 deep-sea fungi were grown in MEB for 3 days at 1 bar and 30°C. Vegetative mycelium prior to the onset of sporulation was homogenized with sterile glass beads. A known weight of finely broken mycelial suspension was inoculated in 5 mL of MEB in pouches made with sterilized gas permeable polypropylene sheets and sealed without trapping any air bubbles. The pouches were suspended in a deep-sea culture vessel (Tsurumi & Seiki Co., Japan) and incubated at 30°C/1 bar, 30°/200 bar, 5°/1 bar and 5°/200 bar for comparing the effect of different pressure and temperature conditions on growth. After 20 days, the contents of the pouches were filtered over pre-weighed filter papers, dried to a constant dry weight and the difference between the initial and final biomass determined as mycelial dry weight (Raghukumar and Raghukumar, 1998). The yeasts were similarly grown in YPD broth and the biomass was lyophilized and weighed. The biomass of mycelial fungi and yeasts were also compared in media made with sea water (35 ppt) and distilled water.

Spore germination under simulated deep-sea conditions

Selected four sporulating fungi (NIOCC #F2, F5, F6 and F13) were grown in MEA plates at 1 bar pressure and 30°C, and the spores were collected by gently flooding the plates with sterile seawater. The spore suspension was appropriately diluted with sea water containing 2% Tween 80 and vortexed for 5 min. One mL of this spore suspension was added to 5 mL of the sediment extract medium (Damare and Raghukumar, 2008) prepared with three different salinities, 1.7, 17 and 34 ppt and packed in pouches. These pouches were incubated at 200, 300, 400 and 500 bar pressure at 30°C for 15 days. The percentage of germination was calculated by counting germinated spores in 20 microscope fields.

Statistical analyses

The statistical analyses were carried out in Microsoft Excel program and PRIMER 5 software.

Results

Isolation and distribution

Pressure incubation of fungi prior to isolation yielded maximum recovery of culturable fungi (Table 2). A total of 16 filamentous fungi and 12 yeasts were isolated from deep-sea sediments during the cruise # ABP 26. No fungi were recovered below 35 cm depth of sediment cores, the maximum colony forming units were recovered at 4-6 cm depth. Species richness (2.8) and Shannon Index (1.8 log e) were accordingly highest at this depth. Species diversity was scanty in all the sections of cores, the number ranging from 1 to 4. Among the various media used for isolation of fungi, MEA appeared to be the best medium for maximum recovery of fungi. Dilution plating yielded the maximum diversity of fungi. The sporulating cultures were deposited in Microbial Type Culture Collection, Chandigarh, India under accession number MTCC. The basidiomycete *Tilletiopsis* sp. and the yeast *Sporidiobolus johnsonii* and one ascomycete *Sagenomella* sp. were the only three species recovered from the deepest part of the sediment core, namely 25-35 cm depth .

Diversity

The identification of sporulating cultures carried out by classical morphological taxonomy matched with molecular identification based on either 18S or ITS sequences and only seldom with both these sequences (Table 3). The 18S and ITS sequences of all the cultures were deposited in GenBank and accession numbers obtained (Table 3). Aligned sequences for the phylogenetic trees are submitted to TreeBASE (study accession No. S2215, journal peer reviewers Pin No. 166658). Most of the fungi belonged to the phylum Ascomycota and based on 18S sequences clustered into 7 subgroups, namely *Aspergillus* sp, *Sagenomella* sp, *Exophiala* sp, *Capronia* sp., *Cladosporium* sp., *Acremonium* sp and *Tritirachium* sp. (Fig. 1). Based on ITS sequences they grouped into 6 clusters showing maximum similarity to *Aspergillus* sp, uncultured member of Hypocreaceae family, *Exophiala* sp, an uncultured soil fungus clone (DQ682584.1), Hypocreales and *Trichothecium* sp. (Fig. 2). The two basidiomycetes (NIOCC #F21 and #F33) belonged to *Tilletiopsis* sp. both by 18S as well as ITS sequencing (Figs. 1, 2 and Table 3). Species of filamentous fungi *Sagenomella*, *Exophiala*, *Capronia*, and *Tilletiopsis* species are being reported for the first time from the deep-sea sediments.

The majority of the yeast isolates belonged to the phylum Basidiomycota (Figs. 3 and 4) and only one isolate, NIOCC # Y8 belonged to Ascomycota identified both by 18S and ITS sequencing (Fig. 4 and Table 3). Sequences of 18S as well as ITS gave matching identification of yeasts (Table 3). The

basidiomycetous yeast belonged to the genera *Sporobolomyces* sp, *Sporidiobolus*, *Rhodospiridium*, *Rhodotorula* and *Cryptococcus* sp. A 100 % consensus in identification was seen between 18S and ITS sequences of the isolates NIOCC#Y2, Y3, Y6, Y7 and PY12 up to generic level (Table 3).

Growth characteristics

All of the deep-sea fungi, mycelial as well as the yeasts showed growth under 200 bar pressure and 5°C temperature (Fig. 5A & 5B). A one-way analysis of variance (ANOVA) revealed that biomass produced was significantly different at different hydrostatic pressure and temperature conditions (see the legend for Fig. 5A & 5B). These cultures showed better growth in media prepared with sea water than with distilled water (Fig. 6A & 6B). A one-way ANOVA clearly demonstrated a distinct difference in growth of fungi in seawater versus distilled water (see the legend for Fig. 6A & 6B). However, none of the cultures showed absolute requirement of seawater for growth. All of the sporulating fungi showed sporulation in seawater medium. Spores from four sporulating fungi showed germination of spores under 200-500 bar pressure at 30°C and no dependence on salinity (Fig. 7). A one-way ANOVA showed statistically significant effect of hydrostatic pressure on spore germination but salinity did not show such effect (see the legend for Fig. 7). With increasing hydrostatic pressure, a decrease in spore germination was observed. The four fungi appeared to be euryhaline because the spores germinated from 1.7 to 34 ppt salinity.

Discussion

Distribution

The fungi isolated from deep-sea sediments did not correspond to the fungi generally termed marine fungi (Jones *et al.*, 2009). Ascomycetes dominated among the filamentous fungi whereas basidiomycetes were dominant among the yeast (unicellular fungi) in deep-sea sediments of the Central Indian Ocean. Bass *et al.* (2007) observed that yeast sequences dominated in deep-sea sediments of the Pacific Ocean at 1500-4000 m depth. On the other hand, in the present study as well as that of Damare *et al.* (2006) filamentous fungi were the dominant culturable fungi. It is possible that deep-sea yeasts may require more specialized media for culturing.

The ITS and 18S sequences matched while identifying only some of the non-sporulating and sporulating mycelial cultures (Table 3). On the other hand, there was a 100% consensus between these two techniques while identifying most of the yeast cultures (Table 3). These results indicate that

identification up to species level should be based on more than two techniques. Using a few other fungal specific primers as reported by Pang and Mitchell (2005), this problem of low diversity and sequence matches could be resolved.

The presence of fast growing sporulating forms such as *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Acremonium* sp. and *Fusarium* sp. have been reported as culturable forms (Roth *et al.*, 1964; Raghukumar *et al.*, 1992; Damare *et al.*, 2006) and their molecular sequences have been detected in the oceanic environment (Bass *et al.*, 2007). We are reporting for the first time the presence of *Sagenomella* sp., *Capronia coronata*, *Exophiala* sp. and *Tilletiopsis* sp. from deep-sea sediments. Several of the cultured fungi in our studies showed phylogenetic similarity to fungi reported in marine environment. The 18S sequences of the filamentous fungi NIOCC#F5 and F8 showed 99-100% homology to Pezizomycotina isolate EU594360, originally reported to have been isolated from a marine sponge *Haliclona simulans* (Table 4). The 18S sequences of the isolate NIOCC# F16 and #F30 showed homology to the isolates obtained from deep hydrothermal ecosystem of Mid-Atlantic Ridge and the isolate NIOCC#F13 to *Cladosporium* sp reported from hypersaline environment (Table 4). The ITS sequences of the isolates NIOCC #F5, #F15, #F16, #Y5, and #Y7 showed homology to the cultures either from deep-sea or marine environment (Table 5).

Growth under simulated deep-sea conditions

Although most of the fungi isolated were terrestrial species, they seem to have adapted to growth in the deep-sea and evolved into distinct physiological species. This was inferred from the fact that all the cultures showed good growth at 200 bar pressure and 5°C. All the filamentous fungi and a few yeasts showed better biomass production at 200 bar/5°C than at 200 bar/30°C. Biomass production was much better in seawater medium than in medium prepared with distilled water in filamentous as well as unicellular fungi. However, none of these fungi showed absolute requirement for sea water or NaCl for growth as has been shown for a few deep-sea actinomycetes (Jensen *et al.*, 2005). The fact that the culturable fungi from the deep-sea sediments showed homology to ITS and 18s sequences to those in GenBank does not necessarily imply that they are identical species. It is possible that they are distinct physiologically as was reported for *Aspergillus sydowii*, the fungal pathogen of seafan (Alker *et al.*, 2001). Although a terrestrial fungus, it was metabolically distinct from non-pathogenic terrestrial strains.

Spores of four fungi (NIOCC #F2, F5, F6 and F13) germinated under 200-500 bar pressure at 30°C. Spores from none of these fungi germinated at 5°C under elevated hydrostatic pressure or even at

1 bar at this temperature. It has been demonstrated that low temperature and not the elevated hydrostatic pressure is the limiting factor for spore germination of deep-sea adapted fungi (Damare *et al.*, 2006; 2008). Three terrestrial cultures (obtained from a culture collection centre) showed good mycelial growth under 200 bar pressure at 5° C as well as at 30°C (data not shown) but their spores failed to germinate under these conditions. Therefore, as reported by Damare *et al.* (2008) the effect of hydrostatic pressure and low temperature is different on mycelial fragments and fungal spores

Diversity

The low recovery of culturable fungi from deep-sea sediments was the most striking feature of the sampling carried out in 2006. A total of only 28 fungi, inclusive of filamentous and unicellular yeasts were recovered from 496 sediment samples (5.6% frequency) by methods such as particle plating, dilution plating, pressure incubation and low temperature incubation and four different culture media. At the same geographical positions (10-15°S and 73-76°E) of the Indian Ocean, Damare *et al.* (2006) isolated a total of 181 fungi from 672 sediment samples collected during three cruises from 2001-2003 (9 % frequency). Almost similar methods and culture media for isolation of fungi were used by Damare *et al.* (2006). Such a difference in recovery of fungi may indicate large temporal variation or possibility of extremely patchy distribution of fungi in deep-sea sediments with pockets of high fungal population. Turner (1973) reported sinking waterlogged wood originating from offshore during monsoon runoffs creating “islands of wood” in the deep sea. Such persistent but shifting islands of wood might bring in saprophytic species, serve as dispersal centres and contribute to habitat diversity, niche specialization and enrichment. Similar route for several mussels in the deep-sea vents has been hypothesized (Distel *et al.*, 2000). Patchy distribution of fungi seen in the Central Indian Basin appears to be similar to the scenario of high bacterial population associated with “whale fall” in deep sea (Smith and Baco, 2003). The sinking particulate organic matter from the dynamic surface waters might also be contributing to this vast temporal difference in the almost static deep-sea sediments.

Species diversity was equally low with a total of 12 and 8 distinct species of filamentous and unicellular fungi respectively. Using classical morphology-based identification system Damare *et al.* (2006) reported only 8 identified filamentous fungi and several unidentified sporulating and non-sporulating fungi. We do not know how many of non-sporulating fungi reported by Damare *et al.* (2006) belonged to the same taxa. Using molecular-based identification system in the present study we could overcome some of these problems. Insufficient database for ITS sequences also is one of the reasons for

reduced diversity assessment (Zachow *et al.*, 2009; Anderson *et al.*, 2003). Culture-based diversity assessments suffer from biases in isolate recovery, unknown culture conditions, competitive interference and over-growth by other fungi (Panebianco *et al.*, 2002; Pang and Michell, 2005). These factors might have played a role in underestimating fungal diversity in deep-sea sediments in the present study. Bass *et al.* (2007) also reported low fungal diversity of uncultured fungal sequences from deep-sea sediments. They reported only 18 fungal 18S-types in deep-sea samples. These results indicate that both, the cultured and uncultured fungal diversity appears to be low in deep sea. Our studies on diversity of yet-uncultured fungi in the same samples might throw some more light on this issue. New culture media and methodologies need to be used as is done for bacterial diversity studies.

Using 18S and ITS sequences, all the fungi isolated in the present study were identified, sequences and cultures deposited in respective repositories, thus increasing the data base for fungi from extreme environment. This study is the first report of molecular phylogenetics to assess the diversity of culturable fungi in deep-sea sediments. Molecular identification facilitated in identifying species which are new to the deep-sea habitat. Low diversity of culturable fungi might also be due to the failure to culture the yet-uncultured fungi, a problem similar to the bacterial diversity in deep-sea environment and many other environments. Direct sequencing of environmental samples would improve our understanding of the fungi present in deep seas as unculturable fungi would be revealed and many methods are becoming available (e.g. DGGE, Duong *et al.*, 2006; Seena *et al.*, 2008). We are now working on 18S and ITS amplification of DNA directly from these deep-sea sediments to estimate diversity of such yet-uncultured fungi.

Marine fungi play a role in macroaggregate formation in deep-sea sediments, possibly carbon sequestration (Damare and Raghukumar, 2008), and decomposition of particulate organic matter (Kimura *et al.*, 2001). Marine-derived fungi are also a treasure trove of bioactive molecules (Bugni and Ireland, 2004; Raghukumar, 2008). Therefore continued efforts to improve culturing techniques will help in discovering the “missing fungi” as a source for new molecules.

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References

- Alker, A.P., Smith, G.W., Kim, K. (2001). Characterization of *Aspergillus sydowii* (Thom et Church), a fungal pathogen of Caribbean sea fan corals. *Hydrobiologia* 460: 105-111.
- Anderson, I. C., Campbell, C. D., and Prosser, J. I. (2003). Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. *Environmental Microbiology* 5: 36-47.
- Bass, D., Howe, A., Brown, N., Barton, H., Demidova, M., Michelle, H., Li, L., Sanders, H., Watkinson, S.C., Willock, S., and Richards, T.A. (2007). Yeast forms dominate fungal diversity in the deep oceans. *Proceedings of Royal Society of Biological Sciences* 274: 3069-3077.
- Bills, G.F., and Polishook, J.D. (1994). Abundance and diversity of microfungi in leaf litter of a lowland rain forest in Costa Rica. *Mycologia* 86: 187-198.
- Bugni, T. S., and Ireland, C. M. (2004). Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Natural Products Reports* 21: 143-163.
- Damare, S., Raghukumar, C., and Raghukumar, S. (2006). Fungi in deep-sea sediments of the Central Indian Basin. *Deep-Sea Research Part 1* 53: 14-27.
- Damare, S., Nagarajan, M., and Raghukumar, C. (2008). Spore germination of fungi belonging to *Aspergillus* species under deep-sea conditions. *Deep-Sea Research Part 1* 55: 670-678.
- Damare, S., and Raghukumar, C. (2008). Fungi and Macroaggregation in Deep-Sea Sediments. *Microbial Ecology* 56: 168-177.
- Distel, D.L., Baco, A.R., Chuang, E., Morrill, W., Cavanaugh, C., and Smith C.R. (2000). Marine Ecology: Do mussels take wooden steps to deep-sea vents? *Nature* 403: 725-726.
- Duong, L.M., Jeewon, R., Lumyong, S. and Hyde, K.D (2006). DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. *Fungal Diversity* 23: 121-138.
- Edgecomb, V.P., Kysela, D.T., Teske, A., de Vera Gomez, A., and Sogin, M.L. (2002). Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. *Proceedings of the National Academy of Sciences USA* 99: 7658-7662.
- Eilers, H., Pernthaler, J., Glöckner, F. O., and Amann, R. (2000). Culturability and *in situ* abundance of pelagic bacteria from the North Sea. *Applied and Environmental Microbiology* 69: 3044-3051.
- Jensen, P.R., Gontang, E., Mafnas, C., Mincer, T.J., and Fenical, W. (2005). Culturable marine actinomycete diversity from tropical Pacific Ocean sediments. *Environmental Microbiology* 7: 1039-1048.

- Jones, E.B.G., Sakayaroj, J., Suetrong, S., Somrithipol, S. and Pang, K.L. (2009). Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. *Fungal Diversity* 35: 1-187.
- Kimura, H., Sato, M., Sugiyama, C., and Naganuma, T. (2001). Coupling of thraustochytrids and POM, and of bacterio- and phytoplankton in a semi-enclosed coastal area: implication for different substrate preference by the planktonic decomposers. *Aquatic Microbial Ecology* 25: 293-300
- Li, L., Kato, C., and Horikoshi, K. (1999). Bacterial diversity in deep-sea sediments from different depths. *Biodiversity and Conservation* 8: 659-677.
- López-García, P., Vereshchaka, A. and Moreira, D. (2007). Eukaryotic diversity associated with carbonates and fluid-seawater interface in Lost City hydrothermal field. *Environmental Microbiology* 9: 546-554.
- Panebianco, C., Tam, W.Y., Jones, E.B.G. (2002). The effect of pre-inoculation of balsa wood by selected marine fungi and their effect on subsequent colonization in the sea. *Fungal Diversity* 10: 77-88.
- Pang K-L., Mitchell J.I. (2005). Molecular approaches for assessing fungal diversity in marine substrata. *Botanica Marina* 48: 332-347.
- Raghukumar, C. (2008). Marine fungal biotechnology: an ecological perspective. *Fungal Diversity* 31: 19-35.
- Raghukumar, C., and Raghukumar, S. (1998). Barotolerance of fungi isolated from deep-sea sediments of the Indian Ocean. *Aquatic Microbial Ecology* 15: 153-163.
- Raghukumar, C., Raghukumar, S., Chinnaraj, A., Chandramohan, D. (1992). Endolithic fungi from deep-sea calcareous substrata: isolation and laboratory studies. In: *Oceanography of the Indian Ocean*. (ed. B.N. Desai) Oxford IBH publ., New Delhi: 3-9.
- Raghukumar, C., Raghukumar, S., Sheelu, G., Gupta, S.M., Nath, B.N., and Rao, B.R. (2004). Buried in time: culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean. *Deep-Sea Research Part 1* 51: 1759-1768.
- Raven, J. A., and Falkowski, P. G. (1999). Oceanic sinks for atmospheric CO₂. *Plant, Cell and Environment* 22: 741-755.
- Roth, F. J., Orpurt, P.A., and Ahearn, D.J. (1964). Occurrence and distribution of fungi in a subtropical marine environment. *Canadian Journal of Botany* 42: 375-383.
- Seena, S., Wynberg, N. and Bärlocher, F. (2008). Fungal diversity during leaf decomposition in a stream assessed through clone libraries. *Fungal Diversity* 30: 1-14.

- Smit, E., Leeflang, P., Glandorf, B., Elsas, J.D., and Wernars, K. (1999). Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. *Applied and Environmental Microbiology* 65: 2614-2621.
- Smith, C. R., and Baco A. R., (2003). Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology* 41: 311-354.
- Stoeck, T., and Epstein, S. (2003). Novel eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen-depleted marine environments. *Applied and Environmental Microbiology* 69: 2657-2663.
- Sudhir Kumar, Masatoshi, N., Dudley, J., and Tamura, K. (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9: 299-306.
- Takami, H., Inoue, A., Fuji, F., and Horikoshi, K. (1997). Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS Microbiology Letters* 152: 279-285.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research* 25: 4876-4882.
- Turner, R.D. (1973). Wood-boring bivalves, opportunistic species in the deep-sea. *Science* 180, 1377-1379.
- White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W., (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR-Protocols and applications- a laboratory manual*. Innis, N., Gelfand, D., Sninsky, J., White, T.J. (eds), New York, Academic Press, pp. 315-322.
- Xia, X., and Xie, Z. (2001). DAMBE: software package for data analysis in molecular biology and evolution. *Journal of Heredity* 92: 371-373.
- Zachow, C., Berg, C., Muller, H., Meincke, R., Komon-Zelazowska, M., Druzhinina, I.S., Kubicek, C.P., and Berg, G. (2009). Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. *The ISME Journal* 3: 79-92.

Legends to figures

Fig 1. Neighbor-joining phylogenetic tree for mycelial fungi based on rDNA-18S sequences (~1100 bp). Numbers at nodes indicate bootstrap values of neighbor-joining analysis for 1000 replicates (values below 50% not shown).

Fig. 2. Neighbor-joining phylogenetic tree for mycelial fungi based on rDNA-ITS sequences (~600 bp). Numbers at nodes indicate bootstrap values of neighbor-joining analysis for 1000 replicates (values below 50% not shown).

Fig. 3. Neighbor-joining phylogenetic tree for yeasts based on rDNA-18S sequences (~1100 bp). Numbers at nodes indicate bootstrap values of neighbor-joining analysis for 1000 replicates (values below 50% not shown).

Fig. 4. Neighbor-joining phylogenetic tree for yeasts based on rDNA-ITS sequences (~600 bp). Numbers at nodes indicate bootstrap values of neighbor-joining analysis for 1000 replicates (values below 50% not shown).

Fig. 5A. Biomass (mg dry wt/20 ml) of mycelial fungi after 20 days incubation in malt extract broth under different pressure and temperature conditions (SD values<10%).

Fig. 5B. Biomass (mg dry wt/20 ml) of yeasts after 20 days incubation in yeast extract, peptone, dextrose broth under different pressure and temperature conditions (SD values<10%).

Results of 1-way ANOVA performed on biomass obtained under different growth conditions (Fig. 5A & 5B):

	Fungi	Yeast
F critical	2.8	2.8
F value	25.5	30.5
P value	9.06-E-11***	7.83E-11***
Degrees of freedom	63	47

*** significant at >0.01 %.

Fig. 6A. . Biomass (mg dry wt/10 ml) of mycelial fungi after 6 days incubation in malt extract broth prepared in sea water (SW) and distilled water (DW) (SD values<10%)

Fig. 6B. . Biomass (mg dry wt/10 ml) of yeasts after 6 days incubation in yeast extract peptone and dextrose broth prepared in sea water (SW) and distilled water (DW) (SD values<10%).

Results of 1-Way analysis of variance (ANOVA) performed for the data in Fig. 6A & 6B

	Fungi	Yeast
F critical	4.2	4.3
F value	30.8	13.1
P value	4.9E-06***	0.002***
Degrees of freedom	31	23

*** significant at >0.01 %

Fig. 7. 3D Surface plot graph showing spore germination of four sporulating fungi, (A) NIOCC#F2, *Penicillium* sp. (B) NIOCC#F5, *Cladosporium* sp. (C) NIOCC#F6 *Aspergillus* sp. and (D) NIOCC#F13, *Cladosporium* sp. grown at 30°C under different hydrostatic pressure (200, 300, 400 and 500 bar) and salinity (1.7, 17 and 34 ppt) for 15 days (SD values<12%).

Results of 1-Way ANOVA performed for the culture NIOCC#F2

	Pressure/% germination	Salinity/% germination
F critical	4.3	4.3
F value	82.6	1.3
P value	6.7E-09***	0.3
Degrees of freedom	23	23

*** significant at >0.01 %

Results of 1-way ANOVA performed for the culture NIOCC#F5

	Pressure vs% germination	Salinity vs% germination
F critical	4.3	4.3
F value	78.3	1.9
P value	1.06E-08***	0.2
Degrees of freedom	23	23

*** significant at >0.01 %

Results of 1-way ANOVA performed for the culture NIOCC#F6

	Pressure vs% germination	Salinity vs% germination
F critical	4.3	4.3
F value	92.2	0.02
P value	2.5E-09***	0.9
Degrees of freedom	23	23

*** significant at >0.01 %

Results of 1-Way ANOVA performed for the culture NIOCC#F13

	Pressure vs% germination	Salinity vs% germination
F critical	4.3	4.3
F value	80.5	2.0
P value	8.4E-09***	0.2
Degrees of freedom	23	23

*** significant at >0.01 %

Table 1: Details of sediment cores collected from the Central Indian Basin.

Sr.No.	Box core (BC) No.	Touch Positions		Water depth (m)
		Lat (°S)	Long (°E)	
1	BC-03	12 00.000	76 29.900	5373
2	BC-04	13 00.000	76 29.950	5377
3	BC-13	14 59.919	75 29.849	4894
4	BC-18	12 59.747	74 30.150	5122
5	BC-19	13 00.070	75 29.900	5104
6	BC-20	11 59.900	75 29.900	5223
7	BC-25	11 00.000	75 29.800	5303
8	BC-26	09 59.848	75 30.085	5339
9	BC-27	11 30.130	75 59.600	5274
10	BC-28	11 30.000	75 30.000	5263
11	BC-29	12 29.900	74 59.900	5174
12	BC-30	12 30.056	75 29.983	5189
13	BC-31	12 00.000	74 59.900	5153
14	BC-32	12 29.853	75 59.950	5309
15	BC-33	12 59.980	74 59.960	5265
16	BC-34	13 30.033	75 29.845	5219
17	BC-35	14 30.081	75 29.792	5211
18	BC-36	16 01.887	75 28.770	5042
19	BC-37	16 06.891	75 24.828	3992
20	BC-38	15 57.590	75 31.950	5642

Table 2. Number of fungi isolated by various isolation techniques

	Particle plating	Dilution plating	Pressure incubation at 300 bar/5 ⁰ C	Direct incubation in MEB at 5 ⁰ C
Total no. of sediment samples used	116	188	39	153
No. of fungi isolated	7	12	6	3
% frequency of occurrence	6	6	15	2

MEB=malt extract broth

Table 3. Identification of fungi by different methods and their accession numbers

Isolate No.	Identification			Accession number	
	Microscopic	18S	ITS	18S	ITS
NIOCC#F2	<i>Penicillium</i> sp.	<i>Penicillium</i> sp.	<i>Penicillium citreonigrum</i>	FJ357792	EU729705
NIOCC#F5	<i>Cladosporium</i> sp.	Pezizomycotina sp.	<i>Cladosporium cladosporioides</i>	EU723484	EU729711
NIOCC#F6 (MTCC9336)	<i>Aspergillus</i> sp.	Uncultured marine eukaryote clone U112H09	<i>Aspergillus</i> sp.	EU723485	EU729718
NIOCC#F8	<i>Cladosporium</i> sp.	Pezizomycotina sp.	<i>Cladosporium cladosporioides</i>	EU723486	EU729712
NIOCC#F13 (MTCC9333)	<i>Cladosporium</i> sp.	<i>Cladosporium</i> sp.	<i>Cladosporium</i> sp.	EU723487	EU729719
NIOCC#F15	<i>Sagenomella</i> sp.	<i>Sagenomella sclerotialis</i> sp.	Uncultured fungus clone	EU723488	FJ357782
NIOCC#F16 (MTCC9332)	<i>Rhinocladiella</i> sp.	Ascomycete MV_26C	<i>Exophiala spinifera</i>	EU723489	FJ357783
NIOCC#F21 (MTCC9339)	Sporulating, unidentified sp.	<i>Tilletiopsis albescens</i>	<i>Tilletiopsis albescens</i>	EU723490	FJ357784
NIOCC#F23	<i>Sagenomella</i> sp.	<i>Sagenomella sclerotialis</i> sp.	Uncultured fungus clone	EU723491	FJ357785
NIOCC#F30 (MTCC9337)	<i>Acremonium</i> sp.	Ascomycete MV_25C	Hypocreales sp.	EU723492	EU729707
NIOCC#F33	Sporulating, unidentified sp.	<i>Tilletiopsis albescens</i>	<i>Tilletiopsis albescens</i>	EU723493	EU729706
NIOCC#F46 (MTCC9334)	<i>Exophiala</i> sp.	Ascomycete MV_26C	<i>Exophiala xenobiotica</i>	EU723494	EU729708
NIOCC#F47	<i>Aspergillus</i> sp.	<i>Aspergillus restrictus</i>	<i>Aspergillus caesiellus</i>	EU723495	EU729709

NIOCC#F48 (MTCC9341)	<i>Sagenomella</i> sp.	<i>Sagenomella sclerotialis</i>	<i>Phialemonium dimorphosporum</i>	EU723496	FJ357786
NIOCC#F49 (MTCC9340)	<i>Acremonium</i> sp.	<i>Acremonium</i> sp.	<i>Trichothecium roseum</i>	EU723497	EU729713
NIOCC#F50	Non -sporulating sp.	<i>Capronia coronata</i>	<i>Exophiala dermatitidis</i>	EU723498	EU729710
NIOCC#Y1	Yeast sp.	<i>Rhodotorula cassiicola</i>	<i>Sporobolomyces</i> sp.	EU723499	FJ357787
NIOCC#Y2	Yeast sp.	<i>Sporidiobolus johnsonii</i>	<i>Sporidiobolus salmonicolor</i>	EU723500	FJ357788
NIOCC#Y3	Yeast sp.	<i>Rhodosporidium toruloides</i>	<i>Rhodosporidium toruloides</i>	EU723501	EU729715
NIOCC#Y4	Yeast sp.	<i>Sporidiobolus johnsonii</i>	<i>Sporobolomyces</i> sp.	EU723502	EU729716
NIOCC#Y5	Yeast sp.	<i>Sporidiobolus johnsonii</i>	<i>Rhodotorula</i> sp.	EU723503	FJ357793
NIOCC#Y6	Yeast sp.	<i>Sporidiobolus johnsonii</i>	<i>Sporidiobolus</i> sp.	EU723504	EU72972
NIOCC#Y7	Yeast sp.	<i>Rhodotorula cassiicola</i>	<i>Rhodotorula calyptogenae</i>	EU723505	FJ357789
NIOCC#Y8	Black Yeast sp.	<i>Coniosporium perforans</i>	<i>Sarcinomyces petricola</i>	EU723506	EU729717
NIOCC#Y9	Filamentous Yeast sp.	<i>Graphiola cylindrica</i>	<i>Tilletiopsis oryziicola</i>	EU723507	FJ357790
NIOCC#Y10	Yeast sp.	<i>Rhodotorula mucilaginosa</i>	<i>Cryptococcus albidosimilis</i>	EU723508	EU729721
NIOCC#PY12	Yeast sp.	<i>Cryptococcus vishniacii</i>	<i>Cryptococcus albidosimilis</i>	EU723509	FJ357791
NIOCC#PY13	Yeast sp.	<i>Cryptococcus vishniacii</i>	<i>Rhodotorula mucilaginosa</i>	EU723510	EU729714

MTCC-Microbial Type Culture Collection, Chandigarh, NIOCC= National Institute of Oceanography Culture Collection

Table 4. Phylogenetic affiliations of fungal and yeast isolates on the basis of 18S sequences

Isolate ID	Phylogenetic nearest taxon, phylum (GenBank Accession No)	% identity	Source of phylogentic nearest taxon
<i>Penicillium</i> sp. (NIOCC#F2)	<i>Penicillium phialosporum</i> , Ascomycota (AF245266)	84	Not known
<i>Cladosporium</i> sp. (NIOCC#F5)	Pezizomycotina , Ascomycota (EU594360)	100	<i>Haliclona simulans</i> (a marine sponge)
<i>Aspergillus</i> sp. (NIOCC#F6)	Uncultured marine eukaryote clone UI12H09, Ascomycota (EU446322)	99	“anoxic deep hypersaline L'Atalante Basin, eastern Mediterranean Sea"
<i>Cladosporium</i> sp. (NIOCC#F8)	Pezizomycotina sp., Ascomycota (EU594360)	99	<i>Haliclona simulans</i> (a marine sponge)
<i>Cladosporium</i> sp. (NIOCC#F13)	<i>Cladosporium</i> sp. EXF228, Ascomycota (DQ780930)	99	hypersaline environments
<i>Sagenomella</i> sp. (NIOCC#F15)	<i>Sagenomella sclerotialis</i> , Ascomycota (AB024592)	99	Not known
Ascomycete sp. (NIOCC#F16)	Ascomycete sp. MV_26C, Ascomycota (EF638702)	100	Mid Atlantic Ridge in deep hydrothermal ecosystem
<i>Tilletiopsis</i> sp. (NIOCC#F21)	<i>Tilletiopsis albescens</i> , Basidiomycota (D83188)	99	Not known
<i>Sagenomella</i> sp. (NIOCC#F23)	<i>Sagenomella sclerotialis</i> , Ascomycota (AB024592)	99	Not known
Ascomycete sp. (NIOCC#F30)	Ascomycete sp. MV_25C, Ascomycota (EF638701)	100	Mid Atlantic Ridge in deep hydrothermal ecosystem
<i>Tilletiopsis</i> sp. (NIOCC#F33)	<i>Tilletiopsis albescens</i> , Basidiomycota (D83188)	100	Not known
Ascomycete sp. (NIOCC#F46)	Ascomycete sp. MV_26C, Ascomycota (EF638702)	100	Mid Atlantic Ridge in deep hydrothermal ecosystem
<i>Aspergillus</i> sp. (NIOCC#F47)	<i>Aspergillus restrictus</i> , Ascomycota (AB008407)	100	Culture collection

<i>Sagenomella</i> sp. (NIOCC#F48)	<i>Sagenomella sclerotialis</i> , Ascomycota (AB024592)	99	Culture collection
<i>Capronia coronata</i> (NIOCC#F50)	<i>Capronia coronata</i> , Ascomycota (AJ232939)	99	Not known
<i>Acremonium</i> sp. (NIOCC#PF49)	<i>Acremonium</i> sp., Ascomycota (AY392130)	99	Onychomyces
<i>Rhodotorula</i> sp. (NIOCC#Y1)	<i>Rhodotorula cassiicola</i> , Basidiomycota (AB055191)	99	Thailand
<i>Sporidiobolus johnsonii</i> (NIOCC#Y2)	<i>Sporidiobolus johnsonii</i> , Basidiomycota (L22261)	100	Culture collection
<i>Rhodosporidium toruloides</i> (NIOCC#Y3)	<i>Rhodosporidium toruloides</i> , Basidiomycota (DQ832192)	100	Culture collection
<i>Sporidiobolus johnsonii</i> (NIOCC#Y4)	<i>Sporidiobolus johnsonii</i> , Basidiomycota (L22261)	100	Culture collection
<i>Sporidiobolus johnsonii</i> (NIOCC#Y5)	<i>Sporidiobolus johnsonii</i> , Basidiomycota (L22261)	100	Culture collection
<i>Sporidiobolus johnsonii</i> (NIOCC#Y6)	<i>Sporidiobolus johnsonii</i> , Basidiomycota (L22261)	100	Culture collection
<i>Rhodotorula</i> sp. (NIOCC#Y7)	<i>Rhodotorula cassiicola</i> , Basidiomycota (AB055191)	99	Thailand
<i>Coniosporium perforans</i> (NIOCC#Y8)	<i>Coniosporium perforans</i> , Ascomycota (EF137365)	100	Rocks
<i>Graphiola cylindrica</i> (NIOCC#Y9)	<i>Graphiola cylindrica</i> , Basidiomycota (D63929)	95	Not known

<i>Rhodotorula</i> sp. (NIOCC#Y10)	<i>Rhodotorula mucilaginosa</i> , Basidiomycota (EF218987)	100	Not known
<i>Cryptococcus</i> sp. (NIOCC#PY12)	<i>Cryptococcus vishniacii</i> , Basidiomycota (AB032657)	99	Culture collection
<i>Cryptococcus</i> sp. (NIOCC#PY13)	<i>Cryptococcus vishniacii</i> , Basidiomycota (AB032657)	99	Culture collection

Table 5. Phylogenetic affiliations of fungal and yeast isolates on the basis of ITS sequences

Isolate ID	Phylogentic nearest taxon, Phylum (GenBank Accession No)	% identity	Source of phylogentic nearest taxon
<i>Penicillium</i> sp. (NIOCC#F2)	<i>Penicillium citreonigrum</i> , Ascomycota (AY373908)	99	Dust
<i>Cladosporium</i> sp (NIOCC#F5)	<i>Cladosporium cladosporioides</i> , Ascomycota (EU497957)	100	Deep-sea
<i>Aspergillus</i> sp. (NIOCC#F6)	<i>Aspergillus</i> sp., Ascomycota (EF672304)	99	<i>Coffea arabica</i>
<i>Cladosporium</i> sp. (NIOCC#F8)	<i>Cladosporium cladosporioides</i> , Ascomycota (EF136373)	99	rice wine, wheat Qu
<i>Cladosporium</i> sp. (NIOCC#F13)	<i>Cladosporium</i> sp., Ascomycota (EF105367)	99	toxic mold <i>Stachybotrys chartarum</i>
<i>Sagenomella</i> sp. (NIOCC#F15)	Uncultured fungus clone, Eukaryota (DQ279836)	91	deep-sea marine sediments in South China Sea
Ascomycete sp. (NIOCC#F16)	<i>Exophiala spinifera</i> , Ascomycota (AM176734)	99	Sediment from deep sea of Pacific Ocean
<i>Tilletiopsis</i> sp. (NIOCC#F21)	<i>Tilletiopsis albescens</i> , Basidiomycota (AB025695)	98	Culture collection, Tokyo
<i>Sagenomella</i> sp. (NIOCC#F23)	Doubtful, Ascomycota	-	-
Ascomycete sp. (NIOCC#F30)	Hypocreales sp., Ascomycota (DQ682584)	100	coffee plants with the fungal entomopathogen <i>Beauveria bassiana</i>
<i>Tilletiopsis</i> sp. (NIOCC#F33)	<i>Tilletiopsis albescens</i> , Basidiomycota (AB025695)	98	Culture collection, Tokyo
Ascomycete sp. (NIOCC#F46)	<i>Exophiala xenobiotica</i> , Ascomycota (EF025407)	99	Fungus Testing Laboratory, Texas, US
<i>Aspergillus</i> sp. (NIOCC#F47)	<i>Aspergillus caesiellus</i> , Ascomycota (AY373865)	100	Not known
<i>Sagenomella</i> sp.	<i>Phialemonium dimorphosporum</i> ,	99	Lesions excised from

(NIOCC#F48)	Ascomycota (DQ403199)		<i>Mugil gyrans</i>
<i>Capronia coronata</i> (NIOCC#F50)	<i>Exophiala dermatitidis</i> , Ascomycota (EF568099)	100	Clinical sample
<i>Acremonium</i> sp. (NIOCC#PF49)	<i>Trichothecium roseum</i> , Ascomycota (EU622273)	100	Culture collection, china
<i>Rhodotorula</i> sp. (NIOCC#Y1)	<i>Sporobolomyces</i> sp., Basidiomycota (EU002893)	95	peduncle endophyte
<i>Sporidiobolus johnsonii</i> (NIOCC#Y2)	<i>Sporidiobolus salmonicolor</i> , Basidiomycota (AY015434)	100	Not known
<i>Rhodosporidium toruloides</i> (NIOCC#Y3)	<i>Rhodosporidium toruloides</i> , Basidiomycota (AB073266)	99	Not known
<i>Sporidiobolus johnsonii</i> (NIOCC#Y4)	<i>Sporobolomyces</i> sp., Basidiomycota (EU002893)	100	coffee plants
<i>Sporidiobolus johnsonii</i> (NIOCC#Y5)	<i>Rhodotorula</i> sp., Basidiomycota (AB025984)	99	deep-sea environments around the northwest Pacific Ocean
<i>Sporidiobolus johnsonii</i> (NIOCC#Y6)	<i>Sporidiobolus</i> sp., Basidiomycota (DQ317366)	100	soils and historic wood from the Ross Sea Region of Antarctica
<i>Rhodotorula</i> sp. (NIOCC#Y7)	<i>Rhodotorula calyptogena</i> , Basidiomycota (EU669878)	99	seawater
<i>Coniosporium perforans</i> (NIOCC#Y8)	<i>Sarcinomyces petricola</i> , Ascomycota (AJ244275)	98	Meristematic tissues
<i>Graphiola cylindrica</i> (NIOCC#Y9)	<i>Tilletiopsis oryzicola</i> , Basidiomycota (AB045708)	95	leaves
<i>Rhodotorula</i> sp. (NIOCC#Y10)	<i>Rhodotorula mucilaginos</i> , Basidiomycota (EU781664)	98	stem from 5 year old tree growing in a subtropical monsoon climate at an elevation of

<i>Cryptococcus</i> sp. (NIOCC#PY12)	<i>Cryptococcus albidosimilis</i> , Basidiomycota (AF145331)	100	600-800m Antarctica
<i>Cryptococcus</i> sp. (NIOCC#PY13)	<i>Rhodotorula mucilaginosa</i> , Basidiomycota (EU781664)	99	stem from 5 year old tree growing in a subtropical monsoon climate at an elevation of 600-800m

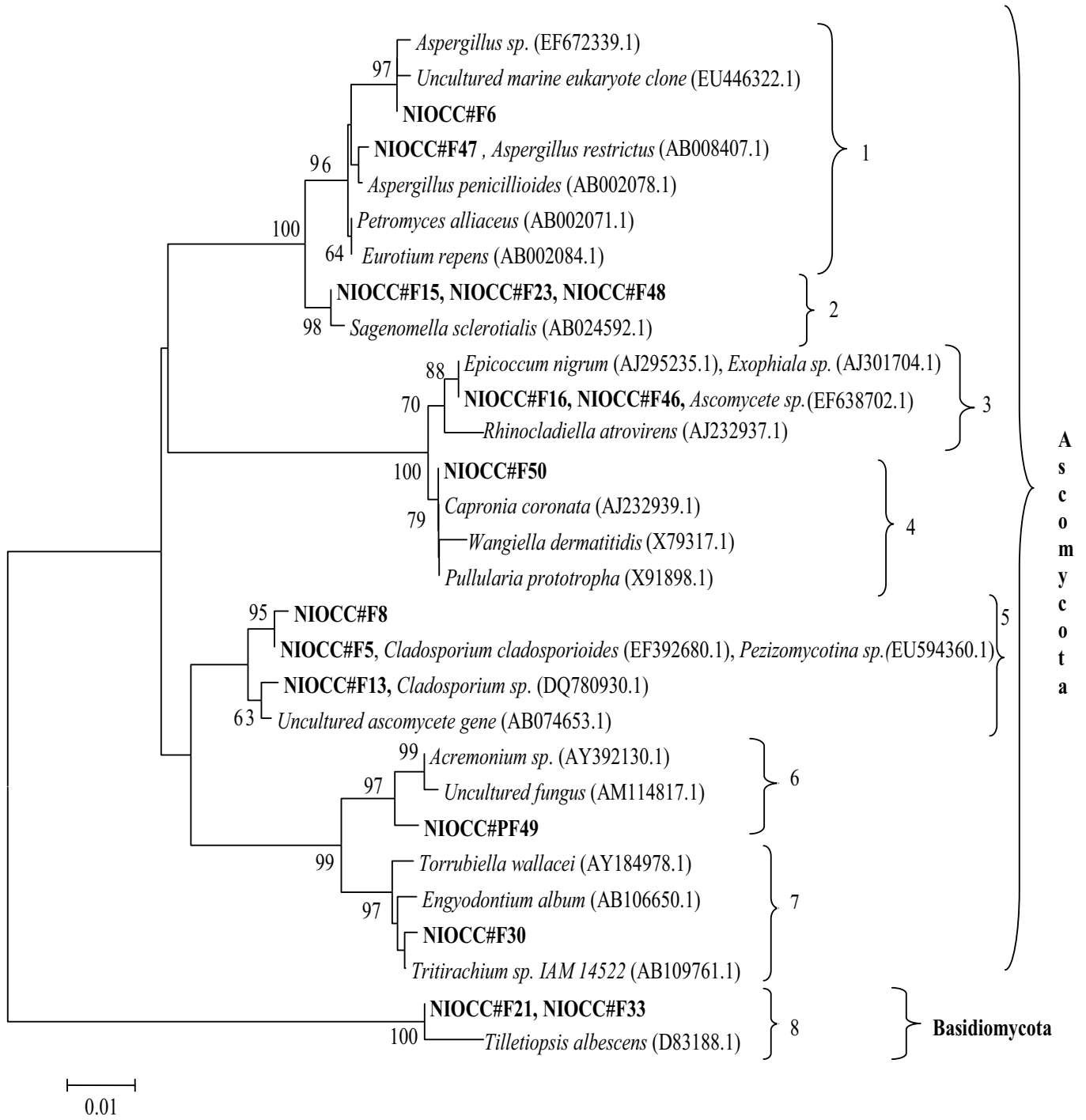


Fig 1.

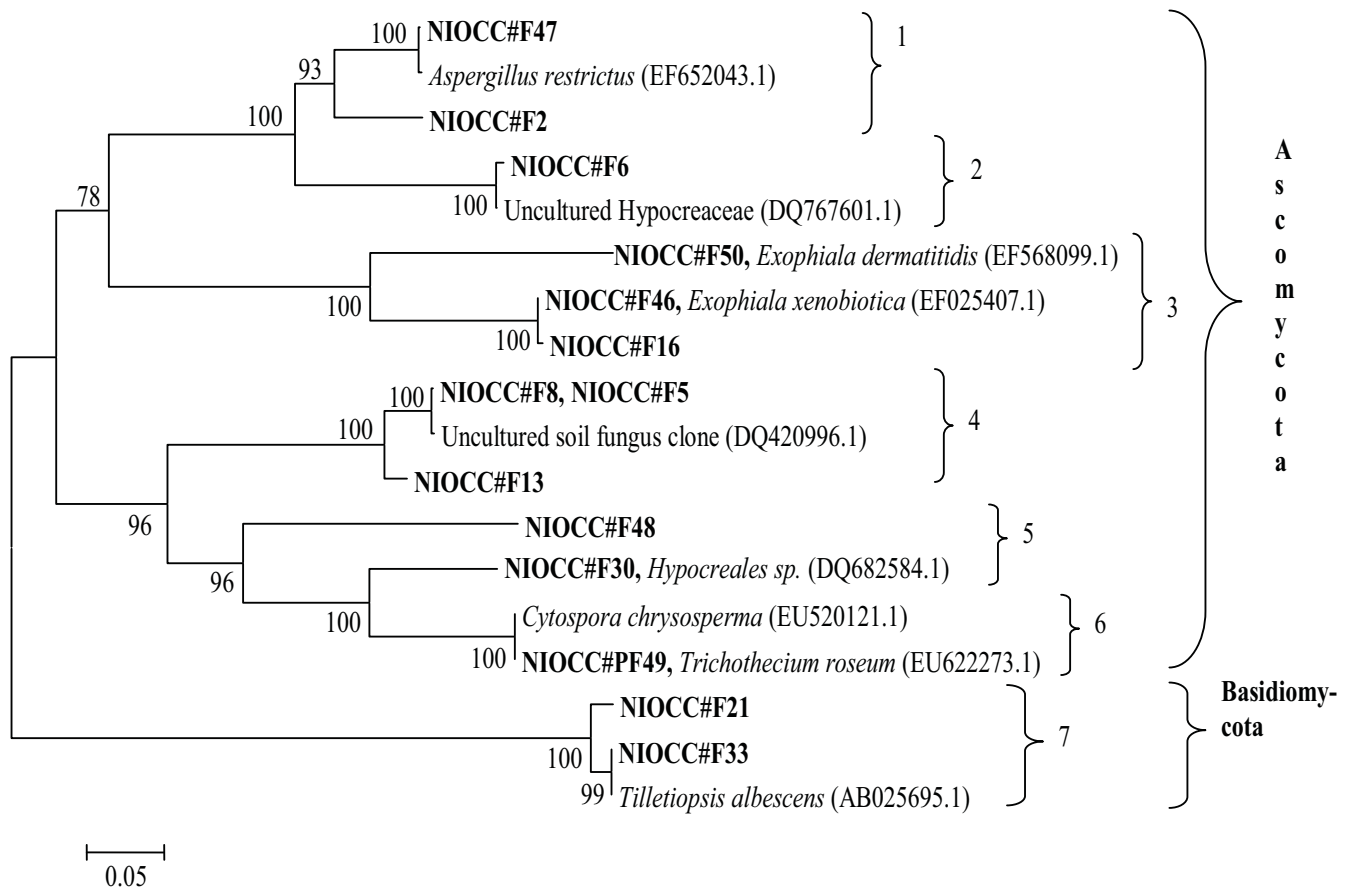


Fig 2.

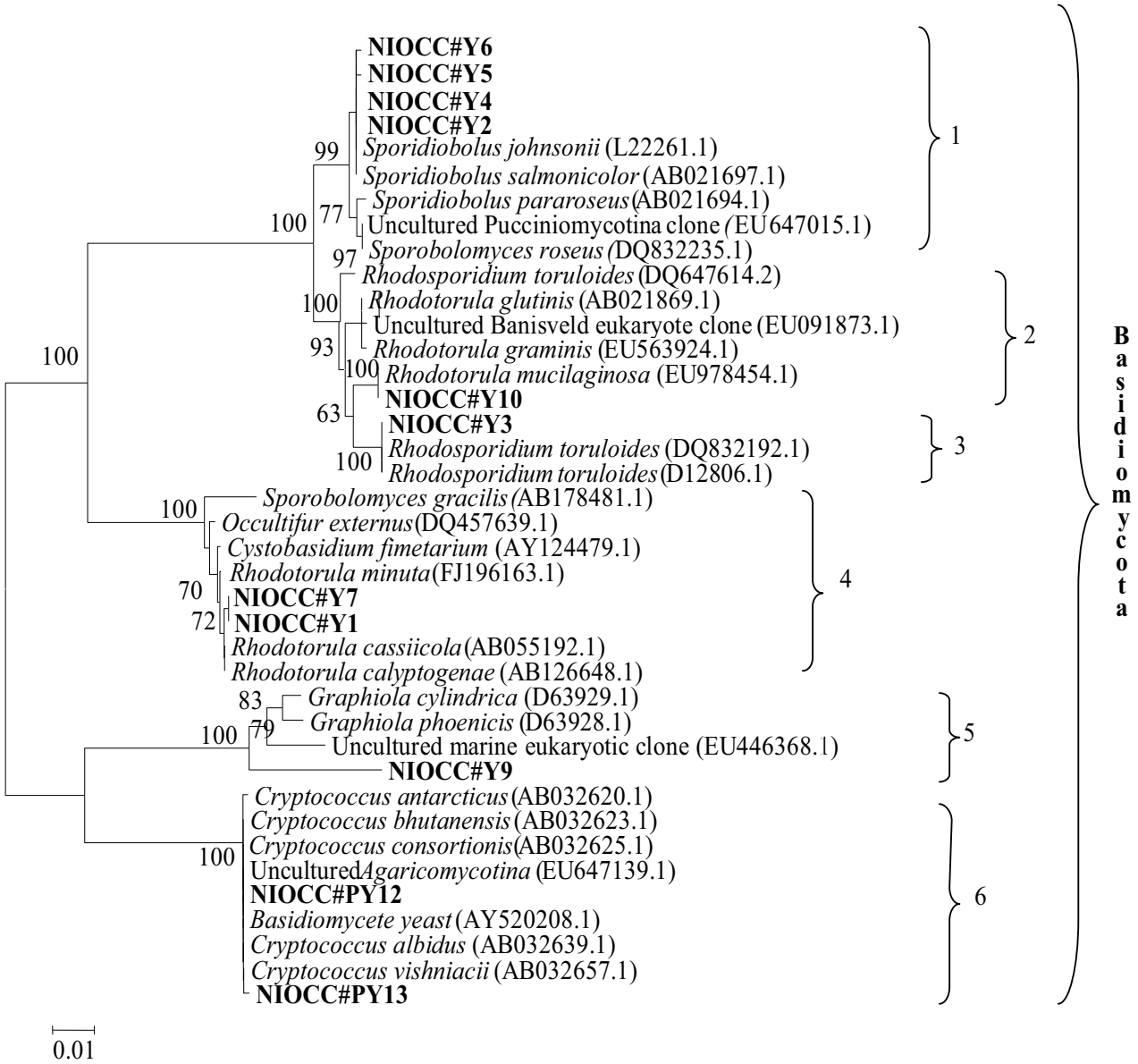


Fig 3.

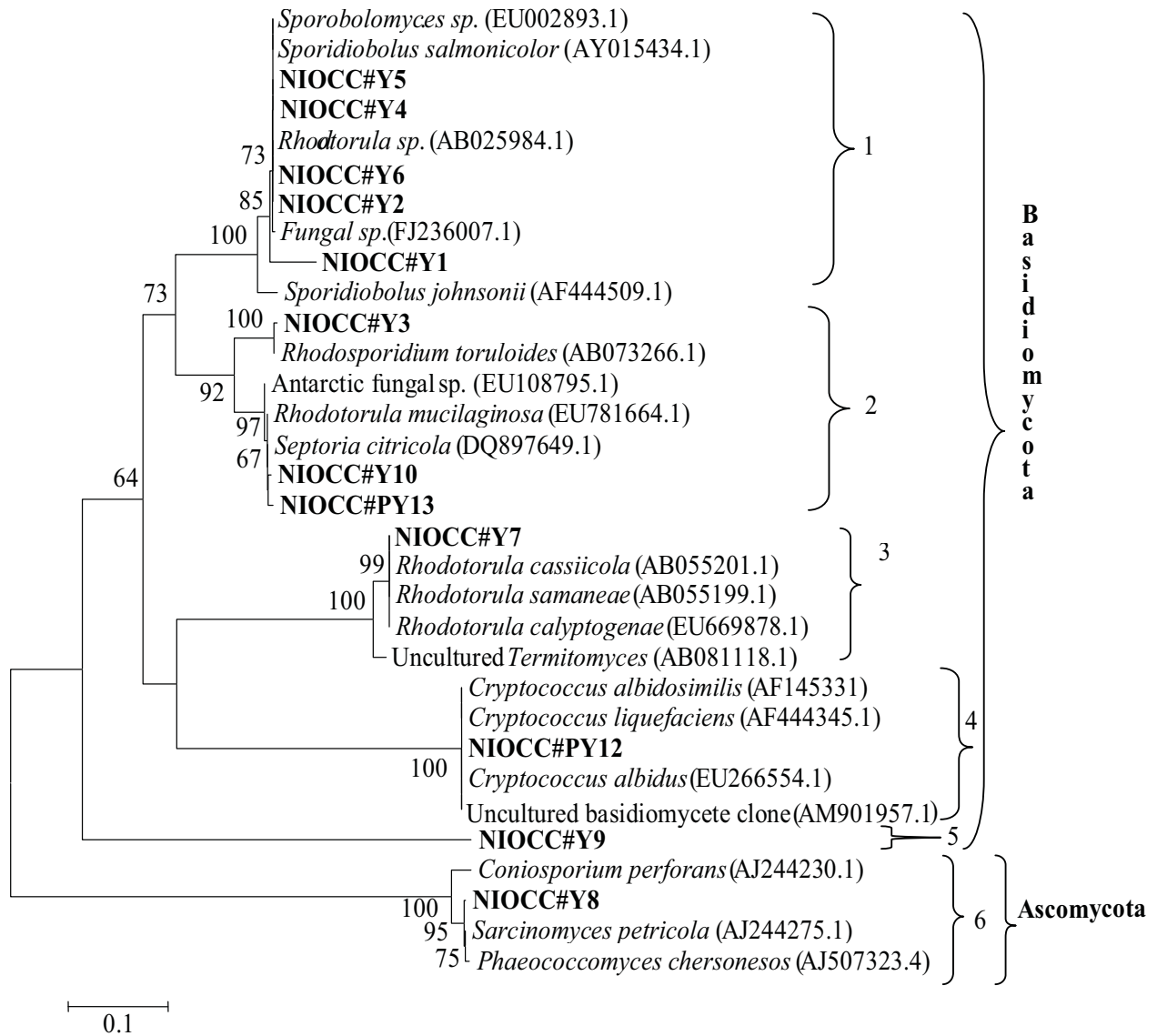


Fig 4.

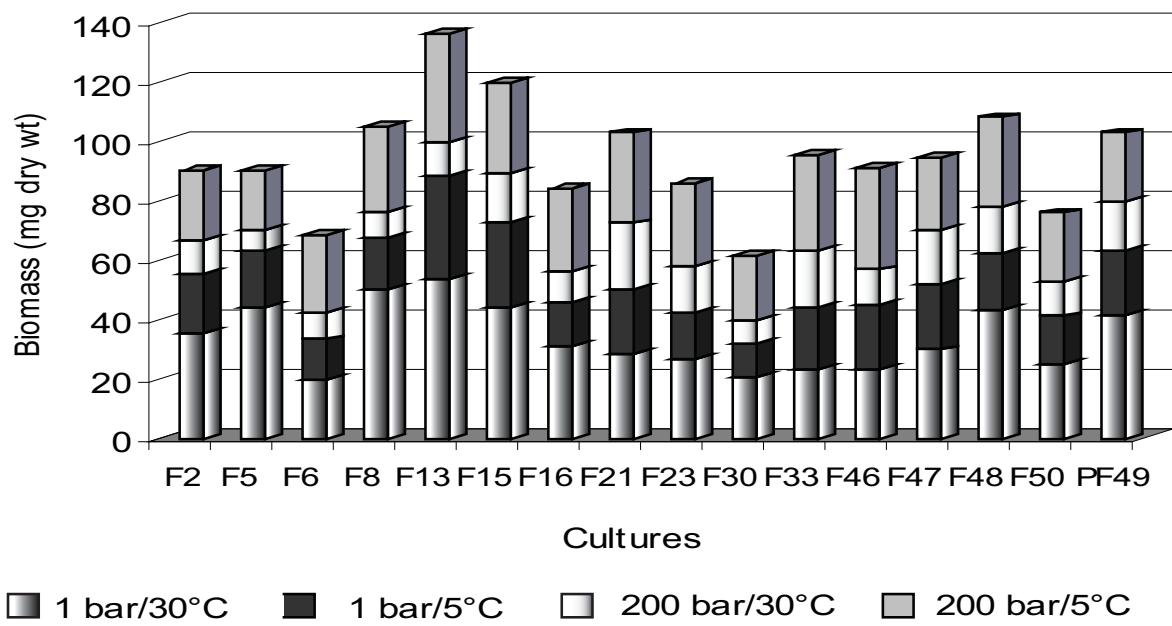


Fig 5A.

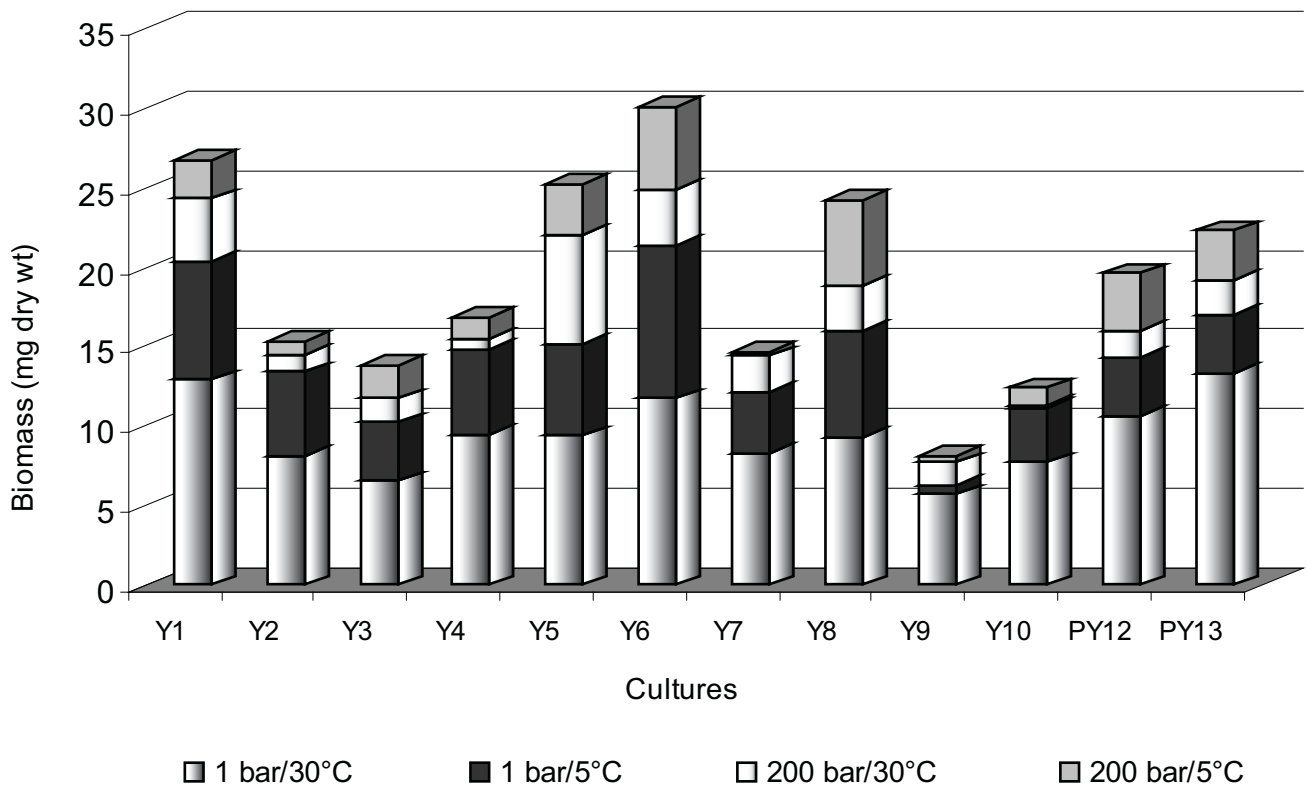


Fig 5B.

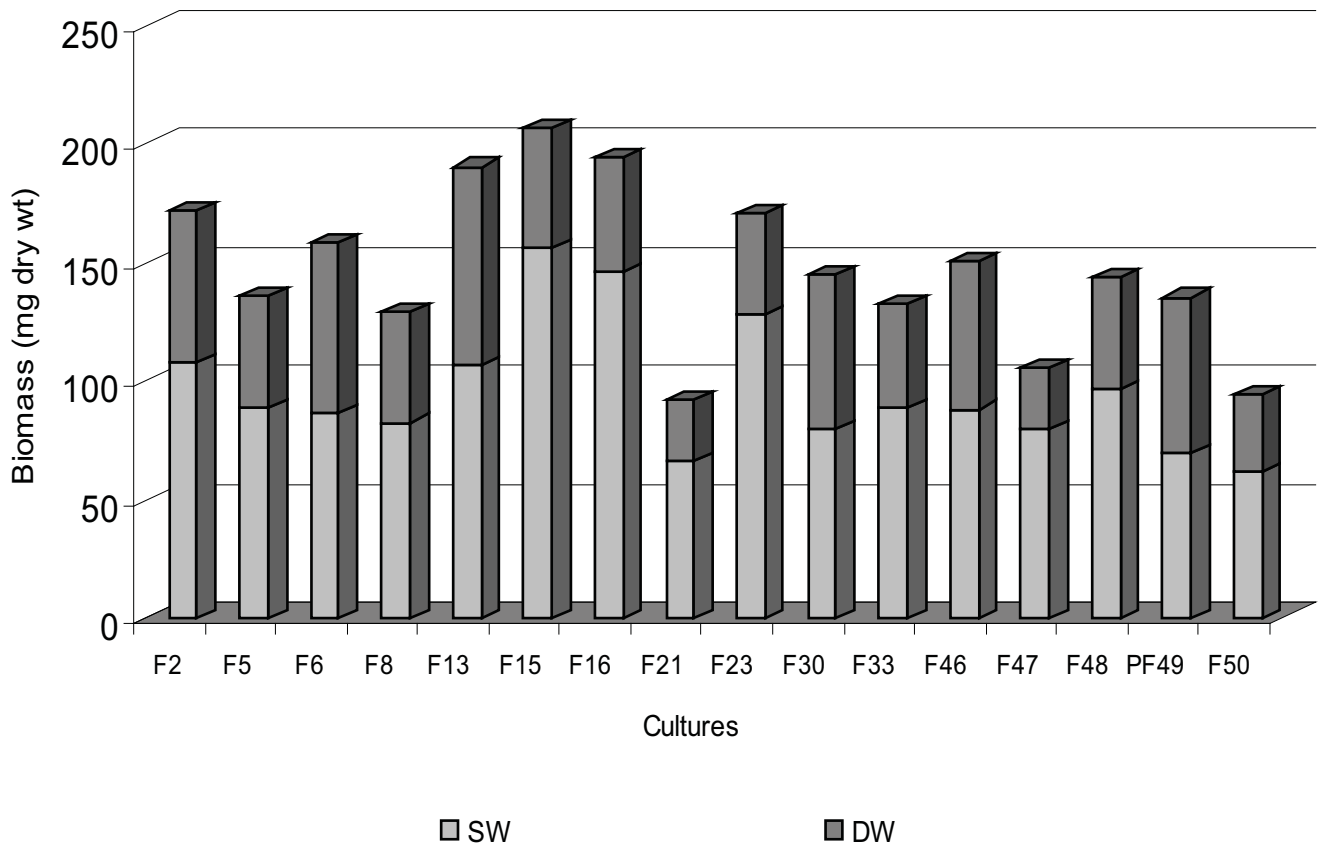


Fig 6A.

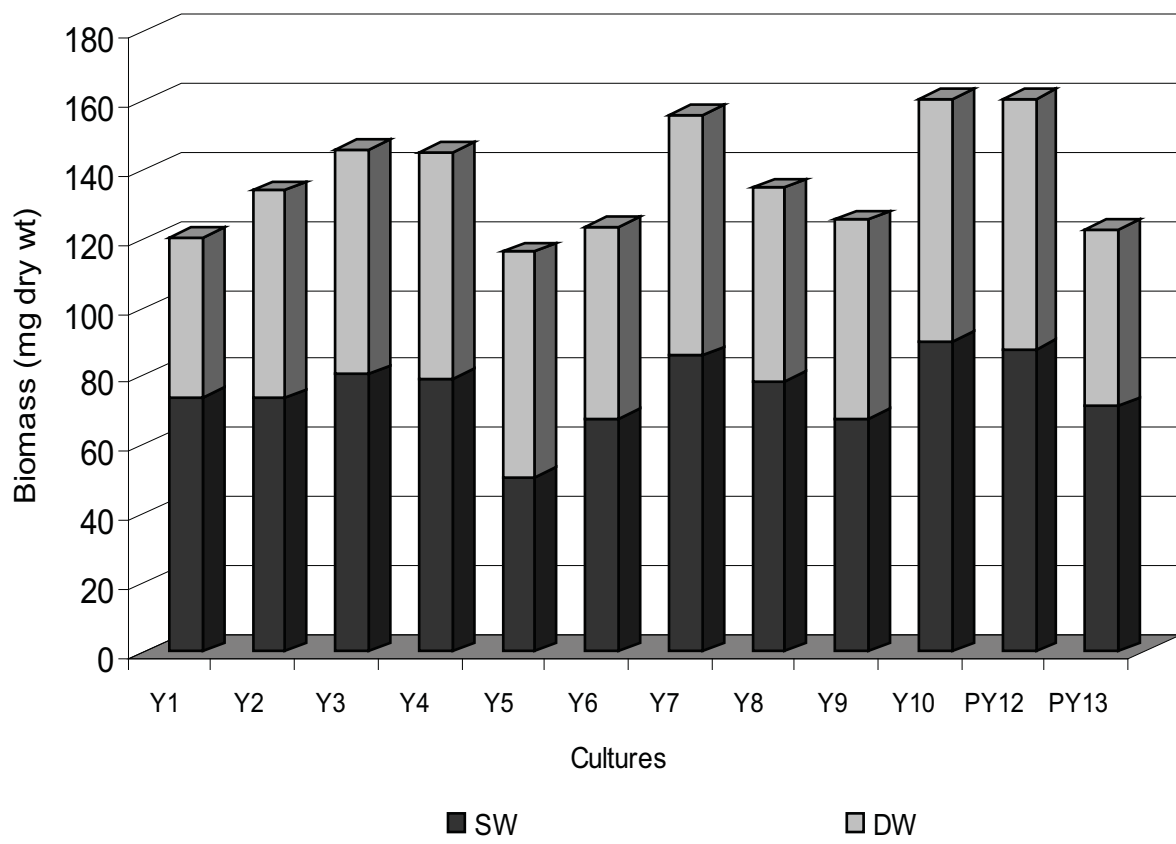
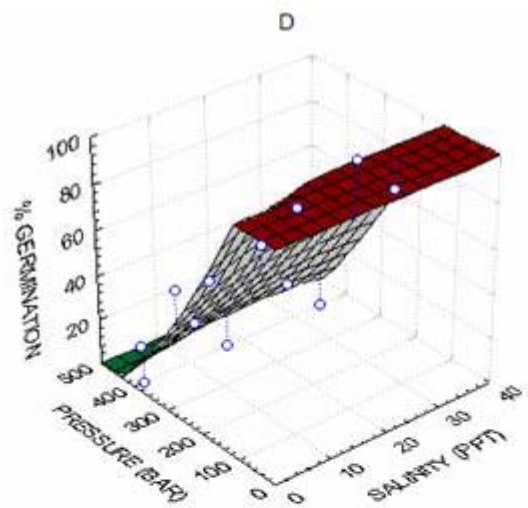
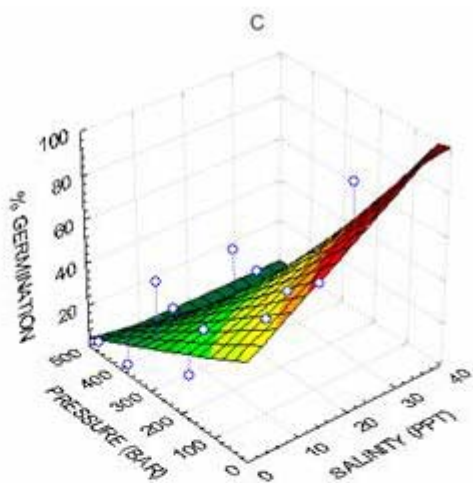
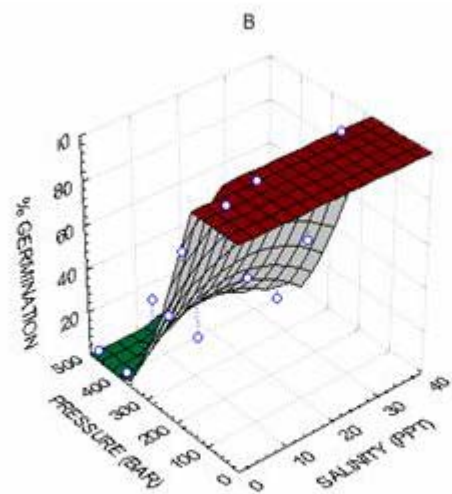
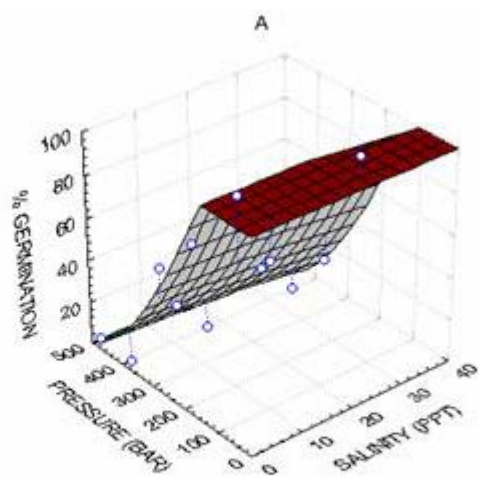


Fig 6B.



9.091
 18.182
 27.273
 36.364
 45.455
 54.545
 63.636
 72.727
 81.818
 90.909
 above

Fig 7.