5.1 Introduction

Fungi are a group of non-motile eukaryotic organisms that have definite cell walls, devoid of chlorophyll and reproduce by means of spores or in some cases by conidia [Carlile et al. 2001]. Though bacteria and archaea are considered to be a major portion of the total living biomass of marine ecosystem [Parkes et al. 2000], much less is known about the abundance of microbial eukaryotes in these systems. Marine fungi which fall under ‘true fungi’ of the Kingdom Fungi or the ‘straminipilan fungi’ of the Kingdom Straminipila [Damare et al. 2012] is one such ecological and physiological group [Hyde et al. 1998], distinct in their geographical dispersal and the substratum on which they inhabit. Among these, obligate marine fungi are those that grow and sporulate exclusively in marine or estuarine habitat while facultative marine fungi are freshwater or terrestrial origin that have undergone
physiological adaptations which allow them to grow in the marine environment [Kohlmeyer and Kohlmeyer 1979]. Facultative marine fungi can be transferred to seawater by wind, rain or runoff soil. Few of them are able to evolve adaptation to marine environment and gradually get transferred to obligate marine forms [Zuluaga-Montero et al. 2010].

Estimates of fungal diversity range from 1.5 million species [Hawksworth 2001] to 3.5–5.1 million species [O'Brien et al. 2005] encompassing a wide range of fungi that possess diverse metabolisms and are adapted for life in heterogeneous environments [Stajich et al. 2009]. However, the share of marine fungi is nearly 1000 to 1500 only. Jones et al. [2009] reported on 530 marine fungi coming under 321 genera, which include 424 Ascomycota (251 genera), 94 mitosporic fungi (61 genera) and 12 Basidiomycota (9 genera).

Fungi (culturable) in the shelf sediments of south western Bay of Bengal

Microflora in the continental shelf sediments of south-western Bay of Bengal

hydrothermal vents [Lopez-Garcia et al. 2003, 2007], and even anoxic marine sediments [Stoeck and Epstein 2003; Stoeck et al. 2003, 2006; Jebaraj et al. 2010]. Invariably when new substrata are surveyed for fungi, a wide range of new genera and species are encountered. However, the fraction of culturable isolates obtained is very low with regard to the overall estimated biodiversity.

In terrestrial environment, fungi are ubiquitous for their role in processing detrital organic matter [Carlile et al. 2001]. Equally, those inhabiting the fresh water and coastal marine ecosystems could play a major role in the detrital food web [Hyde et al. 1998]. However, information on their role in processing the organic matter and thereby biogeochemical cycling [Newell 2001] in the ocean is less, when related with the terrestrial environment [Clipson et al. 2006]. In marine environment, they execute a range of ecological functions to degrade a wide range of recalcitrant biological molecules. Moreover, marine fungi are fast growing as source of potential bioactive resource that could be exploited in biotechnology. Growing number of compounds (e.g., polyketides, terpenoids, alkaloids, peptides, cerebroside analogs) with antimicrobial, antiprotozoal, and cytotoxic activities have been isolated from marine fungi [Bugni and Ireland 2004; Bhadury et al. 2006; Saleem et al. 2007]. Further they are also involved in the acquisition of metals from seafloor basalts and partially responsible for their transfer and accumulation in the biosphere. Oil degrading fungi have been identified in both terrestrial [Capotorti et al. 2004; Reyes-César et al. 2014; Covino et al. 2015; Marco-Urrea et al. 2015; Mineki et al. 2015] and marine...
environments [Da Silva et al. 2003; Cerniglia and Sutherland 2010; Passarini et al. 2011; Hassanshahian et al. 2012].

The distribution of fungi in marine environment is principally determined by the temperature and salinity of the water [Velez et al. 2013]. Lorenz and Molitoris [1992] demonstrated that, with adjusting incubation temperature and salinity to optimum for growth, some marine fungi showed an upward shift. This sort of interaction between salinity and temperature is termed Phoma pattern since it was first illustrated in the marine species of *Phoma*. The pH range for terrestrial fungi is generally between pH 4.5-6.0, whereas facultative marine fungi grow and produce various extracellular enzymes at pH 7-8 [Raghukumar et al. 1994; 1999; 2004, Damare et al. 2006]. Moreover, fungal species diversity may also be controlled by a combination of other factors such as, effects of habitats, substrate availability, competition, dissolved nutrients, osmotic effects, oxygen supply, pollutants, abundance of propagules in the water, hydrostatic pressure etc. [Booth and Kenkel 1986; Jones 2000].

Fungi have been known to exist in marine environments since early times. The earliest study to consider fungal distribution in any real ecological sense was that of Fries [1857]. Various mycologists had reported on the occurrence of marine fungi [Desmazières 1825; Durieu de Maisonuneuve and Montagne 1869; Cesati 1880; Hennings 1908] but, comprehensive studies of the group was carried out by Cotton [1909], Sutherland [1915, 1916 a, b] and Sparrow [1934, 1936] concerning fungi found growing on seaweeds. Although the first autochthonous marine fungus was described and illustrated about 150 years ago, serious
collection of fungi in marine habitats started with Barghoorn and Linder [1944] who described 25 species from submerged wood in New England and California. Roth et al. [1964] isolated marine fungi for the first time from oceanic waters of north western subtropical Atlantic Ocean down to a depth of 4450 m. In 1970s about 100 additional new species were reported mainly by Hohnk (Germany), Meyers (United States), Johnson (United States), Kohlmeyer (Germany), and Jones (Great Britain). However, fungi were not the object of serious biogeographical studies until 1980s [Pirozynski and Walker, 1983]. By then, quantitative data on the occurrence of tropical marine fungi have been published by Koch [1986]; Kohlmeyer [1984]; Zainal and Jones [1984, 1986]. The monograph, Marine Mycology [Kohlmeyer and Kohlmeyer 1979] lists 209 filamentous species. However, twelve years later the number increased to 321 [Kohlmeyer and Volkmann-Kohlmeyer 1991].

For the past few decades marine fungi present in oceanic deep waters and associated sediments have been documented [Fell 1976; Sinclair and Ghiorse 1989; Raghukumar et al. 1992; Raghukumar and Raghukumar 1998; Gonzalez et al. 1998; Raghukumar et al. 2004; Gautschi et al. 2004; Nagahama 2006; Damare et al. 2006; Das et al. 2009; Singh et al. 2010, 2011]. Studies have also been conducted from hydrocasts near hydrothermal plumes from the Mid-Atlantic Ridge [Gadanho and Sampaio 2005], the Pacific sea-floor sediments [Nagahama et al. 2001] and Chagos Trench in the Indian Ocean [Raghukumar et al. 2004]. Takami et al. [1997] showed the presence of
fungi in sediment samples obtained from the Mariana Trench at a depth of 10,500 m in the Pacific Ocean.

Several works have been conducted on the abundance of microfungi from the marine sediments of Indian coast. Damare et al. [2006] have recovered 163 cultivable fungi from the Central Indian Basin deep-sea sediments. Similarly, a total number of 131 species belonging to 77 genera of fungi were isolated from the mangroves of Bay of Bengal by Vittal and Sarma [2006]. In 2009, Das et al. reported filamentous fungal population from the continental slope of Bay of Bengal, India. They could record 16 fungal genera and the fungal population ranged from 3.47-59.51 CFU g⁻¹. In the consecutive year, Singh et al. [2010] isolated 16 filamentous culturable fungi from the deep-sea sediments of the Central Indian Basin. However, 39 species of fungi were isolated from the coastal marine ecosystem along south east coast of India by Babu et al. [2010]. And Nambar and Raveendran [2010] could encounter 82 marine fungi, including 54 Ascomycetes, 2 Basidiomycetes and 26 Mitosporic fungi from the south Indian coastal waters. Later on, population density of 6.224 to 12.432 CFU g⁻¹ filamentous marine fungi from the south east coast of Bay of Bengal was recorded by Samuel et al. [2011]. In the same year, Samuel and Prabakaran [2011] could isolate thirty six species of marine fungi from the sediment samples collected from the intertidal regions of the Adirampattinam coast, south east coast of India. Further, in 2012, Singh et al. investigated fungal population from a depth of 5000m in the Central Indian Basin (CIB) and reported 12 distinct fungal genera.
Fungal biodiversity is the topic that has attracted a great number of researchers over the past 50 years. This work was initially carried out in temperate water fungi, especially wood-inhabiting taxa [Schaumann 1969; Shearer and Burgos, 1987; Cuomo et al. 1988; Grasso et al. 1990] with fewer studies in more recent years [Petersen and Koch 1997; Panebianco et al. 2002; Abdel-Wahab 2011a, b]. The most intensive collections were made by Koch and Petersen [1996], Lintott and Lintott [2002], Jones et al. [1998], Jones [2010] and Abdel-Wahab et al. [2009] who reported 75 (Denmark), 38 (New Zealand), 92 (Friday Harbour, USA) and 41 (Italy) fungi, respectively.

Molecular techniques have been applied for identification and taxonomic studies of fungi. These include restriction fragment length polymorphism (RFLP) [Pederson, 1986], sequencing of ribosomal RNA [Kurtzman and Robnett 1991], restriction enzyme analysis (REA) of genomic DNA [Barbirio et al. 1994], random amplification of polymorphic DNA (RAPD) [Williams et al. 1990] etc. The identification of internal transcribed spacer (ITS) regions of fungal rRNA genes as discriminative targets for molecular analysis of fungal communities and their high sequence variability relative to the flanking sequences makes them valuable for genus- and species-level identification [Buchan et al. 2002].

Several studies documented the fungal diversity in marine environments by the culture-dependent [Nagahama et al. 2001; Raghukumar et al. 2004; Gadinho and Sampaio 2005; Damare et al. 2006; Le Calvez et al. 2009; Burgaud et al. 2009; Connell et al. 2009;
Jebaraj et al. 2010; Singh et al. 2010] and culture-independent methods [Bass et al. 2007; Lopez-Garcia et al. 2007; Lai et al. 2007; Le Calvez et al. 2009; Jebaraj et al. 2010; Nagano et al. 2010; Sauvadet et al. 2010; Singh et al. 2011; Quaiser et al. 2011; Nagahama et al. 2011]. These studies reported that fungi from marine environments mostly belong to the phylum Ascomycota which produces sexual spores within an ascus, and a few species belonging to the Basidiomycota having sexual spores born externally on a basidium [Nagano and Nagahama 2012]. Within the phylum Ascomycota, Eurotiomycetes are the most frequently detected fungi followed by the classes Saccharomycetes, Dothideomycetes and Sordariomycetes. The majority of species belonging to the Eurotiomycetes are members of the *Aspergillus/Penicillium* group. Most marine derived fungi are mitosporic taxa belonging to the genera *Aspergillus, Cladosporium, Fusarium, Gliocladium, Microsphaeriopsis, Paecilomyces, Penicillium, Phoma, Phomopsis, Trichoderma* and *Ulocladium* [Bugni and Ireland 2004]. Less commonly reported species include those that represent the facultative marine genera *Scytalidium, Verticillium* and *Oidiodendron* as well as obligate marine fungi from the genus *Dendriphiella* [Khudyakova et al. 2000]. Five decades of marine mycology have demonstrated that marine fungi are distinct from their terrestrial and freshwater counterparts, both in their taxonomy, morphology and adaptation to an aquatic habitat [Barghoom and Linder 1944; Johnson and Sparrow 1961; Jones 1976; Kohlmeyer and Kohlmeyer 1979].
A few works on fungal diversity were reported from Indian Coast. Das et al. [2009] focused on the diversity of filamentous fungi within the continental slope sediments of Bay of Bengal and found *Aspergillus* as the dominant genus. Fungal community analysis in the deep-sea sediments of the Central Indian Basin (CIB) by culture-independent approach was evaluated by Singh et al. [2011]. A total of 39 fungal operational taxonomic units, with 32 distinct fungal taxa were recovered from 768 clones generated from 16 environmental clone libraries. Their results suggested the existence of cosmopolitan marine fungi in the sediments of CIB. Assessment of mycological diversity of marine sediment of south east coast of Tamil Nadu, India was determined by Samuel et al. [2011]. Diverse filamentous fungi were recovered from the sediment samples of Adirampattinam coastal environs. In 2012 Singh et al. compared the fungal diversity of deep sea sediments of Central Indian Basin (CIB) by both culture-dependent and culture-independent approaches and determined a total of 19 culturable fungi and 46 operational taxonomic units (OTUs) respectively. Later, Saravanan and Sivakumar [2013] concentrated on the diversity of fungi along the coast of Tamil Nadu, India and could identify *Aspergillus* as the common genus represented by 14 species followed by *Penicillium* and *Cladosporium*.

Though marine fungi have been studied for some 100 years, and progressively over the past 50 years, many aspects still remain poorly documented. Survey of marine fungi needs to be continued as many habitats remain unexplored. The study of marine fungi is essential as
they do play a vital role in ecology of marine ecosystems and in the food web of the oceans. They are mainly important in coastal systems, as decomposers of vascular plants [Newell 1993]. Fungi can decompose cellulose and lignin and thus play a prominent part in inland waters as well as in the sea. But they are also to be found among the ‘symbionts’, the ‘parasites’ and the ‘super parasites’. Certain parasitic groups of fungi in the marine habitat cause diseases in sea weeds and aquatic animals. Thus, marine fungi are among the worst enemies as well as best supports to the ecosystem, warranting a special attention for study of their diversity and conservation. Correct identification of strains is a prerequisite for both the utilization and conservation of fungal diversity. This chapter mainly concentrates on the abundance, distribution and diversity of fungi along the continental shelf sediments of Bay of Bengal.

5.2 Materials and Methods
5.2.1 Sample preparation

Sediment samples collected from the shelf regions were serially diluted with sterile seawater blanks. Approximately 10 g of the sediment sample was suspended in 90 ml of sterile seawater and subjected to shaking for 15 minutes. The supernatant was then serially diluted and suitable dilutions ($10^{-3}$ and $10^{-4}$) were used as the inoculum. Total culturable fungi in the sediment samples were determined using culture dependent technique.
5.2.2 Estimation of culturable fungal population

Conventional spread plate method was employed for the estimation of culturable fungi along the shelf sediments. Rose Bengal agar medium supplemented with chloramphenicol (100 mg/L medium) was used for the isolation and enumeration of marine fungi (Plate 5.1). The plates in triplicate were incubated at 28 ± 2 °C for 2-4 weeks. Colonies were counted and expressed as colony forming units (CFU) per gram dry weight of sediment.

**Rose Bengal Agar –Composition (g/L)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>10 g</td>
</tr>
<tr>
<td>Mono potassium phosphate</td>
<td>1 g</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>Sea water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

5.2.2.1 Isolation and preservation of cultures

Colonies with different morphological characteristics (colony reverse colour, colony texture, size and shape) were isolated from plates. The cultures were repeatedly streaked on to malt extract agar plates for purity and preserved in malt extract agar vials overlaid with sterile liquid paraffin. The purified cultures were then resuspended in sterile 0.9% (w/v) saline supplemented with 20% (v/v) glycerol, then stored at −80 °C.
Malt Extract Agar- composition (g/L)

- Peptone : 5.0 g
- Malt extract : 15 g
- Agar : 20 g
- Sea water (50%) : 1000 ml
- pH : 7.2.

5.2.2.2 Microscopic slide preparation

The microscopic examination was done by scotch tape method (Plate 5.2). The sticky side of a piece of clear cellophane tape was first applied to the surface of the colony. The tape was then placed sticky side down on a drop of Lactophenol cotton blue placed on a clean glass slide. The slide was then examined under the microscope. Spores and hyphae that have adhered to the tape could be evaluated through the tape, which acts as a cover slip.

5.2.3 Molecular identification of culturable fungi

Culturable fungi (151 Nos) isolated from the shelf sediments of south western Bay of Bengal were subjected to molecular identification.

5.2.3.1 DNA extraction

Genomic DNA was extracted from the fungal isolates as per standard Proteinase-K digestion method [Bollet et al. 1991]. Cultures were suspended in malt extract broth and incubated in an orbital incubator at 28 °C, 120 rpm for 12-18 hours. For details please refer section 3.2.3.1.
5.2.3.2 Determining quality and quantity of DNA

Refer section 3.2.3.2.

5.2.3.3 Amplification of ITS region

The ITS region of the fungal DNA were amplified using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCGCTTATTGATATGC-3′) [White et al. 1990]. PCR amplifications were performed in 25 μl reaction volume containing 1 x standard Taq buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 3.5 mM MgCl₂, 200 mM dNTPs, 0.4 mM each primer, 1U Taq DNA polymerase (Fermentas, Inc.) and 1–2 μl of DNA template (10–100 ng). The PCR programme used was an initial denaturation of 95 °C for 5 min, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 30 s) and extension (72 °C for 30 s), and a final extension (72 °C for 10 min). Reaction mixtures lacking template DNA were used as negative controls.

5.2.3.4 Agarose Gel Electrophoresis

Refer section 3.2.3.4.

5.2.3.5 Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Amplified ribosomal DNA restriction analysis (ARDRA) was done to group the fungal isolates. PCR products (0.5 – 1.0 μg) were digested using four restriction enzymes Taq1, Hinf1, Sdu1 and Cfo1 (Fermentas, Inc.) in separate reactions. The reaction volume consisted of 20 μl containing 8 μl of the PCR product, 2 μl buffer (50 mM NaCl, 10 mM, Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol) 8 μl of milliQ
and 2 μl (5 units) of each restriction enzyme. The digestions were performed for 16 hours at an incubation temperature of 37 °C for *Hinf* I, *Cfo*I and *Sdu*I and at 65 °C for *Taq*I as recommended by the manufacturer. Gel images were analyzed to construct ARDRA profiles for various fungal isolates. For details please refer section 3.2.3.5.

5.2.3.6 ITS sequencing and sequence analysis

One representative of each ARDRA pattern was selected for sequencing. All the sequences were compared with those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm [Altschul et al. 1990] at the National Centre for Biotechnology information (NCBI), USA (www.ncbi.nlm.nih.gov) and CBS- KNAW Fungal Biodiversity Centre, an institute of Royal Netherlands Academy of Arts and Sciences, Netherlands (www.cbs.knaw.nl/databases). For details please refer section 3.2.3.6.

5.2.3.7 Phylogenetic Analysis

ITS sequences of the fungal isolates and homologous reference sequences retrieved from the NCBI database were aligned using ClustalW [Thompson et al. 1994] with standard parameters (Appendix Table 11). For details please refer section 3.2.3.7.

5.2.3.8 Nucleotide Sequence Accession Numbers

The sequences determined in this study were deposited in the GenBank database using the web based data submission tool, BankIt (http://www.ncbi.nlm.nih.gov/BankIt).
5.2.4 Statistical methods for community analysis

Statistical tools used for community analysis were same as that employed for culturable bacteria. For details please refer section 3.2.4.

5.3 Results

5.3.1 Abundance and distribution of culturable fungi

The fungal population in the shelf sediments of Bay of Bengal was meagre and shifts from 0.13 to 3.1 CFU g\(^{-1}\) dry wt. (Fig.5.1), with an average of 0.47 CFU g\(^{-1}\) dry wt. Significant (p<0.05) depth wise variation in abundance was noticed within the sediment layers. Population was hiked towards the greater depths of the shelf (200 m) (Fig 5.2). Abundance at 50 m depth was in the range of 0.15 – 0.53 CFU g\(^{-1}\) dry wt. (mean ± SD; 0.22 ± 0.13 CFU g\(^{-1}\) dry wt.), at 100 m it ranged from 0.13- 3.1 CFU g\(^{-1}\) dry wt. (mean ± SD; 0.67 ± 1.08 CFU g\(^{-1}\) dry wt.) and at 200 m, it was 0.19-1.1 CFU g\(^{-1}\) dry wt. (mean ± SD; 0.52 ± 0.3 CFU g\(^{-1}\) dry wt.). Least abundance was indicated from the 100 m depth off Karaikal and highest from the same depth off Cheyyur.

Latitudinal variation in abundance was noticed within the shelf sediments. The population revealed a hike towards the northern latitudes though the variation was statistically insignificant (Fig.5.3). Cheyyur was the single dominant transect with highest population followed by Singaraykonda. The average fungal population off Karaikal was 0.16 ± 0.03 CFU g\(^{-1}\) dry wt., off Cuddalore 0.19 ± 0.0 CFU g\(^{-1}\) dry wt., off Cheyyur 1.45 ± 1.22 CFU g\(^{-1}\) dry wt., off Chennai 0.35 ± 0.17 CFU g\(^{-1}\)
dry wt., off Thamminapatnam 0.24 ± 0.12 CFU g⁻¹ dry wt., and off Singarayakonda was 0.44 ± 0.22 CFU g⁻¹ dry wt. (Appendix Table 6).

**Fig. 5.1.** Culturable fungal population present in shelf sediments of south western Bay of Bengal

**Fig. 5.2.** Culturable fungal population at various depths (50, 100 and 200 m) in the shelf sediments of south western Bay of Bengal
Fungi (culturable) in the shelf sediments of south western Bay of Bengal

Fig. 5.3. Map showing the distribution of culturable fungi in the shelf sediments of south western Bay of Bengal

Plate 5.1. Rose Bengal agar plate showing fungal colonies
5.3.2 Molecular identification of the culturable fungi by ITS sequence analysis

5.3.2.1 Amplification of the ITS region

The PCR amplification of ITS region using universal primers ITS1 and ITS4 yielded a single amplicon of approximately 600-700 bp for all the fungal isolates (Fig. 5.4).

![Agarose gel electrophoresis](image)

Fig. 5.4. Agarose gel electrophoresis (1.5%) of PCR amplified ITS region of fungi in the shelf sediments of south western Bay of Bengal

5.3.2.2 Amplified Ribosomal DNA Restriction (ARDRA) Analysis

ARDRA was carried out on the ITS amplicons of each sample using tetra cutter restriction endonuclease, *Taq*1, *Hinf*1, *Sdu*1 and *Cfo*1 (Fig. 5.5, 5.6, 5.7 and 5.8). Fragments smaller than 100 bp were not considered. A total of 26 ARDRA profiles, or haplotypes were obtained on combination of all the restriction patterns obtained using four enzyme cutters. When treated individually, cluster analysis based on restriction digestion using *Taq*1, *Hinf*1, *Sdu*1 and *Cfo*1 enzyme produced 20, 18, 20 and 21 different clusters respectively. From the 26 different ARDRA
groups thus produced (Fig. 5.9) on combination of these restriction patterns, the nucleotide sequence of ITS region were determined which corresponds to 26 different fungal species. The restriction profile obtained using Taq1, Hinf1, Sdu1 and Cfo1 are given in Table 5.1.

![ARDRA patterns of Taq1 restriction on ITS amplicons of fungal isolates](image)

**Fig. 5.5.** ARDRA patterns of Taq1 restriction on ITS amplicons of fungal isolates

![ARDRA patterns of Hinf1 restriction on ITS amplicons of fungal isolates](image)

**Fig. 5.6.** ARDRA patterns of Hinf1 restriction on ITS amplicons of fungal isolates
Fig. 5.7. ARDRA patterns of Sdu1 restriction on ITS amplicons of fungal isolates

Fig. 5.8. ARDRA patterns of Cfo1 restriction on ITS amplicons of fungal isolates
### Table 5.1. ARDRA profile of representative fungal isolates using restriction enzymes *Taq1*, *Hinf1*, *Sdu1* and *Cfo1*

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th><em>Taq1</em></th>
<th><em>Hinf1</em></th>
<th><em>Sdu1</em></th>
<th><em>Cfo1</em></th>
<th>Species Identity</th>
<th>GenBank Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>JF 1</td>
<td>225+200</td>
<td>275+250</td>
<td>400+150</td>
<td>300+100</td>
<td><em>Fusarium solani</em></td>
<td>KT151576.1</td>
</tr>
<tr>
<td>JF 6</td>
<td>160+155+100</td>
<td>300+250</td>
<td>260+125</td>
<td>180+175+150</td>
<td><em>Penicillium dipodomycota</em></td>
<td>KT151579.1</td>
</tr>
<tr>
<td>JF 7</td>
<td>225+150+100</td>
<td>300+175+100</td>
<td>300+175+100</td>
<td>250+175</td>
<td><em>Aspergillus versicolor</em></td>
<td>KT151568.1</td>
</tr>
<tr>
<td>JF 10</td>
<td>250+235</td>
<td>300+150</td>
<td>350+100</td>
<td>200+100+100</td>
<td><em>Auxarthron</em> sp.</td>
<td>KT151569.1</td>
</tr>
<tr>
<td>JF 11</td>
<td>200+155+150</td>
<td>300+250</td>
<td>250+175</td>
<td>180+175+100</td>
<td><em>Penicillium citrinum</em></td>
<td>KM226953.1</td>
</tr>
<tr>
<td>JF 12</td>
<td>150+100</td>
<td>250+225</td>
<td>475</td>
<td>300+130+100</td>
<td><em>Westerdykella dispersa</em></td>
<td>KT151564.1</td>
</tr>
<tr>
<td>JF 13</td>
<td>225+160+130</td>
<td>300+275</td>
<td>250+175</td>
<td>300+175</td>
<td><em>Aspergillus allahabadi</em></td>
<td>KT151566.1</td>
</tr>
<tr>
<td>JF 14</td>
<td>250+160+150</td>
<td>300+125+100</td>
<td>250+175</td>
<td>350+175</td>
<td><em>Aspergillus terreus</em></td>
<td>KT151570.1</td>
</tr>
<tr>
<td>JF 15</td>
<td>200+150+100</td>
<td>300+150+100</td>
<td>300+150</td>
<td>180+175</td>
<td><em>Aspergillus austroafricanus</em></td>
<td>KT151567.1</td>
</tr>
<tr>
<td>JF 17</td>
<td>250+150</td>
<td>225+200+100</td>
<td>325</td>
<td>200+160+150</td>
<td><em>Penicillium</em> sp.</td>
<td>KT151574.1</td>
</tr>
<tr>
<td>JF 18</td>
<td>250+275</td>
<td>300+275</td>
<td>475</td>
<td>350+275</td>
<td><em>Rigidoporus vinctus</em></td>
<td>KT151575.1</td>
</tr>
<tr>
<td>JF 19</td>
<td>250+150+125</td>
<td>300+250</td>
<td>350+175</td>
<td>270+285</td>
<td><em>Aspergillus cristatus</em></td>
<td>KT151565.1</td>
</tr>
<tr>
<td>JF 20</td>
<td>400+150</td>
<td>350+100</td>
<td>600</td>
<td>275+190+175</td>
<td><em>Daluria eschscholbi</em></td>
<td>KT151578.1</td>
</tr>
<tr>
<td>JF 23</td>
<td>225+150</td>
<td>275+200</td>
<td>425+125</td>
<td>300+175</td>
<td><em>Chloosporium sphaeroaspernum</em></td>
<td>KT151571.1</td>
</tr>
<tr>
<td>JF 26</td>
<td>150+125</td>
<td>300+275</td>
<td>275+150</td>
<td>200+175+100</td>
<td><em>Neosartorya fischeri</em></td>
<td>KT151572.1</td>
</tr>
<tr>
<td>JF 31</td>
<td>250+150+125</td>
<td>300+275</td>
<td>300+175</td>
<td>200+175+125</td>
<td><em>Aspergillus nigri</em></td>
<td>KT151581.1</td>
</tr>
<tr>
<td>JF 37</td>
<td>175+155+150</td>
<td>300+290</td>
<td>250+200</td>
<td>180+175+150</td>
<td><em>Penicillium chrysogenum</em></td>
<td>KT151573.1</td>
</tr>
<tr>
<td>JF 38</td>
<td>250+160</td>
<td>300+200+100</td>
<td>450+175</td>
<td>175+125+100</td>
<td><em>Scopulariopsis brevicaulis</em></td>
<td>KT151577.1</td>
</tr>
<tr>
<td>JF 39</td>
<td>250+160</td>
<td>315+300</td>
<td>340+175+100</td>
<td>300+200+100</td>
<td><em>Sagenomella</em> sp.</td>
<td>KT151580.1</td>
</tr>
<tr>
<td>JF 45</td>
<td>225+150+100</td>
<td>300+125+100</td>
<td>400+175</td>
<td>250+175+100</td>
<td><em>Aspergillus sydowii</em></td>
<td>KT175219.1</td>
</tr>
<tr>
<td>JF 60</td>
<td>250+150+125</td>
<td>300</td>
<td>250+175</td>
<td>200+175+150</td>
<td><em>Penicillium brevfieldianum</em></td>
<td>KT175220.1</td>
</tr>
<tr>
<td>JN 15</td>
<td>250+225</td>
<td>275+150</td>
<td>400+125</td>
<td>200+100</td>
<td><em>Emericellopsis terricola</em></td>
<td>KT151582.1</td>
</tr>
<tr>
<td>JN 16</td>
<td>200+150+125</td>
<td>300</td>
<td>350+160</td>
<td>275+260</td>
<td><em>Eurotiun herbariorum</em></td>
<td>KT151583.1</td>
</tr>
<tr>
<td>JN 26</td>
<td>250+150+140</td>
<td>200+100+275</td>
<td>450</td>
<td>250+160+150</td>
<td><em>Talaromyces indigoticus</em></td>
<td>KT151584.1</td>
</tr>
</tbody>
</table>
5.3.2.3 Analysis of ITS sequences of fungal isolates

ITS sequencing of the representative isolates from each ARDRA profile deduced the sequence for all the 26 isolates. Sequences were aligned and the closest match was detected using BLAST. A substantial fraction of the isolates were closely related to previously documented fungal species, with an average identity of 99.5%. Sequence similarities of identified strains compared to the nearest phylogenetic neighbour is shown in the Table 5.2. The sequences determined were deposited in the GenBank database under accession numbers, KM226953.1, KT151564.1-KT151584.1, KT175219.1-KT175220.1 (24 Nos.).

The strains identified by ITS sequence analysis included twenty six species grouped into two phylum including four classes and eight
taxonomic orders: Eurotiales, Onygenales, Pleosporales, Capnodiales, Xylariales, Microascales, Hypocreales and Polyporales. The two representative phylum under which these orders belonged were Ascomycota and Basidiomycota.

Out of the four taxonomic classes, Eurotiomycetes, Dothideomycetes and Sordariomycetes belonged to Ascomycota representing twenty five species and the Agaricomycetes represented one species. About 94% of the species belonged to the phylum Ascomycota whereas, the contribution of phylum Basidiomycota were only 6% (Fig.5.10). Class Eurotiomycetes (70%) contribute significantly to the dominance of Ascomycota along with Sordariomycetes (17%) and Dothideomycetes (7%) (Fig.5.11). The abundance of Eurotiomycetes was unique throughout all depths though its share varies like 74%, 78% and 64% at 50, 100 and 200 m depth respectively. Interestingly, Sordariomycetes were equally represented at all depths (16-18%) while Dothideomycetes constitute 4% at 50 m and 100 m depth whereas it prominently increased to 14% at 200 m. The contribution of Agaricomycetes which belonged to the class Basidiomycota was 5-6% at 50 m and 200 m depth though it was completely absent from 100 m depth (Fig.5.12, 5.13 and 5.14).
Fig. 5.10. Percentage contribution of different fungi in the shelf sediments of south western Bay of Bengal

Fig. 5.11. Percentage contribution of different taxonomic classes in the shelf sediments of south western Bay of Bengal

Fig. 5.12. Percentage contribution of different taxonomic classes at 50 m depth of the shelf sediments of south western Bay of Bengal
The different genera identified from the shelf sediments include *Penicillium*, *Eupenicillium*, *Aspergillus*, *Eurotium*, *Talaromyces*, *Neosartorya*, *Sagenomella*, *Auxarthron*, *Westerdykella*, *Cladosporium*, *Daldinia*, *Scopulariopsis*, *Emericellopsis*, *Fusarium* and *Rigidoporus*. Among these fifteen genera, *Aspergillus* (30%) was the dominant genera followed by *Penicillium* (17%), *Fusarium* (10%) and *Sagenomella* (8%) (Fig.5.15). *Eupenicillium*, *Scopulariopsis*, *Auxarthron* and *Daldinia* make the least abundant forms. Interesting variation in their composition...
was observed latitudinally. Class Agaricomycetes representing *Rigidoporus* was completely absent from Karaikal, Cheyyur and Thamminapatnam. *Westerdykella* and *Cladosporium* which belongs to class Dothideomycetes was rare to Singarayakonda coast. Eurotiomycetes were ubiquitously present throughout the transect whereas Sordariomycetes representatives were less distributed within the transects.

Fig. 5.15. Percentage contribution of different fungal genera identified within the shelf sediments of Bay of Bengal

All the representative species affiliated with aforementioned fifteen genera under the four classes are discussed below.

**Eurotiomycetes**

Majority of the species identified belonged to the class Eurotiomycetes. The representatives constituting this class include *Penicillium citrinum*, *Penicillium chrysogenum*, *Penicillium dipodomyicola*, *Penicillium brefeldianum*, *Penicillium* sp., *Eupenicillium javanicum*, *Aspergillus*
Fungi (culturable) in the shelf sediments of south western Bay of Bengal

allahabadii, Aspergillus austroafricanus, Aspergillus versicolor, Aspergillus terreus, Aspergillus niger, Eurotium cristatum, Eurotium herbariorum, Talaromyces indigoticus, Neosartorya fischeri and Sagenomella sp. of order Eurotiales and Auxarthron sp. of Onygenales.

Among these Penicillium dipodomyicola, Eupenicillium javanicum, Aspergillus allahabadii and Aspergillus austroafricanus were newly reported from the shelf sediments of Bay of Bengal. Few like Sagenomella sp. and Auxarthron sp. exhibiting 99% similarity to type strains EU140821.1 and KC470857.1 respectively, were new reports from the Bay.

Sordariomycetes

The species belonging to class include Daldinia schschoizii of order Xylariales, Scopulariopsis brevicaulis of order Microascales and Emericellopsis terricola, Fusarium solani of order Hypocreales. Daldinia eschscholzii exhibiting 100% to the type strain FR852577.1 and Emericellopsis terricola to the type strain KF156303.1 are new reports from Bay of Bengal.

Dothideomycetes

Only two species Westerdykella dispersa and Cladosporium sphaerospermum belonged to this class. Westerdykella dispersa showing 100% similarity to the type strain NR111187.1 was a new report from the shelf sediments of Bay of Bengal.

Agaricomycetes

The only species belonging to this class was Rigidoporus vinctus, a new report from the shelf sediments of Bay of Bengal.
## Table 5.2. NCBI BLAST results of fungal strains isolated from shelf sediments of south western Bay of Bengal

<table>
<thead>
<tr>
<th>Isolate No</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Max identity</th>
<th>Reference Accession no.</th>
<th>BLAST results</th>
</tr>
</thead>
<tbody>
<tr>
<td>JF 1</td>
<td>953</td>
<td>953</td>
<td>97%</td>
<td>0</td>
<td>100%</td>
<td>HQ026747.1</td>
<td><em>Fusarium solani</em></td>
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<tr>
<td>JF 6</td>
<td>1524</td>
<td>1524</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>DQ339570.1</td>
<td><em>Penicillium dipodomyicola</em></td>
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<tr>
<td>JF 7</td>
<td>893</td>
<td>893</td>
<td>99%</td>
<td>0</td>
<td>99%</td>
<td>NR131277.1</td>
<td><em>Aspergillus versicolor</em></td>
</tr>
<tr>
<td>JF 10</td>
<td>942</td>
<td>942</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>KC470857.1</td>
<td><em>Auxarthron sp.</em></td>
</tr>
<tr>
<td>JF 11</td>
<td>1086</td>
<td>1086</td>
<td>90%</td>
<td>0</td>
<td>100%</td>
<td>AF033422.1</td>
<td><em>Penicillium citrinum</em></td>
</tr>
<tr>
<td>JF 12</td>
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<td>939</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>NR111187.1</td>
<td><em>Westerdykella dispersa</em></td>
</tr>
<tr>
<td>JF 13</td>
<td>1014</td>
<td>1014</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>EF669601.1</td>
<td><em>Aspergillus allahabadii</em></td>
</tr>
<tr>
<td>JF 14</td>
<td>966</td>
<td>966</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>NR131276.1</td>
<td><em>Aspergillus terreus</em></td>
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<tr>
<td>JF 15</td>
<td>941</td>
<td>941</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>JQ301891.1</td>
<td><em>Aspergillus austroafricanus</em></td>
</tr>
<tr>
<td>JF 17</td>
<td>1365</td>
<td>1365</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>EU639449.1</td>
<td><em>Penicillium sp.</em></td>
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<tr>
<td>JF 18</td>
<td>1105</td>
<td>1105</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>JQ717317.1</td>
<td><em>Rigidoporus vinctus</em></td>
</tr>
<tr>
<td>JF 19</td>
<td>928</td>
<td>928</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>JX869560.1</td>
<td><em>Aspergillus cristatus</em></td>
</tr>
<tr>
<td>JF 20</td>
<td>963</td>
<td>963</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>FR852577.1</td>
<td><em>Daldinia eschscholzii</em></td>
</tr>
<tr>
<td>JF 23</td>
<td>918</td>
<td>918</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>JX156365.1</td>
<td><em>Cladosporium sphaerospernum</em></td>
</tr>
<tr>
<td>JF 26</td>
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<td>983</td>
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<td>0</td>
<td>100%</td>
<td>AY373895.1</td>
<td><em>Neosartorya fischeri</em></td>
</tr>
<tr>
<td>JF 31</td>
<td>1040</td>
<td>1040</td>
<td>100%</td>
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<td>100%</td>
<td>FJ878651.1</td>
<td><em>Aspergillus niger</em></td>
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<tr>
<td>JF 37</td>
<td>1448</td>
<td>1448</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>GQ458038.1</td>
<td><em>Penicillium chrysogenum</em></td>
</tr>
<tr>
<td>JF 38</td>
<td>970</td>
<td>970</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>EU821476.1</td>
<td><em>Scopulariopsis brevicaulis</em></td>
</tr>
<tr>
<td>JF 39</td>
<td>1005</td>
<td>1005</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>EU140821.1</td>
<td><em>Sagenomella sp.</em></td>
</tr>
<tr>
<td>JF 45</td>
<td>917</td>
<td>917</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>JN851052.1</td>
<td><em>Aspergillus sydowii</em></td>
</tr>
<tr>
<td>JF 60</td>
<td>883</td>
<td>883</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>AF033435.1</td>
<td><em>Penicillium brefeldianum</em></td>
</tr>
<tr>
<td>JF 137</td>
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<td>911</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>KT224830.1</td>
<td><em>Eupenicillium javanicum</em></td>
</tr>
<tr>
<td>JF 119</td>
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<td>928</td>
<td>100%</td>
<td>0</td>
<td>100%</td>
<td>GU784865.1</td>
<td><em>Eurotium cristatum</em></td>
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<tr>
<td>JN 15</td>
<td>970</td>
<td>970</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>KF156303.1</td>
<td><em>Emeriella tericola</em></td>
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<tr>
<td>JN 16</td>
<td>1367</td>
<td>1367</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>EF652078.1</td>
<td><em>Eurotium herbariorum</em></td>
</tr>
<tr>
<td>JN 26</td>
<td>793</td>
<td>973</td>
<td>100%</td>
<td>0</td>
<td>99%</td>
<td>JN899331.1</td>
<td><em>Talaromyces indigoticus</em></td>
</tr>
</tbody>
</table>

### 5.3.2.4 Diversity and occurrence of isolated strains

The most abundant species identified from the shelf sediments of Bay of Bengal was *Penicillium citrinum* (9%) and *Aspergillus cristatus*. 
(9%) which was followed by *Fusarium solani* (8%) and *Sagenomella* sp. (7%) (Fig.5.16). Least abundant species include *Pencillium* sp. (1%) and *Eurotium herbariorum* (1%). Dominance of *Penicillium citrinum* (11%) and *Aspergillus cristatus* (9%) again dominated the inner shelf along with *Fusarium solani* (9%) and *Aspergillus versicolor* (7%) (Fig.5.17). At 100 m depth, *Fusarium solani* (12%) was the dominant species followed by *Penicillium citrinum* (10%) and *Sagenomella* sp. (10%) (Fig.5.18) whereas, *Cladosporium sphaerospermum* (10%), *Fusarium solani* (8%), *Aspergillus cristatus* (8%) and *Sagenomella* sp. (8%) predominated at 200 m depth (Fig.5.19). The species composition of culturable fungi at different stations of the study area is shown in Appendix Table 9.

**Fig. 5.16.** Percentage contribution of different fungal species in the shelf sediments of south western Bay of Bengal
Fig. 5.17. Percentage contribution of different fungal species in the shelf sediments of south western Bay of Bengal at 50 m depth

Fig. 5.18. Percentage contribution of different fungal species in the shelf sediments of south western Bay of Bengal at 100 m depth
Fungi (culturable) in the shelf sediments of south western Bay of Bengal

Fig. 5.19. Percentage contribution of different fungal species in the shelf sediments of south western Bay of Bengal at 200 m depth

Penicillium dipodomyicola

Neosartorya fischeri
Chapter 5

Aspergillus versicolor

Eurotium cristatum

Penicillium citrinum
Fungi (culturable) in the shelf sediments of south western Bay of Bengal

*Aspergillus allahabadii*

*Rigidoporus vinctus*

*Daldinia eschscholzii*
Plate 5.2. Macroscopic and microscopic observation of some of the identified fungus

*Emericellopsis terricola*

*Talaromyces indigoticus*

*Fusarium solani*
5.3.2.5 Phylogenetic analysis of fungal ITS region

The 26 different fungal sequences that were PCR amplified and sequenced were aligned to the most similar ones available in database. The alignments were used to construct the phylogenetic trees to show relationships between the fungal species identified from the shelf sediments to their closest phylogenetic neighbours. Phylogenetic analysis based on the ITS sequence of the 26 representative isolates confirmed their affiliation with the nearest phylogenetic neighbour, as indicated by BLAST of the ITS sequence. Most phylogenetic clusters were composed by only one or two isolates. Isolates exhibiting 97% or <97% similarity with the nearest phylogenetic neighbour at the ITS sequence level were considered as one group and those which exhibited >97% similarity were grouped into one group.

As seen in the phylogenetic tree, by depicting the bootstrap values, the 26 isolates were sorted into 4 main clusters, corresponding to at least 4 different taxonomic classes, Eurotiomycetes, Dothideomycetes, Sordariomycetes and Agaricomycetes. The phylogenetic relationship between 12 species belonging to the class Eurotiomycetes, 3 species from the class Sordariomycetes, and one each from Dothideomycetes and Agaricomycetes to their closest phylogenetic neighbours at 50 m depth is shown in Fig 5.20. The relationship between 14 species belonging to the class Eurotiomycetes, two from Sordariomycetes and one of Sordariomycetes to their closest phylogenetic neighbours at 100 m depth is shown in Fig 5.21. Similarly, the phylogeny of 12 species belonging to the class Eurotiomycetes, 3 species from the class Sordariomycetes, and two
from Dothideomycetes and one of Agaricomycetes to their closest phylogenetic neighbours at 200 m depth is shown in Fig 5.22.

Fig. 5.20. Neighbour-joining phylogenetic tree based on partial sequences of ITS, showing the relationships among fungi isolated from the 50 m depth region of south western Bay of Bengal. Bootstrap values are shown as percentages from 1000 replications at branch points.
Fungi (culturable) in the shelf sediments of south western Bay of Bengal

Fig. 5.21. Neighbour-joining phylogenetic tree based on partial sequences of ITS, showing the relationships among fungi isolated from the 100 m depth region of south western Bay of Bengal. Bootstrap values are shown as percentages from 1000 replications at branch points.
Fig. 5.22. Neighbour-joining phylogenetic tree based on partial sequences of ITS, showing the relationships among fungi isolated from the 200 m depth region of south western Bay of Bengal. Bootstrap values are shown as percentages from 1000 replications at branch points.
5.3.3 Shannon–Wiener index

Fungal species composition presented above is consistent with Shannon–Wiener data. Diversity values fluctuated between 2.32-3.43 (Table 5.3). The community structure reached the highest diversity in 50 m depth off Chennai and the lowest at 50 m and 100 m depth of Thamminapatnam. Reduced Shannon diversity at the inshore regions off Thamminapatnam resulted when the community was composed only by five species. Highest Shannon index was the result of 9 different species such as *Penicillium citrinum*, *Penicillium chrysogenum*, *Penicillium dipodomyicola*, *Aspergillus versicolor*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus sydowii*, *Cladosporium sphaerospermum*, *Daldinia eschscholzii*, *Fusarium solani* and *Rigidoporus vinctus*. Within other stations substantial differences in the fungal community composition were not observed.
Table 5.3. Diversity indices of culturable fungi present in the shelf sediments of south western Bay of Bengal (d- species richness, J'- species evenness, H'(log2)-species diversity, 1-λ'- species dominance) (A-50 m, B- 100 m and C- 200 m)

<table>
<thead>
<tr>
<th>Sample</th>
<th>d</th>
<th>J'</th>
<th>H'(log2)</th>
<th>1-Lambda'</th>
</tr>
</thead>
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<tr>
<td>KRKL A</td>
<td>2.916</td>
<td>0.993</td>
<td>2.788</td>
<td>0.978</td>
</tr>
<tr>
<td>KRKL B</td>
<td>3.083</td>
<td>1.000</td>
<td>2.807</td>
<td>1.000</td>
</tr>
<tr>
<td>KRKL C</td>
<td>2.860</td>
<td>0.987</td>
<td>2.771</td>
<td>0.968</td>
</tr>
<tr>
<td>CDLR A</td>
<td>3.568</td>
<td>0.996</td>
<td>3.160</td>
<td>0.992</td>
</tr>
<tr>
<td>CDLR B</td>
<td>2.995</td>
<td>0.995</td>
<td>2.795</td>
<td>0.987</td>
</tr>
<tr>
<td>CDLR C</td>
<td>2.995</td>
<td>0.995</td>
<td>2.795</td>
<td>0.987</td>
</tr>
<tr>
<td>CHRY A</td>
<td>3.568</td>
<td>0.996</td>
<td>3.160</td>
<td>0.992</td>
</tr>
<tr>
<td>CHRY B</td>
<td>3.366</td>
<td>1.000</td>
<td>3.000</td>
<td>1.000</td>
</tr>
<tr>
<td>CHRY C</td>
<td>2.690</td>
<td>0.994</td>
<td>2.571</td>
<td>0.983</td>
</tr>
<tr>
<td>CHNI A</td>
<td>4.061</td>
<td>0.993</td>
<td>3.435</td>
<td>0.989</td>
</tr>
<tr>
<td>CHNI B</td>
<td>3.439</td>
<td>0.993</td>
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<td>0.997</td>
<td>3.313</td>
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<td>1.000</td>
<td>2.322</td>
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<tr>
<td>TPTM B</td>
<td>2.485</td>
<td>1.000</td>
<td>2.322</td>
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</tr>
<tr>
<td>TPTM C</td>
<td>3.366</td>
<td>1.000</td>
<td>3.000</td>
<td>1.000</td>
</tr>
<tr>
<td>SKDA A</td>
<td>3.083</td>
<td>1.000</td>
<td>2.807</td>
<td>1.000</td>
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<td>2.585</td>
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</tr>
<tr>
<td>SKDA C</td>
<td>2.690</td>
<td>0.994</td>
<td>2.571</td>
<td>0.983</td>
</tr>
</tbody>
</table>

5.3.4 Bubble plot and Cluster analysis

Bubble plot analysis (Fig.5.23) was done to know the distribution and occurrence of dominant genera along the shelf sediments of south western Bay of Bengal. The representatives selected were *Penicillium citrinum*, *Aspergillus cristatus*, *Fusarium solani* and *Talaromyces*. 
indigoticus as dominant species whereas, Eurotium herbariorum, Scopulariopsis brevicaulis, Rigidoporus vinctus and Westerdykella dispersa as least representatives.

The most dominant species Penicillium citrinum, Aspergillus cristatus and Fusarium solani were much aggregated towards the southern regions of the study area while the other dominant species Talaromyces indigoticus showed its abundance towards northern areas. The least abundant species Eurotium herbariorum was seen only at 100 m depth of Cheyyur and Chennai. Scopulariopsis brevicaulis could be identified from 200 m depth of Karaikal and Cheyyur and 50 m depth of Cuddalore. Rigidoporus vinctus the only representative from Basidiomycota were seen from 50 m and 200 m depths of Chennai and Singarayakonda and also from 50 m depth of Cuddalore. Westerdykella dispersa was identified from the deeper fractions of Cuddalore, Cheyyur and Chennai.

Hierarchical clustering analysis delineates the fungal communities of the sampling stations into two main groups (Fig. 5.24). However the grouping does not follow any significant pattern demonstrating an uneven distribution of species at all depths.
Chapter 5

Transform: Square root
Resemblance: S17 Bray-Curtis similarity
Penicillium citrinum
0.5
2
3.5
5
KRKL A
KRKL B
CDLR A
CDLR B
CDLR C
CHYR A
CHYR B
CHYR C
CHNI A
CHNI B
CHNI C
TPTM A
TPTM B
TPTM C
SKDA A
SKDA B
SKDA C

Aspergillus cristatus
0.5
2
3.5
5
KRKL A
KRKL B
CDLR A
CDLR B
CDLR C
CHYR A
CHYR B
CHYR C
CHNI A
CHNI B
CHNI C
TPTM A
TPTM B
TPTM C
SKDA A
SKDA B
SKDA C

2D Stress: 0.25
Fungi (cultur able) in the shelf sediments of south western Bay of Bengal

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Transform: Square root
Resemblance: S17 Bray Curtis similarity

Fusarium solani

- KRKL A
- KRKL B
- KRKL C
- CDLR A
- CDLR B
- CDLR C
- CHYR A
- CHYR B
- CHYR C
- CHNI A
- CHNI B
- CHNI C
- TPTM A
- TPTM B
- TPTM C
- SKDA A
- SKDA B
- SKDA C

2D Stress: 0.25

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Talaromyces indigoticus

- KRKL A
- KRKL B
- KRKL C
- CDLR A
- CDLR B
- CDLR C
- CHYR A
- CHYR B
- CHYR C
- CHNI A
- CHNI B
- CHNI C
- TPTM A
- TPTM B
- TPTM C
- SKDA A
- SKDA B
- SKDA C

2D Stress: 0.25
Transform: Square root
Resemblance: S17 Bray-Curtis similarity

**Eurotium herbariorum**

0.5

2

3.5

5

**Scopulariopsis brevicaulis**

0.5

2

3.5

5
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Fig. 5.23. Bubble plots showing the distribution of important genera identified from the shelf sediments.

Microflora in the continental shelf sediments of south-western Bay of Bengal
5.3.5 Dominance Plot

The k-dominance curve of species abundance data pooled for each depth is presented in Fig 5.25. Eighty percent of total organisms were represented by 10 species at all depths. The study revealed an average diversity along the continental shelf, irrespective of depth.
5.4 Discussion

Fungi are found ubiquitously in the marine environment where they have a variety of niches to inhabit. In these systems, they execute a wide range of important ecological functions especially in the decomposition and mineralization of organic matter [Hyde et al. 1998]. Only a few studies on fungi have been carried out in marine sediments over the last several decades [Nagahama 2006].

Population density of marine fungi from the shelf sediments of Bay of Bengal ranged from 0.13 to 3.1 CFU g\(^{-1}\) dry wt. However, these records were lower than that observed from the continental slope sediments of Bay of Bengal [Das et al. 2009], deep subsurface sediments [Sinclair and Ghiorse 1989], tropical sand belt off Kanyakumari, India [Upadhyay et al. 1978] and coastal sediments off Tamil Nadu, India [Samuel et al. 2011]. The decrease of filamentous fungi may be due to the prevalence of facultative forms over true forms since; most of the filamentous marine fungi are host specific and uncultivable in a common medium [Hyde et al. 1987]. Even there are reports on the abundance of these facultative forms in the deep sea [Raghukumar et al. 2004]. Jones et al. [2009] stated that the fungi isolated from deep-sea sediments did not correspond to the fungi usually labelled as marine fungi. Further traditional cultivation based studies for fungal diversity analysis and lack of growth media and culture conditions suitable for all members of the community are responsible for the under representation of total fungal population [Anderson et al. 2003].
The number of marine fungi isolated from the shelf sediments of Bay of Bengal was comparable with those documented from coastal waters of Hong Kong [Jones and Vrijmoed 2003], tropical marine fungi of Brunei [Hyde and Jones 1988], deep sea sediments of Indian Ocean [Damare et al. 2006], south Indian coastal waters [Nambar and Raveendran 2010] and deep sea sediments of South China Sea [Zhang et al. 2013]. A significant variation was noticed from majority of marine habitats which inhabit only few isolates. Only 52 were found from the coastal beaches of Mexico [Gonzalez et al. 1998], 16 from Central Indian Basin [Singh et al. 2010], and 39 from coastal marine ecosystem along south east coast of India [Babu et al. 2010]. Similarly, Schmidt and Shearer [2004] found that different oceans supported varying numbers of fungal population. The number of fungi at each site varied: Atlantic Ocean: 12-46 per site; Indian Ocean: 12-64 and the Pacific Ocean: 17-87. And in the present study it was 151.

The abundance, distribution and diversity of fungal species identified from the shelf sediments of south western Bay of Bengal was not significantly different at the various depth profiles. Similar results were reported from the deep sea sediments of Indian Ocean by Damare et al. [2006]. However, an increase in fungal population towards greater depths was noticed during the present study. The sinking particulate organic matter from the dynamic surface waters might contribute to the abundance of population [Singh et al. 2010]. Further, it may be due to the large temporal variation or possibility of extremely patchy distribution of fungi in marine sediments with pockets of high fungal population at certain regions [Singh et al. 2010].
Similarly, variation in population was noticed between the transects chosen and the fungal population hiked towards the northern transects. Interestingly, Das et al. [2009] also reported the same fact from the continental slope sediments of Bay of Bengal. Fungi may be transported to the marine environment both in the form of dormant spores or actively growing mycelia through many ways. The presence of ‘islands of wood’ in the deep-sea has been described by Turner [1973], which seem to be due to sinking of waterlogged wood washed offshore during monsoons. Large particulate organic matter, such as decaying leaves and wood, may be carried offshore and eventually sink. Since rivers could also discharge spores or remains of fungal tissues from freshwater and terrestrial environments [Kohlmeyer and Kohlmeyer 1979] to the ocean, one could argue that the observed genotype patterns of fungi in coastal waters and sediments may include resistant propagules or surviving mycelium that have been transported to the marine environment.

In the present study, the Dikaryotic forms of fungi such as, Ascomycota and Basidiomycota, were the representative phylogenetic sequences identified using ITS primers as observed by O'Brien et al. [2005] and Singh et al. [2011] using the same primer sets. The presence of these phylogenetic groups from the marine environment was further illustrated by Lai et al. [2007] from methane hydrate-bearing deep-sea marine sediments in South China Sea and several other studies [Biddle et al. 2005; Biddle and Teske 2008]. However, the most common fungal group identified was the Ascomycota with 23 species, and it reflects observations
by other workers [Hyde and Jones 1988; Kohlmeyer and Volkmann-Kohlemeyer 1991; Hyde et al. 2000; Sridhar and Prasannarai 2001; Jones et al. 2006; Singh et al. 2010; Pindi 2011; Soumya et al. 2013]. Studies have shown that Ascomycota are cosmopolitan fungi that can occur as single cells or as long tubular filaments that divided into cellular segments, called hyphae [Edgcomb et al. 2011]. These can be isolated from various marine sediments and are heterotrophic forms obtaining nutrition from dead or living organisms and consuming almost any carbonaceous substrate [Griffin 1994]. Further it has been well documented that Ascomycetes embrace a group of obligate marine fungi that immensely contribute to marine ecosystem and represents variety of saprobes, pathogens and symbiont of coastal and deep-see habitats [Kohlmeyer and Kohlmeyer 1979; Shi et al. 2011; Bhadury et al. 2011].

Fungal sequences belonging to the class Eurotiomycetes, Dothideomycetes and Sordariomycetes and Agaricomycetes were identified from the shelf. This was comparable with those reported from the deep sea sediments of Central India Ocean Basin except in the abundance of Saccharomycetes and Wallemiomycetes from this basin [Singh et al. 2011]. Their absence in the present study suggests that their distribution may be site-specific. Majority of the species identified belong to the class Eurotiomycetes (70%) followed by Sordariomycetes (17%) and Dothideomycetes (7%). There are reports stating that Eurotiomycetes of the phylum Ascomycota as the most frequently detected fungal taxa from deep-sea environments, followed by the classes Saccharomycete, Dothideomycete and Sordariomycete [Jaber et al. 2012]. Abundance of
Eurotiomycetes in marine sediments was further reported from the deep sediments of Indian Ocean by Singh et al. [2010]. They found that the species belonged to this class were affiliated with a number of environmental sequences obtained from a hypersaline anoxic Mediterranean deep-sea basin [Alexander et al. 2009], anoxic coastal sediments [Dawson and Pace 2002], acidic mine drainages [Baker et al. 2004], hydrothermal vent habitats [López-García et al. 2007] and boiling springs lake sediment [Wilson et al. 2008]. This strongly point out that the species coming under this class includes extremophilic fungi that are capable to thrive in the most extreme aquatic environments.

Majority of cultured fungi identified from the study area showed phylogenetic similarity to fungi documented from several other habitats of marine environment. These include species described from deep sea sediments of Central Indian Ocean Basin [Damare et al. 2006; Singh et al. 2010], subtropical marine environment [Roth et al. 1964], deep sea calcareous sediments [Raghukumar et al. 1992] and sediments and waters of the Gulf of Aqaba, Red Sea [Jaber et al. 2012].

The generic composition of the present study showed the presence of terrestrial fungi within the shelf sediments of Bay of Bengal supporting the earlier hypothesis [Damare et al. 2006; 2008] and the isolation of terrestrial fungal colonies in the present study might have resulted either from dormant spores or actively growing mycelia. Studies conducted by Raghukumar and Raghukumar [1998] from the deep sea sediments of Indian Ocean also reported adaptation and activity of terrestrial fungi under marine ecosystem as facultatives or indwellers or
residents. Studies conducted by Singh et al. [2011] also observed that a large proportion of fungal sequences recovered from the deep sea sediments matched with terrestrial taxa and reported that they are transported into the deep sea and subsequently adapt to the extreme conditions.

The majority of species belonging to the Eurotiomycete include *Aspergillus* and *Penicillium*, which are globally distributed and ubiquitous in marine as well as terrestrial environments [Nagano et al. 2012]. In the present study, among the fifteen genera identified *Aspergillus* (30%) was the dominant one followed by *Penicillium* (17%), *Fusarium* (10%) and *Sagenomella* (8%). The dominance of *Aspergillus* in marine environment has been illustrated in many studies like those reported by Damare et al. [2006] from the deep sea sediments of Indian Ocean, Samuel et al. [2011] from marine sediment of south east coast of Tamilnadu, India, Jaber et al. [2012] from sediments and waters of the Gulf of Aqaba, Red Sea, Saravanan and Sivakumar [2013] from the marine systems of east coast of Tamil Nadu, India. The presence of *Aspergillus* has been detected for both culture-dependent [Damare et al. 2006; Burgaud et al. 2009] and culture-independent methods from various marine habitats [Bass et al. 2007; Takishita et al. 2007; Lai et al. 2007; Jebaraj et al. 2010]. Similarly, Jaber et al. [2012] isolated many species of these mycelial fungi from marine habitats. Ubiquitous existence of *A. sydowii, A. niger, A. wentii, A. flavus, A. insulicola* and *A. awamori* in marine environment have been established by both molecular and culture-dependent techniques [Kohlmeyer and Kohlmeyer
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1979; Raghukumar et al. 2004; Kales et al. 2007; Wang et al. 2008; Bhadury et al. 2011]. However, in the present study these representatives were not detected further, only five species such as Aspergillus allahabadii, Aspergillus austroafricanus, Aspergillus versicolor, Aspergillus terreus, Aspergillus niger were isolated. But it showed similarity to the species identified from the continental slope sediments of Bay of Bengal [Das et al. 2009]. In addition, there are several other reports on the evidence of the distribution of Aspergillus along the Indian coast [Prabhakaran and Gupta 1990; Sponga et al. 1999; Varoglu and Crews 2000; Ramesh et al. 2006; Catherine and Raghukumar 2009].

In the present study, the species identified within the genus Penicillium include Penicillium citrinum, Penicillium chrysogenum, Penicillium dipodomyicola, and an unidentified Penicillium sp. The genus Penicillium was reported to be the prevailing strain in the marine environment followed by Aspergillus and Cladosporium [Salvo et al. 2005]. It has been previously described as a marine-derived fungus [Chen et al. 2012] and this terrestrial species even grow profoundly in sediments of Mariana Trench at a depth of about 11,500 m [Takami 1999]. Eupenicillium javanicum which was previously known as Penicillium javanicum has also been identified from the shelf and this species was a first report to the study area. Similar species was reported from the sediments and waters of the Gulf of Aqaba, Red Sea by Jaber et al. [2012]. Penicillium and Aspergillus are considered as versatile ubiquitously distributed species that are capable of anaerobic denitrification [Takasaki et al. 2004]. Further, both are rich sources of chemically diverse natural
products with a broad range of biological activities [Gautschi et al. 2004]. Few species belonging to these exhibited relatively high antibacterial and antifungal activity [Zhang et al. 2013]. Fungi like Sagenomella were also profoundly abundant in the shelf sediments of Bay of Bengal. This species was first reported from deep-sea by Singh et al. [2010].

A large number of species belonging to Sordariomycetes were reported from the marine sediments [Singh et al. 2010]. A prominent genera Fusarium belonging to this class was isolated in the present study. Studies have shown that Fusarium serves as a model to study fungal denitrification under low-oxygen conditions [Takaya et al. 1999; Daiber et al. 2005]. Cladosporium, belonging to the class Dothideomycetes, was also profusely present in the outer shelf sediments of the study area. Several workers have shown the ubiquitous presence of this genus from the marine sediments [Damare et al. 2006; Singh et al. 2010; Soumya et al. 2013]. However, in the current study, representation of phylum Basidiomycota by a single species, Rigidoporus vinctus, adds on to the previous observations that they are less diverse than Ascomycota [Bass et al. 2007; Nagano et al. 2010; Singh et al. 2010].

Similar to the studies conducted by Singh et al. [2011] in the deep sea sediments of Central Indian Basin, this study also suggest that fungal diversity in the shelf sediments of Bay of Bengal may be heterogeneous as some of the species were restricted to a few stations. Bubble plot analysis demonstrated that the least abundant species Eurotium herbariorum was seen only at 100 m depth of Cheyyur and Chennai. Scopulariopsis brevicaulis could be identified from 200 m depth of Karaikal and Cheyyur
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and 50 m depth of Cuddalore. *Rigidoporus vinctus* the only representative from Basidiomycota were seen from 50 m and 200 m depths of Chennai and Singarayakonda and also from 50 m depth of Cuddalore.

In the present study, Shannon–Wiener index was found to higher than those reported by Damare et al. [2006] from the deep sea sediments of Indian Ocean and Grishkan et al. 2003 from hypersaline Dead Sea coastal area, Israel but, lower than those of Prasannarai and Sridhar [2001, 2003], Maria and Sridhar [2002], Ananda and Sridhar [2004] studied along west coast of India. Shannon–Wiener index in the continental slope sediments of Bay of Bengal was in the range of 0.92 to 3.69 and in the deep sea sediments of Indian Ocean it was 1.07–2.06. In the present study the Shannon–Wiener index ranged from 2.32-3.43, which proves that the fungal species inhabiting continental shelf of Bay of Bengal was more or less similar to that of its slope sediments and is more diverse than that of deep sea.

Marine-derived fungi are always a treasure of bioactive molecules [Bugni and Ireland 2004; Raghukumar 2008]. The different species identified from the shelf sediments are potent source of bioactive compounds and are also important tools in industrial and biotechnological applications. Besides, marine fungi play a major role in carbon sequestration [Damare and Raghukumar 2008], and decomposition of particulate organic matter [Kimura et al. 2001] in marine sediments. Studies reported that the major fungal phyla/subphyla involved in the biodegradation of oil include the Ascomycota, Basidiomycota, and Mucoromycotina, with specific fungal genera including, *Aspergillus,*
Cephalosporium, Penicillium, Geotrichum, Talaromyces, Cladosporium, Fusarium, Alternaria, Mucor etc. [Harms et al. 2011]. Many of these genera were also encountered in the present study. Studies have pointed out that a thorough understanding of various niches occupied by marine fungi is necessary in discovering the “missing fungi” as a source for new molecules [Singh et al. 2010]. Hence the present effort to understand the diversity of marine fungi inhabiting the shelf sediments of south east coast of India is remarkable. As a whole the continental shelf of Bay of Bengal is rich in in terms of fungal biodiversity, despite the low population. Nevertheless, the result obtained from the present study on species abundance, distribution and diversity will provide a benchmark data for the fungal wealth in the continental shelf sediments of Bay of Bengal.