Ph.D. Thesis

HETEROTROPHIC BACTERIA FROM THE CONTINENTAL SLOPE SEDIMENTS OF ARABIAN SEA

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HETEROTROPHIC BACTERIA FROM THE CONTINENTAL SLOPE SEDIMENTS OF ARABIAN SEA

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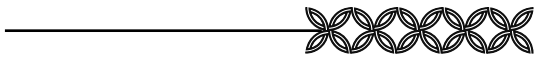

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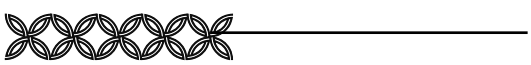

Certificate

*This is to certify that the thesis entitled "**Heterotrophic Bacteria from the Continental Slope Sediments of Arabian Sea**", is an authentic record of research work carried out by **Mr. Neil Scolastin Correya (Reg. No. 2758)** under my supervision and guidance in the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** and no part thereof has been presented before for the award of any degree, diploma or associateship in any University.*

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Declaration

*I hereby do declare that the thesis entitled "**Heterotrophic Bacteria from the Continental Slope Sediments of Arabian Sea**", is a genuine record of research work done by me under the supervision of **Dr. Rosamma Philip**, Asst. Professor, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Kochi- 682016, and that no part of this work, has previously formed the basis for the award of any degree, diploma associateship, fellowship or any other similar title of any University or Institution.*

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Neil Scolastin Correya

Contents

ABBREVIATIONS AND EXPANSIONS

%	-	Percentage
˚C	-	Degree Celsius
µl	-	Microliter
ca.	-	Approximately
bp	-	Base Pair
G P	-	Gram Positive
G N	-	Gram Negative
H’	-	Shannon-Wiener Diversity
J’	-	Pielou’s Evenness
d	-	Species Richness
wt.	-	Weight
v/v	-	Volume by Volume
DF	-	Degrees of Freedom
et al.	-	Et alli (Latin word, meaning ‘and others)
cfu	-	Colony Forming Units
G+C	-	Guanine+Cytosine
mM	-	MilliMolar
OM	-	Organic Matter
pH	-	Hydrogen Ion Concentration
SD	-	Standard Deviation
MW	-	Molecular Weight
$g\ l^{-1}$	-	Gram per Liter
$mg\ g^{-1}$	-	Milligram per Gram
rpm	-	Revolution per Minute
psu	-	Practical Salinity Unit
PRT	-	Protein
LPD	-	Lipid
CHO	-	Carbohydrate
TOC	-	Total Organic Carbon
LOM	-	Labile Organic Matter

TOM	-	Total Organic Matter
BPC	-	Biopolymeric Carbon
DNA	-	Deoxyribonucleic Acid
POM	-	Particulate Organic Matter
CTD	-	Conductivity Temperature Depth
THB	-	Total Heterotrophic Bacteria
OMZ	-	Oxygen Minimum Zone
PCA	-	Principal Component Analysis
PCR	-	Polymerase Chain Reaction
MDS	-	Multi Dimensional Scalling
DOM	-	Dissolved Organic Matter
dNTP	-	Deoxyribonucleotide Triphosphate
FAME	-	Fatty Acid Methyl Esterase
FORV	-	Fishery and Oceanography Research Vessel
JGOFS	-	Joint Global Oceanographic Flux Studies
MoES	-	Ministry of Earth Sciences
rDNA	-	Ribosomal Deoxyribonucleic Acid
EDTA	-	Ethylene Diamine Tetra Acetic Acid
Tris HCl	-	Tris(hydroxymethyl)aminomethane Hydrochloric acid
ANOVA	-	Analysis of Variance
ARDRA	-	Amplified Ribosomal DNA Restriction Analysis

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Chapter 1

GENERAL INTRODUCTION

The marine environment is indubitably the largest contiguous habitat on Earth. Because of its vast volume and area, the influence of the world ocean on global climate is profound and plays an important role in human welfare and destiny. The marine environment encompasses several habitats, from the sea surface layer down through the bulk water column, which extends >10,000 meters depth, and further down to the habitats on and under the sea floor. Compared to surface habitats, which have relatively high kinetic energy, deep-ocean circulation is very sluggish. By comparison, life in the deep sea is characterized by a relatively constant physical and chemical environment. Deep water occupying the world ocean basin is a potential natural resource based on its properties such as low temperature, high pressure and relatively unexplored properties. So, a judicious assessment of the marine resources and its management are essential to ensure sustainable development of the country's ocean resources.

Marine sediments are complex environments that are affected by both physiological and biological factors, water movements and burrowing animals. They encompass a large extent of aggregates falling from the surface waters. In aquatic ecosystems, the flux of organic matter to the bottom sediments depend on primary productivity at the ocean surface and water depth. Over 50% of the earth's surface is covered by deep-sea sediments that are primarily formed through the continual deposition of particles from the productive pelagic waters (Vetriani *et al*., 1999). These aggregates are regarded as 'hot spots' of microbial activity in the ocean (Simon *et al.,* 2002). This represents a good nutritional substrate for heterotrophic bacteria and favours bacterial growth.

Deep-sea surface sediments harbour bacteria and fungi that sink from the overlying waters (Takami *et al.,* 1997). Sediment bacteria play an important role in benthic ecosystems (Deming and Baross, 1993; Kuwae and Hosokawa, 1999). They may comprise a large fraction of the total benthic biomass, contributing significantly to the turnover of organic matter within the sediment (Billen *et al.,* 1990; Schallenberg and Kalff, 1993). Surface sediment bacteria play a noteworthy ecological and biogeochemical role in marine ecosystems due to their high abundance relative to the overlying water column and they play a key role in the decomposition of the organic matter, nutrient cycling and carbon flux. Their biodiversity is structured and determined by the temporal and spatial variability of physiochemical and biotic parameters and thus, can reflect environmental conditions (Urakawa *et al.,* 1999; Zhang *et al.,* 2008a). Therefore, any shift in nutrient, environmental and pollution profiles in the benthic-pelagic ecosystems will directly impact bacterial community that in turn affect nutrient cycles and other related communities. Despite their importance, our knowledge of the bacteria that inhabit surface sediments is very limited, especially in the heterogeneous deep marine ecosystems (Wu *et al.,* 2004).

Bacteria within coastal and shelf sediments play an important role in global biogeochemical cycles, as they are the ultimate sink of most terrestrially derived compounds and a high proportion of marine particle flux. Despite a growing understanding of the global biogeochemical importance of these sediment habitats (Berelson *et al*., 1990; Blackburn and Blackburn, 1993; Vanduyl *et al*., 1993; Codispoti *et al*., 2001), little is known on the bacterial communities inhabiting them (Cifuentes *et al*., 2000; Todorov *et al*., 2000; Madrid *et al*., 2001; Kim *et al*., 2004), nor the factors influencing their distribution (Bowman *et al*., 2005; Polymenakou *et al*., 2005). The estimation of bacterial abundance as well as their genetic diversity is therefore the most fundamental objective of aquatic microbial ecology. Various types of bacteria reside in sediments, including aerobic

heterotrophs, chemolithotrophs, more specifically, hydrogen-oxidizing bacteria, sulfur-oxidizing bacteria, iron oxidizing bacteria, nitrifying bacteria, nitrate respiring bacteria, metal respiring bacteria, sulfur and sulfate-reducing bacteria, methanogens, acetogens, methanotrophs and syntrophic bacteria (Zhang *et al.,* 2008b).

'Heterotrophy' involving all animals and many microbes is a process by which autotrophically synthesised organic compounds are transformed and respired. When bacteria decompose the organic compounds, oxygen is utilized and organic molecules ultimately disappear. Despite their small size, heterotrophic bacteria are far more important and linked to both water column and sediment (benthic) processes. They degrade and respire an enormous variety of organic compounds. In order to bring the importance of heterotrophic bacteria and their processes in the marine environment into focus, an understanding on their abundance, distribution, production and, their involvement in nutrient cycling and food web is essential. Information on abundance and activity of heterotrophic bacteria is very useful to recognize their varied roles including bacterial utilization of labile fraction of dissolved organic matter (DOM), microbial loop and cycling of bio-essential elements. In the marine environment, they are able to increase or decrease their activity over wide ranges of chemical and physical settings than any other group of organisms.

Heterotrophic bacteria in an environment depend on the availability of growth-supporting organic matter and micronutrients (Sayler *et al.,* 1975). The heterotrophic organisms are responsible for the utilization of the extensive pool of dissolved organic carbon, thus making it available for the different food webs (Williams, 1981; Ducklow *et al.,* 1993; Hoppe *et al.,* 1993; Pfannkuche, 1993). Therefore, the microbial community is assumed to play a key role in the lower levels of the food web (Lucea *et al.,* 2005; Tanaka and Rassoulzadegan, 2004). Heterotrophic bacterial degradation promotes organic material transformation and

mineralization processes in sediments and in the overlying waters. They breakdown organic substances to products like ammonia, carbon dioxide, phosphate and silicate. Most of the organic matter that reaches the sediment is mineralized and further recycled by benthic microbial communities. It releases dissolved organic and inorganic substances. The major part of the carbon flow is channeled through the bacteria and the benthic microbial loop (Danovaro *et al.,* 2000). Thus, heterotrophic bacteria are the major agents in shaping the organic composition of the ocean (Raghukumar *et al.,* 2001a). These heterotrophic bacteria comprise the bulk of microbial population inhabiting the water column and are responsible for much of the biological transformation of organic matter and production of carbon dioxide in the oceans (Sherr and Sherr, 1996).

The conversion of organic material into bacterial biomass is the initial step of the detritus food web and a key factor for life in the deeper ocean (Meyer-Reil, 1990; Hoppe, 1991). However, due to the lack of organic nutrients, the deep ocean is characterized by low productivity rate and biomass. Unlike specialised locations, like hydrothermal vents, where chemoautotrophy can fuel extraordinary food web, the nutrition of heterotrophic life in the deep ocean depends on the leftovers of particulate organic matter produced in the euphotic zone (Gage and Tyler, 1991). Apart from that, the living bacteria represent the major protein resource for sediment ingesting animals (Newell and Field, 1983). Hence, the benthic bacteria represent a significant food source for deep sea benthic consumers (protozoans, meio and macrobenthos) (Fenchel, 1969; Rieper, 1978; Danovaro *et al.,* 1993). However, few studies have been tried to assess what factors control their variability in natural systems and to what extent the use of standard theoretical factors can seriously affect microbial estimates (Polymenakou *et al.*, 2005; Fajun *et al*., 2010).

Heterotrophic bacteria are usually discussed in the biological processes as a single group, even though the diversity of bacteria able to mineralize organic matter is not yet well known. In spite of the importance of these bacterial groups in

the transformation of organic matter, little is known about the qualitative structure of the bacterial communities and their role in biogeochemical cycles. It is not clear whether the mineralization of DOM is dominant in selected groups of bacteria and whether changes in the spectrum of hydrolytic enzymes between seasons may be caused by the succession of different species (Martinez *et al.,* 1996). Bacteria respond to polymeric organic matter supply by regulating the synthesis and activity of hydrolytic enzymes, which results in the release of simple monomers available for bacterial uptake. Like other functional parameters, the microbial activity can be inhibited or increased by physical/chemical environmental changes, biological variations or input of allochthonous materials (Hoppe, 1983; Chrost, 1993).

Benthic ecosystems are greatly dependent on the supply of organic material, most of which enters the sediment as polymeric organic compounds. Prior to incorporation into microbial cells, the polymeric material has to be decomposed by extracellular enzymes which are secreted from living cells or liberated through the lysis of cells. By enzymatic hydrolysis, polymeric organic compounds are degraded to oligomers or monomers which can be taken up by microbial cells to meet their energy requirements and to build up biomass. Prokaryotes play a major role as biomass producers and transformers of organic matter in the sea (Azam and Cho, 1987). These processes require extensive extracellular substrate hydrolysis by different ectoenzymes, activities that play a key role in microbial ecology (Hoppe, 1991; Vetter *et al.,* 1998). Microheterotrophs especially bacteria are the only biological populations capable of significantly altering both dissolved organic carbon and particulate organic carbon (Chrost, 1990) and because of this they have a great impact on the cycling of organic matter and on energy flux. Heterotrophic prokaryotes produces extracellular enzymes to hydrolyze high molecular weight dissolved organic matter (DOM) into low molecular weight (MW) compounds (<600 MW), which is the threshold of DOM that can be taken up by prokaryotes (Weiss *et al.,* 1991).

Most of the organic matter in marine ecosystems consist of compounds of a high molecular weight and polymeric structure i.e. mainly protein, starch, lipid, pectin, cellulose, chitin, nucleic acid or lignin (Poremba, 1995; Arnosti *et al.,* 1998; Unanue *et al.,* 1999). For heterotrophic bacteria, high molecular weight biopolymers constitute an important source of carbon, nitrogen and energy for biosynthesis or respiration (Brown and Goulderr, 1996). As polymeric molecules are too large to be directly incorporated into bacterial cells (Hoppe *et al.,* 2002), they have to be decomposed by extracellular enzymes into simpler compounds (Unanue *et al.,* 1999) that can easily diffuse into the periplasmic space (Mallet and Debroas, 1999). Many heterotrophic bacteria are known to carry genetic and metabolic potentials to synthesize and control extracellular enzymes, which can degrade and modify a large variety of natural polymers in water basins (Munster and Chrost, 1990; Mudryk and Skorczewski, 2004)

Cell-surface or 'ecto' enzymes of aquatic microbes, together with 'extracellular' enzymes dissolved or absorbed to other particles than their original ones (Chrost, 1991), are important catalysts in the decomposition of dissolved organic matter and particulate organic matter. Most of these ectoenzymes are found on the cell-surface or in the periplasmic space of heterotrophic bacteria (Martinez and Azam, 1993), and are common in aquatic isolates (Martinez *et al.,* 1996). Extracellular enzymes, that react outside the intracellular compartment, are mainly hydrolases (glycosidases, peptidases, esterases) i.e. enzymes that cleave C-O and C-N bonds that link monomers. Extracellular enzymes can also catalyze oxidative reactions, typically cleaving C-C and C-O bonds. Strict-sense extracellular enzymes occur in free form and catalyse reactions detached from their producers. Extracellular enzymes are proportionally more important in the decomposition of particulate or colloidal material in the dark ocean (Baltar *et al.,* 2010). Variations in ectohydrolase profiles (types and levels of activity) reflect the trophic status of the environment, (Karner *et al.,* 1992; Hoppe *et al.,* 1998) and has been argued that

such variations could be caused by shifts in the dominant species or in the level of enzyme expression by the same species in response to changes in the spectrum of organic substrates (Martinez *et al.,* 1996).

There are numerous investigations on heterotrophic bacterial abundance, activities and processes from Pacific, Atlantic, Antarctic and Indian Oceans. While their abundance and role are realized substantially from the Pacific and Atlantic, it is relatively scarce in one of the biologically unique regions, the Arabian Sea. Both central and eastern regions of the Arabian Sea are biologically highly productive and changes in primary production are quite large on a seasonal basis (Banse, 1994; Madhupratap *et al.,* 1996; Sarma *et al.,* 2003). Physical forcing such as upwelling, winter cooling (Prasannakumar *et al.,* 2001) and semi-annual reversal of monsoonal winds (Banse, 1994; Smith *et al.,* 1998a) usher in such phenomenal dynamics in productivity. Thus, in the Arabian Sea, seasonal cycles in biological production greatly influence the biogeochemistry (Smith *et al.,* 1998b; Hansell and Peltzer, 1998; Burkill, 1999) and sedimentation rates (Nair *et al.,* 1989; Haake *et al.,* 1993). Further, just about two million square kilometer area in the northern Arabian Sea is uniquely distinct with an extremely well developed oxygen minimum zone (Swallow, 1984; Warren, 1994).

Several studies have been carried out to characterize heterotrophic bacteria in different coastal areas and ocean sites of temperate, tropical and polar zones. However, knowledge about the heterotrophic marine bacteria from continental slope region of Peninsular India is still insufficient. Hence we focused our study on estimation and characterization of the heterotrophic (culturable) bacteria in the continental slope sediment of the Arabian Sea.

Objectives of the study

- General hydrography and sediment properties of the slope regions of the Arabian Sea

- Quantitative and qualitative composition of the total heterotrophic bacteria (THB) in the continental slope sediments of west coast of India.
- Extracellular hydrolytic enzyme production potential of the bacterial isolates.
- Molecular characterization and phylogenetic analysis of the dominant bacterial forms in the marine sediments.

Chapter 2

HYDROGRAPHY AND SEDIMENT PROPERTIES

Contents

2.1. INTRODUCTION

The Arabian Sea covering about 1% of the global ocean surface located in the northwest Indian Ocean is bound by India (to the east), Iran (to the north) and the Arabian Peninsula (in the west). Defined solely by its latitudinal position, the Arabian Sea would be considered a tropical oceanic system. It is also one of the most complex and productive regions of the world oceans and has been an area of intense scientific interest for several decades (Smith *et al.,* 1991). The Arabian Sea is distinct from the other low-latitude seas by being semi enclosed and under the influence of monsoonal winds, which reverse their direction and give rise to two distinct seasons i.e. Southwest (Summer) and Northeast (Winter) monsoon.

Physico-chemical variations existing in different zones of the aquatic ecosystem provide significantly varying habitats for different life forms. Understanding the ecosystem would give us a better picture of the ecological parameters which yield insights into the basic detrimental environmental factors of the flora and fauna. Although continental margins account only for ca. 15 percent of total ocean area and 25 percent of total ocean primary production, today more than 90 percent of all organic carbon burial occurs in sediments built up by particle

deposition on continental shelves, slopes, and in deltas (Hartnett *et al.,* 1998). Lateral transport of particles at the continental margin is largely controlled by the hydrodynamics within the benthic boundary layer (McCave, 1984). As hydrodynamic processes are highly active at the ocean margins the transport of both lithogenic sediments and organic material is enhanced and controlled by local topography and hydrography (Huthnance *et al.,* 2001).

Circulation in the Arabian Sea is controlled by the alternate heating and cooling of the Indian sub-continent. Shetye *et al.* (1990) provided general information on the hydrography and circulation of the south west and north east monsoon along the west coast of India. Some of the earlier studies on the hydrography of the eastern Arabian Sea also have described the different water masses formed in the Arabian Sea (Rochford, 1964; Wyrtki, 1971). Prasanna Kumar and Prasad (1999) gave an account of the formation and seasonal spreading of Arabian Sea High Salinity Water (ASHSW) towards the southern Arabian Sea. The orientation of the coastlines render upwelling of deeper water on both sides of the Arabian Sea. Cool, nutrient-rich subsurface water is present on the shelf from Cochin to northern regions throughout the summer extending in some areas until mid-December (Banse, 1968).

The basin of the Arabian Sea is about 6 x 10^6 km^2, of which at least 25% is under a coastal regime (Shetye *et al.,* 1994). It is believed to be globally significant as far as biogeochemical fluxes are concerned. The characteristics of the shelf and slope sediments change with latitude and reflect the balance between input from the Indus river in the north, the Narmada and Tapti rivers in the central area and local runoff from small mountain rivers in the south (Rao *et al.,* 1995). The Indus river is the largest source of sediments to the Arabian Sea originating in the Himalayas and flowing through the semi-arid and arid soils of the Thar desert in western Pakistan and northwestern India (Rao *et al.,* 1995). In the central region, the Western Ghat mountains span from Mumbai to Cochin along the western coast

of India. In addition to the Narmada and Tapti rivers draining into the Gulf of Cambay, sixteen small rivers drain the Western Ghats depositing sediments on to the continental shelf during the summer monsoon season. The climate of the west coast of India is dominated by the intense seasonal contrast between the summer monsoon season and the winter monsoon season. Consequently, runoff from both the local mountain rivers and the Indus river has a seasonal cycle of sedimentary deposition along the continental shelf and slope.

The Arabian Sea accounts for an estimated 30% of the world's margins that are impacted by extreme hypoxia (<0.2 ml l^{-1}) (Helly and Levin, 2004). The enhanced accumulation of organic matter in upper slope sediments under current conditions of monsoon-driven upwelling and extreme oxygen depletion, combined with vertical flux of carbon across the entire basin, due both to high productivity and reduced remineralization within the water column (Haake *et al.,* 1996; Luff *et al.,* 2000). Therefore the Arabian Sea sediments represent a disproportionately significant long-term carbon sink. Our understanding of this complex and variable ocean region is still evolving.

In recent years there are considerable interest in understanding the ecology and biogeochemistry of Arabian Sea as the southern and central parts of Arabian Sea support a large fishery industry. Two Mediterranean seas, the Red Sea and the Persian Gulf join the northern part of the Indian Ocean and have remarkable influence on its salinity and consequently on density structure. The Red Sea water and the Persian Gulf water injected into the Arabian Sea at sub-surface levels leave this semi-enclosed basin more saline than any other part of the Indian Ocean. Consequently, runoff from both the local mountain rivers and the Indus river also has a considerable influence on the sedimentary deposition along the continental margin areas.

The physical, chemical and biological dynamics of the upper ocean in this region are tightly coupled to seasonal variability in monsoonal atmospheric

forcing. Seasonal reversal of winds leads to strong upwelling of nutrient-rich water from the depths along the narrow continental shelf resulting in high surface productivity and high export particle flux from the euphotic zone (Qasim, 1982; Sen Gupta and Naqvi, 1984; Nair *et al.,* 1989). In the deeper area, the temporal variations are less pronounced. Understanding the relationship between the deep-sea benthic system and the upper ocean processes is essential in forming a comprehensive view of the biogeochemical cycling in the Arabian Sea. An intense oxygen minimum characterizes the entire Arabian Sea at intermediate water depths (Wyrtki, 1971). The low oxygen conditions in this layer have been ascribed to a combination of sluggish circulation at intermediate depths due to the presence of a land-mass to the north and high rate of oxygen utilization reflecting a high settling flux of organic matter due to very high primary production in the region (Sen Gupta and Naqvi, 1984).

The Arabian Sea's unusual features have drawn attention from oceanographers and other scientists since the late 1800s. Water-column processes, including the seasonally reversing monsoon-driven circulation and the associated upwelling and productivity, as well as a basin-wide, mid-water layer of intense oxygen depletion have been the foci of many studies. However, the importance of benthic processes in the Arabian Sea has also been recognized. Previous studies has revealed that processes and biogeochemical fluxes occurring across the bottom layer and within the surficial sediments of the world oceans are also of great importance, especially at the ocean margins (Walsh, 1991). The different margins along the Arabian Sea shows widely contrasting oceanographic conditions (e.g. circulation, upwelling, lithogenic input, seasonality) combined with regionally high productivity resulting in a correspondingly variable supply of organic material to the benthic boundary layer (Luff *et al.,* 2000). Studies of Arabian Sea sediments and benthic processes, especially across the margins serve to clarify the role of the

sediments in determining water column chemistry and thus the importance of benthic–pelagic coupling in the Arabian Sea.

Studies associated with the John Murray Expedition (1933–1934), were the first to report an unusual mid-depth zone off Arabia (between the approximate depths of 100 and 1200m) wherein they recovered sediments apparently free of organisms (Sewell, 1934). These early studies were followed by surveys from India and other countries adjoining the Indian Ocean starting in the early 1950s. Later extensive surveys across the Arabian Sea were conducted while International Indian Ocean Expedition during 1962–1965 (Ulrich, 1968; von Stackelberg, 1972). The result of the water-column studies gradually improved the understanding of the Arabian Sea's unusual oceanographic features, including its monsoon-driven circulation and productivity (Wyrtki, 1971).

The array of pelagic investigations, gave rise to a growing recognition of the significance of biogeochemical processes occurring in the Arabian Sea region. As a result, the Arabian Sea became a key study site for the Joint Global Oceanographic Flux Study (JGOFS) program in the 1990s, which encompassed research expeditions from the US, UK, Netherlands, Germany, India and Pakistan. The majority of Arabian Sea JGOFS research was focused on the water column despite the recognized significance of benthic biogeochemical processes, especially on continental margins as described by Walsh (1991). The significance of benthic processes in the Arabian Sea has been recognized by the oceanographic community and is reflected in the considerable number of expeditions since the late 1980's, during which important benthic process studies were conducted to parallel the pelagic studies of the International JGOFS program.

Early Arabian Sea surveys led to a series of reports from the 1970s to the early 1990s that attributed the organic-rich deposits to enhanced preservation of organic matter due to the absence of oxygen. The distribution of organic rich deposits in the OMZ displayed an apparent mismatch with the regional distribution

of pelagic primary production (von Stackelberg, 1972; Kolla *et al.,* 1981; Slater and Kroopnick, 1984; Paropkari *et al.,* 1993). While sufficient data have been accumulated to show the existence of seasonal productivity and high organic matter accumulation in the shelf areas of the west coast of India, not much information is available with regard to the actual estimates of organic matter distribution in the continental slope regions. Deep sea, as was considered in the past is by no means the stable and monotonous environment sustained by the constant drizzling of particles to the ocean floor. An interdisciplinary approach was attempted in the northern Arabian Sea to investigate the coupling of particulate organic carbon with the benthic organic carbon degradation process in the deep sea sediments during a German research program the BIGSET (Biogeochemical transport of matter and energy in the deep sea, 1996; Pfannkuche and Lochte, 2000). Conversely, contemporary studies (Pedersen *et al.,* 1992) postulated that the quantity and quality of organic matter deposited across the margins (Off Oman) was controlled primarily by a combination of hydrographic and bathymetric influences and upwelling driven productivity.

Organic matter is an unusually complicated heterogeneous mixture of organic material mostly composed of plant and microbial residues and it contains mono to polymeric molecules of organic substances i.e. lignin, proteins, polysaccharides (cellulose, hemicellulose, chitin, peptidoglycan), lipids and other aliphatic materials (waxes, fatty acids, cutins, suberins, terpenoids). The distribution of individual organic compounds can provide information about the sources of organic matter as well as intensities of organic matter decomposition. (Cowie and Hedges, 1994). Quantity and quality of deep-sea sediment organic matter (OM) are largely dependent upon several factors including origin, composition and biochemical transformations that occur on organic particles during their descent to the ocean floor (Billett *et al.,* 1983; Lochte and Turley, 1988; Danovaro *et al.,* 1999). Defining organic matter quality means

discriminating between labile and refractory organic compounds (Mayer, 1989). Its composition, indeed, is important both in a biogeochemical perspective as OM degradation rates might affect its burial in the sediments (Hartnett *et al.,* 1998).

The possible cause of organic matter enrichment in marine sediments have already been a central issue of numerous investigations. Many of these studies revealed concentration maxima of organic carbon at intermediate water depths on continental slopes (Premuzic *et al.,* 1982) where an oxygen minimum zone (OMZ) in the water column impinges on the sea floor. In contrast other investigators have reported organic matter enrichment due to upwelling induced high primary productivity, sediment texture, grain size, and mineral surface area (Pedersen and Calvert, 1990). In the sediment, organic matter quality rather than quantity can be related to benthic abundance (Fabiano and Danovaro, 1999) and activity (Albertelli *et al.,* 1999). This is accomplished with the use of biochemical indices (e.g. lipid and protein concentration, protein/carbohydrate ratio) of organic matter rather than with carbon or organic matter concentration alone (Dell'Anno *et al.,* 2000; Medernach *et al.,* 2001). Thus the biochemical analysis of sediment is an efficient tool to provide insight into benthic faunal ecology (e.g. abundance, diversity, and activity) (Fabiano and Danovaro, 1999; Rossi *et al.,* 2003).

Grandel *et al.* (2000) concluded that there was strong evidence for overall pelagic–benthic coupling of processes, regional trends in the bulk organic matter composition and more significantly the labile organic matter as reflected by surface ocean productivity and by the supply of additional materials to the sediments, possibly by particles reaching from the margins or adjacent topographic highs (Luff *et al.,* 2000). Sedimentation across the entire basin is directly or indirectly influenced by the monsoon, with regional differences in upwelling-driven productivity, but also changes through time in the extent of upwelling and the intensity of the OMZ. Continental slopes are the sites of accumulation of high concentrations of organic matter. Sedimentation rate and primary productivity are

recognized as important factors controlling the quantity of OM buried in marine sediments (RullkoEtter, 1999). The role of adsorption of organic matter on mineral surfaces was emphasized in a number of studies (Keil *et al.,* 1994; Mayer, 1994). It was suggested that organic matter settling through the water column and that in surface sediments may be protected against oxidation and microbial degradation by sorption on mineral surfaces (Keil *et al.,* 1994; Bergamashi *et al.,* 1997).

The quality and quantity of organic matter in marine sediments are of special interest since organic matter accumulated in sediments is the source that may fuel the benthic realm despite seasonal restrictions of organic matter production in the euphotic zone. The nutritive value of the organic content of the sediment has been suggested as a key factor that may explain the extreme richness of the benthic biomass (Gili *et al.,* 2001). The distribution of individual organic compounds can provide information about the sources of organic matter as well as pathways and intensities of organic matter decomposition (Cowie and Hedges, 1994). Understanding the relationship between biomass and the sediment quality will provide important insight towards the development of a comprehensive profile of the benthic ecosystem.

2.2. MATERIALS AND METHODS

2.2.1. Study Area

The present investigation was confined to the upper continental slope in the Arabian sea extending from Off Cape Comorin (6°57'96" N and 77°21'96" E) to Off Porbandar (21°29'99" N and 67°46'96" E) region of the west coast of India (Fig. 2.1). Forty eight stations (48) distributed along 16 transects in the continental slope regions (200-1000m depths) were selected for the study. Each transect was situated one degree latitude apart. The transects selected were Cape Comorin, Thiruvananthapuram, Kollam, Kochi, Ponnani, Kannur, Mangalore, Coondapore,

Karwar, Goa, Ratnagiri, Dabhol, Mumbai I, Mumbai II, Veraval and Porbandar. Sampling was conducted at 200, 500 and 1000m depths along each transect. The details of stations are presented in Table 2.1.

2.2.2. Sample Collection

Oceanographic surveys were carried out on-board Fishery and Oceanographic Research Vessel (FORV) *Sagar Sampada*, Ministry of Earth Sciences, Govt. of India (Fig. 2.2). Totally three cruises were conducted to collect two sets of samples i.e. Sampling I (Cruise Nos. 228 and 233) and Sampling II (Cruise No. 254). The first set of sampling was completed by two cruises of FORV *Sagar Sampada* Cruise No.228 (Fig. 2.3) and Cruise No. 233 (Fig. 2.4) conducted during August-September 2005 and March-April 2006 respectively. FORV *Sagar Sampada* Cruise No.228 covered 27 stations (9 transects) and Cruise No. 233 covered 21 stations (7 transects) extending from Cape comorin to Mangalore. Sampling II was conducted during FORV *Sagar Sampada* Cruise No.254 during May-June 2007 covering all the 48 stations of the 16 transects along the west coast extending from Cape comorin to Porbandar (Fig. 2.5). Hydrographical parameters of the bottom water (Temperature, Salinity, Dissolved Oxygen) were recorded using CTD (Sea bird, USA) (Fig. 2.6a). The sediment samples were collected using a Smith Mc-Intyre Grab (Fig. 2.6b). Sediment samples were transferred aseptically into sterile polythene bags for microbiological analysis. Temperature and pH of the sediment was noted using a mercury thermometer and a portable pH meter respectively. Another fraction of the sediment was stored at -20 ˚C in a freezer for biochemical analysis.

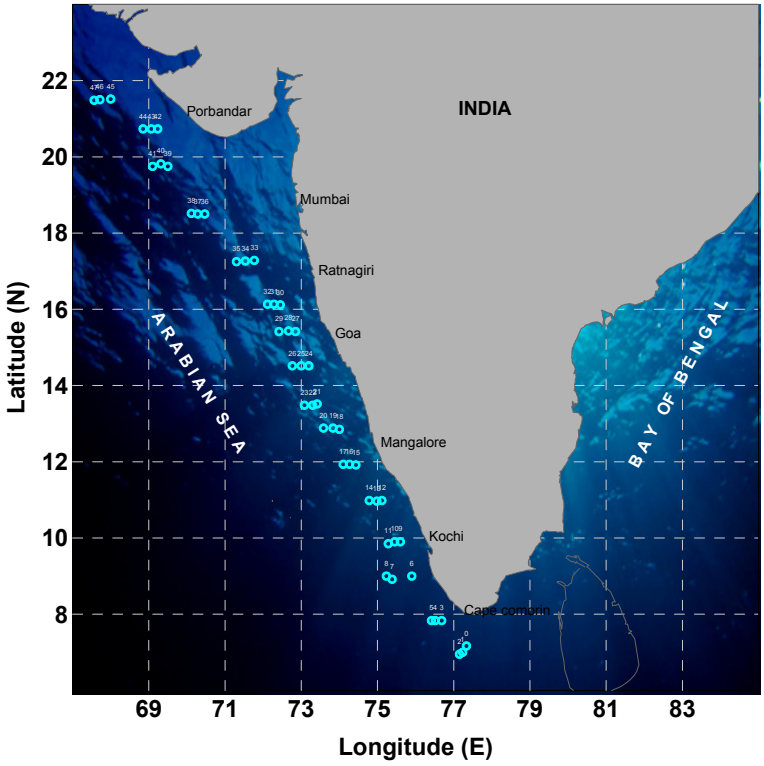


Figure 2.1. Map showing the location of sampling stations in the Arabian Sea.

Fig 2.2. FORV *Sagar Sampada* (CMLRE, MoES, Govt. of India).

Table 2.1. Table showing the details of the sampling stations in the Arabian Sea during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

Transect	Station	Cruis	Sampling I (Cruise #228&233)			Sampling II (Cruise #254)		
			Depth (m)	Latitude (N)	Longitude (E)	Depth (m)	Latitude (N)	Longitude (E)
	0	Cruise No # 233	207	7 10 N	77 20 E	215	7 09 N	77 20 E
Cape comorin	1		536	7 00 N	77 20 E	486	7 01 N	77 15 E
	2		999	6 57 N	77 21 E	897	7 06 N	77 15 E
	3		205	7 50 N	76 41 E	216	8 28 N	76 28 E
Thiruvanan-thapuram	4		584	7 50 N	76 37 E	490	8 28 N	76 16 E
	5		976	7 50 N	76 37 E	983	8 28 N	75 26 E
	6		267	9 00 N	75 54 E	236	8 42 N	75 44 E
Kollam	7		490	8 55 N	75 29 E	466	8 42 N	75 32 E
	8		1050	9 00 N	75 26 E	925	8 42 N	75 26 E
	9		180	9 54 N	75 36 E	275	9 50 N	75 34 E
Kochi	10		500	9 54 N	75 33 E	500	9 50 N	75 26 E
	11		1095	9 51 N	75 29 E	835	9 50 N	75 16 E
	12		226	10 59 N	75 07 E	220	10 50 N	75 10 E
Ponnani	13		515	10 58 N	75 05 E	520	10 50 N	75 00 E
	14		992	10 59 N	74 59 E	856	10 50 N	74 52 E
	15		202	11 55 N	74 26 E	215	11 56 N	74 28 E
Kannur	16		523	11 56 N	74 22 E	503	11 56 N	74 22 E
	17		958	11 56 N	74 18 E	880	11 56 N	74 15 E
	18		200	12 51 N	74 00 E	219	12 46 N	73 56 E
Mangalore	19		494	12 53 N	73 56 E	508	12 46 N	73 50 E
	20		1000	12 53 N	73 47 E	829	12 46 N	73 38 E
	21	Cruise No # 228	220	13 31 N	73 25 E	220	13 58 N	73 24 E
Coondapore	22		520	13 29 N	73 24 E	519	13 58 N	73 15 E
	23		1040	13 29 N	73 17 E	863	13 58 N	73 09 E
	24		216	14 31 N	73 12 E	215	14 32 N	73 10 E
Karwar	25		416	14 31 N	73 06 E	501	14 32 N	73 00 E
	26		1000	14 31 N	72 58 E	847	14 32 N	72 52 E
	27		230	15 25 N	72 51 E	200	15 25 N	72 48 E
Goa	28		520	15 26 N	72 46 E	498	15 25 N	72 39 E
	29		1050	15 25 N	72 37 E	839	15 25 N	72 31 E
	30		210	16 07 N	72 27 E	197	16 18 N	72 25 E
Ratnagiri	31		470	16 08 N	72 23 E	488	16 18 N	72 14 E
	32		950	16 08 N	72 19 E	855	16 18 N	72 04 E
	33		220	17 17 N	71 46 E	206	17 24 N	71 20 E
Dabhol	34		588	17 16 N	71 38 E	558	17 24 N	71 12 E
	35		1010	17 15 N	71 30 E	919	17 24 N	71 08 E
	36		200	18 30 N	70 28 E	259	18 30 N	70 26 E
Mumbai I	37		520	18 30 N	70 23 E	536	18 30 N	70 14 E
	38		1000	18 31 N	70 19 E	862	18 30 N	70 06 E
	39		200	19 45 N	69 30 E	203	19 34 N	69 48 E
Mumbai II	40		490	19 49 N	69 25 E	517	19 34 N	69 27 E
	41		1010	19 45 N	69 18 E	856	19 34 N	69 22 E
	42		202	20 44 N	69 14 E	203	20 30 N	69 43 E
Veraval	43		530	20 44 N	69 10 E	541	20 30 N	69 31 E
	44		1006	20 44 N	69 03 E	856	20 30 N	69 19 E
	45		215	21 31 N	68 00 E	204	21 30 N	68 00 E
Porbander	46		500	21 30 N	67 49 E	508	21 30 N	67 42 E
	47		1025	21 29 N	67 46 E	940	21 30 N	67 28 E

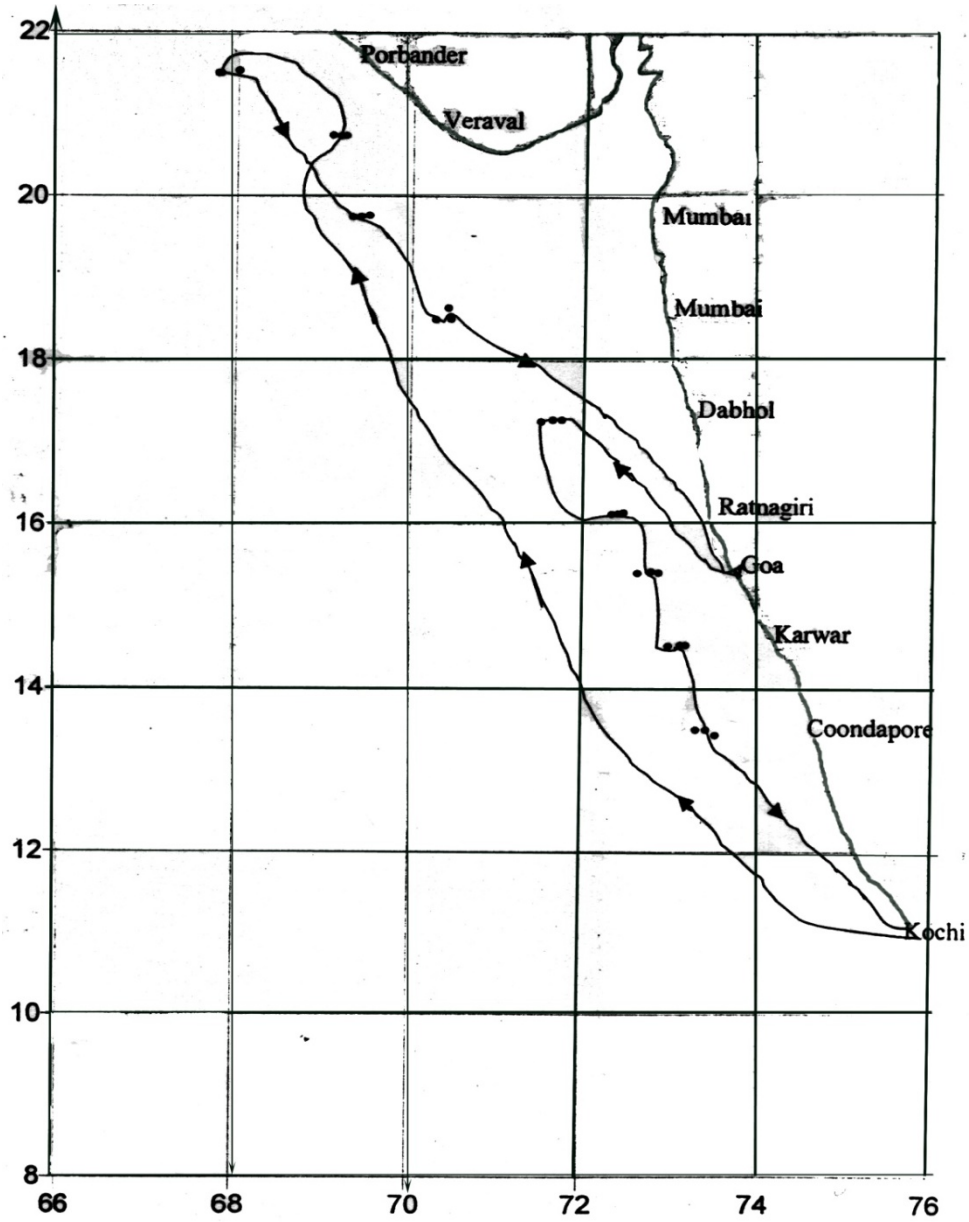


Fig 2.3. Map showing details of the cruise track undertaken for sampling along the west coast of India during Cruise No.228.

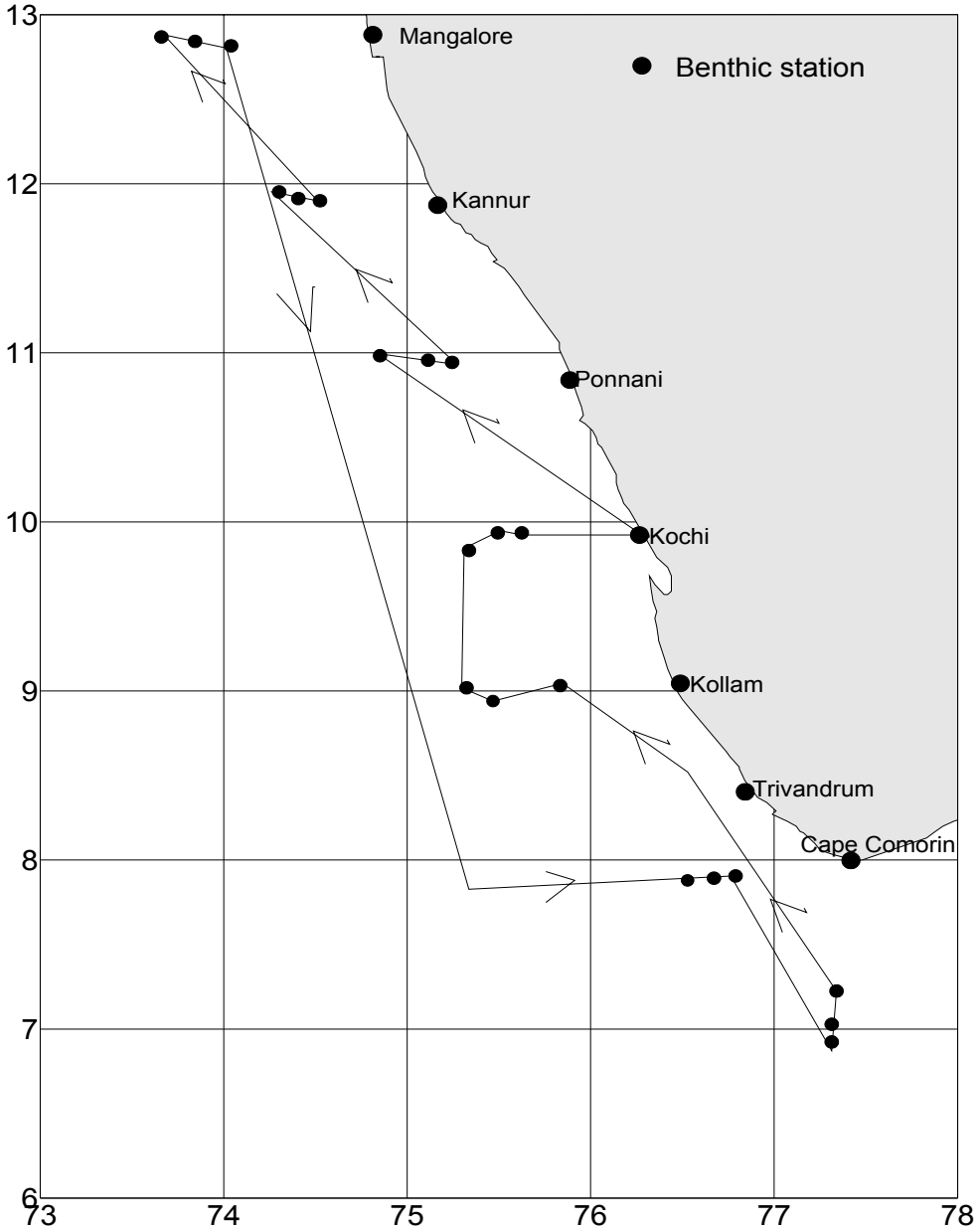


Fig 2.4. Map showing details of the cruise track undertaken for sampling along the west coast of India during Cruise No. 233.

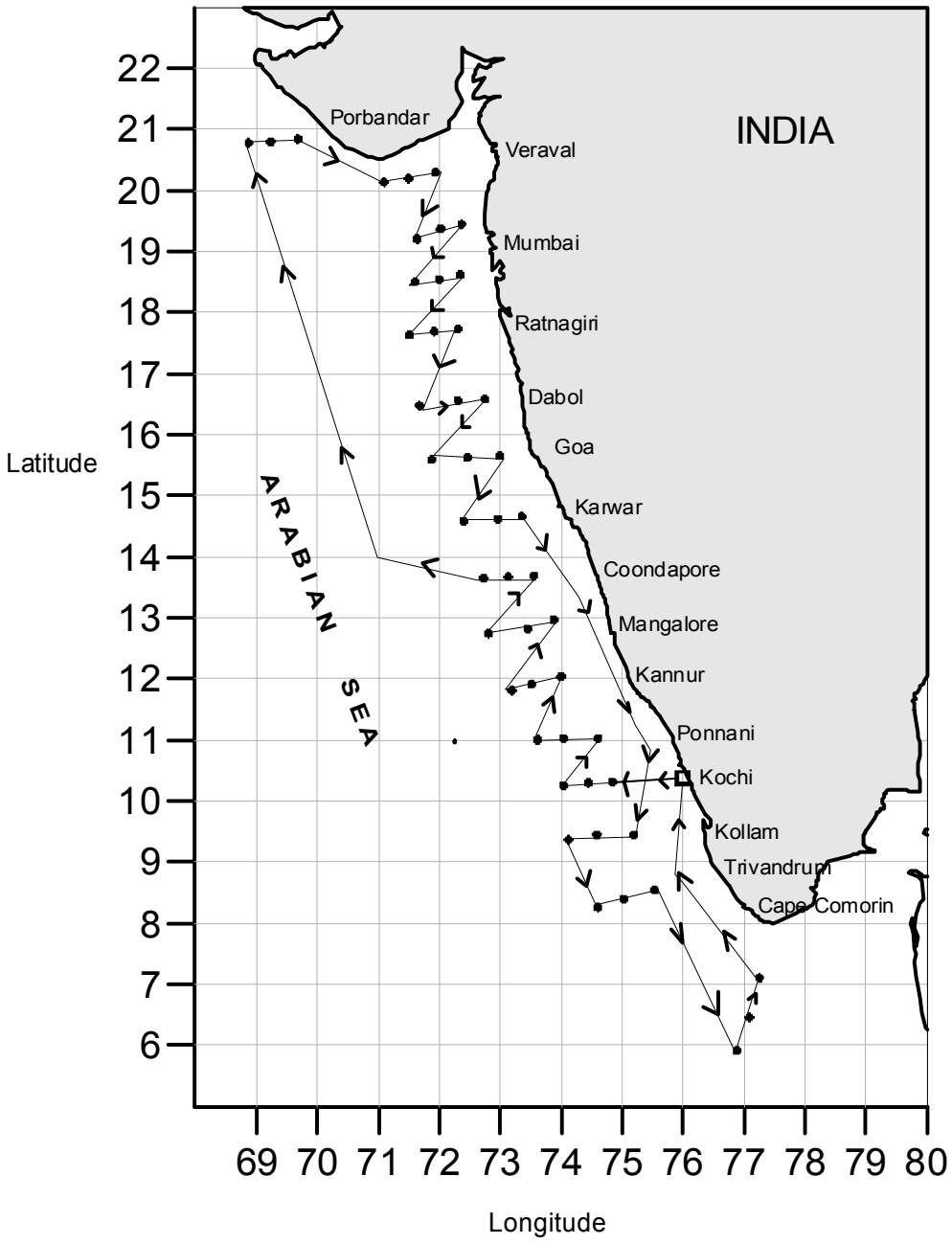


Fig 2.5. Map showing details of the cruise track undertaken for sampling along the west coast of India during Cruise No. 254.

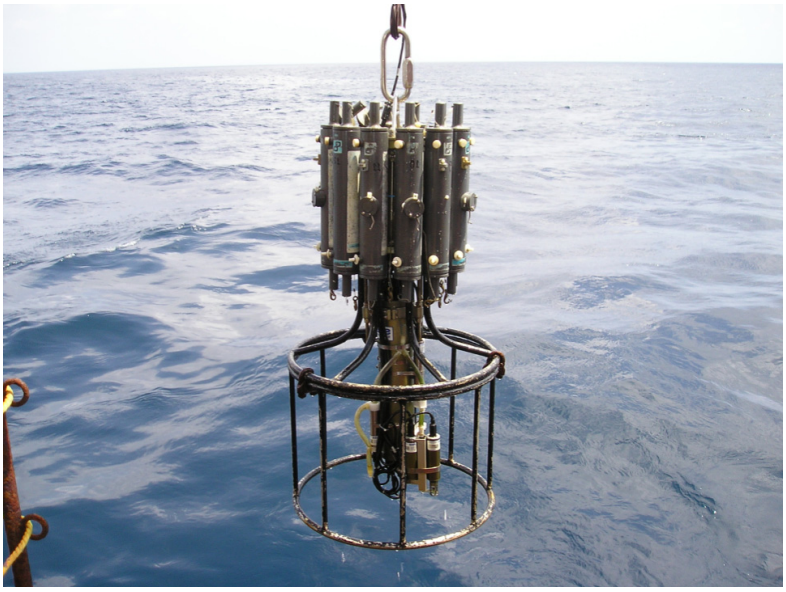


Fig 2.6a. CTD Sampling device.

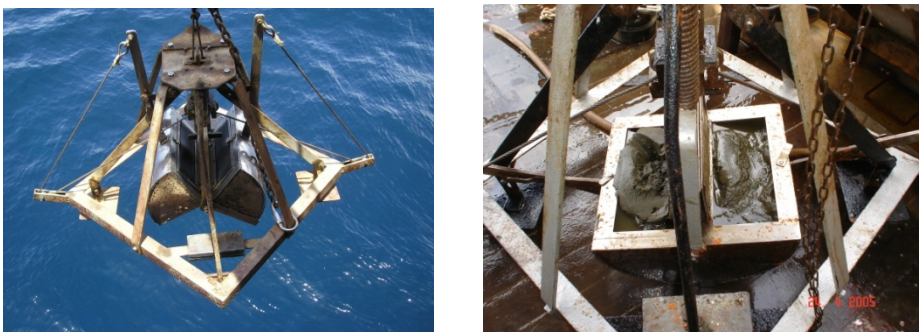


Fig 2.6b. Smith-McIntyre grab used for sediment sampling

2.2.3. Sediment Texture Analysis

Sediment was air dried and separated the fine fraction (<150µm) by wet sieving. The size of the fine fraction was determined by using Particle size analyzer (Sympatec, Germany). The sand, silt and clay fractions were calculated and expressed as percentage.

2.2.4. Total Organic Matter

The sediment samples were air dried and homogenized for the determination of Total Organic Carbon content (TOC) which was measured by chromic acid oxidation method followed by titration with ammonium ferrous sulfate (El Wakeel and Riley, 1956). Total Organic Matter (TOM) was obtained from TOC using a conversion factor of 1.72 according to Wiseman and Bennette (1940) and expressed as percentage organic matter in sediment.

2.2.5. Labile Organic Matter

i) Determination of Proteins.

Protein analysis of the sediments was carried out according to Lowry *et al.* (1956) using bovine serum albumin as standard.

ii) Determination of Carbohydrates.

Carbohydrate in sediments was analysed spectrophotometrically according to Dubois *et al.* (1956). Carbohydrate concentrations were calculated from calibration curves of D-glucose and expressed per gram dry weight of sediment.

iii) Determination of Lipids.

Total lipids were extracted from sediment samples by direct elution with chloroform and methanol (1:2 v/v) following the procedure of Bligh and Dyer (1959) and analysed according to sulphovanillin method of Barnes and Blackstock (1973). Lipid concentrations were calculated from calibration curves of Cholesterol and expressed as per gram dry weight of sediment.

2.2.6. Biopolymeric Carbon (BPC).

Biopolymeric carbon is defined as the sum of the carbon equivalents of total carbohydrates, proteins and lipids and was calculated utilizing conversion factors 0.4, 0.49 and 0.75 respectively (Fabiano *et al.,* 1995). The result was expressed as percentage biopolymeric carbon per gram dry weight of the sediment.

2.2.7. Statistical Analysis.

Data were analysed by both univariate and multivariate statistical methods with respect to the hydrography and sediment variables using statistical softwares ORIGIN 7.0, SPSS 10.0 and PRIMER-6 (Clarke and Gorley, 2001). Variations in environmental variables on a depthwise scale were examined by a one-way factorial analysis of variance (ANOVA). A post-hoc Tukey's test was adopted to determine if there were significant differences among the depths. Probabilities (p) of <0.05 were considered to be significant. A Student's *t*-test (independent two-sample test) was used to assess the variations between samplings. Nonparametric multivariate techniques (Clarke, 1993; Clarke and Ainsworth, 1993; Clarke and Gorley, 2006; Anderson *et al.,* 2008) were further applied to both hydrography and sediment data. The Principal Component Analysis (PCA, normalised data) was carried out in relation to environmental factors based on Bray-Curtis similarity (Bray and Curtis, 1957) after suitable transformation of data. Similarity among the stations were analysed based on environmental data by hierarchical agglomerative Cluster analysis and nonmetric-multidimensional scaling (MDS) based on Bray-Curtis similarities and the results were plotted into ordination graphs.

2.3. RESULTS

2.3.1. Hydrography Parameters

The hydrography parameters recorded are presented in Appendix Table 2.A1.

i) Temperature: Latitudinal variation in bottom water temperature was not clearly evident, even though a slight increase was observed in the bottom water temperature from southern to northern regions during both the sampling. During the period of Sampling I in the 200m depth region the bottom water temperature ranged from 14.46 to 16.13 ˚C. In 500m depth stations, it ranged between 9.87 and 13.23 ˚C and in 1000m depth stations, it varied between 6.86 and 10.20 ˚C. During Sampling II (Cruise No. 254) the bottom water temperature in 200m depth varied between 12.84 and 17.33 ˚C, 10.62 and 13.09 ˚C and 7.26 and 10.21 ˚C at 200, 500 and 1000m depths respectively. The bottom water temperature of both the sampling period is shown in Fig. 2.7.

However considerable variations were observed between each depth sectors. The mean temperatures (±SD) during Sampling I (Cruise No. 228 & 233) at 200, 500 and 1000m were 15.17±0.59, 11.61± 0.86 ˚C and 8.32±1.13 ˚C respectively. During Sampling II (Cruise No. 254) the mean temperature at various depths were 15.23±1.25 ˚C, 11.69±0.82 ˚C and 9.08±0.89 ˚C at 200, 500 and 1000m depths respectively as shown in Fig. 2.8.

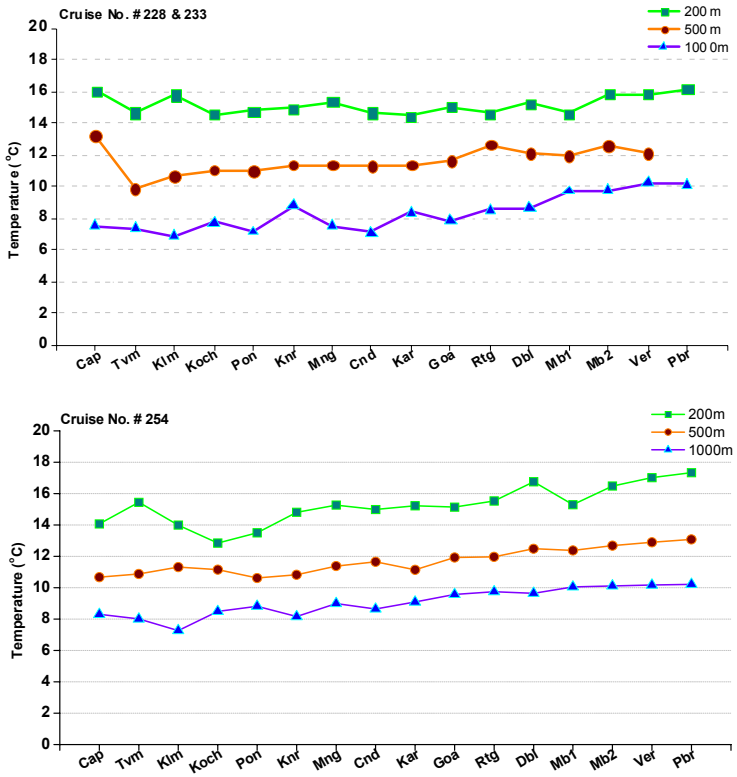


Fig. 2.7. Bottom water temperature at various depths (200, 500 and 1000m) in the slope regions of Arabian Sea observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

According to the one-way ANOVA results, the bottom water temperature showed significant differences between the depths during Sampling I (one-way ANOVA: DF=2; F=238.87; $p<0.05$) and during Sampling II (one-way ANOVA: DF=2; F=150.76; $p<0.05$). The result from the independent two-sample (student's) *t*-test, showed that no significant difference were observed between sampling ($p>0.05$).

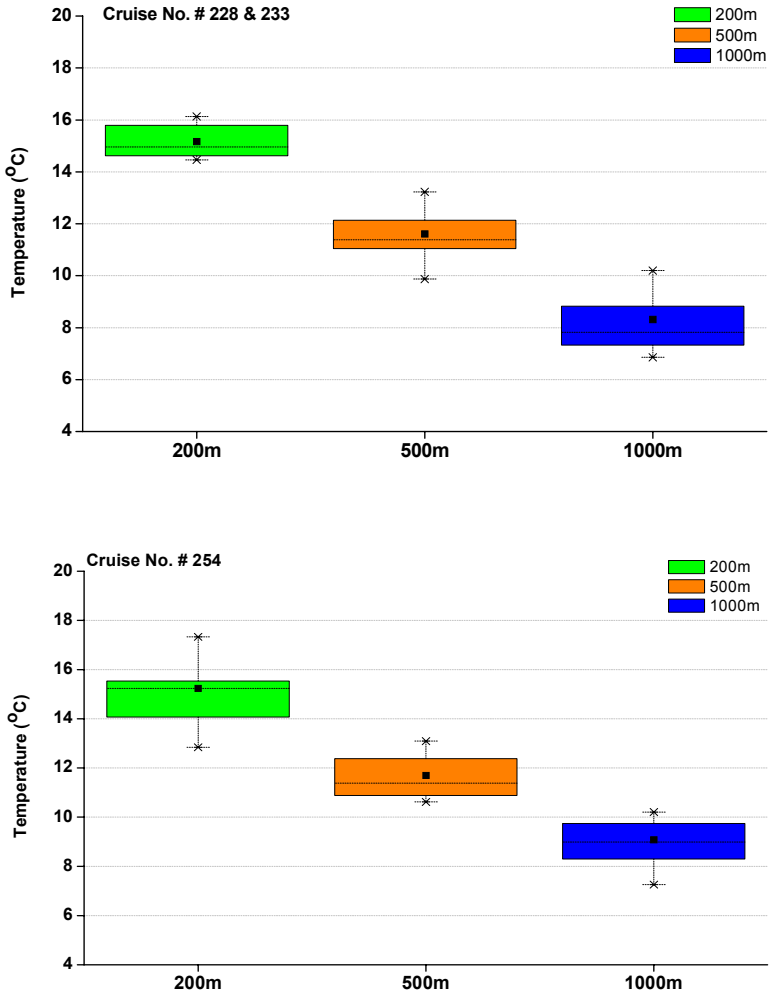


Fig 2.8. Depthwise variation of bottom water temperature observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

ii) Salinity: The bottom water salinity during the period of sampling I ranged between 34.97 and 35.84 psu and during sampling II between 35.02 to 36.03 psu. Though salinity variations were not prominent as temperature, an increase in salinity at stations lying in the northern region were observed at all the depths (Fig. 2.9). The depthwise variations were more profound in the northern region than the southern region during both the cruises. Depthwise variation of bottom water salinity are shown in Fig. 2.10.

Distribution pattern of salinity in each depth region showed that during Sampling I the salinity reported were 35.33±0.24, 35.36±0.20 and 35.20±0.19 psu at 200, 500 and 1000m depth respectively. During Sampling II the mean salinity was 35.40±0.33 psu at 200m depth 35.38±0.23 psu at 500m depth and 35.26±0.18 psu at 1000m depth stations.

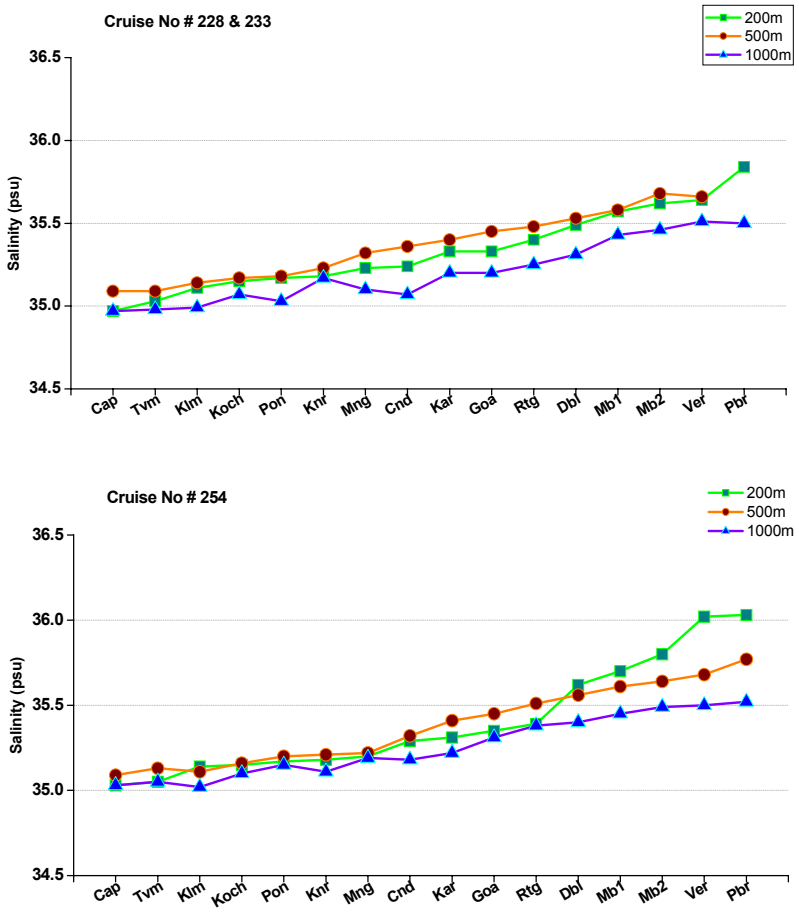


Fig 2.9. Latitudinal variation of bottom water salinity observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

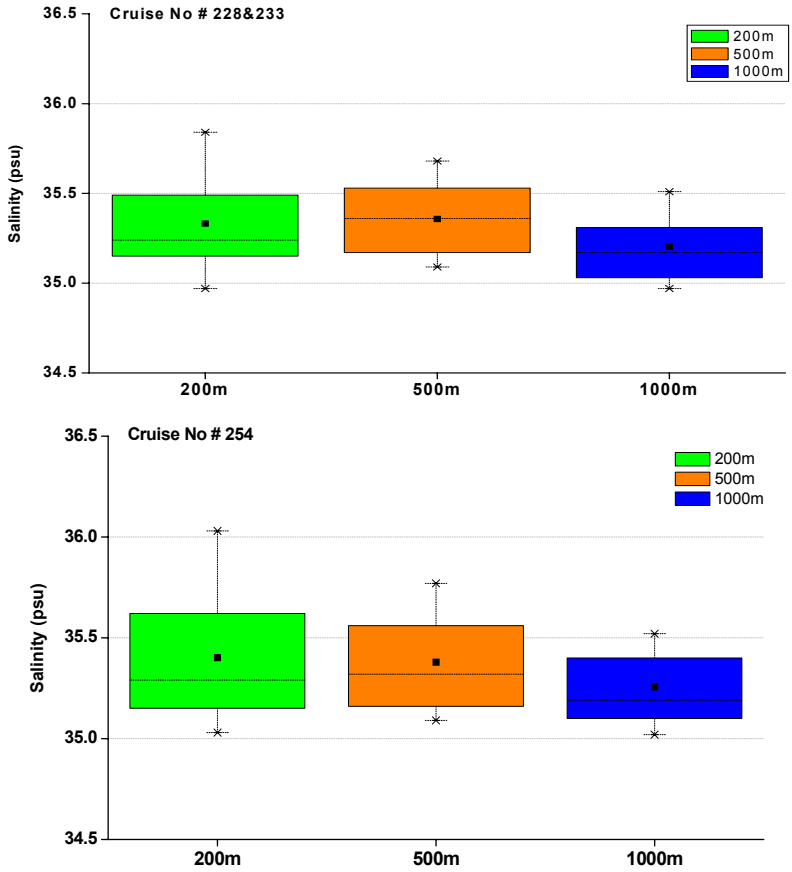


Fig 2.10. Depthwise variation of bottom water salinity observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

According to the one-way ANOVA there was no significant difference in the bottom water salinity between the depths during Sampling I (one-way ANOVA: DF=2; F=2.37; p=0.105) and during Sampling II (one-way ANOVA: DF=2; F=1.53; p=0.227). The result from the independent two-sample (student's) *t*-test showed that the bottom water salinity was not significantly different between the two samplings ($p>0.05$).

iii) Dissolved Oxygen: A remarkable feature of dissolved oxygen was the negligible levels detected at several locations along the north west coast. Within each depth range dissolved oxygen varied considerably. However, the bottom water close to 1000 m depth stations were relatively more oxygenated compared to 500m depth regions. The

distribution (Fig. 2.11) shows that dissolved oxygen of the bottom water during Sampling I (Cruise No. 228&233) varied between 0.23 and 1.40 ml l^{-1}. In 200m depth stations, it ranged between 0.23 to 1.40 ml l^{-1} with a mean (±SD) of 0.66±0.34 ml l^{-1}, 0.16 and 0.88 ml l^{-1} at 500m with a mean (±SD) of 0.54± 0.19 ml l^{-1} and in 1000m depth stations, it varied between 0.29 and 1.27 ml l^{-1} with a mean (±SD) of 0.74±0.28 ml l^{-1}.

The dissolved oxygen of the bottom water during Sampling II (Cruise No. 254) varied between 0.10 and 1.09 ml l^{-1}. In 200m depth stations, it ranged between 0.10 and 1.07 ml l^{-1} with a mean (±SD) of 0.32±0.26 ml l^{-1}. In the 500m depth stations, it ranged between 0.07 and 0.73 ml l^{-1} with a mean (±SD) of 0.33±0.22 ml l^{-1} and in 1000m depth stations, it varied between 0.12 and 1.09 ml l^{-1} with a mean (±SD) of 0.49±0.26 ml l^{-1}. Depthwise distribution of bottom water DO are shown in Fig. 2.12.

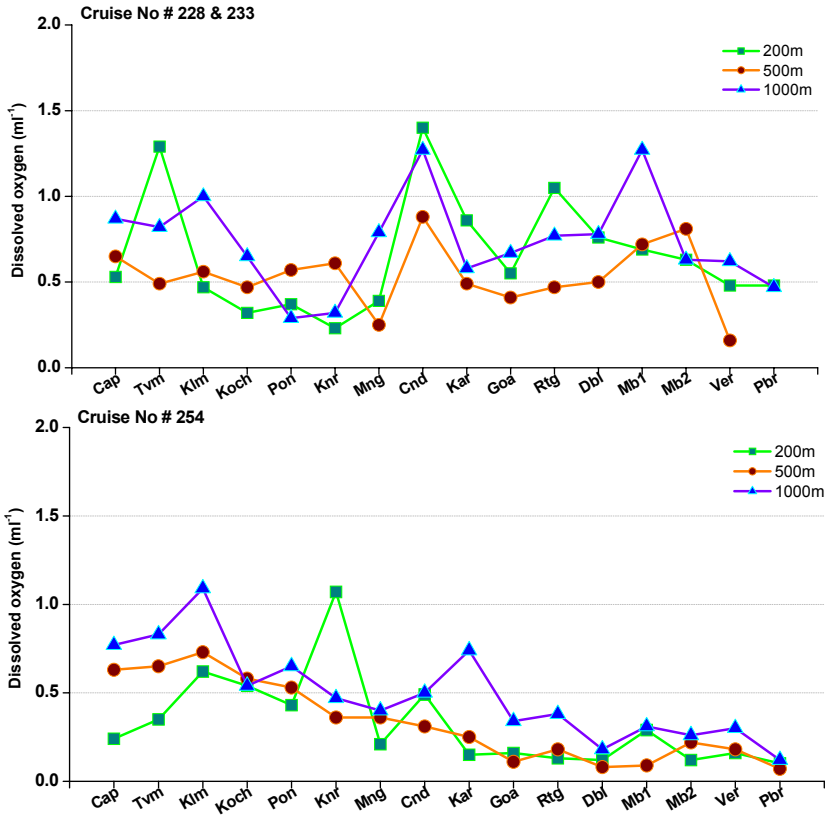


Fig 2.11. Latitudinal variation of bottom water Dissolved Oxygen observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

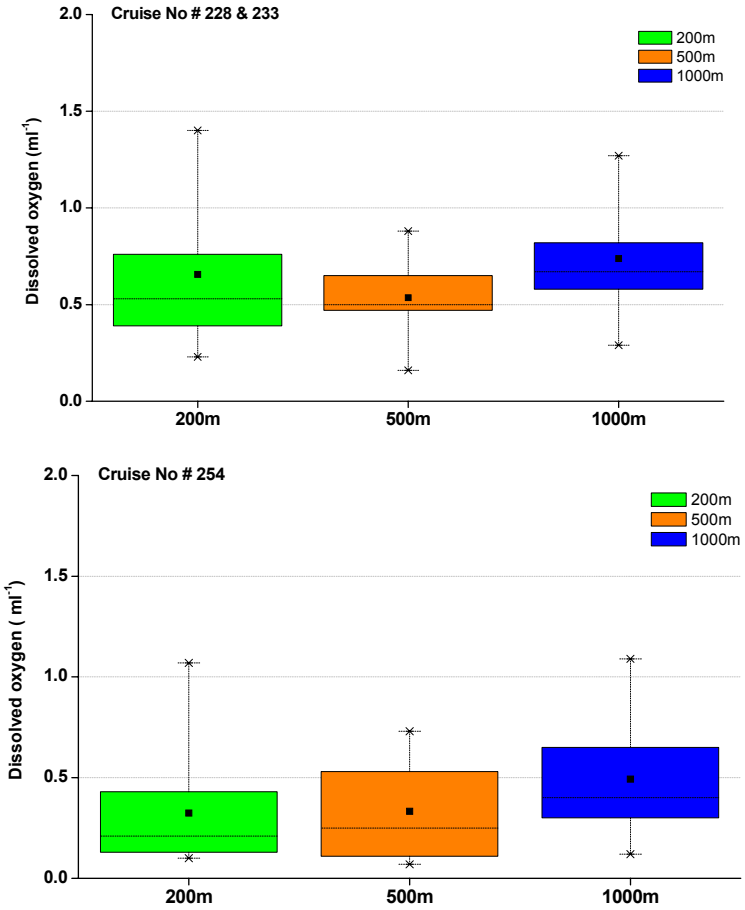


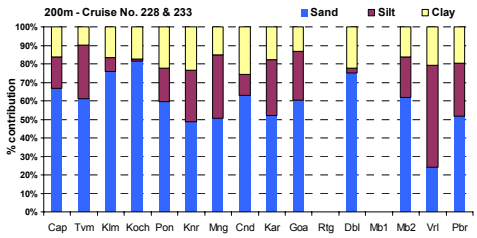
Fig 2.12. Depthwise variation of Dissolved Oxygen in bottom water observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

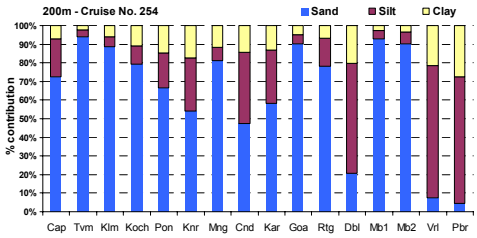
According to the one-way ANOVA results there was no significant difference in the bottom water dissolved oxygen between the depths during Sampling I (one-way ANOVA: DF=2; F=2.05; p=0.141) and Sampling II (one-way ANOVA: DF=2; F=2.31; p=0.111). However a significant difference in the bottom water dissolved oxygen between the two samplings were observed from the independent two-sample (student's) *t*-test ($P < 0.05$).

2.3.2. Sediment Texture

The percentage composition of the sediment fractions (sand, silt and clay) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254) are presented in Appendix Table 2.A2 and Fig. 2.13-2.15. The textural types of the sediments recorded were clay, silty clay, sandy clay, clayey silt and sandy silt in both the cruises. Generally the southern transects were more coarser in texture compared to the northern region. Southern transects mostly showed silty sand, whereas northern transects were found to be silty. Depthwise distribution also showed remarkable variation in texture as majority of the stations were clayey silt or silty. While 200m depth stations were mostly sandy and silty sand in nature, the higher depth stations were generally sandy silt or clayey silt. Cruisewise variation in the sediment texture was observed in the 200m stations of the northern region.

In general during Sampling I at 200m depth range, the sediment was sandy except Off Veraval which was silty in nature. At 500m stations the sediment were clayey silt in nature except a zone of sandy texture at the southernmost region from Cape to Kollam. In the northern region, Station 25 (Off Karwar), 34 (Off Dabhol) and 46 (Off Porbandar) showed sandy nature compared to the adjoining regions. At 1000m depth, most of the stations were clayey silt in nature except Off Ratnagiri which was sandy. During Sampling II, 200m depth range was generally sandy except Off Veraval and Porbandar in the northern region which was clayey silt in nature. In 500 and 1000m depth ranges, the substratum was clayey silt except a few stations in the southern most region.





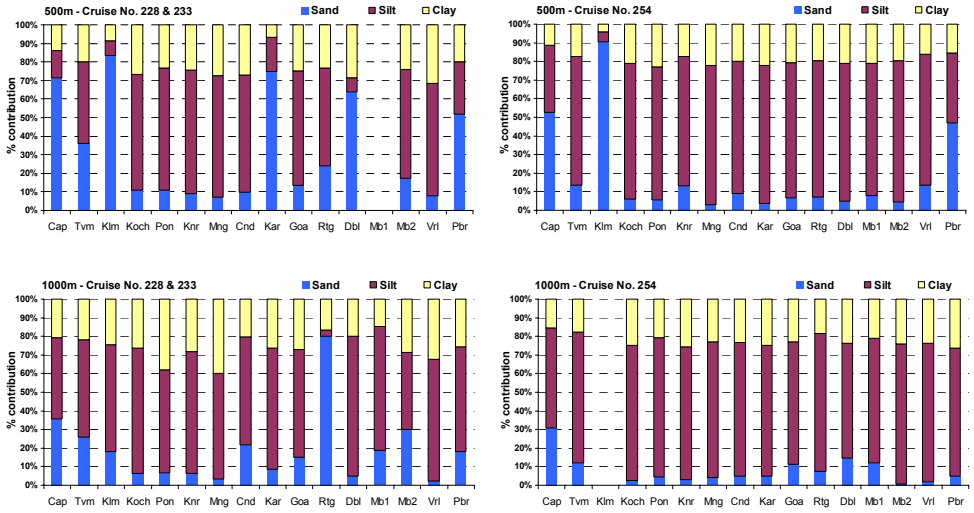


Fig 2.13. Texture of slope sediments at various depths (200,500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

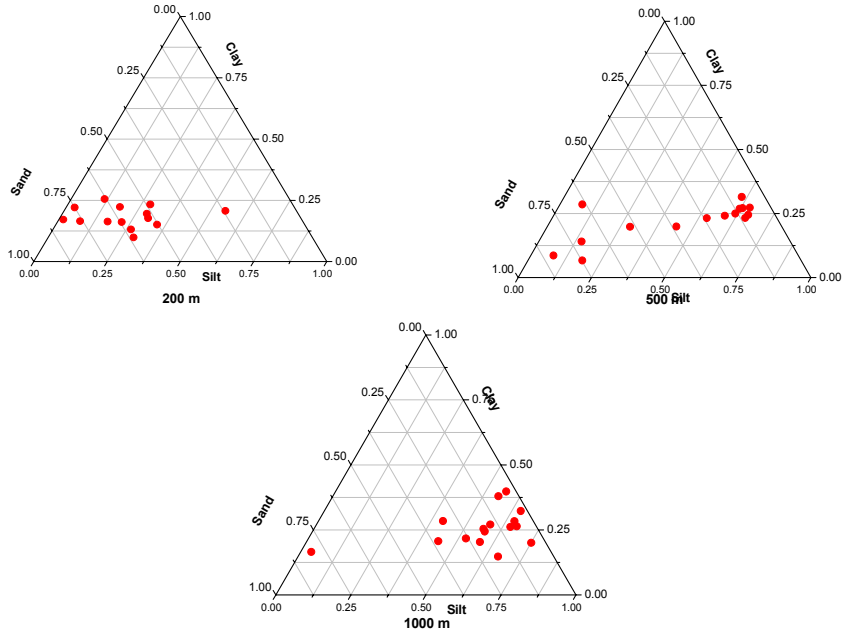


Fig 2.14. Depthwise variation of sediment texture in the slope regions of Arabian Sea during Sampling I (Cruise No. 228&233).

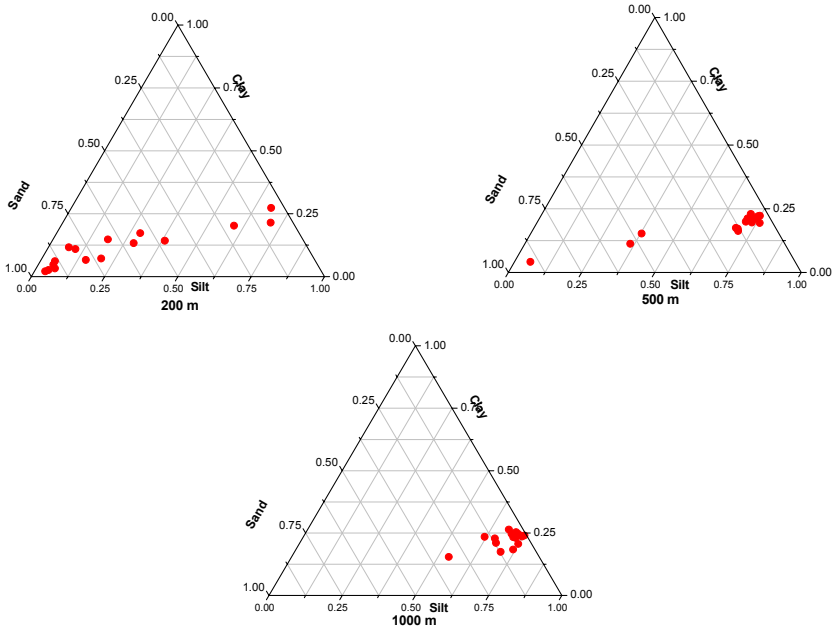


Fig 2.15. Depthwise variation of sediment texture in the slope regions of Arabian Sea during Sampling II (Cruise No. 254).

According to the ANOVA results grain size properties of the sediment including Sand, Silt and clay content showed significant differences between the depths during Sampling I (one-way ANOVA: DF=2; $p<0.05$; $F_{sand}=13.63$; $F_{silt}=12.71$; $F_{clay}=5.12$) and during Sampling II (one-way ANOVA: DF=2; $p<0.05$; $F_{sand}=26.74$; $F_{silt}=29.29$; $F_{clay}=15.50$). Although the results from the independent two-sample (student's) *t*-test, to test the variation between sampling showed no significant difference in the sand content between sampling ($p>0.05$). However, there was a significant difference in silt and clay content between sampling ($p< 0.05$).

2.3.3. Total Organic Matter

Generally the organic matter (OM) in the sediment increased as the depth increased, the maximum being at 1000m followed by 500m and 200m depths. There was an increasing trend in OM towards the northern region. The organic

matter (OM) of sediments ranged between 0.83 and 12.88% (Cruise No. 228&233). An increase in OM was observed towards higher depths in all the transects under study. Stations at 200m depths showed comparatively low amount of organic matter than 500 and 1000m stations. The mean (±SD) value for OM in 200, 500 and 1000m depth ranges were 2.56±1.26, 6.3±3.46 and 7.14±2.59 % respectively.

The OM was comparatively higher in northern region with the highest value being recorded Off Mumbai II, in 200m depth stations it ranged between 0.83 and 5.52%. There was a gradual increase towards the northern region with the lower values being recorded Off Trivandrum, Kochi and Mangalore. In 500m depth range the OM varied between 1.72 and 12.82%. Considerably higher values were observed Off Kochi, Ponnani, Goa, Ratnagiri and Mumbai II. Though there was not much variations in the OM between 500 and 1000m depth range stations, higher values could be observed in the northern regions at 1000m (Fig. 2.16).

During Sampling II (Cruise No. 254) also organic matter was found to be higher in the northern transects, however, the values recorded during this sampling was found to be slightly higher than that observed during the first sampling. Considerably higher values were observed Off Ponnani, Ratnagiri, Coondapor-Karwar and Veraval-Porbandar regions. Depthwise distribution of OM are shown in Fig. 2.17. Generally the organic matter ranged from 0.95 (Off Cape comorin, 200m) to 17.56% (Off Mumbai II, 500m). It ranged between 0.95 to 11.63% with a mean (±SD) of 4.72±3.18% at 200m stations, 2.02% to 17.56% with a mean (±SD) of 10.21±4.23% at 500m stations and 3.32% to 11.87% with a mean (±SD) of 8.84±2.77% at 1000m stations.

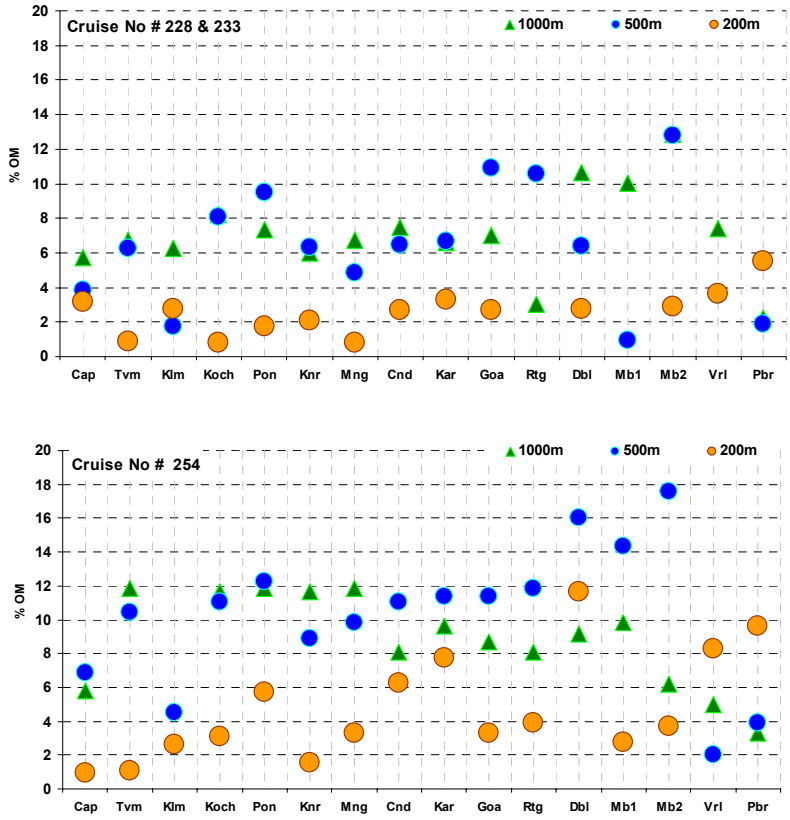


Fig. 2.16. Percentage of organic matter observed in the slope sediments of Arabian Sea during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

According to the one-way ANOVA results, the organic matter concentration showed significant differences between the depths during Sampling I (one-way ANOVA: DF=2; F=12.45; $p<0.05$) and Sampling II (one-way ANOVA: DF=2; F=10.89; $p<0.05$). The result from the independent two-sample (student's) *t*-test also showed that there exist a significant difference in organic matter between sampling ($p<0.05$).

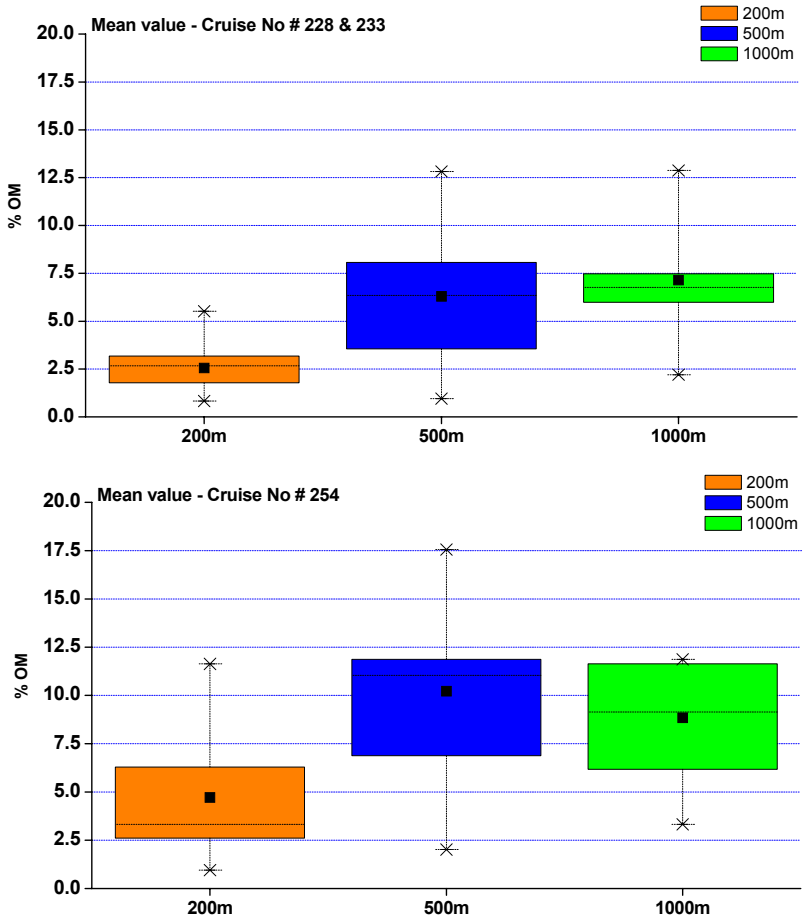


Fig.2.17. Depthwise variation of percentage Organic matter observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

2.3.4. Labile Organic Matter (LOM)

Analysis of the quality and bioavailability of the organic matter has shown that both spatial and temporal variations occur in the distribution of biopolymeric fractions in the surficial sediments of the western Indian continental margin. In general the organic matter showed high lipid and protein fraction in the sediments. Composition of protein, carbohydrate and lipids in the surface sediments of the region showed that the

deeper regions had the highest concentrations. In most cases, lipids followed by proteins were the principal contributors to the labile organic matter.

The labile organic matter (protein, carbohydrate and lipids) in sediment during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254) are presented in Fig. 2.18a and Fig. 2.18b respectively. Depthwise distribution showed remarkable variation amongst individual fractions of LOM. The protein component of LOM in sediments (Cruise No. 228 & 233) ranged between 0.68-10.53 mg g^{-1} dry wt. sediment. Protein concentration in the sediment was 2.26±1.08 mg g^{-1} at 200m, 6.35±2.74 mg g^{-1} at 500m and 6.41±1.75 mg g^{-1} dry wt. sediment at 1000m stations. The carbohydrate content of sediment ranged between 0.05-1.02 mg g^{-1}. Carbohydrate content showed a mean value of 0.19±0.1 mg g^{-1} at 200m region whereas at 500m it was 0.61±0.27 mg g^{-1} dry wt. sediment and at 1000m region 0.59±0.15 mg g^{-1} dry wt. sediment. The lipid content of sediment ranged between 1.97-13.06 mg g^{-1} dry wt. sediment. Lipid content was 3.29±0.96 mg g^{-1}, 7.65±3.03 mg g^{-1} and 6.91±2.20 mg g^{-1} dry wt. sediment at 200, 500 and 1000m stations respectively. Region wise distribution has revealed that LOM was higher towards the northern region.

During Sampling II (Cruise No. 254) the protein content of LOM in sediments ranged between 0.13-7.43 mg g^{-1} dry wt of sediment. Protein content in sediment at various depths were 1.11±0.89, 3.45±1.67 and 3.08±1.92 mg g^{-1} dry wt. sediment at 200m, 500m and 1000m respectively. The carbohydrate content in sediments ranged between 0.06-1.03 mg g^{-1}. Carbohydrate content was 0.28±0.22 mg g^{-1} at 200m, 0.59±0.29 mg g^{-1} at 500m and 0.53±0.21mg g^{-1} at 1000m stations. The lipid content in sediments ranged between 3.08 mg g^{-1} and 8.08 mg g^{-1} dry wt of sediment. The mean value was 4.64±0.96 mg g^{-1} at 200m 4.88±1.29 mg g^{-1} at

500m stations and 4.40±1.40 mg g^{-1} at 1000m. Depthwise distribution of labile organic matter of both the samplings are shown in Fig. 2.19a and Fig. 2.19b.

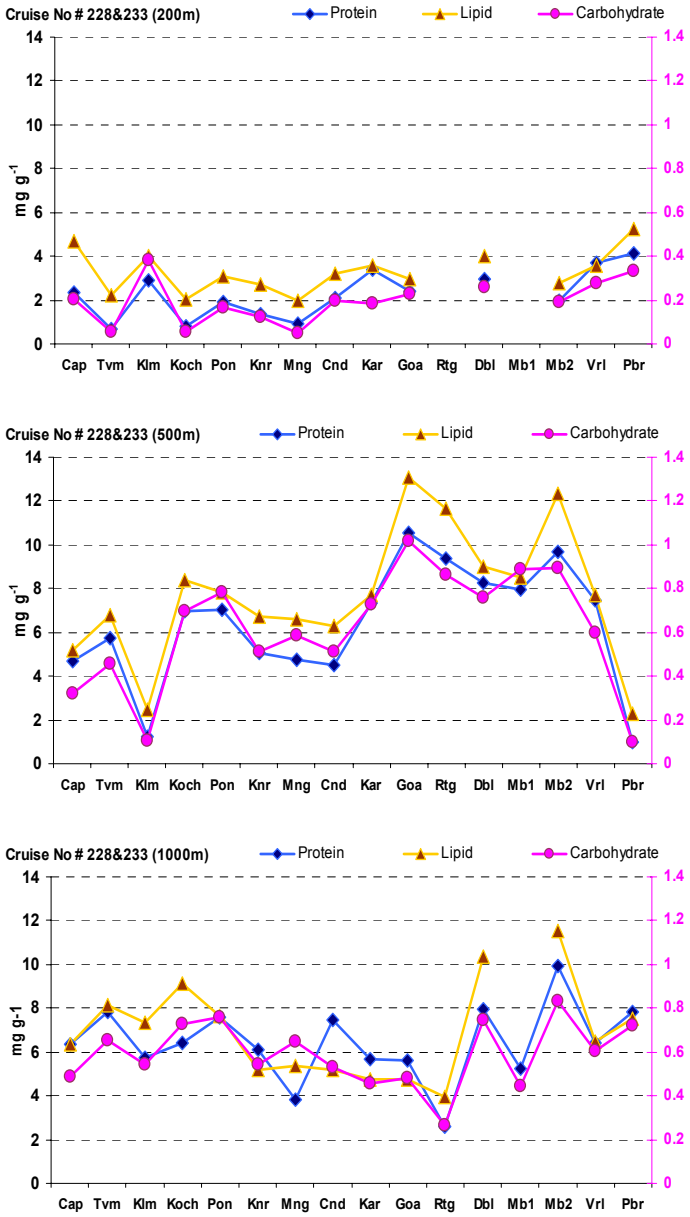


Fig.2.18a. Latitudinal variation of sediment labile organic matter observed during Sampling I (Cruise No. 228&233).

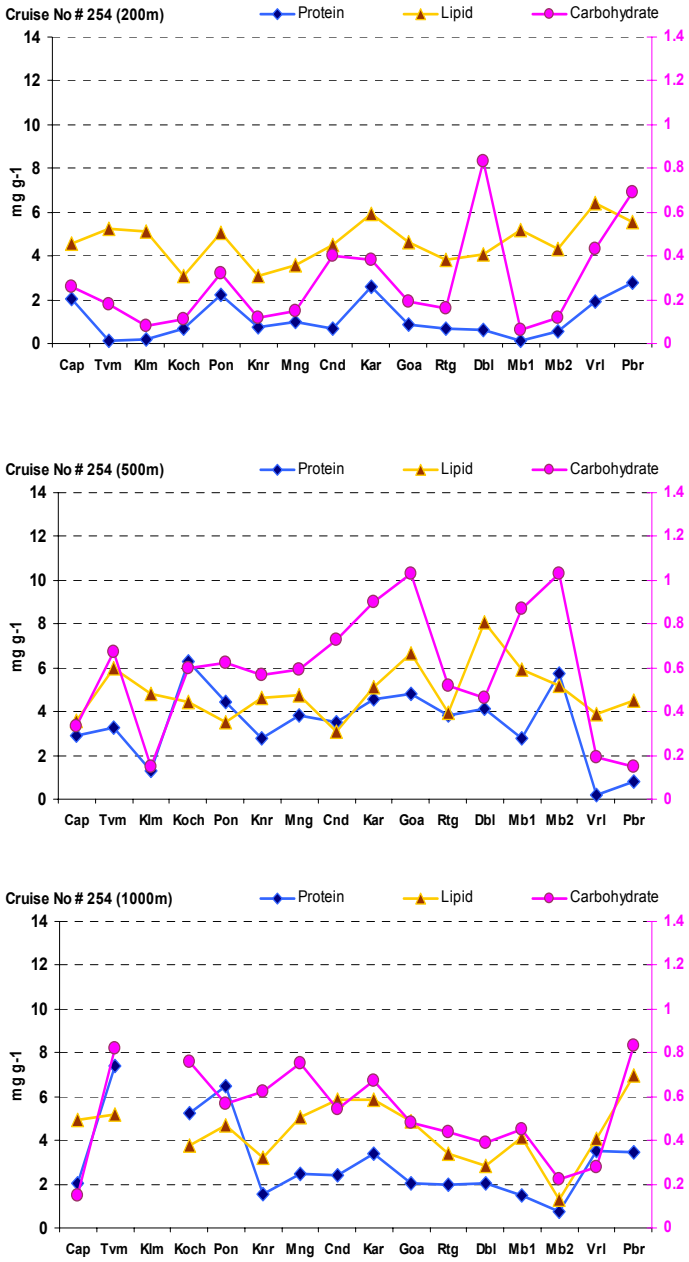


Fig.2.18b. Latitudinal variation of sediment labile organic matter observed during Sampling II (Cruise No. 254).

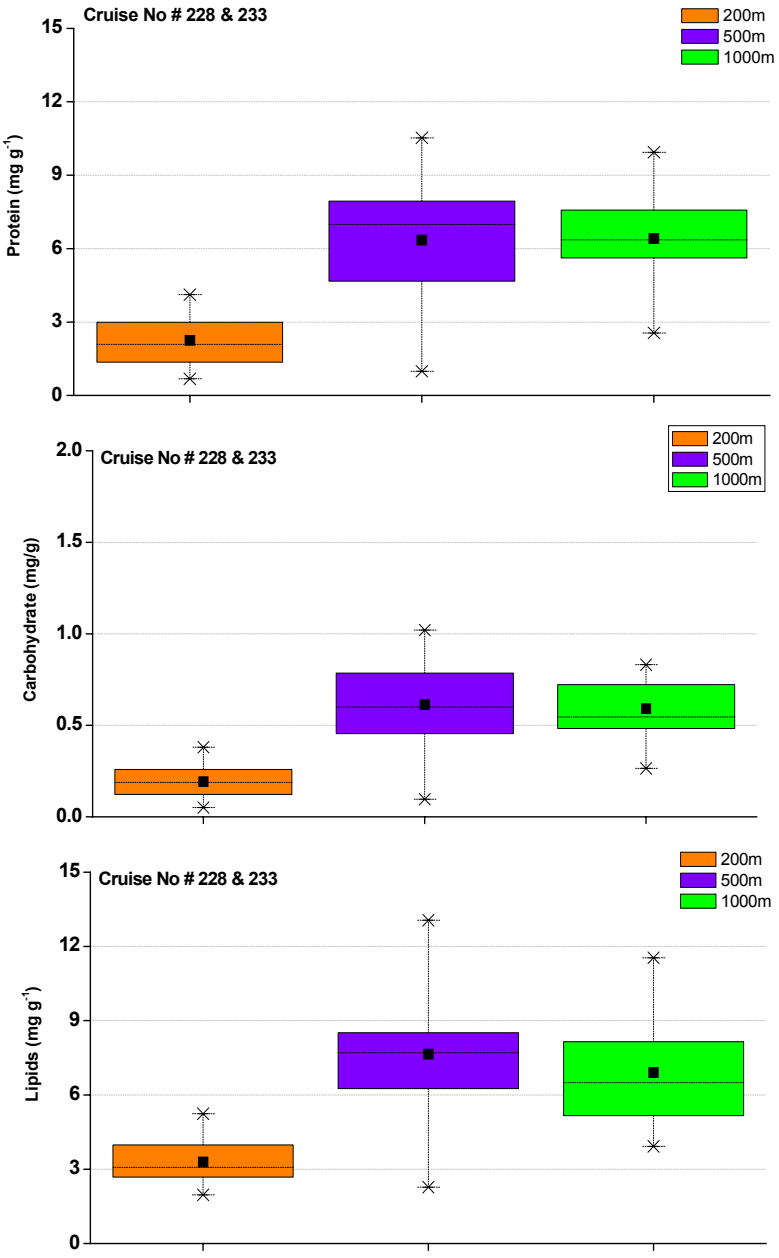


Fig. 2.19a. Depthwise variation of sediment labile organic matter at various depths observed during Sampling I (Cruise No. 228&233).

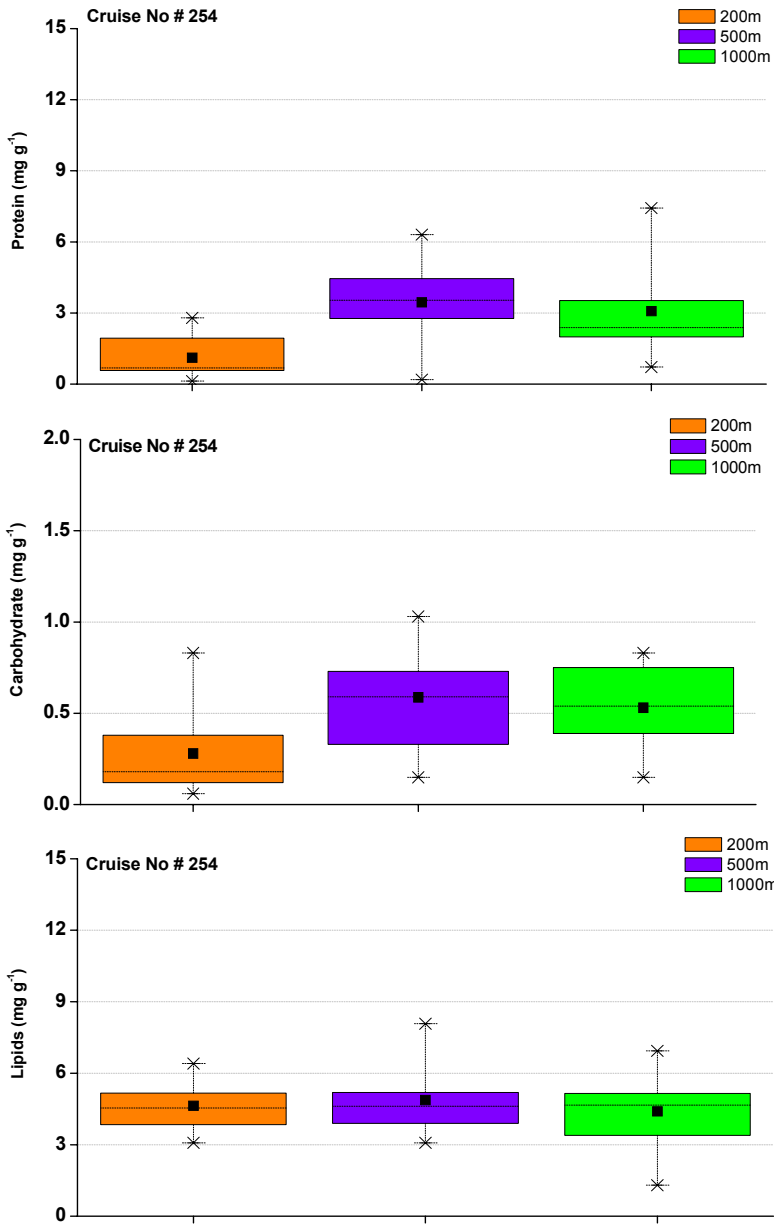


Fig. 2.19b. Depthwise variation of sediment labile organic matter at various depths observed during Sampling II (Cruise No. 254).

According to the ANOVA results labile organic matter fractions including protein and carbohydrate content showed significant differences between the depths during Sampling I (one-way ANOVA: DF=2; $F_{protein}$=20.49 and $F_{carbohydrate}$=22.31; $p<0.05$) and during Sampling II (one-way ANOVA: DF=2; $F_{protein}$=10.47 and $F_{carbohydrate}$=7.22; $p<0.05$) whereas in the case of lipid fraction significant difference between depths were noted only during Sampling I (one-way ANOVA: DF=2; F_{lipid}=15.36; $p<0.05$). The results from the independent two-sample (student's) *t*-test, showed that although a significant difference in protein and lipid content were observed between sampling ($p<0.05$) there was no significant difference in the carbohydrate content between samplings ($p>0.05$).

2.3.5. Biopolymeric Carbon Fraction

The percentage of the biopolymeric carbon fraction was found to be between 0.2 and 1.54% during Sampling I and between 0.15 to 0.83% of the dry wt of the sediment during Sampling II **(**Fig. 2.20). It showed an increasing trend towards deeper depths. Regionwise distribution also showed that the biopolymeric carbon was comparatively higher towards the north, except Off Veraval and Porbandar during Sampling I. Biopolymeric carbon content showed a contribution of 20-25% to the organic carbon (OC) present. The depthwise distribution of BPC has shown that 0.37, 0.9 and 0.86% were observed at 200, 500 and 1000m depth respectively (Fig. 2.21). However, temporal changes in the biopolymeric carbon fraction were observed and in the second sampling, a slight decrease was observed at the northern stations. This decrease was more pronounced at the 500 and 1000m depth regions than 200m. Mean values BPC observed were 0.42, 0.56 and 0.51% for 200, 500 and 1000m depths respectively.

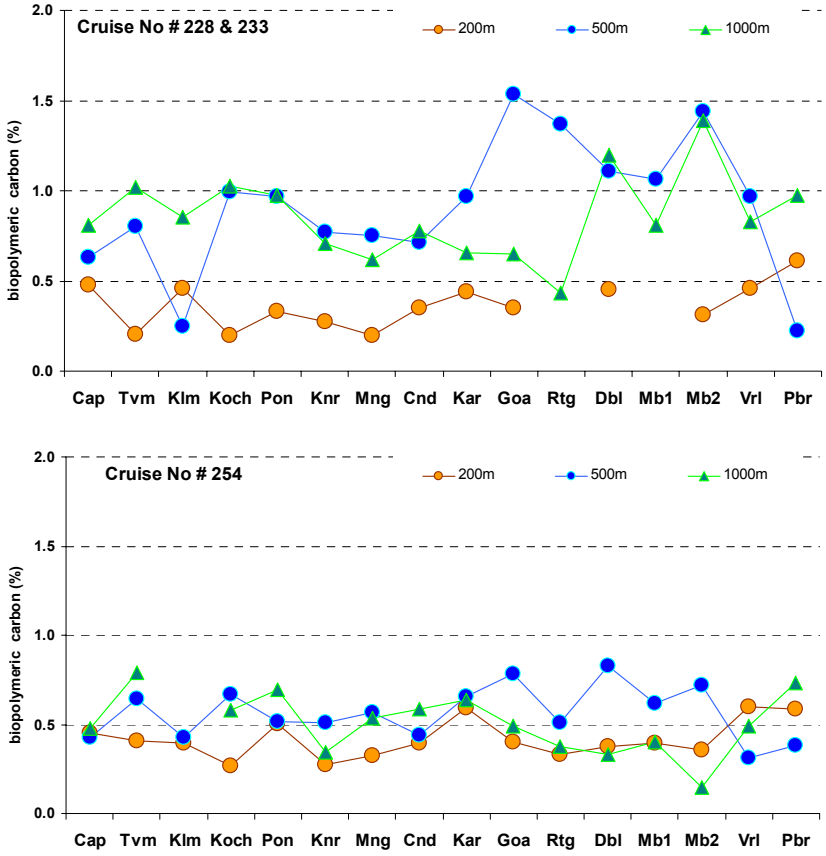


Fig.2.20. Latitudinal variation of Biopolymeric carbon at various depths (200, 500 and 100m) observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

According to the one-way ANOVA results, the Biopolymeric carbon fraction of the organic matter showed significant differences between the depths during Sampling I (one-way ANOVA: DF=2; F=20.16; $p<0.05$) and Sampling II (one-way ANOVA: DF=2; F=4.20; $p<0.05$). The results from the independent two-sample (student's) *t*-test also noted a significant difference between the two samplings ($p<0.05$). The results of sediment biochemical and labile organic matter are illustrated in Appendix tables 2.A3 and 2.A4.

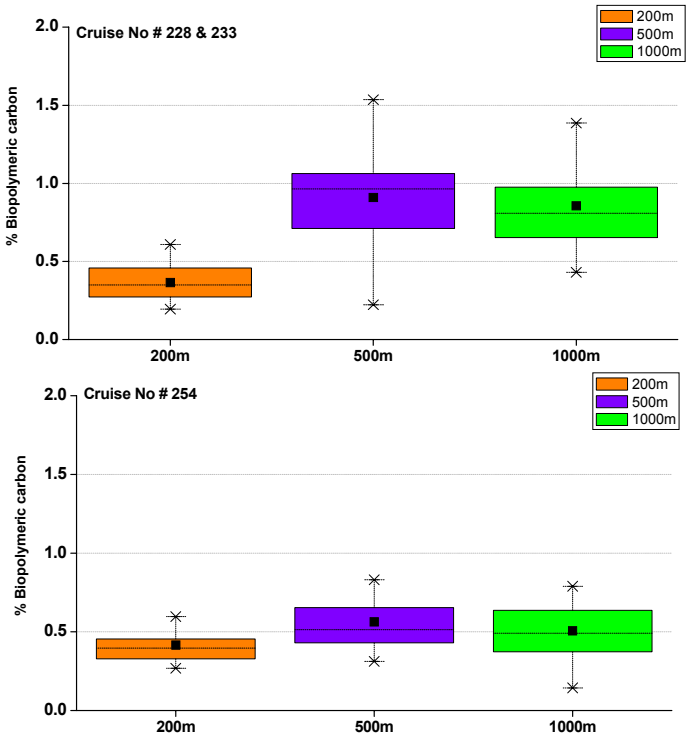


Fig.2.21. Depthwise variation of Biopolymeric carbon observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

2.3.6. Cluster Analysis and Multi Dimensional Scaling (MDS) Ordination

Hierarchical cluster analysis and non metric MDS ordination were used to compare the various environmental parameters for the sediment samples attained in both the sampling. Hierarchical cluster analysis generated similarity matrix based on environmental variables clearly differentiated stations in 200m depth ranges with the stations from the deeper depths. Result of cluster analysis indicated that the samples fall into two major groups at 95% plotted on a Bray-curtis similarity scale (Fig. 2.22). The first group comprised most of the sample locations in 200m depth region. However in the second group, the samples were distributed homogenously between 500m and 1000m depth ranges. MDS represented the samples collected as points in a map. Samples from 200m depth region (Fig. 2.23) lie closer and have more similarity in their physico-chemical parameters.

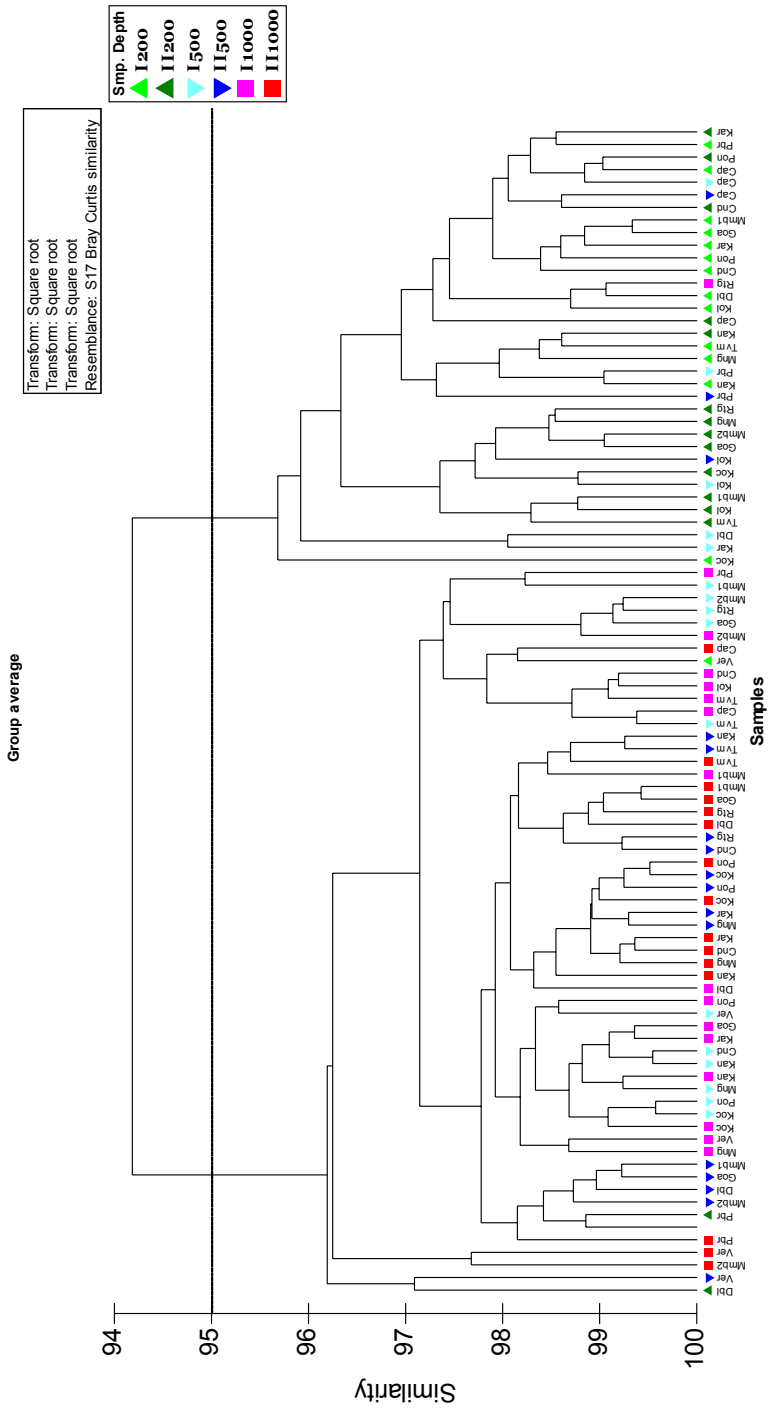


Fig.2.22. Cluster analysis dendrogram based on environmental characteristics observed during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

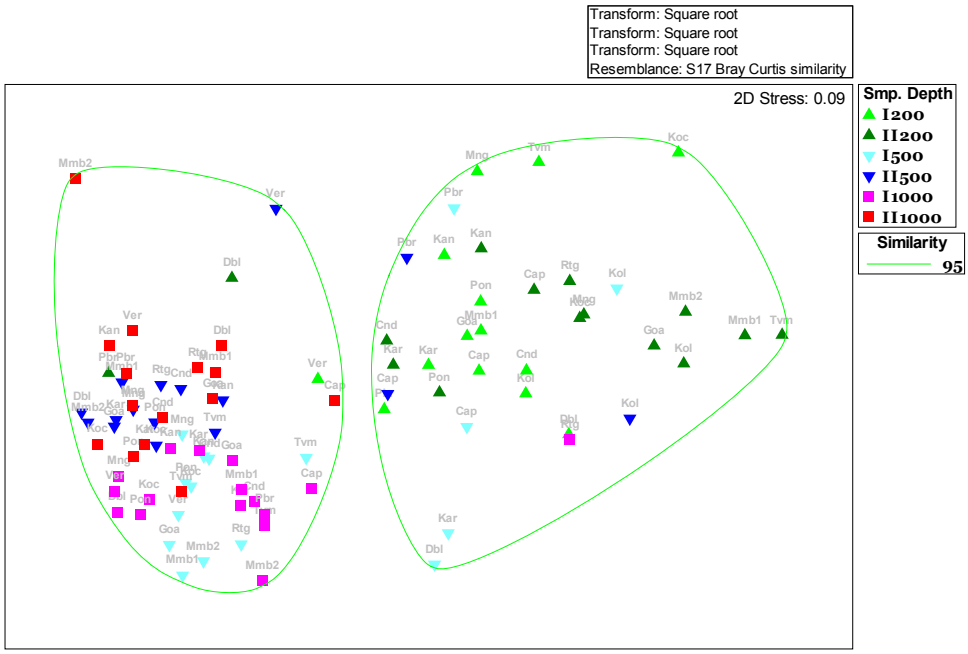


Fig. 2.23. Non metric Multidimentional scalling (MDS) ordination based on environmental characteristics collected during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

2.3.7. Principal Component Analysis.

The Principal Component Analysis (PCA, normalised data) was carried out for ordination of the sample locations in relation to physico-chemical factors. Table 2.2 shows the Eigen values of each principal component and their varibility. Table 2.3 shows the contribution of environmental parameters to each Principal Components.

Table 2.2. Eigen values distributed for principal components and their respective variability plotted as percentage.

Eigen values:

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
Eigenvalue	8.57E-2	1.22E-2	7.43E-3	5.37E-3	4.16E-3	3.03E-3	1.02E-3	8.1E-4	4.7E-4
Variability(%)	71.3	10.1	6.2	4.5	3.5	2.5	0.8	0.7	0.4
Cumulative %	71.3	81.4	87.6	92.1	95.6	98.1	98.9	99.6	100.0

Table 2.3. Contribution of environmental variables to each Principal Components

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Temperature	0.08	0.103	0.086	-0.099	0.209	0.021	-0.154	0.942	-0.095
Salinity	-0.001	0.003	0.005	-0.001	0.009	-0.001	-0.006	0.016	-0.01
DO	0.014	-0.369	-0.48	0.108	-0.726	0.041	-0.192	0.229	0.056
Sand	0.698	-0.394	0.068	-0.538	0.081	0.203	-0.02	-0.097	0.077
Silt	-0.553	0.059	-0.138	-0.813	-0.079	-0.069	0.003	-0.013	-0.005
Clay	-0.229	-0.164	-0.403	0.113	0.463	0.633	-0.349	-0.087	0.016
Protein-C	-0.186	-0.588	-0.142	0.094	0.26	-0.12	0.664	0.146	-0.124
Carbohydrate-C	-0.152	-0.176	0.19	0.051	0.083	-0.1	-0.075	0.087	0.935
Lipid-C	-0.057	-0.274	0.133	0.015	0.136	-0.388	-0.538	-0.102	-0.211
Biopolymeric-C	-0.098	-0.369	0.091	0.046	0.149	-0.381	-0.283	-0.045	-0.135
Organic Matter (%)	-0.28	-0.28	0.703	0.039	-0.294	0.48	-0.017	0.029	-0.17

The Principal Component Analysis (PCA) showed higher similarity of environmental characteristics within respective depth regions (Fig 2.25). For the PCA, eigen values were developed based on a correlation matrix. The variability of all physico-chemical factors with respect to both the cruises were evaluated. The results indicated that 81.4% of the total variance in biogeochemical parameters can be represented by only two principal components (PC1 and PC2). PC1 explained 71.3% of the total variance and was strongly dependent on sand, silt and clay, whereas PC2 explained 10.1% of the total variance and relied mostly on Protein carbon content, Sand, Dissolved oxygen and Biopolymeric carbon. Sand, temperature and dissolved oxygen showed a positive correlation with PC I, while silt, clay, protein, carbohydrates and lipids showed a negative correlation. With PC

II temperature, salinity and silt showed a positive correlation, whereas all other factors were negatively correlated. Resemblance curves clearly separated stations in 200m depth range from deeper depths at 95% similarity.

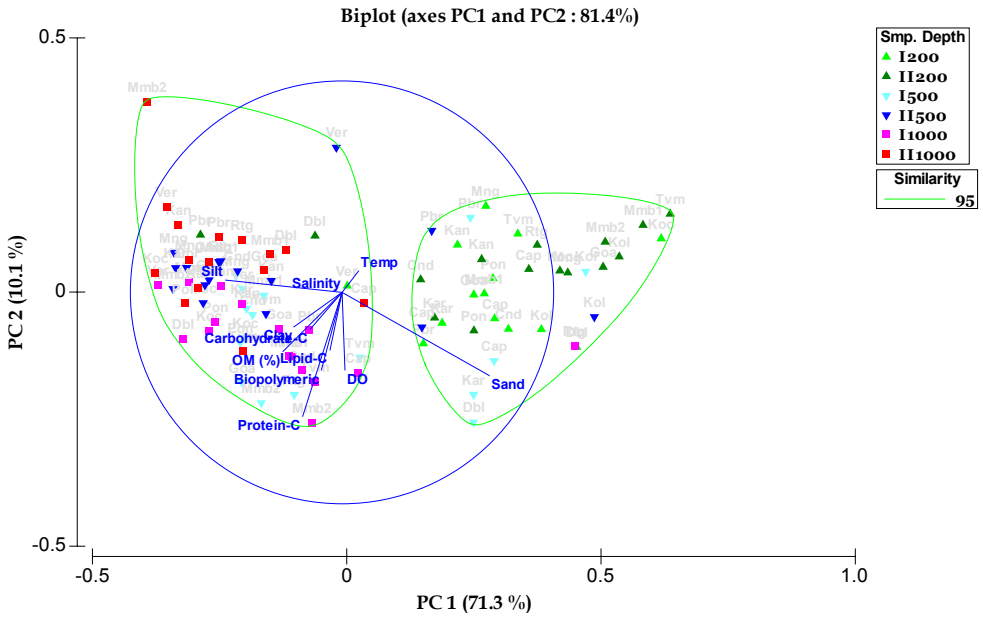


Fig. 2.25. Principal Component Analysis.

2.4. DISCUSSION

Ocean margins and continental slope regimes are natural boundaries common to oceanic and shelf sea domains. These are areas of enhanced productivity and biologically mediated transport of materials within the benthic boundary layer providing strong links between these two realms. The Arabian Sea in the north-western Indian Ocean is believed to be globally significant as far as biogeochemical fluxes are concerned. Water-column processes including the seasonally reversing monsoon-driven circulation and the associated upwelling and productivity/oxygen depletion have been the foci of many studies. The Present study is confined to the continental slope of west coast of India (eastern Arabian Sea) located at the northwestern Indian Ocean. The continental slope has long been

recognized as zones of matter and energy transfer between the continental shelf and the deep ocean (Griggs *et al.,* 1969). Studies on the physical, chemical and biological processes at ocean margins, the shelf break and the continental slope would help to determine the transport of materials from the shelf to the deep sea (Flach *et al.,* 2002). Understanding the relationship between the deep-sea benthic system and the local ocean processes is essential in forming a comprehensive view of the biogeochemical cycling in the Arabian Sea.

In the present study a decrease in water temperature with depth was observed during both the cruises. However latitudinal variation in temperature was limited, though a slight increase was observed towards the northern region. In deeper waters of Arabian Sea an increase in temperature was noticed from south to north by Qasim (1982) albeit a decrease of temperature noticed in the surface waters. Recently, the study on the Indian Ocean circulation (Benny and Mizuno, 2000) has also showed a warm water advection from equator towards Dwaraka region. A slightly higher temperature recorded in the southern most Arabian Sea may be related to the intrusion of the Arabian Sea mini warm pool developed prior to the onset of southwest monsoon season (Seetaramayya, 1984).

Among the hydrographical parameters, salinity showed least variation latitudinal as well as on a depth-wise scale. However a slight increase could be observed towards the northern region concomitant with a reduction in dissolved oxygen during the sampling period. The higher salinity noticed in the northwest coast can be due to the inflow of waters of higher salinity from the north along the coast (Wyrtki, 1971). According to Schott and Fischer (2000), there is a southward transport of Persian Gulf water below the surface layer in the 200-500 m depth range and that of Red Sea water below 500m. The Persian Gulf water mass is warmer and more saline which reaches the northern region and have higher temperature than the southern region in this depth range (Wyrtki, 1971). The Red Sea water and the Persian Gulf water that is injected into the Arabian Sea at sub-

surface levels leave this semi-enclosed basin more saline than any other part of the Indian Ocean. The lower salinity of southern region might be due to the incursion of low saline waters from Bay of Bengal to the south west coast (Darbyshire, 1967; Wyrtki, 1971). Hareesh Kumar and Mathew (1997) noticed that the maximum northward extension of this low saline water could be traced upto 17ºN.

The level of dissolved oxygen during the sampling period showed a decrease with depth in the upper water column till mid depths (600-800m) whereas the deeper layers at 1000m depth ranges were more oxygenated. However, the variations were more profound regionally than depthwise. Many of the stations in all depth ranges showed dissolved oxygen values <1 ml l^{-1} and even <0.5 ml l^{-1} were detected. The northern stations showed considerable depletion of oxygen. This depletion of oxygen in the northern latitudes may be associated with the oxygen minimum layer described by Sen Gupta *et al.* (1977, 1980) and Qasim (1982). Permanently hypoxic water masses in the open ocean, referred to as oxygen minimum zones, impinge on a much larger seafloor surface area along continental margins of the eastern Pacific, Indian and western Atlantic Oceans (Helly and Lewin, 2004). Earlier Ingole and Koslow (2005) reported a decreasing pattern in the oxygen concentrations towards the northern region in the Arabian Sea and Bay of Bengal. The region experiences upwelling during the Southwest monsoon, and the oxygen deficient waters are brought close to the surface. Sometimes the entire shelf is covered by waters with O_2 <0.5 ml l^{-1} (Naqvi *et al.,* 2002). The occurrence of coastal upwelling seasonally also makes the region highly fertile and the existence of Asian landmass forming the northern boundary prevents quick renewal of subsurface layers. Consequently, dissolved oxygen gets severely depleted below the thermocline and reducing conditions prevail at intermediate depths (ca. 150-1200m) (Wyrtki, 1971; Cowie, 2005). Higher nutrients and lower oxygen concentrations occur in the bottom layer as compared to the overlying water column in deep waters of the Arabian Sea, suggesting that considerable quantities

of organic matter reach the deep sea floor and get oxidized in the bottom layer (Sen Gupta and Naqvi, 1984). Cowie *et al.* (1999) indicated that the oxygen availability is an important factor in determining sedimentary organic matter content.

Sediment texture in the continental slope region showed a heterogenous pattern of distribution. It was found that in many stations clayey silt fractions dominated the continental slope regions. As expected, the fine sediment fraction increased with depth. Generally, sediment grain size varies as a function of water depth (Bennett *et al.*, 1999). This was in concomitant with the studies from the continental margin of other ocean sites including Wedell Sea region, off Antarctica (Isla *et al.,* 2006). The present study showed that sand fraction in the sediments was negatively correlated with depth. The sediment texture at 200m depth was sandy, and the deeper stations showed less sand content. In the present study, the silt fraction of the sediment was found to be positively correlated with the depth. A major quantity of sediment load is delivered to the oceans by the rivers. The input of terrigenous sediments show strong latitudinal trends which results in a distinct pattern of increasing accumulation rates from north to south. Enormous quantity of sediments are transported to the oceans by the rivers in India which carry about 5% of global runoff (Chakrapani *et al.,* 1995). Waters of the rivers along with silt and suspended sediments spread laterally and along shore on reaching the shelf area. Consequently, relatively coarser particles are deposited at/or near the confluences while finer material like clay gets transported away from the confluence to the deeper parts. Further, Thorne *et al.* (1993) reported that the texture of sediment delivered at the river mouth was silty clay with only 15-20% of usually fine to very fine sand. Coarse fraction studies along the west coast of India by Murty *et al*. (1968) also have revealed that there existed a distinct zonation with regard to their distribution, and much of the slope sediments were covered with silty clay or clayey silt fractions. Regional studies of the marine sediments from the Northern Indian Ocean (Kolla *et al.,* 198l) have shown the influence of major rivers of the west coast of India on deep sea sedimentation. As described by Rao *et al.* (1995) it

appears that the Indus derived sediments are transported onto the outer shelf of western India and to a lesser extent to the continental slope regime. The influence of the Indus borne sediments on the continental slope decreases from north to south and cross shelf transport processes dominate the south western continental margin.

The distribution of organic matter on the western Indian continental margin during the present investigation showed that in general the organic matter in the sediment increased as the depth increased. At 200m the organic matter was comparatively lesser than the 500 and 1000m depth zones. Calvert *et al*. (1995) has reported that the inner shelf sediments of eastern Arabian sea probably have low organic matter content because of the heavy dilution of marine organic material by relatively organic-poor clays. Latitudinal variation in the amount of organic matter between the stations at 200m depth region was not prominent. However at 500m and 1000m depth ranges, the organic matter showed a gradual increase towards the northern region. The organic matter in the sediments ranged between 0.83 to 17% which was fairly higher when compared with the oceanic realm. Paropkari *et al.* (1987, 1994) have reported organic carbon enrichments of 5-8% with maxima up to 12-16% even in areas where bioproductivity is low to moderate.

The organic matter content in sediment at any depth is a function of several factors like productivity of the region, time of burial, rates of sedimentation and *in situ* biological and chemical activities. Organic matter which is not stored on the shelves, is generally transferred by waves and currents over the shelf edge onto the continental slope and canyons (Fisher *et al.,* 1996). Therefore, continental slopes are important depocenters of organic carbon (Jahnke, 1990). Depthwise distribution of the organic matter reflects the existence of variable conditions along the continental region Off the west coast of India through different periods. The sediment organic carbon contents in the Atlantic slope also increased with increasing water depth and decreasing grain size (Bennett *et al.,* 1999).

It is widely accepted that in sediments, the organic matter is mainly attached to the fine grained fraction. Organic matter in the sediment depends on the texture of the sediments and higher organic matter content is associated with finer fractions of sediments than coarser ones. It was further reported that the smallest sediment size fraction had more organic material than the largest size (Ramamurthy *et al.,* 1979) and the clayey sediments which offer larger surface area for the adsorption of organic matter would accumulate more organic carbon (Rajamanickam and Setty, 1973). Ransom *et al.* (1998) also found that organic matter is mainly attached to clay minerals. As a result clay and silty clay have relatively higher organic matter content than sand and clayey sand (Paropakari, 1979; Paropakari *et al.,* 1978; De Haas, 2002).

The sediment organic carbon maximum on the continental slope of western India is widely believed to be due to the preferential preservation of deposited organic matter at water depths where intense oxygen minimum intersects the sea floor. Organic carbon reach maximum concentrations at 200 to 1600m depth, where most of the depth is influenced by a strong oxygen minimum zone. Most of the organic matter that reaches the sea floor is mineralized by benthic organisms. Structural characteristics of benthic communities can give important indirect and time integrated information about the quantity and quality of organic materials settling at the sea floor and the burial of organic material in the sediments (Flach and Heip, 1996).

The biochemical composition of the sedimentary organic matter could be assumed as an estimate of the material potentially available to benthic biota (Danovaro *et al.,* 1993; Fabiano *et al.,* 1995).Sedimentary protein, carbohydrate and lipid concentrations reported in this study were high when compared to deep-sea values collected worldwide (Danovaro *et al.,* 1993; Boetius *et al.,* 1996; Tselepides *et al.,* 2000), indicating that the region is characterized by trophic conditions typical of shelf environments. This is not surprising since the eastern

Arabin sea is characterized by a large phytodetritus deposition to the sea floor (Rice *et al.,* 1994; Thiel *et al.,* 1989).

Biopolymeric carbon is mainly composed of protein and lipid carbon (Fabiano and Danovaro, 1999; Pusceddu *et al.,* 2000). Rapid sedimentation promotes the burial of fresh, highly reactive organic matter, while in areas of slow sedimentation, much of the organic matter that reaches the sea floor decomposes before it can become deeply buried, even in oxygen-depleted environments (Schuffert *et al.,* 1994). The contribution of carbohydrates to total organic carbon in the surficial sediments is found to be low as was previously noticed by Alagarasamy (2003) from regions along the Oman margin. However this is inconsistent with the observation by Luna *et al.* (2004) who reported carbohydrates as the dominant biochemical class of organic components in the deep sea sediment of the Mediterranean region. He further stated that a decrease in the carbohydrate concentrations was noticed up to 10cm depth, whereas the protein and lipid content increased in the deepest region (8-10cm depth).

Furthermore, lipid concentration has been associated to the most labile fraction of sedimentary organics and it was considered as a good index to describe the energetic (or food) quality of the organic contents in the sediment (Fabiano and Pusceddu, 1998). In the highly productive upwelling region off central Chile very high protein, lipid and carbohydrate contents were noticed in comparison to many other regions of the world (Neira *et al.,* 2001). The high lipid contents in that region were also attributed to the flux of diatoms to the seabed (Neira *et al.,* 2001). Diatoms which are responsible for the high primary production in the Arabian Sea, are also important carriers of lipids to the sediment upon sinking (Budge and Parrish, 1998; Ramos *et al.,* 2003). Consequently, diatoms in the Arabian Sea should be also responsible for the high nutritive quality found in sediments.

The high nutritive quality of the sediment suggests that primary production exports from the euphotic zone are intense and not fully degraded, enabling the

organic matter to keep its good nutritive condition and the microbial community may also contribute to maintain such a "healthy" condition of the sediment (White *et al.,* 1984; Orejas *et al.,* 2003). However, further studies on microbial activity and organic matter degradation should be performed to elucidate the importance of the microbiota for the nutritional value of the surface sediment. Hence, it seems that local differences in primary production exert a control on the biochemical quality of the surface sediment on the continental margin area. The type (origin) of organic matter constituents and their (related) lability towards oxic degradation, mineral surface area, mineral composition, and possibly the secondary productivity by (sediment) bacteria also appear to have an influence on the accumulation and composition of organic matter in this area and in some cases to explain discrepancies with published observations for other continental margin settings.

Chapter 3

OCCURRENCE AND DISTRIBUTION OF TOTAL HETEROTROPHIC BACTERIA

Contents

3.1. INTRODUCTION

Marine sediments constitute a large global carbon reservoir and an environment with numerous microorganisms (Whitman *et al.,* 1998). Approximately one-third of oceanic primary production occurs in neritic and coastal environments that cover less than one-tenth of the ocean area. Due to the shallow water column in these areas, up to 50% of the organic matter produced as phytoplankton biomass settles to the seafloor and the majority is degraded by sedimentary microorganisms, suggesting a key role of the microbial population for the global recycling of organic matter. Many studies were conducted to understand how these microorganisms are distributed in the environment and how they influence biogeochemical processes (D'Hondt *et al.,* 2004). The microbial ecology of marine sediments is not well understood and the limited number of studies focusing on sediment microorganisms has detected a mixture of both aerobic and anaerobic microbial groups.

Considering the importance of the microorganisms, their distribution and abundance have received considerable attention in the past. Observations made during the Galathea Expedition of 1950-1952 demonstrated the presence of living bacteria in sediment samples obtained at depths greater than 6000m. Prior to that,

bacteria at depths higher than 6000m, have not been extensively studied for several reasons, the most obvious being the sampling difficulties. However, deep sea bacteria were first collected by Certes (1884) as a result of the Travaillier and Talisman Expeditions conducted during 1882-1883. Later, Fischer (1894) obtained few colonies from samples collected at depths of >1100m during a transatlantic crossing by a passenger ship in 1886. Thereafter, improvements in the knowledge of deep sea biology progressed slowly and a rapid development started largely due to the sterling efforts of C.E. ZoBell and co-workers. The investigators were concerned primarily with establishing the existence and the numbers of bacteria at great depths and attention was directed towards the biochemical and physiological capabilities. To date, deep sea microbiological studies have been carried out in a wide range of depths (Jannasch *et al.,* 1971; Jannasch and Taylor, 1984) and it was emphasized that about 75% of the integrated bacterial biomass from surface waters to deep sea sediments is found in the top 10cm sediment (Lochte, 1992). The fact was later established by Whitman *et al.* (1998) who reported that the bacteria in oceanic and coastal sediments constitute around 76% (ca. $3.8x10^{30}$) of all global bacteria (ca. $5x10^{30}$) with around 13% (ca. $6.6x10^{29}$) of the total global fraction being found in the upper 10cm of deep sea sediments. In the case of bacterial population difficulty is commonly encountered in evaluating the abundance of bacteria in sediments adequately (Epstein and Rossel, 1995).

While earlier works on marine microbial studies were concentrated on the water column chiefly during JGOFS (Joint Global Ocean Flux Studies; 1992-97), work on the microbiological aspects from benthic environment of Arabian Sea are scanty. Nair *et al.* (1978) studied the sediment bacterial population and physiological responses of the bacterial isolates from the west coast (Arabian Sea) and south east coast (Bay of Bengal) of India. Palaniappan and Krishnamurthy (1985) studied the heterotrophic bacterial flora from both the Arabian Sea and Bay of Bengal. Lakshmanaperumalsamy *et al*. (1986) reported that generally, microbial

populations are more abundant in muddy sediments than in sandy ones depending on the granulometry of particles. Chandrika and Girijavallabhan (1990) studied the quantitative and qualitative distribution of heterotrophic bacteria from northern Indian Ocean in relation to environmental parameters. Ramaiah *et al.* (2004) studied the abundance of pollution indicator bacterial population from Mumbai coast (Arabian Sea). Goltekar *et al.* (2006) studied the irretrievability of marine heterotrophic bacteria from coastal to the deep regions of Arabian Sea and Bay of Bengal. Recently an attempt to evaluate the diversity of aerobic culturable heterotrophic bacteria inhabiting the OMZ (sediment) in the eastern Arabian Sea was made by Divya *et al.* (2010). Chandramohan *et al.* (1987) reported bacterial abundance from the sediments and ferromanganese nodules from the Indian Ocean Basin. Nair *et al.* (2000) studied heterotrophic bacterial population in relation to sediment variables from Central Indian Ocean and Raghukumar *et al.* (2006) studied the spatial and seasonal variability of microbial and biochemical parameters in deep sea sediments of Central Indian Ocean Basin.

The abundance and distribution of total heterotrophic bacteria have a direct bearing on other forms of nutrients in different compartments of the environment. Several studies have already been carried out to characterize heterotrophic bacteria in ocean sites and in different coastal areas of temperate, tropical and polar zones (Billen *et al.,* 1990; Ducklow *et al.,* 1993; Hopkinson *et al.,* 2002). The bacterial population depend on changes in water temperature, salinity, abundance of organic nutrients, and on other physico-chemical parameters (Azam *et al.,* 1983; Fong *et al.,* 1993). However, it has been recognized that bacterial population may be considerably modified by interactions with biotic factors (Martin and Bianchi, 1980).

Decay of wood and cellophane in the deep sea (250-5000m) by bacteria was studied to understand the role of deep sea bacteria in the degradation process (Kohlmeyer, 1980). Further, Newell and Field (1983) observed that living bacteria

represent the main protein resource for sediment ingesting animals. Bianchi and Giuliano (1996) studied the viable bacteria from Mediterranean Sea. Patching and Eardly (1997) described the biomass and activity of barophilic bacterial communities at various depths in the water column and in the sediment contact water at two deep sites in the eastern Atlantic. Bak and Nieuwland (1997) studied the seasonal variation in bacterial and flagellate communities of deep sea sediments and reported that densities of microbes vary along spatial gradients and, in general, decrease with depth. Findlay and Watling (1998) reported the role of environmental factors and benthic bacterial interactions in the structure of a marine benthic microbial community. Pfannkuche and Soltwedel (1998) investigated the standing stock, activity and effects of food pulses on the benthic microorganisms in the continental slope of Celtic Sea. The microbial biomass from the deep sediment of Aegean Sea were studied in relation to the labile organic matter by Danovaro *et al.* (1999). Yanagibayashi *et al.* (1999) studied the barophilic microbial community in the samples collected from the sediments of Japan Trench at a depth of 6292m. Danovaro *et al.* (2000) and Bernard *et al.* (2000) studied the diversity of culturable heterotrophic bacteria from Mediterranean Sea. However, it was opined that microorganisms are common in subsurface sediments but not ubiquitous, cell numbers frequently decline with depth through the active soil horizons but then exhibit no clear numerical trend other than patchy distribution (Stevens, 2002). Queric *et al.* (2004) studied the bacterial abundance and activity along the transect (1250-5600m) of Vestnesa Ridge to the Molloy Hole. Fabiano *et al.* (2004) studied the trophic state of the benthic marine environment and assessed the benthic health of ecosystems based on the use of the microbenthic loop. The vertical distribution of free-living bacteria was investigated at deep sites of >4000m in the Arabian Sea by Koppelmann *et al.* (2005). Microbial diversity from the deep sea sites was reported by Xu *et al.* (2005). Metabolically active bacterial cells have recently been quantified in deep marine sediments using oligonucleotide hybridization probes targeting active cells and their rRNA (Teske, 2005). Zoppini *et al.* (2005) described

the temporal trend of the bacterial activity, abundance and composition in the aggregated and dissolved organic matter under different trophic conditions in the Northern Adriatic Sea.

Bacterial heterotrophy has been extensively measured over most oceanic regions (Ducklow and Carlson, 1992). Heterotrophic bacteria present in the water and sediment of marine and brackish water environments and their role in the decomposition of organic matter and regeneration of minerals have been investigated by Azam and Hodson (1977) and Pomeroy (1979). In addition, the distribution of heterotrophic bacterial population has been suggested to be a precise tool in the identification of water mass and currents (Kriss *et al.,* 1960).

3.2. MATERIAL AND METHODS

3.2.1. Cultivation of Heterotrophic Bacteria

Sediment samples were collected using Smith Mc-Intyre Grab onboard FORV *Sagar Sampada*. Sediment sub-samples were aseptically transferred into sterile and labeled polypropylene bags for isolation of heterotrophic bacteria. The samples were immediately shifted to the Microbiology Laboratory onboard FORV *Sagar Sampada*. The samples were analyzed after suitable dilutions with sterile sea water (10^{-3} and 10^{-4}) and plating onto ZoBell's 2216E Marine Agar medium by spread plate method.

Composition of ZoBell's 2216E Marine Agar Medium - (g l^{-1})

Peptone	:	5.0g
Yeast extract	:	1.0g
Ferric phosphate	:	0.02g
Agar	:	20.0g
Sea water (35psu)	:	1000ml
pH	:	7.2

ZoBell's 2216E agar medium was prepared and sterilized by autoclaving at 121 ˚C for 15 minutes. The sterilized medium was poured into sterile petri dishes and after surface drying inoculation was done using a sterile glass spreader. The plates were kept for incubation at 22±2 ˚C for 5-7 days, colonies were counted and expressed as cfu g^{-1} dry weight sediment.

3.2.2. Isolation and Preservation of Culture

Approximately twenty colonies were isolated at random from each sample. The cultures were repeatedly streaked on nutrient agar plates for purity and preserved in sterile nutrient agar vials overlaid with sterile liquid paraffin.

Composition of Nutrient Agar Medium - (g l^{-1})

Peptone	:	5.0g
Beef extract	:	3.0g
Ferric phosphate	:	0.02g
Agar	:	20g
Sea water (35psu)	:	1000ml
pH	:	7.2

3.2.3 Gram Staining

The bacterial cultures were subcultured on nutrient agar slants and smears were prepared on clean glass slides within 16-18 hours. The smear was air dried and flame fixed for staining. The primary stain, crystal violet was added to the smear and allowed to stand for one minute. Then the slides were rinsed gently in running water. Then Lugol's iodine solution was added as mordant and allowed to

stand for one minute. Washed the slides and treated with the decolorizer for 30 seconds. Then the counter stain safranin was added after washing the smear and allowed to stand for one minute. Rinsed the smear, allowed to air dry and observed under oil immersion objective. Cultures which appeared violet or purple were recorded as Gram-positives and pink as Gram-negatives.

3.2.4. Statistical Analysis

The data were analysed by univariate and multivariate statistical methods. Depthwise variations were compared separately for each sampling by one-way ANOVA, followed by post-hoc pairwise comparisons (Tukey's test) using ORIGIN v.5.0. Effects were considered significant when $p<0.05$. A Student's *t*-test (independent two-sample test) was used to assess the variations between samplings. A correlation martrix was generated to find the correlation coefficient between microbial populations and environmental factors. To select the variables that best explain the distribution of hetertrophic bacteria in the sediment, the BIOENV analysis was carried out (Spearman rank correlation method) as integrated in PRIMER v.6. (Clarke and Ainsworth, 1993).

3.3. RESULTS

3.3.1. Distribution of Total Heterotrophic Bacteria (THB)

Total (culturable) heterotrophic bacteria (THB) in the surface sediments of the upper continental slope of Arabian Sea showed a distinct zonation with regard to their distribution along the continental margin of India. The THB counts showed a progressive increase from South to North during the sampling and a decrease with increase in depth. Taking into account the heterogenous nature of the

substratum, total heterotrophic bacterial (THB) densities in the sediments showed that the distribution pattern of THB is patchy along the different regions. THB in the sediments at different depth ranges were highly variable, and it was difficult to find any clear distribution pattern. However, the culturable heterotrophic bacterial densities were usually higher at 200m depth ranges than the deeper regions.

3.3.1.1 Sampling - I (Cruise No. 228 & 233)

During Sampling I (Cruise No. 228 and 233), of all the stations examined the lowest density was observed Off Trivandrum, at 500m (2.31×10^2 cfu g^{-1} dry sediment weight) and the highest Off Kannur at 200m (95.57×10^4 cfu g^{-1} dry weight sediment). The distribution pattern of THB was found patchy with different zonation through several peaks along the various depth regions. At 500 and 1000m depth ranges higher values were observed at regions Off Ponnani to Porbander except Off Karwar and Dabhol.

i) Latitudinal variation: Generally the population was higher Off Ponnani and Kannur and lower Off Trivandrum and Kochi. At 200m depth stations the population varied from 2.83×10^3 (Off Kochi) to 95.57×10^4 (off Kannur) cfu g^{-1} dry weight sediment. At 500m depth stations, the minimum (2.31×10^2 cfu g^{-1} dry sediment weight) was observed Off Trivandrum and the maximum (75.38×10^3 cfu g^{-1} dry sediment weight) Off Kannur and in 1000m depth stations, the minimum (8.26×10^2 cfu g^{-1} dry sediment weight) was observed Off Karwar and the maximum (87.06×10^3 cfu g^{-1} dry sediment weight) Off Mumbai 1 during cruise No. 228&233 (Fig.3.1).

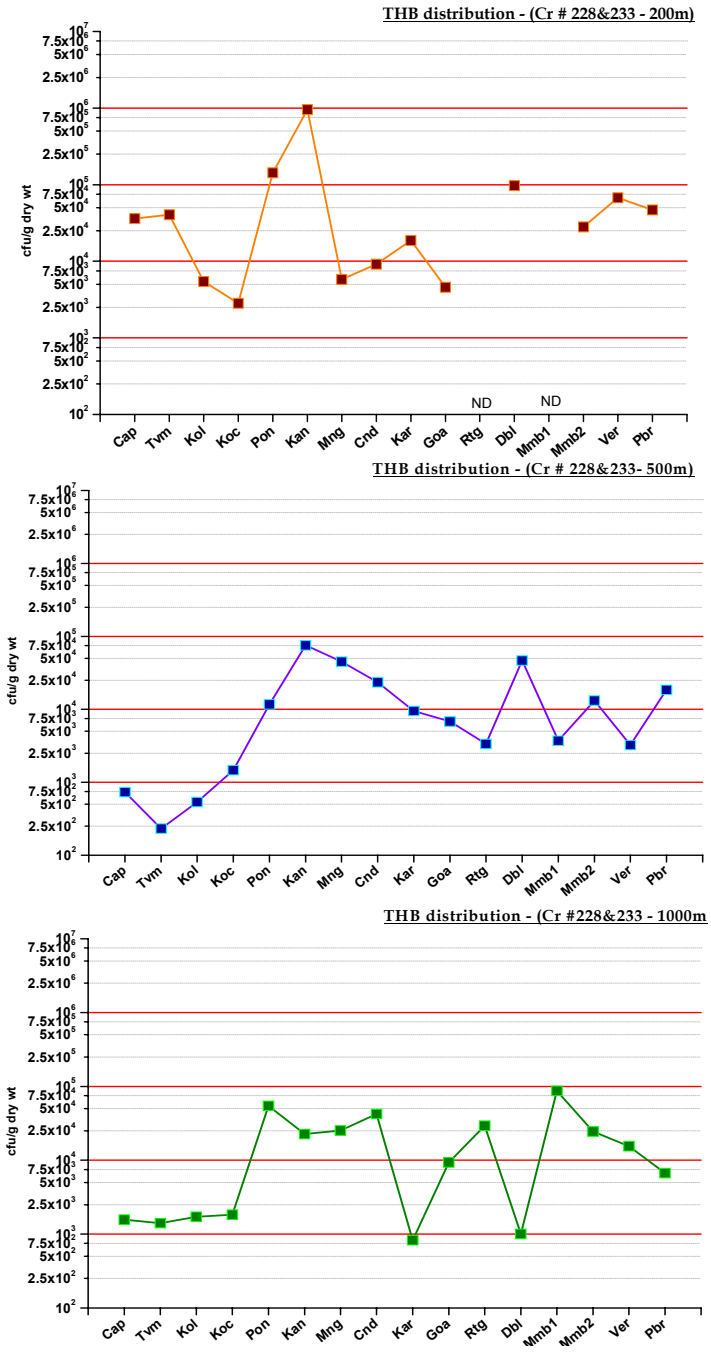


Fig. 3.1. Total heterotrophic bacterial population at various depths (200, 500 and 1000m) in the slope sediments of Arabian Sea during Sampling I (Cruise. No. 228&233)

ii) Depth-wise variation: The bacterial population was found to be maximum at 200m depth stations (104.42±248.34 x 10^3 cfu g^{-1} dry sediment weight) followed by 1000m (20.36±24.11 x 10^3 cfu g^{-1} dry sediment weight) and 500m (16.48±21.5 x 10^3 cfu g^{-1} dry sediment weight) depth. (Fig. 3.2)

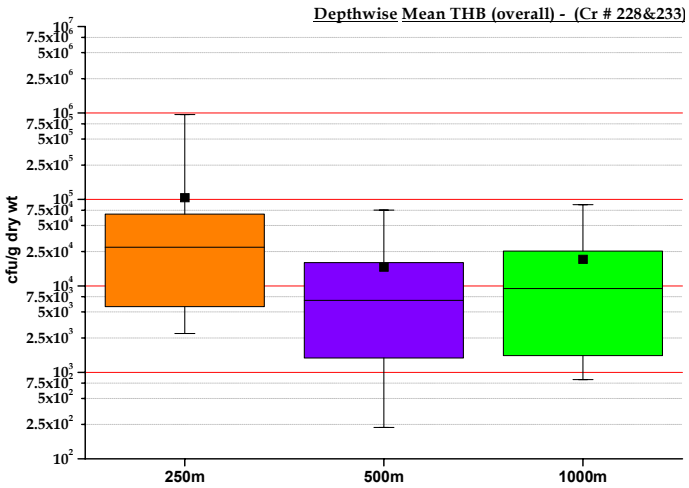


Fig 3.2. Depthwise distribution of total heterotrophic bacteria (mean) in the slope sediments of Arabian Sea during Sampling I (Cruise. No. 228&233).

3.3.1.2. Sampling - II (Cruise No. 254)

Distribution pattern of THB had no particular distinctive zonation, though several peaks were observed along different depth regions during Cruise No.254 (Fig. 3.3). At 200m depth ranges low values were observed along Trivandrum to Kochi, Mangalore to Ratnagiri and Mumbai II. At 500m depth also, low values were observed at regions between Kollam to Ponnani, Coondapoore to Ratnagiri and Mumbai II. At 1000m depth lower values were observed at regions Off Karwar and Goa. The southern regions were more patchy and an increasing trend was seen towards northern region from Goa onwards.

i) Latitudinal variation: In the second set of Sampling carried out during Cruise No. 254, heterotrophic bacterial population at 200m depth stations varied from 1.47 x 10^3 cfu g^{-1} (Off Karwar) to 26.72 x 10^3 cfu g^{-1} (Off Kannur) dry weight of sediment at 500m depth stations it ranged from 4.66 x 10^2 cfu g^{-1} (Off Kochi) to 10.49 x 10^3 cfu g^{-1} (Off Ratnagiri) dry sediment weight and in 1000m depth stations, from 3.32 x 10^2 cfu g^{-1} (Off Karwar) to 16.09 x 10^3 cfu g^{-1} (Off Kochi) dry sediment weight.

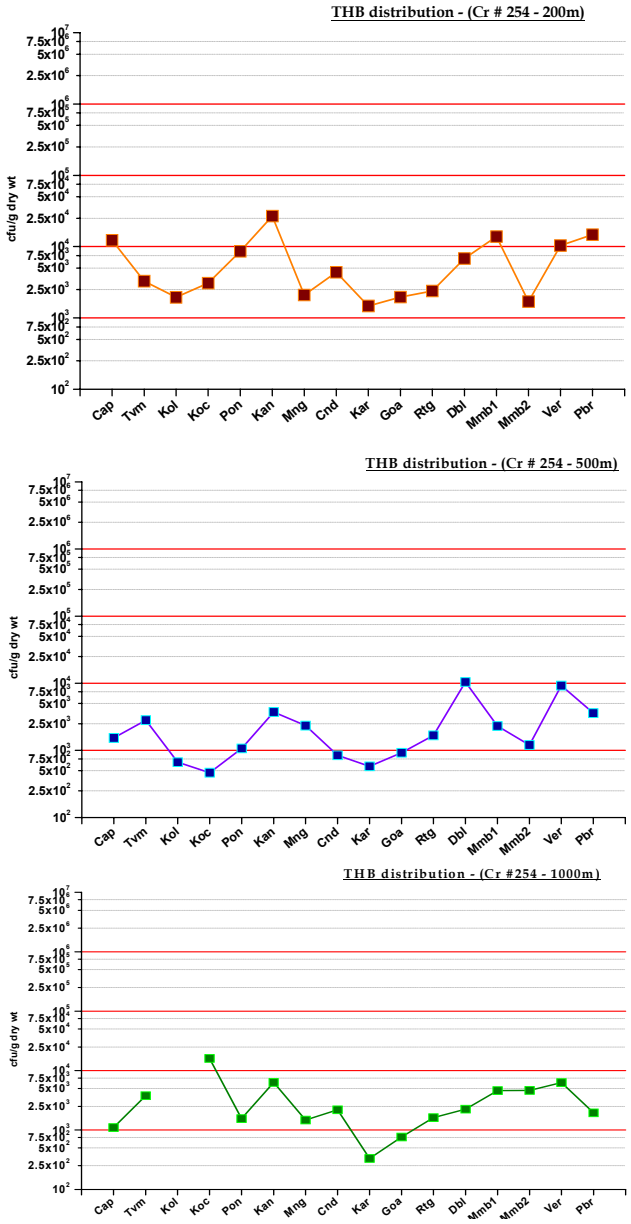


Fig. 3.3. Latitudinal distribution of total heterotrophic bacteria in the slope sediments of Arabian Sea during Cruise. No # 254 (Sampling II).

ii) Depth-wise variation: Mean bacterial population in relation to depth showed higher bacterial population at 200m depth stations (7.02±7.16 x 10^3 cfu g^{-1} dry

sediment weight) than at 1000m (3.66±3.94 x 10^3 cfu g^{-1} dry sediment weight) and 500m (2.72±2.98 x 10^3 cfu g^{-1} dry sediment weight) stations. (Fig 3.4)

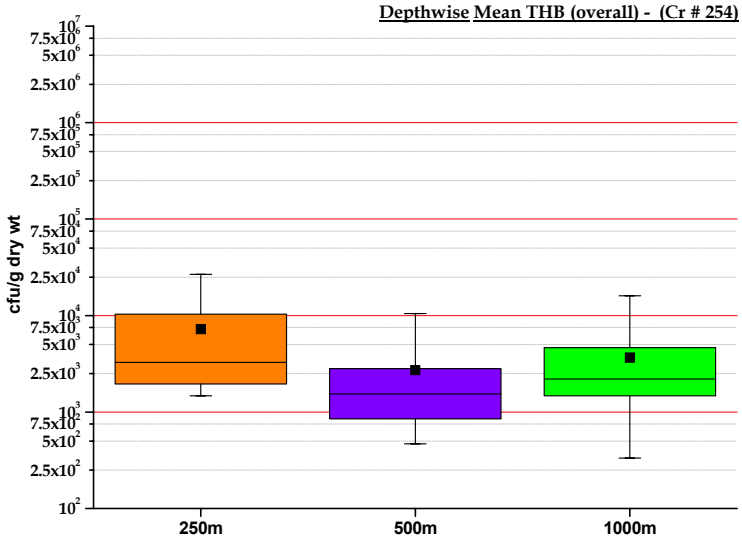


Fig 3.4. Depthwise distribution of total heterotrophic bacteria during Cruise. No # 254 (Sampling II).

3.3.2. Bacterial Morphotypes - Gram-Positives /Gram-Negatives

3.3.2.1. Sampling - I (Cruise No. 228 & 233)

The study showed that Gram-positive bacteria predominated the sediments in the study area. At 200m depth range the G P and G N forms were 57.16 and 42.84%, at 500m depth range 69.36 and 30.64% and at 1000m 70.58 and 22.35%.

3.3.2.2. Sampling - II (Cruise No. 254)

During Sampling II, Gram-positive forms predominated in the slope sediments. In the 200m depth range the Gram-positives comprised about 50-91% with a mean value recorded as 74.39 and 25.61% for G P and G N ones. Whereas in the case of 500m depth stations the abundance recorded were in the range 37.5-100% with a mean value recorded as 77.66 and 22.34% for G P and G N forms. While for stations at 1000m depth range the values ranged between 26-100%. The average values for G P and G N forms were 69.6 and 30.4% respectively. Latitudinal distribution of Gram-

positives and Gram-negatives are shown in Fig. 3.5a and Fig. 3.5b. Fig. 3.6. illustrates the depthwise distribution of Gram-positives and Gram-negatives forms.

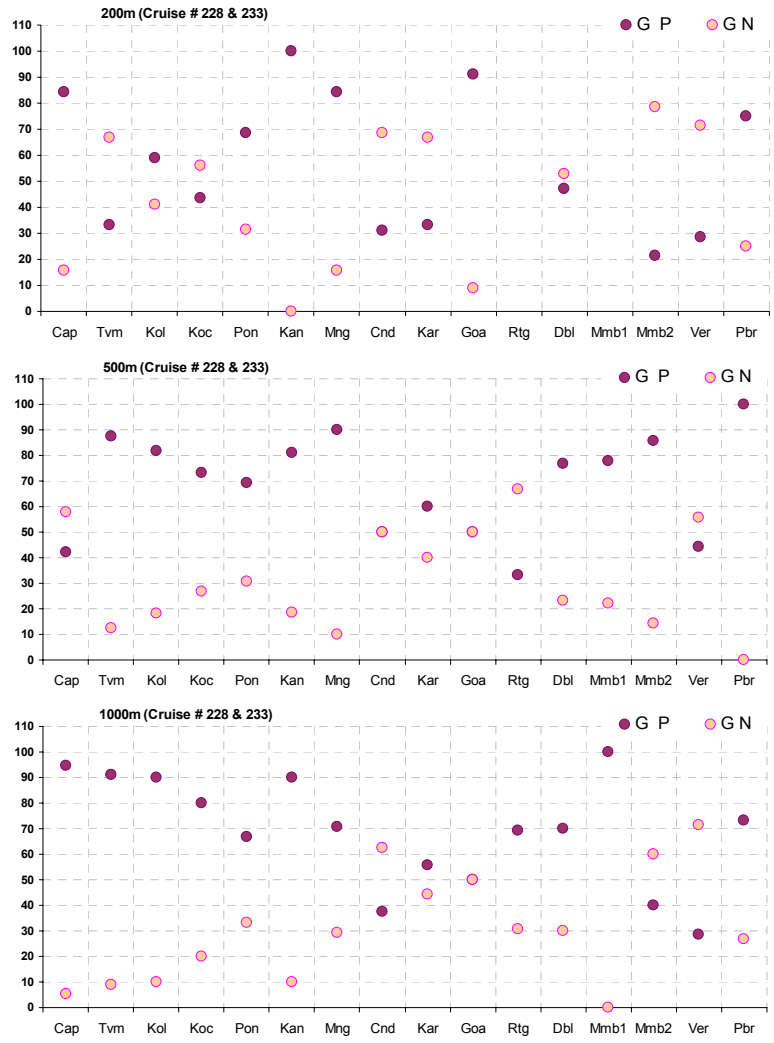


Fig.3.5a. Distribution of Gram-positive and Gram-negative heterotrophic bacteria in the slope sediments of Arabian Sea during Sampling I (Cruise. No. 228&233).

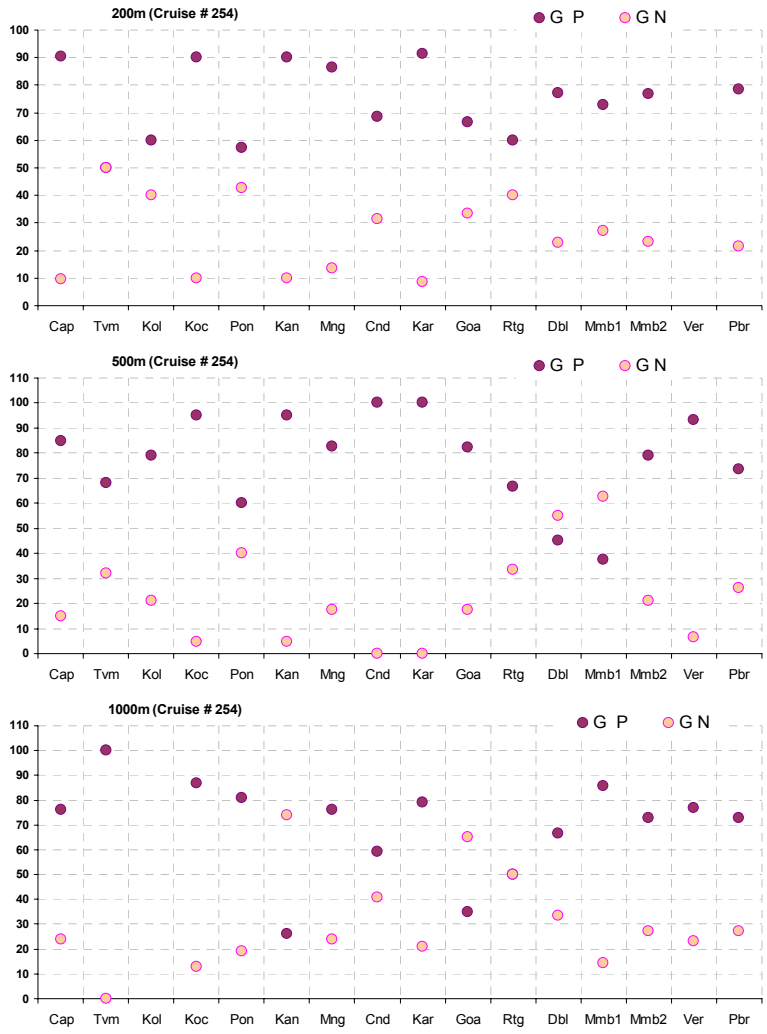


Fig.3.5b. Distribution of Gram-positive and Gram-negative heterotrophic bacteria in the slope sediments of Arabian Sea during Sampling II (Cruise No. 254).

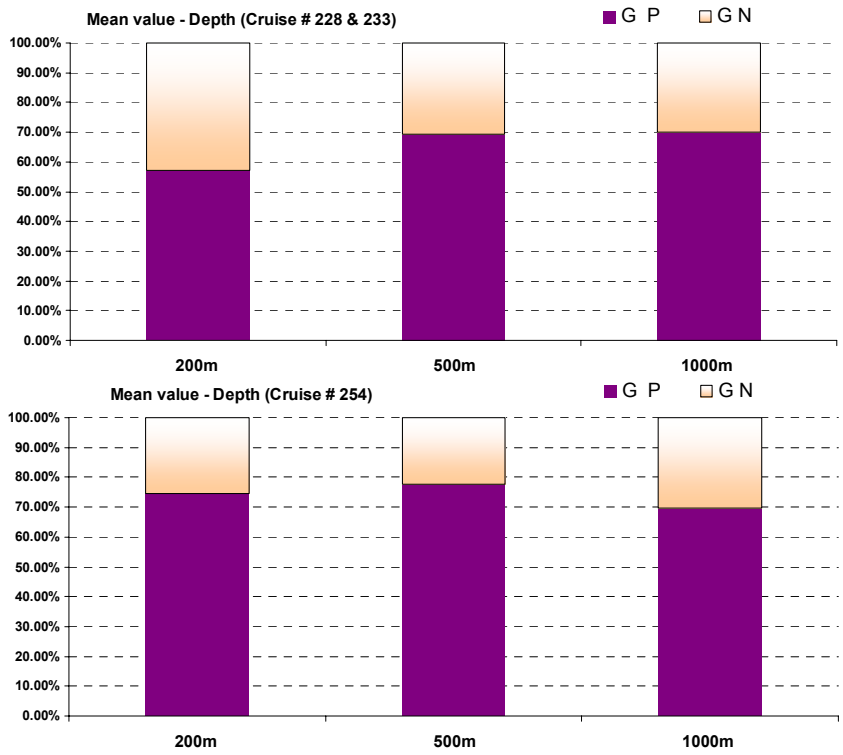


Fig. 3.6. Depthwise distribution of Gram-positive and Gram-negative heterotrophic bacteria in the slope sediments of Arabian Sea during Sampling I (Cruise. No. 228&233) and Sampling II (Cruise No. 254).

The data recorded for microbial variables from the slope sediments at various depths (200, 500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254) are presented in Appendix Table 3.A1.

3.3.3. Statistical Analysis

i) Total Heterotrophic Bacteria

According to the one-way ANOVA results there was no significant difference in THB between the depths during Sampling I (one-way ANOVA: DF=2; F=1.9; p=0.162) and during Sampling II (one-way ANOVA: DF=2; F=3.15; p=0.053). The result from the independent two-sample (student's) *t*-test showed that THB data were not significantly different between sampling ($p>0.05$).

ii) Gram-Positive Forms

According to the one-way ANOVA results there was no significant difference in percentage of Gram-positive forms of heterotrophic bacteria between various depths during Sampling I (one-way ANOVA: DF=2; F=1.34; p = 0.271) and Sampling II (one-way ANOVA: DF=2; F=0.82; p=0.45). However, the result from the independent two-sample (student's) *t*-test showed that the percentage of Gram positive forms were significantly different between samplings ($p<0.05$).

iii) Gram-Negative Forms

According to the one-way ANOVA results there was no significant difference in the percentage of Gram-negative forms of heterotrophic bacteria between the depths during Sampling I (one-way ANOVA: DF=2; F=1.34; p=0.271) and during Sampling II (one-way ANOVA: DF=2; F=0.82; p=0.45). However, the result from the independent two-sample (student's) *t*-test showed that the percentage of Gram-negative forms were significantly different between samplings ($p<0.05$).

iv) BIO-ENV Analysis

The relation between measured environmental variables and heterotrophic bacteria was investigated by BIOENV analysis using PRIMER software and the Pearson correlation coefficient matrix. The correlation matrices for different sampling periods are presented in Appendix Table 3.A2 to 3.A3. BIOENV analyzed the correlation between Bray-Curtis similarity matrices for environmental variables and biotic compartments. These correlations are repeated for all possible combinations of the measured environmental variables and the set of environmental variables that produce the highest correlation are those that most accurately explain the distribution of heterotrophic bacteria in the sediments.

Table 3.3. Summary of results from the BIOENV analysis by Bray-Curtis similarity based on THB, Gram-positive and Gram-negative bacterial community. The combination of environmental variables generating the highest rank correlations between similarity matrices of community and environmental data (CHO-C: Carbohydrate Carbon; PRT-C: Protein Carbon; LPD-C: Lipid Carbon).

Best results

BIOENV analysis		**200m**	**500m**	**1000m**	**Data All**
THB	Variable 1	Salinity	D Oxygen	D Oxygen	Salinity
	Variable 2	D Oxygen	Silt	Clay	D Oxygen
	Variable 3	Clay	CHO-C	PRT-C	CHO-C
	Variable 4	PRT-C	LPD-C	CHO-C	
	Highest rank correlation	**0.185**	**0.121**	**0.284**	**0.047**
G positive	Variable 1	Salinity	D Oxygen	Salinity	Salinity
	Variable 2	D Oxygen	LPD-C	D Oxygen	D Oxygen
	Variable 3	PRT-C		PRT-C	CHO-C
	Variable 4	LPD-C			LPD-C
	Highest rank correlation	**0.221**	**0.113**	**0.038**	**0.086**
G negative	Variable 1	D Oxygen	D Oxygen	Salinity	D Oxygen
	Variable 2	LPD-C	CHO-C	D Oxygen	CHO-C
	Variable 3	BPC	LPD-C	CHO-C	LPD-C
	Variable 4				
	Highest rank correlation	**0.136**	**0.1459**	**0.172**	**0.066**

As recommended by Clarke and Ainsworth (1993), all measured environmental parameters were included in the BIOENV analysis without any prior transformations (Table 3.3). In the 200m and 500m depth regions, the highest rank correlation with THB data (R=0.185 and 0.221 respectively) were obtained with salinity and DO exerting equally with additional variables Clay and PRT-C in 200m and CHO-C and LPD-C in 500m depth regions. In 1000m depth region, the results relied on four variables to obtain the highest rank correlation (R=0.284). In the case of Gram-positives DO was detrimental for all the depth zones, while additionally PRT-C, LPD-C and salinity contributed in each depth region to obtain the highest rank correlation. For Gram-negatives also DO was the major variable and LPD-C, CHO-C and BPC contributed to obtain the highest rank correlation. These results point to the key role of the geographical differences of these three regions, combined with the complex local hydrological and substratum properties which would explain the observed variability in bacterial community composition.

3.4. DISCUSSION

The sediment-water interface is a zone of high microbial abundance and processes occurring are of great biogeochemical importance especially in the continental margin creating a unique sedimentary environment in the Arabian Sea (Walsh, 1991). Bacteria in this sediment-water interface are of great ecological importance as they are responsible in decomposing, mineralizing and subsequent recycling of organic matter. The organic matter in these sediments are chiefly contributed by the detritus exported from the overlying water column along with considerable fluvial and aeolian inputs (Cowie, 2005).

Arabian Sea is well known for high primary productivity and the limited dissolved oxygen concentrations (<0.5 ml l^{-1} to non-detectable levels) in the water column which mainly impinges on the continental slope extends from water depths of 150 to 1500m (Wyrtki, 1971; Kamykowski and Zentera, 1990; Helly and Levin, 2004; Cowie, 2005). The sediment underlying this region is rich in organic matter and a primary site of sedimentary organic carbon burial with high sedimentation rates (Hedges and Keil, 1995). Sediments are highly heterogeneous containing varied proportions of both organic and inorganic materials. By virtue, organic particles get modified by bacterioplankton during sinking to deeper depths and therefore these settled organic matter do not contain any of its original components. Furthermore, the arrangement of these materials in sediments depend largely on the energy regime of the depositional environment, sediment input, effect on benthic communities *etc.*. Sediment bacteria play an important role in benthic ecosystems and may comprise a large fraction of the total benthic biomass, contributing significantly to the turnover of organic matter within the sediment (Billen *et al*., 1990; Schallenberg and Kalff, 1993). The ability of marine bacteria to utilize these resources found in various habitats will ultimately have a significant effect upon their distribution in the sea. The present investigation contributes to the knowledge of culturable heterotrophic bacteria in the sediments overlying 200m to 1000m

region of the continental slope of the Arabian Sea by the quantitative and qualitative description on the variations during the two sampling period.

For the marine environment, colony forming units (cfu) provide an inadequate description of the relative abundance of bacteria, because traditional cultivation methods do not mimic the real environmental conditions under which natural populations flourish (Ward *et al.,* 1990). Besides, it has been pointed out that only 1% of the cells present in natural seawater can be cultured and isolated on standard laboratory media (Buck, 1979) as they occur in a state of dormancy (Stevenson, 1978) or under starvation (Roszak and Colwell, 1987) or inactive (Bianchi and Giuliano, 1996). However, the cells that are metabolically active (Schut *et al.,* 1993) can be recovered to a large extent on agar plates. Therefore, culturing organisms remains an important step in the process of understanding the biology and ecology of microbial species (Connon and Giovannoni, 2002). Pinhassi *et al.* (1997) have shown that the culturable colony forming units (cfu) on agar plates can form a large fraction from the marine environment and can be as high as 29-100%.

Our investigation on microbial abundance in the sediments along the Arabian Sea has illustrated that the THB values were in the range 10^2-10^6 cfu g^{-1} dry weight of the sediment. These values were also in agreement with the total heterotrophic viable bacteria observed from different areas by other investigators which were found to be in the range 10^2-10^9 cfu g^{-1} (Litchfield and Floodgate, 1975; Meyer-Reil *et al.,* 1978; Nair and Loka Bharathi, 1982; Loka Bharathi and Nair, 2005). Lower counts were also observed by Maeda and Taga (1973), Austin *et al.* (1979), Alongi (1987), Delille *et al.* (1990), Loka Bharathi and Chandramohan (1990), Ramaiah *et al.* (1996) from different ocean sites. High values in the range of 10^5-10^6 cfu were also recorded from Dapeng Bay in the South China Sea during the investigations by Jiang *et al*. (2010) on the community structure and the relationships of culturable heterotrophic bacteria with

environmental factors. However these values from sediment were higher than those reported from the water column in the Arabian sea during the survey conducted by National Institute of Oceanography, Goa through the studies of Nair and co-workers (1979).

Prokaryotic abundance in the water overlying regions of eastern Meditteranean Sea were in the range of 10^4-10^5 from as reported by Yokokawa *et al*. (2010) and further demonstrated that vertical abundance below 100m declined exponentially with depth. ZoBell (1968) has indicated that bacteria are widely and unevenly distributed in deeper waters. Horizontal and vertical distributions of bacterial population in sediments are influenced by various factors, such as the physico-chemical nature of sediments and the presence of high organic matter concentrations. In aquatic ecosystems, the flux of organic matter to the bottom depends on primary productivity at the ocean surface and on water depth. This represents a good nutritional substrate for heterotrophic bacteria and favours bacterial growth. Deep sea is characterized by the absence of light, temperature of 2-3 °C and a high hydrostatic pressure, the concentration of organic energy is low, which is mirrored by the scantiness of organic residues in sediments. This exerts a negative selective pressure on the microbial flora and consequently, the bacterial concentration (10^4-10^5 g^{-1}) in oceans.

Their abundance and distribution highlight the basic property of the sediment, and any change, either on a short term or long term, is recognizable. This aspect is particularly fundamental in deep sea sediments where changes are limited in space and time, unlike the surficial waters or coastal sediments, where the system is more dynamic and turnover of elements much faster (Raghukumar *et al.,* 2001b). The fine scale distribution is the result of a combination of physical factors and biological interaction in the sediment. Information on the variability of bacterial population in deep sea sediments are very much limited (Turley and Lochte, 1990). In addition, microbial activity in the very top layer of deep sea

sediments can be expected to be orders of magnitude higher than in the overlying water column due to sedimentation (Jannasch and Taylor, 1984).

The distribution of bacterial population depends on changes in water temperature, salinity, abundance of organic nutrients and on other physico-chemical parameters (Jorgensen and Desmaraias, 1988; Henreke and De Tanga, 1990; Alavandi, 1990). The effect of temperature on microorganisms is well documented (Rose, 1967; Morita, 1974). However, Velankar (1955) reported the absence of any relationship between temperature and bacterial population. Chandrika and Nair (1994) reported high correlation between bacterial counts and temperature supporting the view of Gundersen *et al.* (1972) illustrating that the distribution of grain size, nutrients and temperature play an important role in the distribution of aerobic heterotrophic bacteria. The distribution of the bacterial population may also be controlled by the oxygen supply in the sediment as in the case of benthic organisms in the Arabian Sea (Heinz and Hemleben, 2003). The present investigation has also recorded low population from southern and a few stations in the northern regions than the central and central north region. The reason for this lesser value of bacterial population could probably be due to the coarser sediments including rocky areas in the southern region than the northern stations where the texture was finer. However, the low primary productivity of the northern region than the south and central west coast of India (Krey and Babenerd, 1976; Madhupratap and Parulekar, 1993) can have an indirect bearing on the bacterial population of the region. Bacterial community composition is not always influenced by physico-chemical parameters only, but may be affected by biotic factors such as primary production, grazing (Pernthaler *et al.,* 1997; Schafer *et al.,* 2000) and viral infection (Fuhrman, 1999). Besides, bacteria represent an important food source for protozoan and metazoan meiofauna that appear to be size-selective and capable of influencing bacterial abundance and distribution (Kemp, 1988; Epstein and Shiaris, 1992). To draw a general conclusion on the influence of

physico-chemical parameters on the heterotrophic bacterial population, these aspects also are to be considered.

Our investigation on changes of microbial structure along the continental slope cannot take into account all the factors determining the deposition and concentration of organic matter at the sea floor (e.g. terrestrial or advective inputs, productivity of the surface water layer, bottom currents), but it presents detailed information on changes of bacterial abundance and composition in the sediment as they are influenced by water depth and other related factors. As bacteria have short generation time, and are sensitive to changes in the physical and chemical environment, they may be the first component of the marine habitat to be affected by any perturbation. Only a few reports concern the population of deep-sea bacteria and the impact of environmental disturbance on them (Gordon and Giovannoni, 1996).

Sediment texture in the west coast was of mixed nature. At 200m, the texture was sandy and the organic matter was comparatively lesser than 500 and 1000m depths. At 500m stations the texture was silty sand to clayey silt. At 1000m depth, the sediment was clayey silt with more organic matter. The texture of the sediment in the present study was found to be clayey silt in the northern stations and silty sand in the southern stations. Also there was a steady increase in organic matter from 200 to 1000m depth regions. Higher population was mainly encountered with clayey sediments than in sandy sediments as reported earlier by Nair *et al.* (1978). Sandy sediments have organic contents lower than those of muddy sediments and support less surface area for bacterial attachment compared to clayey sediments. Bacterial population had a direct relationship with organic carbon and organic matter, and as the organic matter was more in finer sediments, the bacterial population associated with clayey sediments along each depth range were also higher as reported by Nair *et al.* (1978). Higher count of heterotrophic bacteria was observed from Arabian Sea in the clayey sediments by Dhevendaran

et al. (1987). Raghukumar *et al.* (2001a) also supported the present findings and reported that the clayey sediments of the deep sea harbour higher bacterial numbers.

Distribution of the total heterotrophic bacteria did not show any particular trend eventhough a decrease in population was noticed in a depthwise scale. However the type of distribution was not remarkable. Alongi (1992) have reported a rapid decline in bacterial population with increasing water depth in most shelf and slope environment. Small-scale patchiness in microbial abundance from the deep sea sediments as described by Bak and Nieuwland (1997) was also noticed in the present study. Hewson *et al.* (2006) found that bacterial community composition was remarkably heterogeneous in the oligotrophic deep sea of North Pacific and Atlantic Oceans. In an investigation on the community structures of cold and low-nutrient adapted heterotrophic sediment bacteria from the deep eastern tropical Atlantic, Ruger and Tan (1992) have found high quantities of (69 x 10^4 and 71 x 10^3 cfu g^{-1}) in relatively shallow sediment samples of depths 123m and 303m respectively in contrast to the colder sediments from depths between 1510 and 2815m of the same region (3.2 x 10^3 cfu g^{-1}). At depths greater than about 3000m, cfu were generally low. Vertical distribution of heterotrophic bacteria in the water column Off Marmugao (Goa) to Cape comorin was conducted and their biochemical activities were studied in the Arabian Sea by Nair *et al*. (1979). They recorded population count from nil to 10^3 cfu ml^{-1}. The viable population recorded a relatively smaller value at 500m depth region of the area and most of the deepwater region were with lesser poulation. Counts of 140-190 cfu ml^{-1} were recovered from depths of 1000m. A general trend in the decline of bacterial population with depth was observed in our study also.

Many studies, especially at mid-latitudes, have also demonstrated a coupling between pelagic and benthic processes, whereby benthic organic matter accumulation and metabolism are linked to flux events derived from phytoplankton

blooms in the upper ocean (Graf, 1992; Pfannkuche *et al.,* 1999). However, information on bacterial abundance in slope environments (>200m) is too limited to draw a general conclusion about factors controlling their distribution (Deming and Yager, 1992; Danovaro *et al.,* 2000). Moreover, the lack of quantitative information on active and inactive cells (Zweifel and Hagstrom, 1995), does not allow the evaluation of "background level" of inactive bacteria that could also contribute to the bacterial population. Prokaryote abundance in bathypelagic water column varies typically decreasing with depth (Reinthaler *et al.,* 2006; Arıstegui *et al.,* 2009). In general, depth-integrated prokaryote abundance in the deep sea sites increases with increasing surface productivity and the sinking flux of organic matter. In the Arabian Sea, Hansell and Ducklow (2003) found that the mean annual prokaryote abundance upto ca. 2000m depth was positively correlated with the annual organic matter flux at that depth. These findings are consistent with the notion that prokaryote biomass is regulated by the availability of organic carbon in the deep sea. Nagata *et al.* (2000) found that depth-integrated heterotrophic production (1000–4000m) generally follows the regional trend of prokaryote abundance in the Pacific, suggesting that carbon consumption by prokaryotes was generally coupled with sinking particulate organic carbon (POC). In contrast, Hansell and Ducklow (2003) reported that the variation in heterotrophic production in the Arabian Sea was complex and was not correlated with POC flux.

However, one possible explanation for the distribution of bacterial population in the surface fraction of the sediment is that due to sedimentation, microbial activity in the top layer of deep sea sediments can be expected to be orders of magnitude higher than that of the water column directly above, as well as in the sediment layers centimeters below the surface (Jannasch and Taylor, 1984). Benthic bacteria represent a key step in the benthic food web because they are capable of exploiting organic inputs (Deming and Baross, 1993) and enhancing organic matter quality in the sediments by protein enrichment (Danovaro, 1996).

However, the benthic responses vary depending on the quantity and quality of the sedimenting particulate organic matter (Pfannkuche *et al.,* 1999), nutrient cycling which may be the reason for discrepancy in heterotrophic bacterial population at different stations during the two cruises. Further, temporal changes in microbial population composition also can occur, often very rapidly and may be induced by the biota and/or driven by external forces (Bull, 2004). On the other hand, the spatial variations of bacterial population in the different stations did not show any particular trend coinciding with the result of Nair *et al.* (1979). ZoBell (1968) indicated that bacteria which are widely distributed in deeper waters do not show any particular trend.

Further, temporal changes in the composition microbial population also can occur, often very rapidly and may be induced by the biota and/or driven by external forces (Bull, 2004). But, the temporal changes in the bacterial population are likely to be more pronounced in the coastal environment than the open ocean or deep sea (Chandrika and Nair, 1994). On the other hand, the spatial variations of bacterial population in the different stations did not show any particular trend coinciding with the result of Nair *et al.* (1979). ZoBell (1968) indicated that bacteria which are widely distributed in deeper waters do not show any particular trend.

Many bacteria might be apparently unculturable due to the presence of bacteria in aggregates, selective effects of the media, and the presence of inactive cells and lysogenic viruses (Weinbauer and Suttle, 1996). Though they constitute a small fraction of total bacteria, which might be considered trophically dependent on particulate organic matter, they serve an important role in higher trophic levels. Information on the variability of bacterial population in deep sea sediments are very much limited (Turley and Lochte, 1990). Small-scale patchiness in microbial communities were previously reported from the deep sea sediments by Bak and Nieuwland (1997) was noticed in the present study also. Distribution of bacteria had no particular trend and there was no correlation between bacterial population

and depth which has also been previously reported (Nair *et al.,* 1978; Bianchi *et al.,* 2003). Most shelf and slope environments show a rapid decline in bacterial population with increasing water depth (Alongi, 1992).

Although marine ecosystems harbour Gram-negative group, Ruger *et al.* (2000) have isolated Gram-positive forms in deep-sea sediments including Antarctic, Arctic, and Atlantic. Chandramohan *et al.* (1987) during their investigation in the Indian Ocean sediments also have reported that Gram-positive forms as a dominant group in the deep-sea beds associated with polymetallic nodules. Maruyama *et al.* (1997) have also remarked that Gram-positive forms dominate deep-sea sediments of the Pacific Ocean. In the Central Indian Ocean Basin (CIB) regions Gram-negative forms were more pronounced before disturbance of the sediment, whereas Gram-positive forms increased after the sediments were disturbed. Any changes in the environment result in changes in community structure. In an another study Gowing and Wishner (1992) reported that active bioturbation by the benthic fauna in the sediment could be the source of Gram-positive bacteria, as gut contents of the deep sea fauna are also predominated by Gram-positive bacteria. Apparently the Gram-positive bacteria are more successful survivors and colonizers.

We have observed that Gram-positive bacteria can represent a large percentage of the colony-forming bacteria obtained from near-shore, tropical marine samples and that their relative abundance varies depending on the type of sample collected. It is generally accepted that colony-forming bacteria represent a small fraction of the total bacteria (Kogure *et al.,* 1979; Simidu *et al.,* 1983). This generalization does not appear to apply to all marine samples, because similar colony-forming and total bacterial counts have been reported for surface associated bacteria (Lewis, 1985). The observation that bacteria with Gram-positive cell wall (which have long been neglected in studies of marine bacteria) can represent a large percentage of the culturable bacteria in certain marine samples, sets the stage

for future study of the ecological roles of these bacteria in marine systems. Additional studies, possibly employing techniques in molecular biology, may help elucidate the true numbers of Gram-positive bacteria in marine samples.

Based on their relative abundance, Gram-positive bacteria have been shown to represent an important component of the culturable, tropical marine microbiota. The results presented in our study indicate that Gram-positive bacteria can represent a large percentage of the colony-forming, heterotrophic bacteria associated with sediments in contrast to the earlier studies of marine bacteria, wherein it was apparent that most seawater bacteria are Gram-negative (ZoBell and Upham, 1944; Chandrika, 1996). Although the nature of this association remains unclear, it can be concluded that the distribution and relative abundance of Gram-positive bacteria in specific marine habitats warrants additional study. Several investigations, both spatial and temporal, have been carried out on culturable heterotrophic bacteria in Indian waters (Nair *et al*., 1992, 1993, 1994). Pigmented bacteria were found to be more abundant in offshore waters compared to near shore regions (Chandramohan, 1997).

Chapter 4

GENERIC COMPOSITION OF TOTAL HETEROTROPHIC BACTERIA (CULTURABLE) IN THE SLOPE SEDIMENTS OF ARABIAN SEA

Contents

4.1. INTRODUCTION

Deep-sea sediments represent one of the largest ecosystem on earth where bacteria account for most of the benthic biomass and play a major role in biogeochemical cycles (Dixon and Turley, 2002). Despite the recognized relevance of benthic bacteria in carbon cycling and nutrient regeneration on global scale, our comprehension of the benthic ecosystem functioning is constrained by the lack of adequate information on benthic bacterial diversity and metabolism (Turley, 2000; Luna and Danovaro, 2003). Factors responsible for the distribution of bacterial diversity in the deep sea are yet to be studied. This is largely the result of extremely scanty information for deep-sea benthic environments (Moyer *et al*., 1998), and of the fact that most of these investigations have been mainly addressed to understand phylogenetic relationships rather than to identify patterns and constraints of benthic bacterial diversity.

Communities of heterotrophic bacteria are associated with oxidation of deposited organic matter, regeneration of inorganic nutrients, and food web support (Fenchel *et al.,* 1998*).* Cultivated isolates and biogeochemical field measurements have provided limited information about the role of microbes in marine and

freshwater sediments (Capone and Kiene, 1988). Historically, natural abundance and diversity of microbial populations were estimated by routine culture techniques. Studies from several types of habitats estimate that more than 99% of microscopic organisms cannot be cultivated by routine techniques (Amann *et al.,* 1995; Pace, 1997). Recent research suggests that environmental conditions at distinct geographic locations select for a distinct assemblage of prokaryotic organisms. Studies that described the distribution and genetic diversity of bacteria are particularly important in coastal and estuarine ecosystems (Zedler, 2000). Description of the genetic diversity of microbes that inhabit deep sea sites may lead to the development of a novel metric for assessing habitat disturbance and recovery.

Heterotrophic bacterial action promotes organic matter degradation, decomposition and mineralization processes in sediments and overlying water, releasing dissolved organic and inorganic substances. Their activities and relative abundance reflect the hydrological structure and nutrient levels in the marine environment (Oppenheimer, 1963). The heterotrophic organisms are responsible for the utilization of the extensive pool of dissolved organic carbon, thus making it available for the different food webs (Ducklow *et al.,* 1993). Thus, heterotrophic bacteria are the major agents in shaping the organic composition of the oceans (Raghukumar *et al.,* 2001b).

The abundance and distribution of heterotrophic bacteria have a direct bearing on other forms of nutrients in different compartments of the environment. So far, several studies have been carried out to characterize heterotrophic bacteria in ocean sites and in different coastal areas of temperate, tropical and polar zones. Heterotrophic bacteria present in the water and sediment of marine and brackish water environments and their role in the decomposition of organic matter and regeneration of minerals have been investigated by Azam and Hodson (1977) and Pomeroy (1979). In addition, the distribution of bacterial population has been suggested to be a precise tool in the identification of water mass and currents (Kriss

et al., 1960). Determination of the extent of diversity is mainly restricted to cultured prokaryotes because the vast majority of strains are not yet accessible for subsequent research (Stackebrandt, 2001).

Observations made during the Galathea Expedition (1950-1952) demonstrated the presence of living bacteria in sediment samples obtained at depths greater than 6000m. However, deep sea bacteria were first collected by Certes (1884) during the Travaillier and Talisman Expeditions (882-1883). Certes (1884) recovered barotolerant (pressure tolerant) bacteria from depths of 5000m and suggested that such microorganisms may exist in a state of suspended animation. Later, Fischer (1894) obtained few colonies from samples collected at depths of >1100m during a trans-Atlantic crossing by a passenger ship in 1886. Thereafter, improvements in the knowledge of deep sea biology progressed slowly and took a new dimension largely due to the sterling efforts of ZoBell and co-workers. Initially, the investigators were concerned primarily with establishing the existence and the numbers of bacteria extant at great depths and attention was directed towards the biochemical and physiological capabilities. It was in 1968, when Quigley and Colwell reported for the first time information regarding the characterization, identification and classification of deep sea bacterial cultures isolated from sediment samples obtained from the Philippine Trench (9854 and 9443m) and Challenger Deep (10373m) in the Mariana Trench of the Pacific Ocean. To date, deep sea microbiological studies have been carried out in a wide range of depths (Jannasch and Taylor, 1984) and it was emphasized that about 75% of the integrated bacterial biomass from surface waters to deep sea sediments is found in the top 10 cm of sediment (Lochte, 1992).

Attempts to isolate environmentally adapted (baroduric and barophilic) bacteria from deep ocean sources have proved more successful. Such isolates have been obtained from deep water and sediment samples by ZoBell and Morita (1957), Kato *et al.* (1995) and Patching and Eardly (1997). Kato *et al.* (1996) reviewed the isolation of extremophiles and characterized several microorganisms

that are adapted to living in the extremes of the deep sea environment. The existence of barophiles led to the speculation that this was the niche for an active and adapted bacterial community whose development had been supported by the food supply generated by their scavenging host. There have been some reports of active deep ocean microbial communities (Deming and Colwell, 1985; Turley and Lochte, 1990). However, very little is known about the quantitative importance of benthic bacteria in deep sea sediment (Deming, 1985; Delille *et al.,* 1990).

Okami and Okazaki (1972) also isolated microorganisms from 58 samples of water and mud of Japan Sea and totally 142 strains were isolated and tested for antimicrobial activity. Austin *et al.* (1979) studied the bacterial flora of Chesapeake Bay and Tokyo Bay and reported several bacterial genera as agents of mineralization and transformation of organic and inorganic matter. The bacterial flora of marine animals collected at depths of 570 to 2446m was examined for population size and generic composition and the barotolerant characteristics of selected bacterial isolates were determined by Ohwada *et al.* (1980). Bianchi and Giuliano (1996) studied the viable bacteria from Mediterranean Sea. Patching and Eardly (1997) described the biomass and activity of barophilic bacterial communities at several depths in the water column and in the sediment contact water at two deep sites in the eastern Atlantic. Bak and Nieuwland (1997) studied the seasonal variation in bacterial and flagellate communities of deep sea sediments and reported that densities of microbes vary along spatial gradients and, in general, decrease with depth. Findlay and Watling (1998) reported the role of environmental factors and benthic bacterial interactions in the structure of a marine benthic microbial community. Pfannkuche and Soltwedel (1998) investigated the standing stock, activity and effects of food pulses on the benthic microorganisms in the continental slope of Celtic Sea.

The microbial biomass from the deep sediment of Aegean Sea were studied in relation to the labile organic matter by Danovaro *et al.* (1999). Yanagibayashi *et al.* (1999) studied the barophilic microbial community in the samples collected

from the sediments of Japan Trench at a depth of 6292m. Danovaro *et al.* (2000) and Bernard *et al.* (2000) studied the diversity of culturable heterotrophic bacteria from Mediterranean Sea. However, it was opined that microorganisms are common in subsurface sediments but not ubiquitous, cell numbers frequently decline with depth through the active soil horizons but then exhibit no clear numerical trend other than patchy distribution (Stevens, 2002). Queric *et al.* (2004) studied the bacterial abundance and activity along the transect (1250-5600m) of Vestnesa Ridge to the Molloy Hole. Fabiano *et al.* (2004) studied the trophic state of the benthic marine environment and assessed the benthic health of ecosystems based on the use of the microbenthic loop. The vertical distribution of free-living bacteria was investigated at deep sites of >4000m in the Arabian Sea by Koppelmann *et al.* (2005). Microbial diversity from the deep sea sites was reported by Xu *et al.* (2005). Metabolically active bacterial cells have recently been quantified in deep marine sediments using oligonucleotide hybridization probes targeting active cells and their rRNA (Teske 2005). Zoppini *et al.* (2005) described the temporal trend of the bacterial activity, abundance and composition in the aggregated and dissolved organic matter under different trophic conditions in the Northern Adriatic Sea.

Nair *et al.* (1978) studied the sediment bacterial population and physiological responses of the bacterial isolates from the coastal regions of India. Chandrika and Girijavallabhan (1990) studied the quantitative and qualitative distribution of heterotrophic bacteria from Bay of Bengal in relation to environmental parameters. Ramaiah *et al.* (1996) reported the culturable heterotrophic bacterial numbers, generic composition and uptake of labeled glucose by microbial assemblages in Bay of Bengal. Ramaiah *et al.* (2004) studied the abundance of pollution indicator bacteria from Mumbai coast (Arabian Sea). Goltekar *et al.* (2006) studied the irretrievability of marine heterotrophic bacteria from coastal to deep regions of Arabian Sea and Bay of Bengal.

We focused on characterizing the culturable heterotrophic bacteria over a depthwise distribution with the objective of evaluating changes in the community

structure involved in the decomposition of organic compounds in the sediments from continental slope region in a latitudinal range extending from Cape comorin to Porbandar in the west coast of India.

4.2. MATERIALS AND METHODS

4.2.1. Isolation of Heterotrophic Bacteria

The bacterial cultures isolated from the slope sediments of Arabian Sea during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No.254) were used for the study. Bacterial strains were categorized into different Gram's reaction types (as described in Chapter 3) prior to the various identification tests.

4.2.2. Identification of the Bacterial Strains

Standard bacteriological procedures such as colony morphology, pigmentation, Gram staining and endospore formation were performed to identify the isolates up to generic level following the scheme given by Oliver (1982) and as per *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994).

4.2.3. Spore Staining

Gram-positive rods were subjected to spore staining. Smears were prepared using 60-72 hours old cultures. The slides were flooded with malachite green and steamed for 5 minutes replacing the malachite green as it evaporates from the slides. The slides were allowed to cool for about 5 minutes and rinsed with water. Then the counter stain safranin was added and kept for one minute and observed under oil immersion microscope. Spores appear green and vegetative cells pink in color.

4.2.4. Biochemical Tests

Marine oxidation-fermentation, Mannitol utilization, Motility, Oxidase, Catalase, Arginine dihydrolase, Pencillin and Polymyxin sensitivity and DNase tests were carried out to identify the culture at generic level.

i) Marine Oxidation Fermentation Test (MOF)

Hugh and Leifson's medium – composition

Dextrose	:	1g
Peptone	:	0.2g
K_2HPO_4	:	0.03g
Phenol red	:	0.3ml of 1% of aqueous solution
Agar	:	2g
Sea water (35psu)	:	100ml
pH	:	7.2

The medium was prepared and about 5ml was distributed in test tubes and sterilized in an autoclave. After sterilization the tubes were kept in a slanting position. The inoculum was stabbed and streaked on the slope and incubated for 48 hours at room temperature (28$\pm$2 ˚C). Oxidative forms show a change of colour from pink to yellow in the slope area alone. Fermentative reaction was observed by change of colour from pink to yellow both at slope and butt region. Gas production was observed by presence of cracks and bubbles in the hard agar of the butt area. Alkaline reactions were noticed by a deep pink colour in the slope area.

ii) Mannitol Motility Test

Composition of the medium (g l^{-1})

Mannitol	:	1.0g
Beef extract	:	3.0g
Peptone	:	10.0g
Phenol red	:	0.1g
Agar	:	4.0g

Sea water (35psu)	:	1000ml
pH	:	7.2

The medium was prepared and about 3 – 4 ml was distributed in test tubes. The tubes were sterilized in an autoclave and left for setting at vertical position. After cooling the bacteria were stab inoculated in to the tubes and incubated at room temperature for 48-72 hours. Change of colour from pink to yellow in the medium shows the utilization of mannitol. Motile bacteria show diffused growth.

iii) Catalase Test

On a clean glass slide a smear of bacterial culture was prepared. A drop of H_2O_2 solution was added to the smear. Effervescence or bubbling was noticed in the case of cultures producing catalase enzyme.

iv) Oxidase Test

Small pieces of filter paper (Whatman No.1) were soaked in 1% tetra methyl paraphenylene diamine dihydrochloride. A small portion of the culture was placed on the test paper with a clean platinum loop. A blue colour appears within 10 – 30 seconds if the culture is capable of producing oxidase enzyme.

v) Arginine Dihydrolase Test

Composition of the medium (g l^{-1})

Peptone	:	1.0g
K_2HPO_4	:	0.3g
Dextrose	:	0.5g
L-Arginine	:	10.0g
Agar	:	3.0g
Bromocresol purple	:	0.016g
Sea water (35psu)	:	1000ml
pH	:	7.2

This test was used to distuinguish between *Vibrio* and *Aeromonas*. The medium contains bromocresol purple as pH indicator. As the dextrose in the medium is fermented, the medium changes from purple to yellow. If both decarboxylation and deamination occurs alkaline end products are formed.

$$\text{Arginine} \rightarrow NH_3 + CO_2 + H_2O$$

Then the medium reversed to purple colour (positive reaction). A control tube with no added amino acid should always be inoculated with tests, as a negative reaction.

vi) DNase Test

Composition of DNA Agar Medium

Peptone	:	5.0g
Beef extract	:	3.0g
Ferric phosphate	:	0.02g
DNA sodium salt	:	2.0g
Agar	:	20g
Sea water (35psu)	:	1000ml
pH	:	7.2

This test was used to distinguish between DNase producing *Alteromonas* from other Gram-negative forms. The Nutrient Agar medium contains 0.2% DNase sodium salt. DNA agar plates were spot inoculated and incubated at room temperature (28$\pm$2 ˚C) overnight. Flooded the plate with 1N HCl. Clearing around the colonies indicates the DNase activity. The HCl reacts with unchanged deoxyribonucleic acid to give a cloudy precipitate.

vii) Antibiotic (Kirby-Bauer) Test

Pencillin-G sensitivity was tested to distinguish between *Pseudomonas* and *Moraxella.* Nutrient agar plates were swabbed with 24 hr old bacterial broth, the antibiotic discs were kept and incubated overnight at room temperature (28±2 ˚C). Clearing zone around the colonies indicated sensitivity to the antibiotic.

4.2.5 Statistical Analysis

The data were analysed by univariate and multivariate statistical methods using statistical software PRIMER-v.6 (Clarke, 1993; Clarke and Gorley, 2006; Anderson et al., 2008). Similarity among the stations were analysed based on bacterial generic composition by hierarchical agglomerative cluster analysis and non metric-multidimensional scalling (MDS) based on Bray-Curtis similarities (Bray and Curtis 1957) and the results were plotted into ordination graphs. A K-dominance curve was plotted to establish the relative dominance of bacterial genera at different depth regions. The Shannon-Wiener diversity index, H'; Peilou's evenness index, J'*;* Margalef's richness, *d* and Species dominance were analyzed using PRIMER-v.6 (Clarke & Gorley, 2001). Diversity indices provides a valuable measure of the community composition.

4.3. RESULTS

A total of about 1472 cultures were isolated from both the cruise samples i.e. 608 cultures during Cruise No. 228&233 and 864 cultures during Cruise No. 254 (Fig. 4.1). All the isolates were sub-cultured and identified by several morphological, physiological and biochemical methods upto generic level. Percentage abundance of the various bacterial genera in different depth ranges during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254) are presented in Appendix (Table 4.A1 to 4.A3).

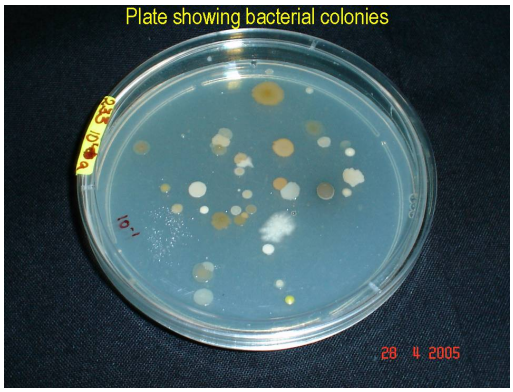


Fig. 4.1. Bacterial colonies developed on ZoBell's Marine agar plates.

4.3.1. Latitudinal Distribution of Heterotrophic Bacteria

i) Sampling - I (Cruise No. 228&233)

Among the heterotrophic bacteria isolated during Sampling I (Cruise No. 228&233) Gram-positive forms dominated over the Gram-negative ones. The percentage composition of the heterotrophic bacteria are shown in Figure 4.2. In 200m depth range *Bacillus* and Coryneforms together constituted over 50% of the microflora in many locations except Trivandrum, Kochi and Coondapore. *Bacillus* strains were not recovered from 200m station Off Ponnani and their occurrence in the northern regions were also less. Presence of Coryneforms were marked from stations typically north i.e. Veraval and Porbandar. A progressive increase in Coryneforms were observed from Coondapore to Veraval at 500m depth range. Yet they were not recovered from Mumbai II and Veraval. Albeit variations in microbial community among the sampling locations *Bacillus* and Coryneforms were frequently isolated from the sediments. The genus *Bacillus* dominated in all the 3 depths examined with an overall percentage of isolation at 200, 500 and 1000m depths as 19.5, 40.1 and 41.9% respectively. While Coryneforms also were recovered from the region in considerable fractions, their contribution in the higher depths were relatively lesser than *Bacillus*.

Among the other Gram-positive forms *Micrococcus* was occasionally isolated from locations, off Ponnani and Kannur at 200m depth range. While at 500m, contribution from *Micrococcus* was significant Off Cape comorin, Trivandrum and Kannur. In 1000m depth range *Micrococcus* was detected off Kochi and from regions Off Mangalore and Coondapore. However in contrast to the stations located in the southern regions genus *Micrococcus* was more abundant in stations lying in the northern region and a high percentage was observed from stations in all 3 depth ranges. Apart from *Micrococcus*, the presence of *Staphylococcus* at 1000m depth range in the northern regions was remarkable particularly in the region Off Dabhol to Mumbai II. Irregular distribution was detected in the case of *Planococcus* Off Kochi (200m), Kollam (500m) and Kannur (1000m) and for *Staphylococcus* isolated from stations off Mangalore (200 and 1000m); Cape comorin and Trivandrum in the 500m depth range and Off Kollam (1000m).

Among the Gram-negative forms, *Pseudomonas* was the predominant genus being isolated more frequently from stations in the 200 and 500m depth range. While in the 1000m depth range their distribution was more confined to the regions Off Kochi and Kannur to Coondapore. Apart from *Pseudomonas*, *Moraxella* species was observed Off Trivandrum and Kollam in the 200m depth range and from stations Off Kollam to Ponnani in 1000m depth range. *Flavobacterium-Flexibacter* were marked by their distribution in regions Off Cape to Kollam and Kollam to Kochi at 200 and 500m depths respectively. *Aeromonas* was also recorded Off Kannur-Mangalore and Manglore- Coondapore in 500 and 1000m depth range. *Acinetobacter*, *Vibrio*, Enterobacteriaceae, *Cytophaga*, *Chromobacterium* and *Photobacterium* were lesser in the region and were not uniformly distributed. *Acientobacter* spp. were isolated from Dabhol-Veraval in 200m depth range and from Coondapore and Goa and Mumbai II and Porbandar in 1000m depth range. Isolation from 500m depth range was scanty. A uniform distribution of *Aeromonas* was noted from most of the stations at various depth

ranges. Enterobacteriaceae were recorded in the 200m depth range between Dabhol-Veraval. At 500m depth *vibrios* were obtained from Karwar to Mumbai I. Vibrios were also isolated Off Mumbai II and Veraval 200 and 1000m depths. Apart from these *Photobacterium*, *Flavobacterium* and *Moraxella* were also occasionally isolated from stations located in the northern region.

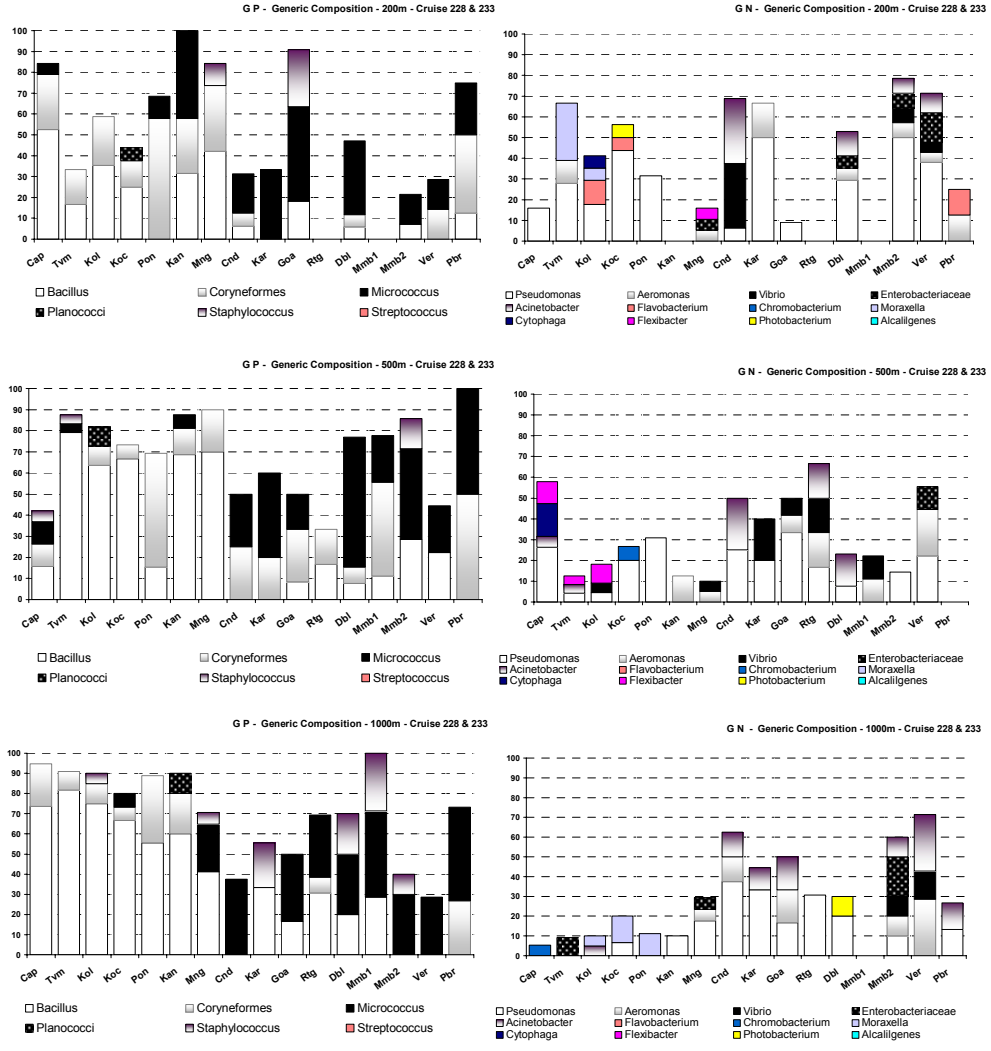


Fig. 4.2. Percentage isolation of bacterial genera belonging to Gram-positive (GP) and Gram-negative (GN) group isolated from different depth regions during the Sampling I (Cruise No. 228&233).

ii) Sampling - II (Cruise No. 254)

During Sampling II (Cruise 254) also Gram-positive bacteria dominated in all the sampling sites than the Gram-negatives. The percentage composition of heterotrophic bacteria are shown in Fig. 4.3. The genus *Bacillus* dominated at 200 and 500m depth range. While Coryneforms formed the predominant group in the 1000m depth range. The percentage occurrence of *Bacillus* and Coryneforms were 40.1 and 28.8%, 46.7 and 28.8% and 33.1 and 28.2% at 200, 500 and 1000m depths respectively. At 200m depth range *Bacillus* formed the major contributor to the resident flora in many stations and recorded a progressive increase towards the northern region recording a highest percentage of isolation Off Mangalore and declined towards the northern region reaching a minimum value off Mumbai II. However, apart from *Bacillus*, Coryneforms formed the second major contributor in this depth range and were isolated frequently from stations in the southern region. Their distribution in the northern region were considerably higher and showed a progressive increase from regions Off Coondapore towards Porbandar. While in the case of 500 m depth range *Bacillus* spp. were isolated from most of the stations with a higher percentage of occurrence recorded Off Trivandrum to Kannur and between Mumbai II to Porbandar, while a decline in its occurrence was recorded within a belt of Kannur to Mumbai I. The Coryneforms were also distributed more or less evenly but were confined more to Cape comorin-Trivandrum and from Kannur to Goa. In the 1000m depth range also coryneforms were confined to the region between Cape comorin to Ponnani and Mumbai I to Veraval, while *Bacillus* showed several peaks and declines in this depth range. Isolation of *Bacillus* spp. were recorded more Off Trivandrum-Kochi, Veraval-

Porbandar and Karwar. *Micrococcus* was occasionally isolated from 200m depth stations *i.e.*, Off Cape comorin, Kochi, Kannur, Ratnagiri and Mumbai I. while at 500m depth range, stations Off Kochi and Dabhol-Mumbai I in the north west showed *Micrococcus* population. In the 1000m depth range this group was recorded from Kannur to Coondapore in the south west and from Dabhol to Mumbai II in the north west region. *Planococcus* were also isolated from Cape, Kannur and Karwar in 200m depth range, while their sporadic distribution in 500m were notable Off Kochi to Goa In the case of 1000m depth also the distribution was irregular and were isolated from few stations Off Kannur to Mumbai I.

In the case of Gram-negative forms *Pseudomonas, Aeromonas* and *Acinetobacter* were the predominant forms. The distribution of pseudomonads were more confined to the south west region in the 200m and 500m depth range while it was also recorded in regions Off Ratnagiri to Mumbai II in the north west region and were isolated in low fraction in most of the stations in 1000m depth range. A remarkable even distribution were observed in the case of *Aeromonas* in 200 and 500m depths and with a higher percentage of isolation recorded at 1000m depth stations distributed in considerable higher range from Coondapore to Veraval. *Acinetobacter* was more ubiquitous and were recorded from most of the stations in all the depth range. While *Moraxella* was recorded Off Karwar-Mumbai I in 200 and 500m depth range while in 1000 m depth they extended from Kannur to Mumbai I. *Vibrio*, Enterobacteriaceae, *Alcaligenes, Flexibacter* were also isolated from various stations.

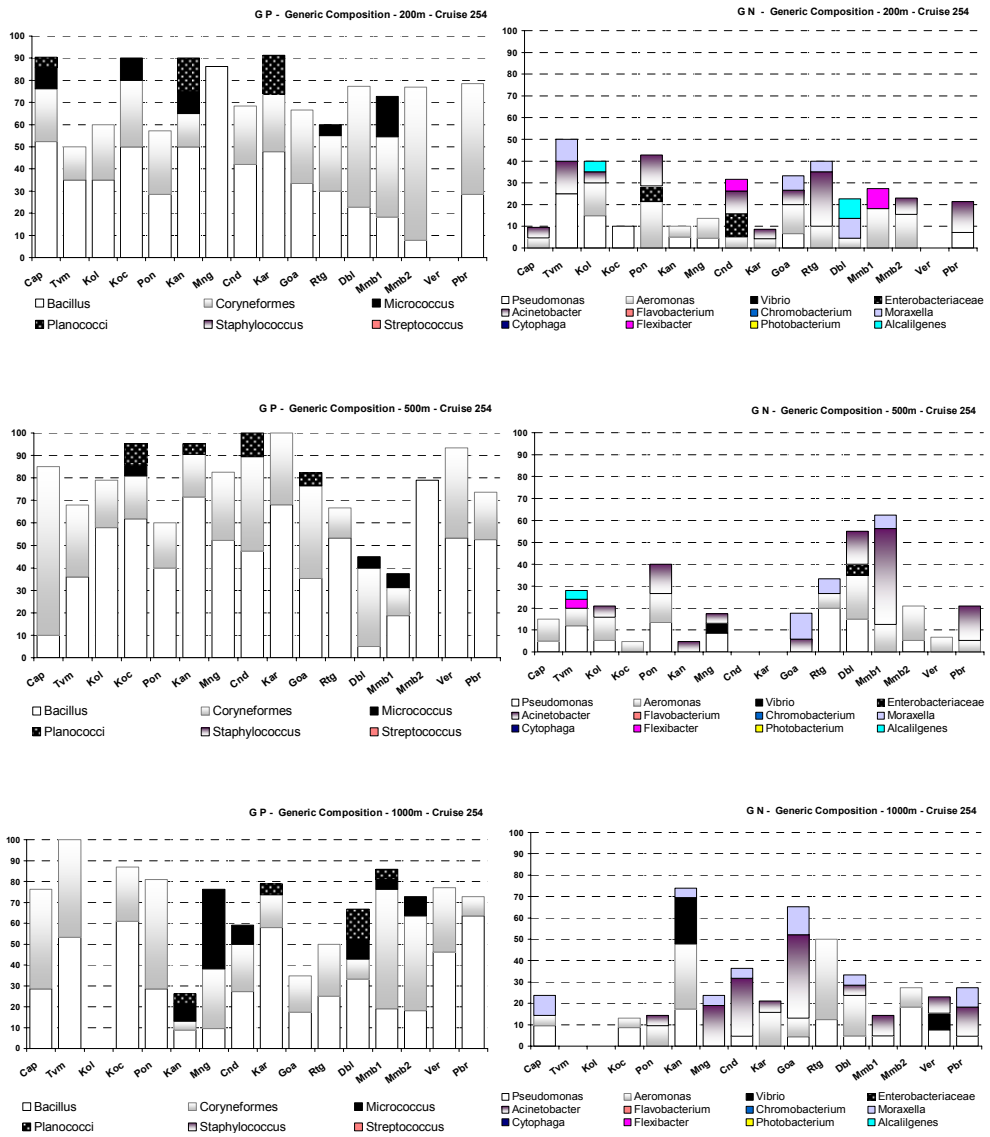


Fig.4.3. Percentage isolation of bacterial genera belonging to Gram-positive (GP) and Gram-negative (GN) group isolated from different depth regions during the Sampling II (Cruise No. 254).

4.3.2. Depthwise Distribution of Heterotrophic Bacteria

i) Sampling - I (Cruise No. 228 & 233)

The overall percentage isolation of different bacterial genera during Sampling 1 (Cruise No. 228&233) is presented in figure 4.4. *Bacillus* was dominant (33.3%) followed by Coryneforms and *Pseudomonas* (15.7% each), *Micrococcus* (15.5%), *Acinetobacter* (4.1%), *Aeromonas* (3.6%), *Staphylococcus* (2.8%), *Vibrio* (2.5%), Enterobacteriaceae (1.8%) *Moraxella* (1.7%), *Flexibacter* (1.0%), *Flavobacterium, Cytophaga* and *Planococcus* (0.7% each) *Photobacterium* and *Chromobacterium* (0.3% each).

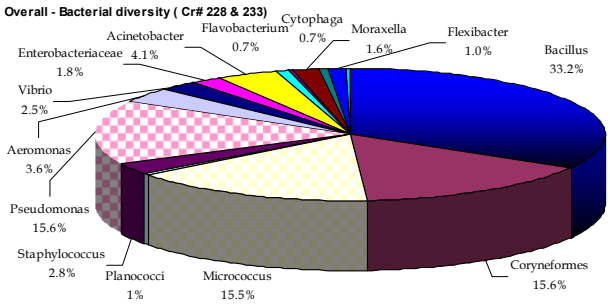


Fig. 4.4. Qualitative analysis of culturable heterotrophic bacterial community from the slope sediments of Arabian Sea during Sampling I (Cruise No. 228&233).

The mean percentage of isolation of the bacterial genera from 200m depth range showed that Genus *Pseudomonas* was dominant (22.3%) followed by Coryneforms (20.0%), *Bacillus* (19.5%), *Micrococcus* (15.5%), *Acinetobacter* (4.5%), *Aeromonas* (3.6%), *Vibrio* (3.2%), Enterobacteriaceae and *Moraxella* (2.7% each), *Staphylococcus* (2.3%), *Flavobacterium* (1.8%) and *Flexibacter, Cytophaga, Planococcus* and *Photobacterium* (0.5%). The mean percentage of isolation of the bacterial genera from 500m depth range showed that *Bacillus* was dominant (40.1%) followed by Coryneforms (16.3%) *Micrococcus* (13.9%), *Pseudomonas* (12.4%), *Aeromonas (4.0%), Vibrio* and *Acinetobacter* (3.0% each), *Flexibacter* (2.5%), *Staphylococcus* and *Cytophaga, (1.5%), Planococcus* (1.0%), Enterobacteriaceae and *Chromobacterium* (0.5% each). The mean percentage of isolation of the bacterial

genera from 1000m depth range showed that *Bacillus* was dominant (41.9%) followed by *Micococcus* (17.2%), *Pseudomonas* (11.3%), Coryneforms (9.7%), *Staphylococcus* and *Acinetobacter* (4.8% each), *Aeromonas* (3.2%), Enterobacteriaceae and *Moraxella* (2.2% each), *Vibrio* (1.1%) *Planococcus, Photobacterium* and *Chromobacterium* (0.5% each). The mean percentage of isolation of the bacterial genera from different depth ranges are shown in Figure 4.5.

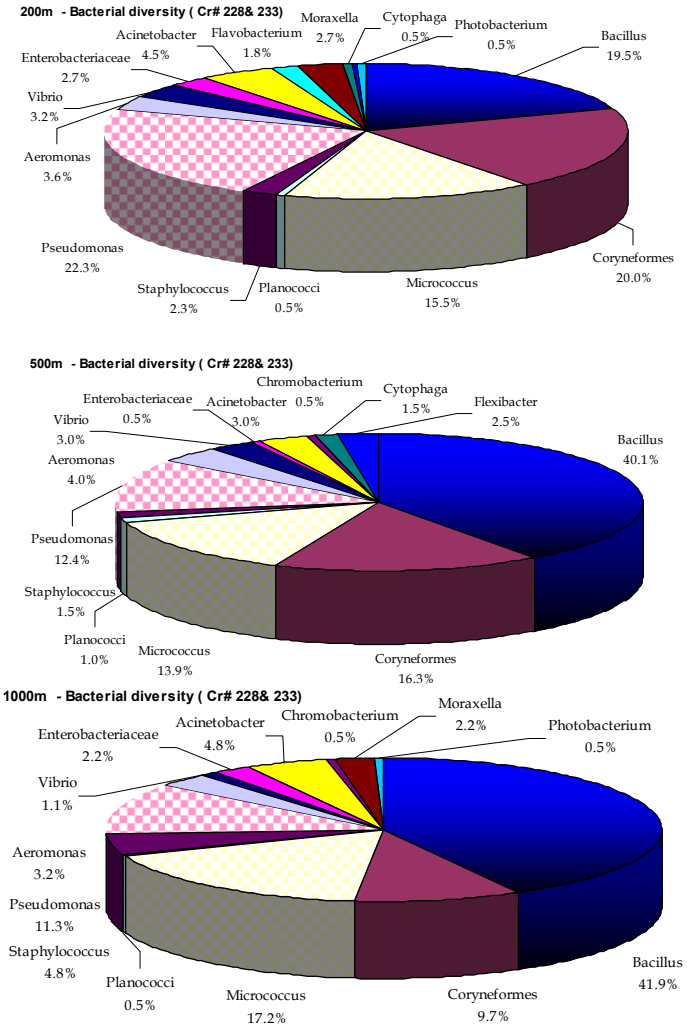


Fig. 4.5. Qualitative analysis of culturable heterotrophic bacteria from the slope sediments at different depth ranges during Sampling I (Cruise No. 228&233).

ii) Sampling - II (Cruise No. 254)

The overall percentage composition of different bacterial genera during Sampling II (Cruise No. 254) is presented in figure 4.6. *Bacillus* was the dominant genus (40.2%) followed by Coryneforms (28.6%), *Aeromonas* and *Acinetobacter* (7.6% each), *Pseudomonas* (5.4%), *Micrococcus* (3.2%), *Moraxella* (2.4%), *Planococcus* (2.3%), *Vibrio* (0.8%), Enterobacteriaceae and *Alcaligenes* (0.5 % each) and *Flexibacter* (0.4%).

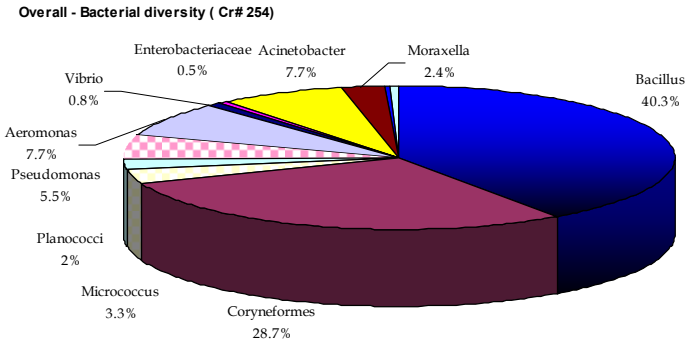


Fig. 4.6. Qualitative analysis of culturable heterotrophic bacteria in the slope sediments during Sampling II (Cruise No. 254)

At 200m depth range also *Bacillus* was the dominant (40.1%) genus followed by Coryneforms (28.8%) *Aeromonas* (7.7%), *Acinetobacter* (6.9%) *Pseudomonas* (5.1%), *Micrococcus* (3.3%), *Planococcus* (2.9%), *Moraxella* (2.2%), Enterobacteriaceae and *Alcaligenes* (1.1% each) and *Flexibacter* (0.7%). *Bacillus* was dominant (47.0%) followed by Coryneforms (28.9%) *Aeromonas* (6.9%) *Acinetobacter* (6.3%) *Pseudomonas* (5.3%), *Planococcus* (2.0%), *Moraxella* (1.3%) *Micococcus* (1.0%), *Alcaligenes, Flexibacter* and Enterobacteriaceae (0.3%) at 500m depth. The mean percentage of isolation of the bacterial genera from 1000m depth range showed that genus *Bacillus* was dominant (31.9%) followed by Coryneforms (28.4%), *Acientobacter* (9.9%), *Aeromonas* (8.5%), *Pseudomonas* (6.0%), *Micrococcus* (5.7%), *Moraxella* (3.9%) *Planococcus* and *Vibrio* (0.7% each) (Figure 4.7). The overall percentage

abundance of various bacterial genera from different depth ranges during both the samplings are presented in Appendix Table 4.A4.

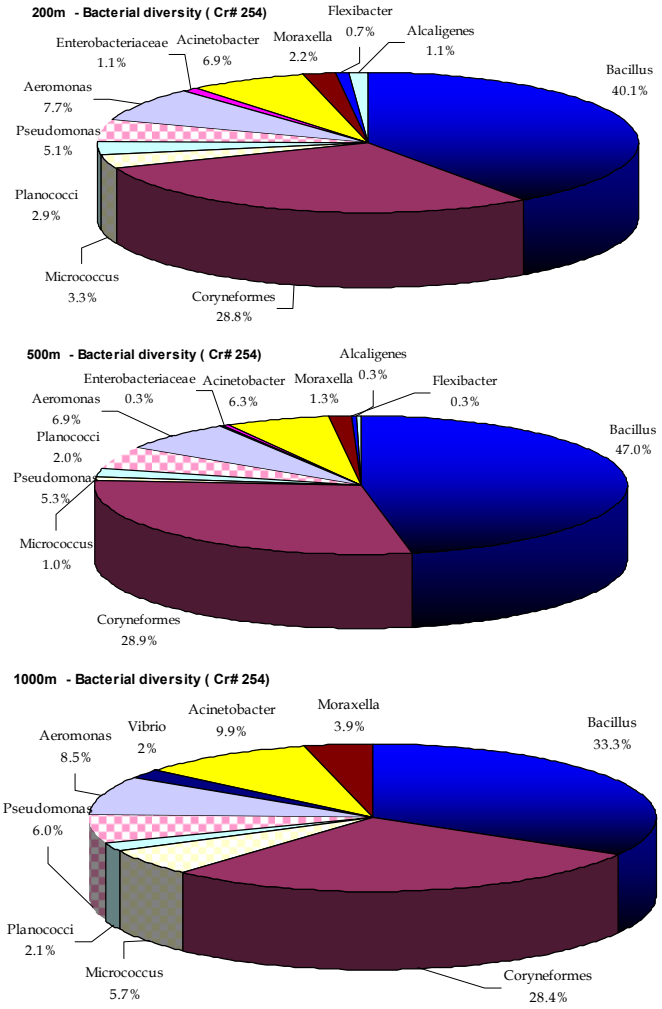


Fig. 4.7. Qualitative analysis of culturable heterotrophic bacteria from different depth ranges in the Arabian Sea during Sampling II (Cruise No. 254).

4.3.3. Multivariate Analysis

i) Cluster Analysis and Non-parametric MDS Ordination

A dendrogram showing station affinities, based on the mean root transformed abundance of bacterial genera found in the study, using the Bray-

Curtis measure of similarity and group-average sorting is presented in Fig.4.8. A broken line drawn at the arbitrary similarity level of 40% delineates 2 major groups of stations: Group 1 divides into 2 homogeneous subgroups at 50% similarity level which have been designated 1A and 1B. Group 1A comprises most of the stations from the south west region , Group 1B comprises stations at 200m depth between Trivandrum, Kollam, Kochi and Ponnani and also 500m stations Off Ponnani and Ratnagiri. Group 2 divides into 3 subgroups at 50% similarity level which have been designated as 2A, 2B and 2C. Group 2A comprises stations in the south west coast lying across 500m Off Cape comorin and Trivandrum and 200m stations off Kannur. It also include 200m stations Off Goa, and the region lying across 1000m depth range Off Karwar - Dabhol, Ratnagiri and Mumbai. Group 2B comprised 500m stations off Mumbai 1 and 200 and 500m depth range Off Porbandar. The group 2C holds remaining stations from the north west region and the stations lying across Coondapore and the 1000m station Off Mangalore. A Multidimensional Scaling (MDS) is also plotted as shown in Figure 4.9. based on similarity/ dissimilarity of the sampling locations in relation to the bacterial diversity during Sampling I.

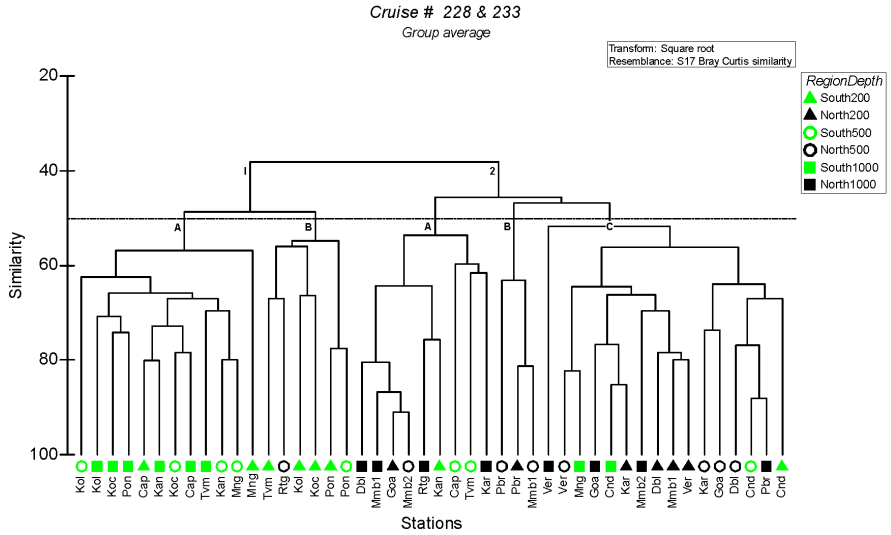


Fig 4.8. Hierarchial Cluster dendrogram plotted based on similarity/ dissimilarity of the sampling locations in relation to the bacterial diversity of the location isolated during Sampling I (Cruise No. 228&233)

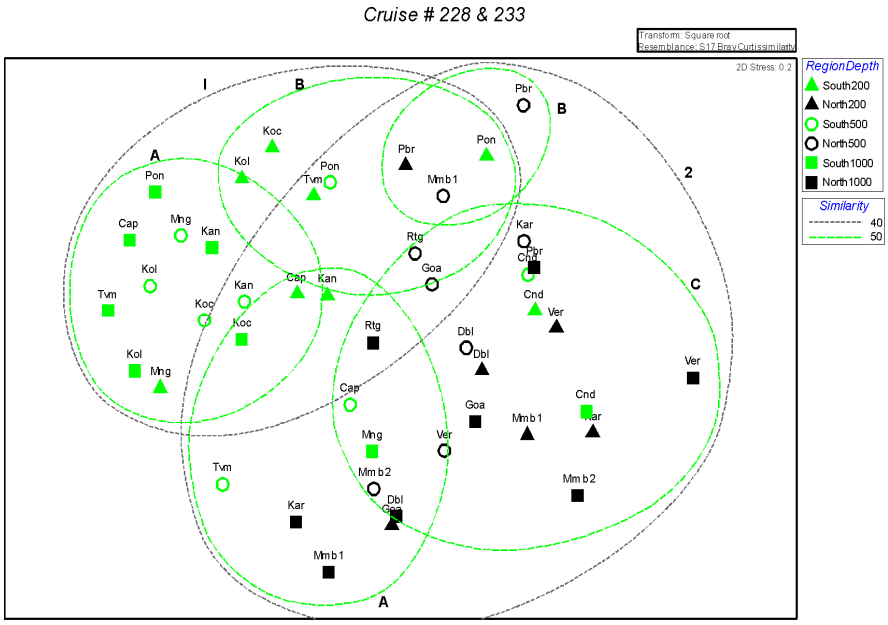


Fig 4.9. Multidimensional Scaling (MDS) plotted based on similarity/ dissimilarity of the sampling locations in relation to the bacterial diversity of the location isolated during Sampling I (Cruise No. 228& 233)

Fig. 4.10. is a dendrogram showing station affinities during Sampling II (Cruise No. 254) based on the mean root-root transformed abundance of bacterial genera found in the study, using the Bray-Curtis measure of similarity and group-average sorting. A broken line drawn at the arbitrary similarity level of 60% delineates 3 groups of stations: Group 1 divides into 3 homogeneous subgroups at 63% similarity level which have been designated 1A, 1B and 1C. Group 1A comprised of 1000 m depth range of Mangalore, Coondapore and Goa, 200m Off Ratnagiri and 500 Off Mumbai I. Group 1B is comprised of 200m depth ranges Off Ponnani, Coondapore, Dabhol, Mumbai I and II and 500m depth regions Off Cape comorin and Dabhol. Group 2 comprised stations Off Mangalore (200m) and Mumbai II (500m) and Group 3 included 1000m station Off Karwar.

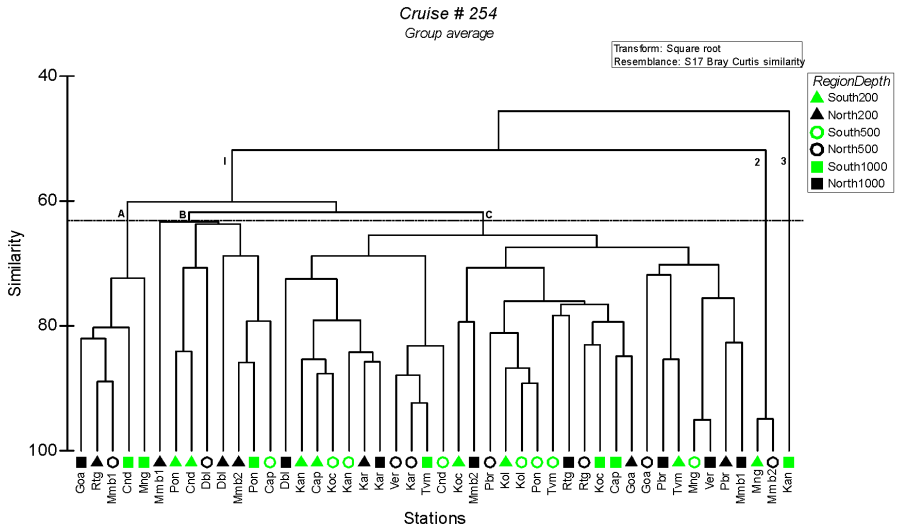


Fig 4.10. Cluster diagram plotted based on similarity/ dissimilarity of the sampling locations in relation to the bacterial diversity of the location (slope sediments of Arabian Sea) during Sampling II (Cruise No. 254)

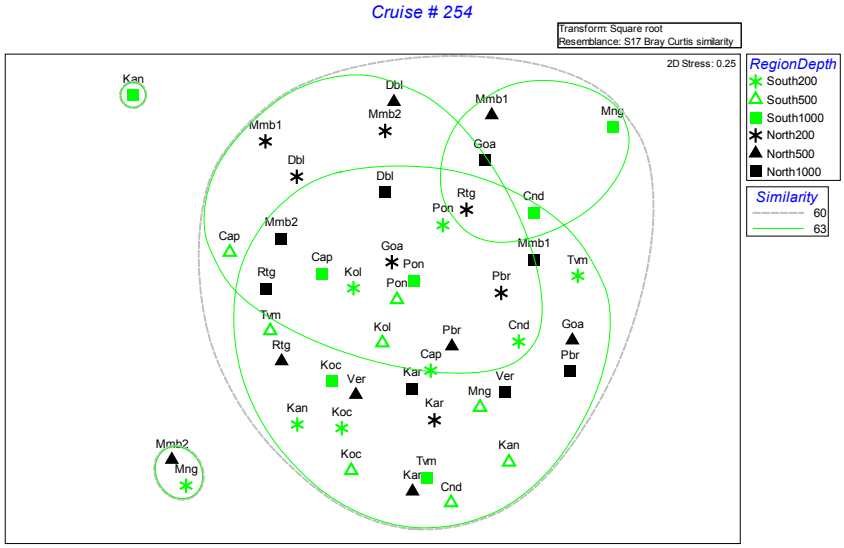


Fig 4.11. Multidimensional Scaling (MDS) plotted based on similarity/ dissimilarity of the sampling locations in relation to the bacterial diversity of the location isolated during Sampling II (Cruise No. 254).

Fig. 4.11. shows the results of multidimensional scaling using the same similarity matrix as above, delineating groups of stations from the dendrogram

(Fig. 4.10.). This analysis gives essentially the same picture as the dendrogram and confirm the groupings defined from the dendrogram.

ii) K-dominance Curve: The K-dominance curve was plotted to establish the relative abundance of different species at different depth regions (Figure 4.12), which gives more information regarding the distribution. K-dominance measures intrinsic diversity and in the present study a particular dominance with respect to the heterotrophic bacterial diversity was not clearly observed in the depth zones under study. Percentage dominance shows that during Sampling I (Cruise No. 228&233) an increase in the dominance was observed as the depth increased. This was more particular with regard to principal contributors to the community in each depth. It shows that a single genus in 200m depth region contributed to nearly 25% of the cumulative abundance. Whereas a single species from 500 and 1000m region contributing nearly 30 and 38% respectively to the cumulative abundance.

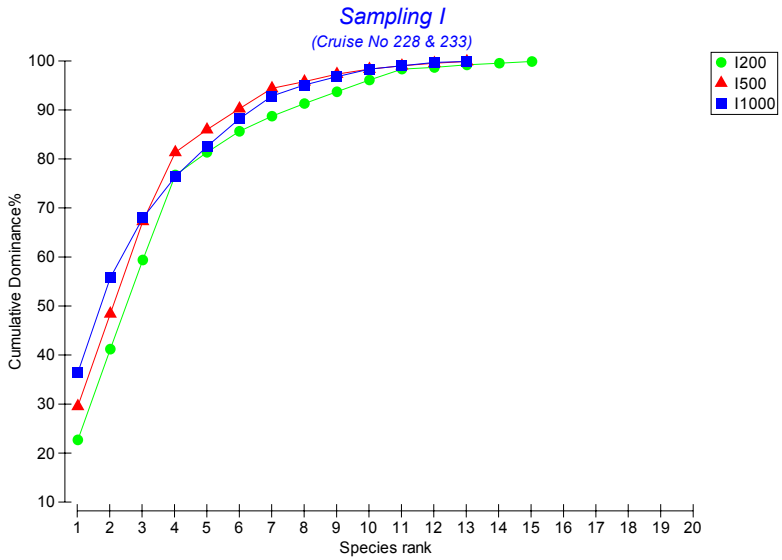


Fig 4.12. K-dominance curve plotted for different depth zones based on the bacterial diversity in the slope sediments of Arabian Sea . Sampling I (Cruise No. 228&233)

During the second set of sampling carried out through Cruise No. 254, the percentage dominance showed that the region occupying 1000m depth range was

seldom affected with a single genera showing values close to 35% to the total cumulative abundance which is in accordance with the first sampling. However, in the region overlying 200m and 500m depth zones a slight increase in the dominance was observed which marked a value at 37 and 46% for 200 and 500m depth zones respectively (Fig. 4.13).

The K- dominance plot was constructed from the mean value of both the sampling combined (Figure 4.14). The result further comprehend that region overlying 200m depth zone was less dominant compared to the greater depth which showed a value of 37 and 36% for 500 and 1000m respectively.

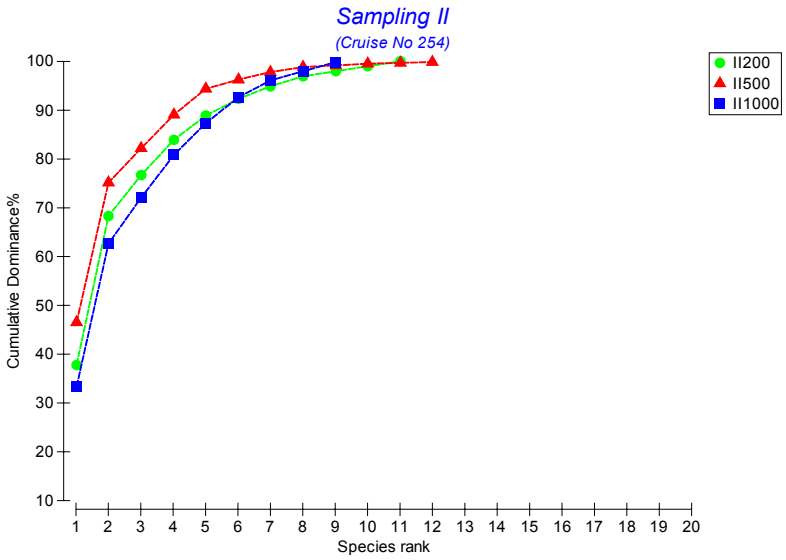


Fig 4.13. K-dominance curve plotted for different depth zones based on the bacterial diversity in the slope sediments of Arabian Sea. Sampling II (Cruise No. 254).

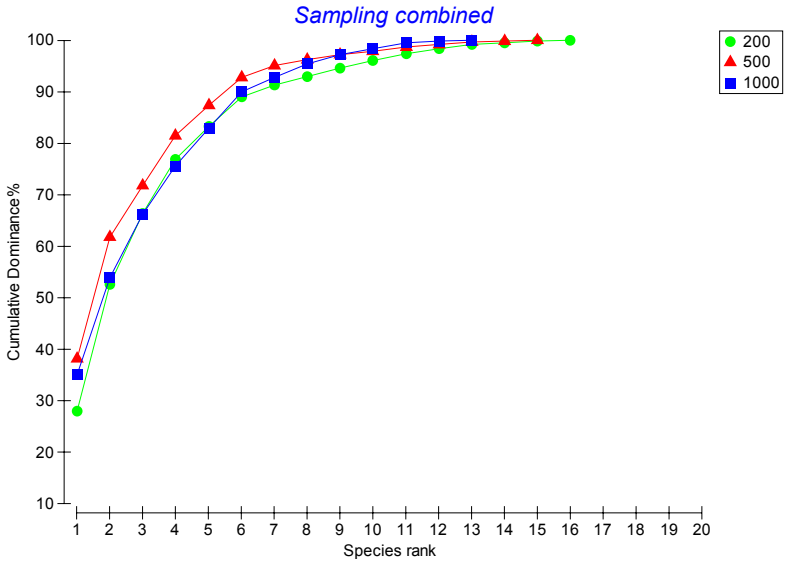


Fig 4.14. K-dominance curve plotted for different depth zones based on bacterial diversity in the slope sediments of Arabian Sea considering both the Samplings (I & II).

iii) Diversity Index Measures

The results of diversity indices of different depth ranges of both the sampling are presented in Table 4.1.

a) Sampling - I (Cruise No. 228&233)

Shannon-Wiener diversity (H'; $\log^2$), Pielou's evenness (J') and Species richness (d) were found to be higher in 200m depth region. While 500m and 1000m depth regions recorded similar values. The diversity ranged from 3.02 at 200m depth region to 2.78 at 500 and 1000m depth regions. Similar results were obtained for Species richness recording 3.04 at 200m depth and 2.60 at 500 and 1000m depth regions. Species evenness was 0.77 at 200m depth and 0.75 at 500 and 1000m depth regions. Dominance was 0.19 at 1000m depth, 0.18 at 500m depth and 0.15 at 200m depth regions (Figure 4.15).

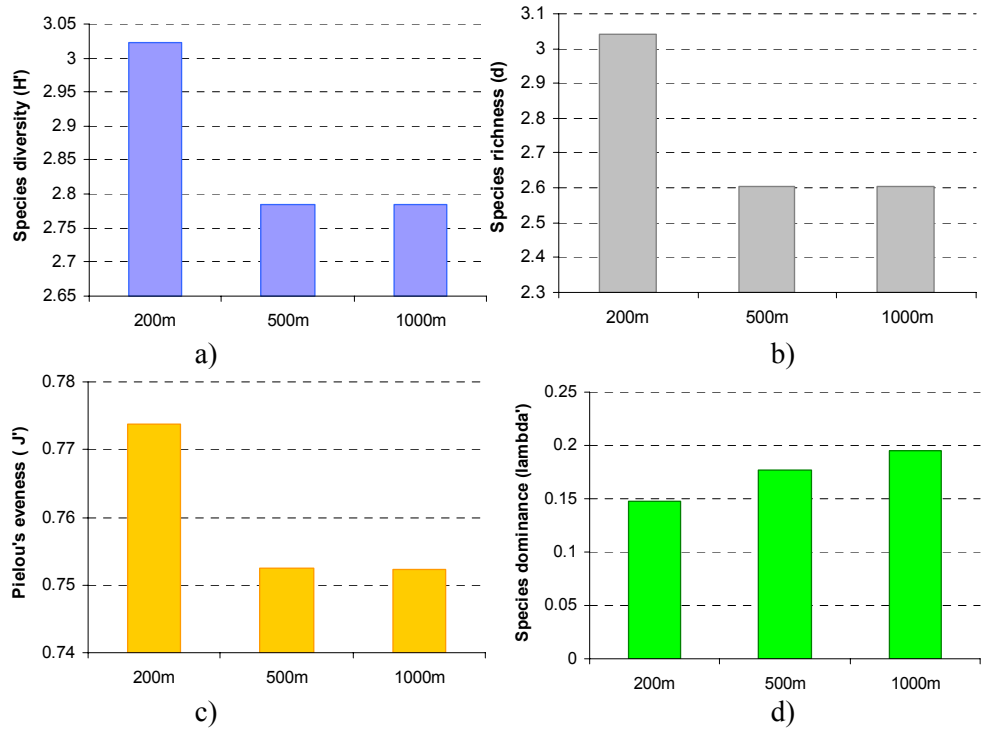


Fig 4.15. Diversity indices (a) Species diversity b) Species richness c) Species evenness and d) Species dominance) plotted for different depth zones based on the composition of heterotrophic bacteria in the slope sediments of Arabian Sea. (Sampling I -Cruise No. 228& 233)

b) Sampling - II (Cruise No. 254)

Shannon Wiener diversity (H'; $\log^2$) and Pielou's evenness (J') showed a similar trend recording higher values at 1000m depth followed by 200 and 500m depth region. The results of the diversity indices are plotted as shown in Figure 4.16. The diversity ranged from 2.54 at 1000m depth region to 2.45 at 200m depth and 2.15 at 500m depth range. Pielou's evenness ranged from 0.80 at 1000m depth region to 0.71 at 200m depth and 0.60 at 500m depth range. Species richness varied from 2.39 (500m) to 1.74 at 1000m depth region. Dominance was highest (0.31) at 500m depth, and lowest (0.22) at 1000m depth region.

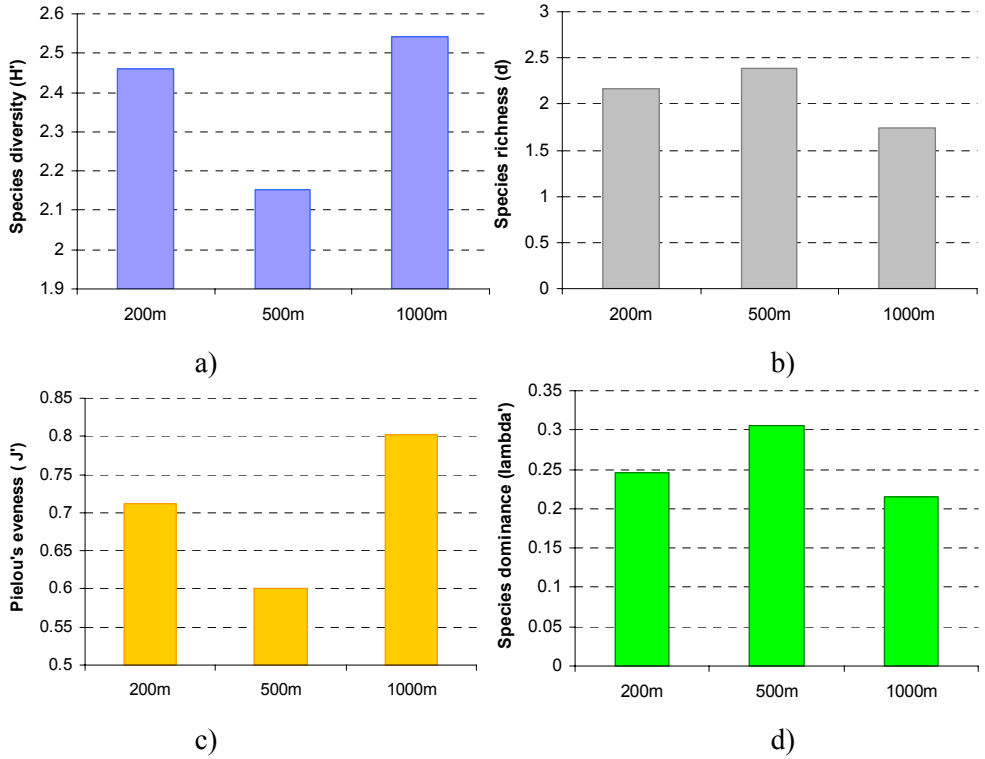


Fig 4.16. Statistical indices (a) Species diversity b) Species richness c) Species evenness and d) Species dominance) plotted for different depth zones based on the distribution of heterotrophic bacteria in the slope sediments of Arabian Sea (Sampling II-Cruise No. 254)

Combining the data of both the samplings has shown that Shannon Wiener diversity (H'; $\log^2$), Pielou's evenness (J') and species richness (d) were found to be higher at 200m depth region. The diversity was maximum (2.92) at 200m depth region and minimum (2.62) at 500m. Species richness varied from 3.26 at 200m depth to 2.60 at 1000m depth region. Species eveness was 0.75 at 1000m depth and 0.67 at 500m depth region. Dominance ranged from 0.22 at 500m depth, and 0.19 at 1000m depth. Whereas at 200m depth region dominance showed a value of 0.17 (Fig. 4.17).

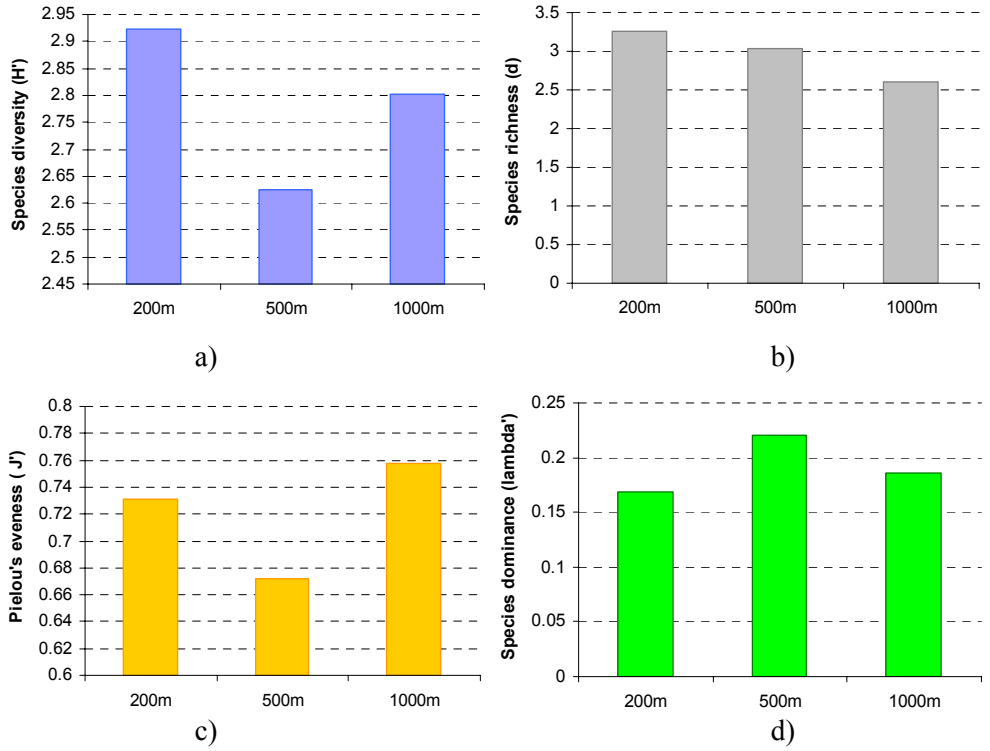


Fig 4.17. Diversity indices (a) Species diversity b) Species richness c) Species evenness and d) Species dominance plotted for different depth zones based on the distribution of heterotrophic bacteria in the slope sediments of Arabian Sea Mean of both the Samplings (I & II).

Table 4.1. Diversity indices of different depth ranges samplingwise and on combining both the samplings

	Sampling I (Cruise No. 228&233)			Sampling II (Cruise No. 254)			Mean		
	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m
S	15	13	13	11	12	9	16	15	13
N	100	100	100	100	100	100	100	100	100
Species richness d	3.040	2.606	2.606	2.171	2.392	1.740	3.257	3.042	2.608
Pielou's eveness J'	0.774	0.753	0.752	0.711	0.600	0.802	0.731	0.672	0.757
Species Diversity H'(log 2)	3.023	2.785	2.784	2.459	2.153	2.543	2.923	2.625	2.802
Species dominance lambda'	0.148	0.177	0.195	0.246	0.305	0.215	0.169	0.220	0.186

4.4. DISCUSSION

In recent years, preserving the world's biodiversity has become a priority in environmental studies. Because of the importance of bacteria in the marine environment it is vital to understand the full extent of bacterial diversity and the role of the most abundant species. The use of both cultivation based and cultivation independent approaches have provided new perspectives on understanding the nature of the bacterial communities in the sea over the past decade (Giovannoni *et al.,* 1990; Suzuki *et al.,* 1997). Marine bacterioplankton represent one of the most thoroughly studied environmental communities on the planet (Giovannoni and Stingl, 2005), yet bacteria inhabiting marine sediments remain largely uncharacterized. This lack of information hinders an effective assessment of marine bacterial diversity and limits our understanding of the fundamental differences between the bacterial populations inhabiting the various ocean ecosystems. Widespread species distribution between different oceanic regions raises the question of how this heterogeneity among certain genera is maintained. Significant changes in the bacterial species composition occur at various temporal and spatial scale and has also demonstrated that stratification may result in more pronounced differences between different depths (Murray *et al.,* 1998). Although only quantitative aspects of the distribution pattern of aerobic heterotrophic bacteria have been considered so far, it has now become obvious that qualitative analyses are also important for describing marine environments. The present investigation contributes to the knowledge of culturable heterotrophic bacteria in the Arabian Sea along the western continental margin of India by qualitative and quantitative description of their community variations occurring in a depthwise and regional scale.

In the present study a cultivation based approach was adopted to describe the standing bacterial community of the sediments of Arabian Sea. As a proper scheme of investigation is still lacking to provide a complete insight into the

structure and activity of microbial communities in nature isolating and cultivating the bacteria and further characterizing them *in vitro* is still an important method in microbial ecology. Accordingly, the descriptions of deep sea bacterial communities presented here are based on microscopical, biochemical and physiological aspects of the isolates from sediment samples collected from continental slope region of the Arabian Sea sediments. On the other hand it is well-known that only a small fraction of the natural microflora can be isolated on agar media and that the greatest part remains hitherto uncultivable. Thus the fact is that uncultivable part consists of unknown bacteria which has become permanently or transiently uncultivable under oligotrophic conditions, and remain in the starved but viable state (Roszak and Colwell, 1987; Wiebe *et al.,* 1992). However, analysis of natural microbial populations by ribosomal RNA gene sequences (Pace *et al.,* 1986), low molecular weight RNA profiles (Hofle 1990), or DNA hybridizations (Voordouw *et al.,* 1991) offer little information about the metabolic potential of the organisms.

Attempts were made here to isolate heterotrophic bacteria in solid ZoBell's Marine agar plates. In an environmental condition with a wide range of poorly described genera of marine bacteria, conventional taxonomic methods could be used for initial studies. Rehnstam *et al.* (1993) contend that even when colony counts are low, they may include those species that are dominant in seawater. In agreement with those of other authors (Kriss, 1963; Chandramohan *et al.,* 1987; Maruyama *et al.,* 1997; Loka Bharathi and Nair, 2005), the results obtained in this study demonstrate that the most common bacteria in the sediments are Gram-positive ones, in contrast to a higher proportion of Gram-negative bacteria commonly isolated from marine waters. Ruger and Tan (1992) during an investigation on the deep sea sediment of tropical Atlantic ocean has shown that the majority of indigenous isolates from sediment surface samples were Gram-negative, however most of the cold-adapted strains from the subsurface oxic layers of sediment and from the surface sample belonged to the Gram-positive type,

showing the same pattern as the mesophilic strains isolated from both sediment layers.

In the present study, one of the dominant genera was found to be *Bacillus* and this genus is readily obtained from marine sediments (Sieburth, 1979). The genus *Bacillus* constitutes a diverse group of rod-shaped, Gram-positive bacteria, belonging to a group of low G+C phylum of *Firmicutes*. It encompasses rod-shaped bacteria capable of aerobically forming resistant endospore which make them ubiquitous in the environment. A high proportion of *Firmicutes* in the sediment in the Arabian Sea was reported earlier by Divya *et al.* (2010) unlike the deep sea sediments of Pacific Ocean where the dominant groups were Gamma-proteobacteria and Alpha-proteobacteria (Kato *et al.,* 1996; DeLong *et al.,* 1997; Wang *et al.,* 2004). The report states that *Bacillus* was the dominant genus in the sediment unlike in Okinawa Trough and southwestern Okhotsk Sea where *Halomonas* was the major group (Dang *et al.,* 2009). *Bacillus* isolated from marine origin are not, in reality, known to display specific traits. Indeed, the ubiquity of the genus members, might explain the fact that they could survive under diverse conditions such as seawater and sediments. While the majority of the identified *Bacillus* spp. are rather terrestrial bacteria blown or deposited as spores into marine sediments, The occurrence of *Bacillus* spp. in the marine environment has been well documented (Ivanova *et al.,* 1999). Many Gram-positive bacteria are known to generate spores under adverse conditions, and in those encountered in marine ecosystems, spore formation is co-regulated with antibiotic production (Marahiel *et al.,* 1993). *Bacillus* was found dominant in the water and sediment samples of Bay of Bengal and Arabian sea (Palaniappan and Krishnamurthy, 1985). This genus was also the most common type among the cold adapted strains from the subsurface oxic layers and the mesophilic strains from the sediment during an investigation led by Ruger and Tan (1992) on the deep sea sediment of tropical Atlantic ocean. He also stated that the cold-adapted bacterial communities from sediment surface were represented by mainly

Alteromonas and *Vibrio* strains with members of the genus *Bacillus* predominating. Evidently, the marine *Bacillus* strains from the deep-sea represent a group of bacteria worth investigating. Detailed descriptions of their phylogenetic taxonomy will be presented in Chapter 6.

Another important group of Gram-positive forms found in the sediment bacterial community of the study area belonged to group coryneforms. The dominance of these non-sporing Gram-positive rod shaped forms belonging to group coryneforms in the deep-sea sediments of the Pacific Ocean have earlier been remarked by Maruyama *et al.* (1997). Loka Bharathi and Nair (2005) demonstrated that disturbance or bioturbation in the sediment substratum evoked an increase in the abundance of G-positive coryneforms among the deep-sea bacteria of Central Indian Ocean Basin. Any changes in the environment result in changes in community structure. Another important group identified during the study was *Micrococcus* which showed higher population in the shallow depth and the difference was significant. The occurrence of this genera in the coastal waters have been earlier reported by several researchers (Dhevendaran *et al.,* 1987; Chandrika and Nair, 1994). Most of the bacteria from stable cold environments like the deep-sea are considered to be Gram-negative (Baross and Morita, 1978). It was surprising that the isolates from the sediment layer of Arabian sea were predominantly Gram-positive. A predominance of Gram-positive sediment bacteria after incubation at room temperature, particularly cocci, was also reported for the abyssal Vema Fault, located around Atlantic Ocean (Bensoussan *et al.* 1984). Similar results on the composition of Gram-positive bacteria were found by Bensoussan *et al.* (1979) in deep-sea sediments of eastern tropical Atlantic region applying the incubation at mesophilic temperature.

Other important heterotrophic bacteria in this study also comprised of strains related to *Pseudomonas, Aeromonas, Acinetobacter,* Enterobacteriaceae, *Moraxella, Vibrio, Flavobacterium, Flexibacter, Cytophaga, Chromobacterium*

and *Photobacterium*. During his study Ramaiah (1994) has reported temporal variations in the generic composition of bacteria from the west coast of India. In addition, Delille (1995) used agar-plate isolations to demonstrate seasonal variation in the culturable fractions of sub Antarctic benthic bacterial communities. *Pseudomonas, Vibrio,* and *Bacillus* showed seasonal periods of dominance throughout a two-year study, which further support the present findings. Members of *Vibrio, Bacillus, Micrococcus, Staphylococcus, Arthrobacter,* Enterobacteriaceae and Coryneforms were also encountered in a bacteriological investigations of the ferromanganese nodules collected from the Indian Ocean region (Chandramohan *et al.*, 1987). The results indicated that the microbial populations associated within these regions were in general comparable to those of the Pacific and Atlantic oceans (Chandramohan *et al.*, 1987). Extensive studies were carried out by Bolter and Rheinheimer (1987) on the spatial and temporal relatedness of heterotrophic bacteria isolated using ZoBell's agar medium from the Baltic Sea. They provided interesting insights into features especially adapted to bacteria from the Baltic Sea and illustrated that in the Baltic region *Pseudomonas, Achromobacter, Flavobacterium*, and Enterobacteriaceae and Coryneforms were reported to be the most important groups.

Pseudomonas represents a fraction of the total microbial flora characterized by high metabolic versatility, and it is known for its capacity to degrade a considerable quantity of synthetic organic compounds. This genus is often encountered in sea water, sediments, phytoplankton and zooplankton (Austin *et al.,* 1979; Nair and Simidu, 1987). Under certain conditions, such as organic matter in solution and temperature in the mesophilic range the capacity of *Pseudomonas* for rapid growth in the absence of complex growth factors is responsible for its predominance (Palleroni, 1984). Their occurrence and role in nutrient cycling and biodegradation of organic matter in deep-sea sediment are well established (Li *et al.,* 1999). *Aeromonas* also occurred considerably among the Gram-negative

bacteria. Aeromonads are the most abundant bacteria found in the aquatic environments. This genus has been reported to be an autochthonous organism occurring in uncontaminated as well as sewage-contaminated waters (Burke *et al.,* 1984). Aeromonads are inhabitants of aquatic environments and also belong to the flora of fish, amphibia and other marine organisms (Shotts *et al.,* 1972; Kueh and Chan, 1985).

Members of the genus *Acinetobacter* are usually isolated in water. There are reports on the distribution of *Acinetobacter* from deeper waters (Vimala *et al.,* 2003). They are capable of degrading aromatic compounds and their metabolic versatility is that of the pseudomonads (Abd-el- haleen *et al.,* 2002). Organisms of the genus *Moraxella* are commonly isolated from animals. Maruyama *et al.* (1997) showed that among the psychrotrophs from deep-sea water there was abundance of *Moraxella,* and deep-sea *Moraxella* were different from surface ones. *Moraxella* plays an important role in nitrogen cycling (Stolp, 1988). The dominance of *Acinetobacter-Moraxella* was due to the presence of aged particulate matter. The dominant communities of heterotrophic bacteria attached to particulate organic carbon changed from *Pseudomonas–Alcaligenes* group to *Acinetobacter–Moraxella* group with age (Baxter and Sieburth, 1984).

The presence of Enterobacteriaceae, *Chromobacterium, Flavobacterium, Flexibacter, Cytophaga, Vibrio* and *Photobacterium* were also remarkable. Enterobacteriaceae were less abundant during the whole sampling period. A similar pattern was seen for *Cytophaga, Pseudomonas* and *Photobacterium*. The enzymatic versatility of these genera is well known and has been suggested as an explanation of their importance in particle turnover (DeLong *et al.,* 1993). *Chromobacterium* is ubiquitous in nature and found in waters from polar regions to the tropics. *Flavobacterium* has been isolated from marine water, ocean sediments and foods; it tolerates low, but prefers higher temperatures and can grow in alkaline environments (Stolp, 1988). This genus has been reported to be involved in the

degradation of pesticides and chitin. In marine environments, *Flexibacter* is abundant near to the shore, on seaweeds and on decaying sea animals. *Cytophaga* group occurrence indicates the supply of fresh macro aggregates (Riemann *et al.,* 2000). As naturally occurring bacteria *Cytophaga* spp. are capable of biodegrdation of natural polymers such as cellulose, pectin, keratin, agar and chitin. *Vibrio* occurs in saline aquatic environments, both free in the water and bound to animate and inanimate surfaces. These microorganisms are readily culturable from sea water, marine animals and seaweeds. Several studies on the role of *Vibrio* spp in the marine environment have shown their importance in biodegradation, nutirient regeneration and biogeochemical cycling (Jorgensen, 1983; Colwell and Hill, 1992). *Photobacterium* are usually found in marine environments, hence the requirement of sodium for growth. They may be free living in the water, or found associated with intestinal contents of marine animals and with the specialized luminous organs of fish (Baumann and Baumann, 1984).

The genus *Flavobacterium* is one of the more commonly represented genera in the marine environment. Thus, the presence of this genus in the present study during cruises was quite justified. This genus has been isolated from seawater and marine sediments by ZoBell (1946). *Flavobacterium* was also isolated by Ohwada (1980) from Atlantic Ocean. Nair *et al.* (1994) found that *Flavobacterium* was confined to the offshore stations in Indian Ocean. Further, it has also been suggested that *Flavobacterium* and *Alcaligenes* genera are common in the marine environment and undergo seasonal fluctuation (Seshadri *et al.,* 2002).

The possible presence of hydrocarbons in the continental margin areas of the north eastern Arabian Sea have already been a central issue of many of the geochemical investigations. Presence of hydrocarbons in the environment brings about adaptive responses in microbial communities resulting in a net increase in the number of hydrocarbon-utilizing organisms. Hydrocarbon degradation depends on the composition of the community. The ability to utilize hydrocarbon substrates is

exhibited by a wide variety of bacterial and fungal genera. However, in the marine environment, bacteria are generally considered to represent the predominant hydrocarbon-degrading element of the microbial community. Based on the number of published reports, the most important hydrocarbon-degrading bacteria in both marine and soil environments are *Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia*, *Pseudomonas* and the coryneforms. In a numerical taxonomy study of petroleum-degrading bacteria from Chesapeake Bay water and sediment, Austin *et al.* (1977) found that *Pseudomonas, Micrococcus, Nocardia* and members of the family Enterobacteriaceae, Actinomyces, and Coryneforms made up 95% of the isolates.

Significant changes in the bacterial species composition occur at various temporal and spatial scale and has also demonstrated that stratification may result in more pronounced differences between different depths (Murray *et al.,* 1998). Moreover, any change in bacterial composition in response to environmental variables could be tracked within a reasonable time frame. The present investigation contributes to the knowledge of culturable heterotrophic bacteria in the Arabian sea along the western continental margin of India by qualitative and quantitative description of their community variations occurring in a depthwise and regional scale. Bacterial diversity indices (Shannon diversity) were calculated from different depth ranges of the west coast of India. Combining the data of both the samplings has shown that Shannon Wiener diversity and species richness were found to be higher at 200m depth region. Species richness varied from 3.26 to 2.60 recording minimum at 1000m depth region. Species eveness was 0.75 at 1000m depth and 0.67 at 500m depth region. Dominance ranging from 0.17 to 0.22 was the highest recorded in the region of 500m depth range. The diversity was maximum (2.92) at 200m depth region and minimum (2.62) at 500m with the values (2.5–3.1) as reported by Schauer, *et al.* (2000) from the Catalan coast in NW Mediterranean but higher than Shannon indices reported from a seasonal study in a eutrophic lake (0.2–0.9; Hofle *et al.,* 1999) and Norwegian Coastal waters (1.8 to

2.4; Larsen *et al.,* 2004). Dave and Desai (2006) studied the microbial diversity from the west coast of India. They found Shannon-Wiener index in the range of 0.048 - 1.334, evenness in the range of 0.070 - 0.829 and Margalef richness in the range of 0.621 - 2.485. Damare *et al.* (2006) reported the diversity indices for filamentous fungi from deep-sea; the Shannon Weiner index varied between 1.07 and 2.06, Pielou's evenness, 0.77 – 0.95 and richness, 1.44 - 2.65. Luna *et al*. (2004) reported that in deep Mediterranean sediments the diversity indices of benthic bacterial communities were in the range of 2.1 to 2.7 for Shannon-Weiner index. Margalef (species richness) was reported to be 0.80 to 0.98 and Pielou evenness between 1.26 and 2.27 in the upper surface sediment.

Bacteriological indices enable assessment of the trophic status of sediment and the extent of disturbance taking place in this region. Moreover, any change in bacterial composition in response to environmental variables could be tracked within a reasonable time frame. Significant changes in the bacterial species composition occur at various temporal and spatial scale (Murray *et al.,* 1998). In deep sea it is not only important to have an understanding of the natural or autochthonous microbial flora in terms of biomass and potential activity, but also in relation to community structure and species composition (Austin *et al.,* 1979). Marine microbiological studies on occurrence, species/generic composition, abundance of viable bacteria have been helpful in realizing the importance of heterotrophic prokaryotes in biogeochemical processes (Ramaiah *et al.,* 1996). The present investigation contributes to the knowledge of culturable heterotrophic bacteria in the Arabian sea along the western continental margin of India by qualitative and quantitative description of their community variations occurring in a depthwise and regional scale.

Chapter 5

HYDROLYTIC ENZYME PRODUCTION OF THE CULTURED BACTERIAL STRAINS

Contents

5.1. INTRODUCTION

Extracellular enzymes produced by sediment bacteria play important roles in deposited and buried organic matter decomposition, nutrient cycling, element transformation and mobilization. In aquatic environments heterotrophic bacteria are key players in the processes of organic matter recycling, decomposition and mineralization (Azam and Cho, 1987). In order to allow transport across the outer membrane, complex substrates must first be hydrolyzed outside the cell into smaller sized molecules (Weiss, 1991; Chrost, 1991). This process is conducted by extracellular enzymes which enable heterotrophic bacteria to obtain substrates suitable for incorporation from a diverse array of complex compounds from the organic matter which are dominated by high-molecular-weight compounds (Arnosti, 2003).

Because extracellular enzymes catalyze the degradation of complex molecules into easily assimilable units (Meyer-Reil, 1991) any factors affecting their activity or disrupting the production or availability of extracellular enzymes will impact the entire remineralisation pathway (Arnosti, 2003). Hence, bacterial extracellular enzymatic activity is regulated at the ecosystem level, by environmental factors and at the micro-environment level by enzyme-substrate

interactions. Extracellular enzymes are proportionally more important in the decomposition of particulate or colloidal material in the dark ocean (Baltar *et al.,* 2010). The activity of extracellular enzymes in the dark ocean is likely related to the composition of sinking particulate organic matter. Previous studies suggest that a substantial fraction of sinking particulate organic matter (POM) is solubilized to dissolved organic matter (DOM), fueling prokaryotic production in the meso and bathypelagic zones (Cho and Azam, 1988). In the spectrum of enzymes studied in the aquatic environment, special attention has been given to ectoenzymes responsible for the hydrolysis of the major components of DOM.

Most of the information on extracellular enzymatic activity in the sea refers to hydrolytic enzymes such as proteases, glucosidases, chitinases, lipases and phosphatases. The biochemical role of extracellular enzymes in the sea is similar to other aquatic environments but the hydrographic conditions in the ocean are characterized by distinct vertical and horizontal zonations. Consequently, patterns of polymer hydrolysis rates change horizontally and vertically, between different water masses. Temporal and spatial variation of hydrolytic potential are detectable in the activity rates of particular extracellular enzymes. It is not clear whether the mineralization of DOM is dominant in selected groups of bacteria and whether changes in the spectrum of hydrolytic enzymes between seasons may be caused by the succession of different species (Martinez *et al.,* 1996). Like other functional parameters the microbial activity can be inhibited or increased by physical and chemical environmental changes, biological variations or input of allochthonous materials (Chrost, 1993).

The differences are related with shifts in the availability of labile organic matter, leading to variations in extracellular enzymatic expression (Baltar, 2010) Extracellular enzyme activity is particularly lower in bottom water layers, being the activity dependent on the rates of sedimentation of detritus produced in the euphotic layers above. Investigations on pure cultures of bacteria have

demonstrated that extracellular enzymes can be released from cells in the presence of the corresponding substrate (Alderkamp *et al.,* 2007), as a function of the growth phase (Antranikian, 1992), in response to bacterial starvation (Albertson *et al.,* 1990), viral lysis (Karner and Rassoulzadegan, 1995), protozoan grazing (Bochdansky *et al.,* 1995), or changes in microbial cell permeability (Chrost, 1991). This complexity of factors interacts in the regulation of bacterial extracellular enzymatic activity, modulating enzyme expression to the influence of environmental physical and chemical parameters.

We focused on characterising the culturable heterotrophic bacteria over seasonal oceanographic surveys with the objective of evaluating changes in the community structure and in the physiological activities involved in the decomposition of the main organic compounds. It is well known that culturable bacteria represent a limited fraction of total count, however cultural methods can still provide some information on potential metabolic activity and on the role of heterotrophic bacteria in the biogeochemical cycles. When particular nutrients are present in limited amounts, microbes can produce enzymes to liberate them from organic matter. Sieburth (1971) and Kjelleberg and Hakansson (1977) have investigated the distribution of bacterial physiological groups in the sea on the basis of selective agar media supplemented with starch, protein, Tween-80 or other polymers. Many microbiologists have tried to identify bacteria on the basis of "physiological groups" for a more biochemically oriented analysis of the composition of a bacterial population.

Jones (1971) showed a seasonal fluctuation in separate populations of protease, amylase and lipase producing bacteria in lakes. This methodological approach was developed, modified and also extensively used by many other microbiologists (ZoBell, 1946; Holding and Collee, 1971). Sizemore and Stevenson (1970) developed a marine agar-milk double layer plate technique for the detection of proteolytic marine bacterial colonies. Paoni and Arroyo (1984)

described a method which makes use of chromogenic substrates (p-nitrophenyl-2-acetamido-2-deoxy-cr-D-galactopyranoside) in detecting glycosidase activity of bacterial colonies on agar plates. Although these methods offer some advantages in comparison with the selective media approach, it is somewhat inconvenient to prepare double-layer agar plates. Analysis of this type could be used to characterize extracellular enzymatic activities of bacteria isolated from such ecological niches as macroalgal surfaces, outer and intestinal surfaces of zooplankton, interfaces etc. There is some indication that enrichment of polymers or continuous excretion of certain compounds lead to stabilization of a corresponding microflora in these environments. For activities and properties of bacteria living in association with benthic macroalgal mats this has been demonstrated by Mow-Robinson (1983).

The number of heterotrophic bacteria (saprophytes) plays an important role as an easily assessable indicator of pollution and of autochthonous organic matter accumulation. In this regard, the identification of 'physiological groups' of saprophytes could lead to a better understanding of organic matter cycles in ecosystems. Despite the uncertainties still involved, the described method can be used as a rapid and sensitive measure of potential extracellular enzymatic properties of saprophytes and could also be a tool in physiological studies.

Thus, the enzymatic activities outside the cells constitute the initial step of organic matter conversion and define the type and quantity of substrates available to the microbial food web in aquatic ecosystems. The activities of various hydrolytic enzymes in aquatic ecosystems have been investigated to describe the microbial activity and dynamics of organic matter conversion (Hoppe *et al.,* 2002). Variations in ectohydrolase profiles (types and levels of activity) reflect the trophic status of the environment, and this changes with the season (Karner *et al.,* 1992). It has been argued that such variations could be caused by shifts in the dominant species or in the level of enzyme expression by the same species in response to changes in the spectrum of organic substrates (Martinez *et al.,* 1996).

Bacteria hydrolyze large polymers such as proteins by means of extracellular enzymes. Proteolytic enzymes catalyze the cleavage of peptide bonds in other proteins. Proteases are degradative enzymes which catalyze the total hydrolysis of proteins. Proteases represent one of the three largest groups of industrial enzymes and account for about 60% of the total worldwide sale of enzymes. The nitrogen cycle in marine sediments has received considerable attention over the last two decades, primarily as a result of its importance in nitrogen cycling in coastal ecosystems (Blackburn and Sorensen, 1988). This nitrogen is delivered primarily in the form of polymeric organic compounds, of which proteinaceous material is the dominant compound class identified to date. The end depolymerization, or hydrolysis, of the organic inputs, is a process that has received relatively little attention. The activity of these enzymes has been studied in soil systems quite intensively for over 20 years (Ladd and Butler, 1972; Nannipieri *et al.,* 1982), but only in the past few years attention has been paid to their role in marine sediments (Meyer-Reil, 1987).

Lipases are hydrolases acting on the carboxyl ester bonds present in acyl glycerols to liberate organic acids and glycerol. Triacyl glycerols are mainly uncharged insoluble lipids, although those with short-chain fatty acids are slightly soluble in water. The physical properties of lipids have caused many difficulties in studying the characteristics of lipolytic enzymes (Jaeger, 1994). A variety of lipases from microbial origin with different enzymological properties and substrate specificities have been found and characterized (Jaeger *et al.,* 1999).

Chitin is a tough leathery insoluble substance of indeterminate chemical structure somewhat resembling cellulose. It is generally believed to be a polymer of glucosamine in which each amino group is acetylated. Chitin is the chief constituent of the exoskeleton of arthropods and it occurs in some mollusks, coelenterates and protozoa as well as in certain fungi. There may be more than one kind of chitin but the observations of van Iterson *et al.* (1936) and others indicate

that animal and fungal chitins are identical. Large quantities of chitin are produced in the oceans of the world each year.

The fragmentary and contradictory literature on the subject fails to indicate to what extent chitin may be utilized as a source of food by animals which may ingest it. Bacteria are probably responsible for the disintegration of much chitin in the sea. ZoBell and Anderson (1936) have reported chitin digestion by mixed cultures from marine sources but have not described the bacteria involved. These observations, together with those of Stuart (1936) on the occurrence of halophilic chitinovorous bacteria in marine salt from Africa, S. America, Spain, California and the West Indies, indicate a worldwide distribution of chitin-digesting bacteria in the sea. Uneven distribution of chitinolytic microorganisms were described by ZoBell and workers during their study in California. Chitinases are glycosyl hydrolases that catalyze the degradation of chitin, a fibrous, insoluble polysaccharide, consisting of β-1,4-N-acetyl-glucosamine residues (GlcNAc). Many chitinolytic bacteria such as *Serratia marcescens* and *Aeromonas* sp., have been reported to produce more than one type of chitinase (Brurberg *et al.,* 1996; Shiro *et al.,* 1996)

5.2. MATERIALS AND METHODS

5.2.1. Bacterial Strains

The bacterial strains (1472 nos) isolated from the slope sediments (200-1000m depth zones) of Arabian Sea were used for the study. The isolates were checked for purity and transferred to nutrient agar vials for further analysis.

5.2.2. Hydrolytic Extracellular Enzyme Production

The microbial isolates were screened for the hydrolytic enzyme production by plate assay. All the isolates were tested for amylase, gelatinase, lipase, DNase and chitinase production. These tests were done using nutrient agar media

supplemented with various substrates such as starch, gelatin and tributyrin, DNA-sodium salt and colloidal chitin at 1% concentration. Composition of the media are given below. Sterile nutrient agar plates were prepared with corresponding substrates, spot inoculation of the isolates were done and incubated the plates for 3-5 days at room temperature (28±2 ˚C). Presence of clearing zone on tributyrin agar and chitin agar indicated positive result. For amylase, gelatinase and DNase reagents were added as given below and the clearing zone formation was noted.

i) **Starch Hydrolysis Test** (Hankin and Anagnostakis, 1975)

Composition of the medium (g l^{-1})

Peptone	:	5.0g
Beef extract	:	3.0g
Soluble Starch	:	10.0g
Agar	:	20g
Seawater	:	1000ml
pH	:	7.2$\pm$2

Flooded the plate with Gram's Iodine solution. The Iodine reacts with starch to give a deep blue coloration. Presence of amylase was visualized by clear zone around the culture due to starch hydrolysis as shown in Fig. 5.1a.

ii) **Gelatin Hydrolysis Test** (Smibert and Kreig, 1994)

Composition of the medium (g l^{-1})

Peptone	:	5.0g
Beef extract	:	3.0g

Gelatin crystals	:	10.0g
Agar	:	20g
Seawater	:	1000ml
pH	:	7.2±2

Flooded the plate with a solution of 15% Mercuric Chloride in 20% HCl. Presence of gelatinase was visualized by clear zone around the culture due to gelatin hydrolysis as shown in Fig. 5.1b.

iii) **Lipid Hydrolysis Test** (Sierra, 1957)

Peptone	:	5.0g
Beef extract	:	3.0g
Tributyrin	:	10ml
Agar	:	20g
Seawater	:	1000ml
pH	:	7.2±2

Presence of lipase was visualized by a clear zone around the culture due to lipid hydrolysis as shown in Fig. 5.1c.

iv) DNA Hydrolysis Test

Composition of DNase medium (g l^{-1})

Peptone	:	5.0g
Beef extract	:	3.0g
DNA sodium salt	:	2.0g

Agar	:	20g
Seawater	:	1000ml
pH	:	7.2±2

Flooded the plate with 1N HCl. Hydrochloric acid reacts with DNA to give a cloudy precipitate. Clearing around the colonies indicated DNase activity.

v) Chitin Hydrolysis Test

Peptone	:	5.0g
Beef extract	:	3.0g
Colloidal chitin	:	10.0g
Agar	:	20g
Seawater	:	1000ml
pH	:	7.2±2

Colloidal Chitin was prepared as reported by Lingappa and Lockwood (1962). Presence of chitinase was visualized by a clear zone around the culture due to chitin hydrolysis as shown in Fig. 5.1d.

5.3. RESULTS

5.3.1. Extracellular Hydrolytic Enzyme Production

The percentage of various extracellular hydrolytic enzyme production from different depth ranges during both the sampling periods are presented in Appendix Table 5.A1. and Fig. 5.2.

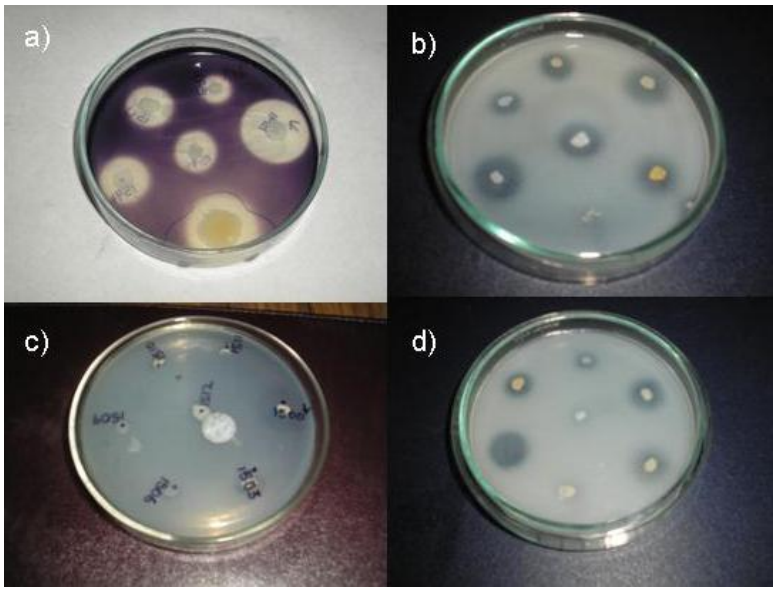


Fig. 5.1. Hydrolytic enzyme production by heterotrophic bacteria showing halo zone around colonies a) Amylase b) Gelatinase c) Lipase and d) Chitinase

i) **Sampling - I (Cruise No. 228&233)**

Over 50% of the isolated strains in 200m depth region showed the capability to hydrolyse gelatin and lipid. The other groups were amylase (33%), DNase (19%) and chitinase (5%) producers. The isolates from 500m depth region showed that gelatinolyitc (57%) and lipolytic (48%) forms were the predominant types in relation to amylase (41%) and DNase (25%) producers. Few chitinolytic forms in this region were also recorded (10%). The 1000m depth region constituted a high percentage of gelatinolyitc (64%) forms followed by lipolytic (56%), amylolytic (39%), DNase producing (20%) and chitinolytic forms (5%). In general, majority of the cultured isolates during this sampling showed high percentage of gelatinolytic and lipolytic forms at all depth zones. The occurrence of amylase and DNase producers were also remarkable. The least abundant forms were chitin hydrolyzing bacteria which recorded >10% in all depth regions.

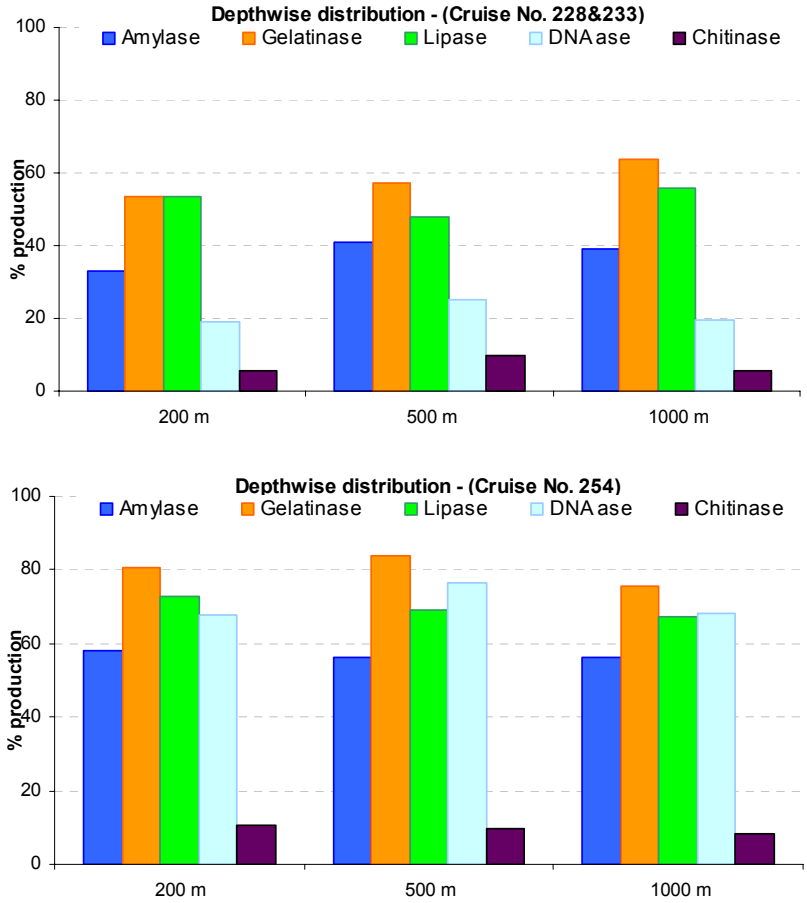


Fig. 5.2 Hydrolytic enzyme production of heterotrophic bacteria (Mean) in different depth ranges - Sampling I (Cruise No. 228& 233) and sampling II (Cruise No. 254)

A decreasing trend was observed in the order Gelatinase > Lipase > Amylase > DNase > Chitinase in case of these hydrolytic enzyme producing bacteria**.** Latitudinal distribution of various extracellular enzyme producing bacteria in different depth ranges isolated during Sampling I and Sampling II are presented in Fig. 5.3a and Fig. 5.3b repectively.

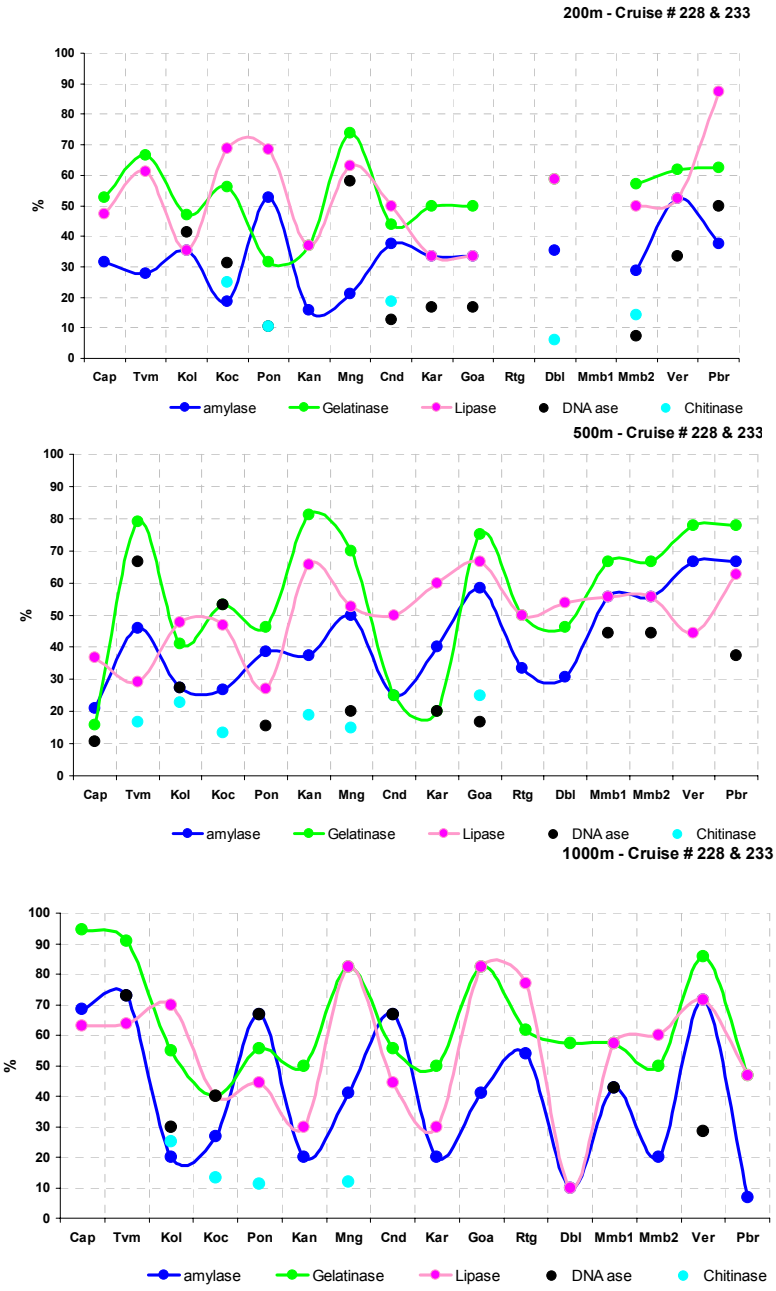


Figure 5.3a. Distribution of various extracellular enzyme producing bacteria at different depth ranges in the slope sediments of Arabian Sea during Sampling I (Cruise Nos. 228&233)

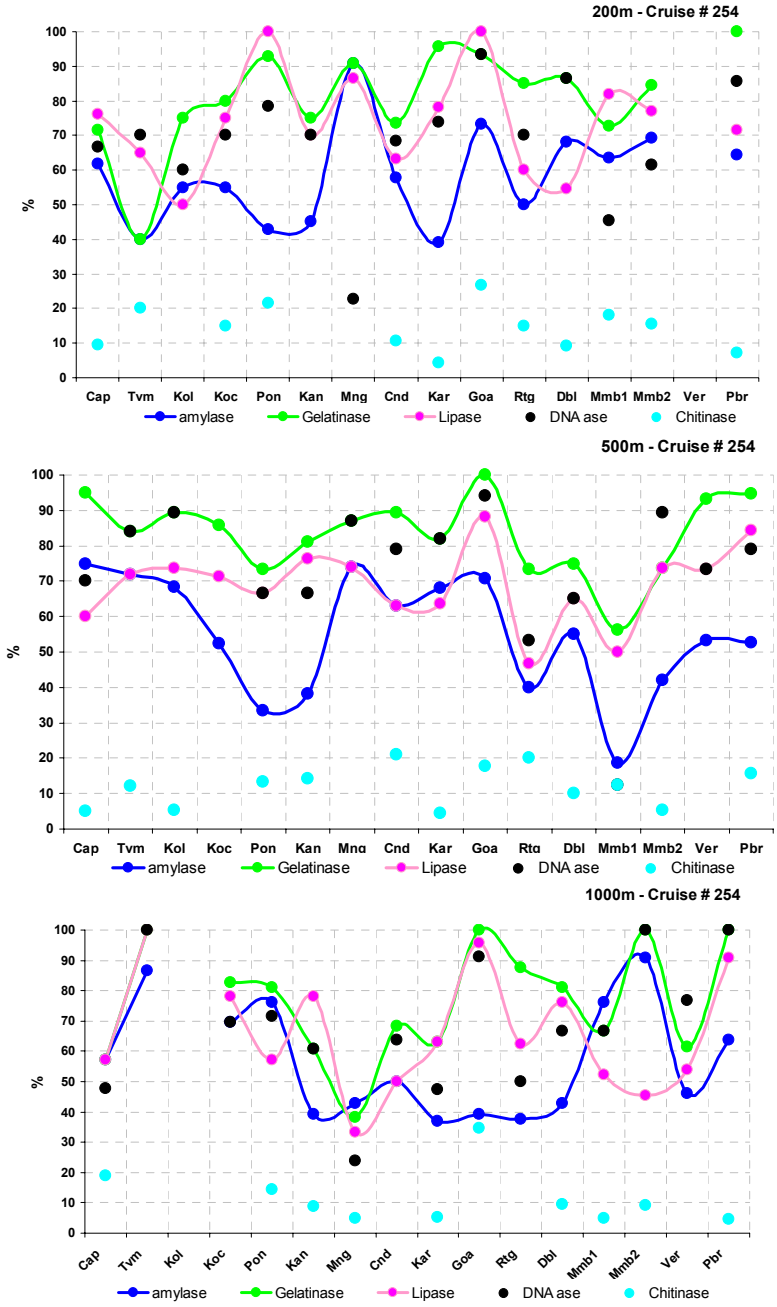


Figure 5.3b. Distribution of various extracellular enzyme producing bacteria at different depth ranges in the slope sediments of Arabian Sea during Sampling II (Cruise No. 254).

ii) Sampling - II (Cruise No. 254)

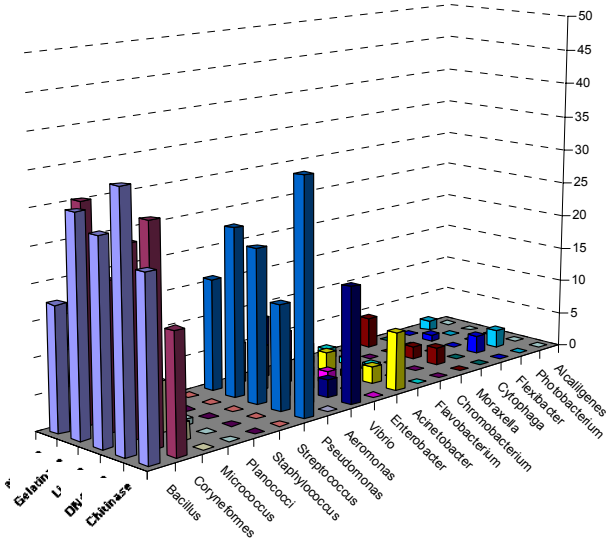
During Sampling II, the heterotrophic bacteria isolated from 200m depth range showed considerably high percentage of amylase, gelatinase, lipase and DNase producers with the mean value recorded as 58, 81, 73 and 68% respectively. As recorded in the Sampling I period, the chitinolytic forms were remarkably less (11%) compared to the other forms. Gelatinolytic forms recorded highest in all the depth regions constituting a mean value of 81, 84 and 75% at 200, 500 and 1000m depth zones respectively. At 200m depth region, lipase (73%) producing forms were highest followed by DNase (68%) and amylase (58%) producers. At 500 m depth range, DNase (76%) producers were maximum followed by lipase (69%), amylase (56%) and chitinase (9%) producing forms. At 1000m depth range DNase (68%) producers were maximum followed by lipase (67%), amylase (56%) and chitinase (8%).

5.3.2. Heterotrophic Bacteria involved in Hydrolytic enzyme production

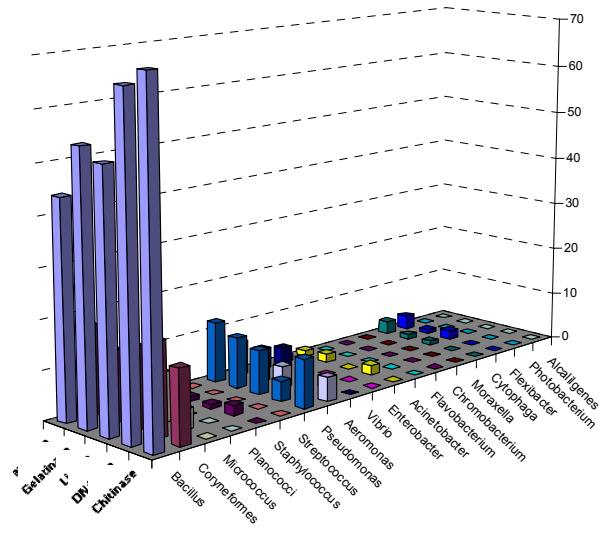
The overall percentage contribution of various genera to the extracellular hydrolytic enzyme production during different sampling periods are presented in Appendix Table 5.A2.

Generic wise contribution of major bacterial groups has shown that at 200m depth range *Bacillus* and Coryneforms together constituted over 50% for the hydrolytic forms (amylase, gelatinase, lipase, DNase). The percentage contribution of various bacterial genera to the extracellular hydrolytic enzyme production during Sampling I is presented in Fig. 5.4. The second major contributor was *Pseudomonas* exhibiting 16, 24, 22 and 15% for amylase, gelatinase, lipase and DNase. The other major contributors for amylase production were *Vibrio* (9%), *Aeromonas* and Enterobacteriaceae (6%). *Pseudomonas* formed the major group for chitinase production in 200m depth range contributing 33% of the chitinolytic forms. The second major contributor being *Bacillus* with 25%. The next major groups were Coryneforms and *Vibrio* forming 17% of chitinase producers at 200m depth range.

At 500m depth range, *Bacillus* was the predominant group involved in the hydrolytic degradation of all the major macromolecules. Its contribution to amylase, gelatinase, lipase, DNase, and chitinase production were 44, 54, 52, 67 and 70%. The second predominant contributor was Coryneforms comprising 14-20%. The third major group was genus *Pseudomonas* constituting 9-12%.



Genericwise contribution- Enzyme 200m (Cr# 228& 233)



Genericwise contribution- Enzyme 500m (Cr# 228 & 233)

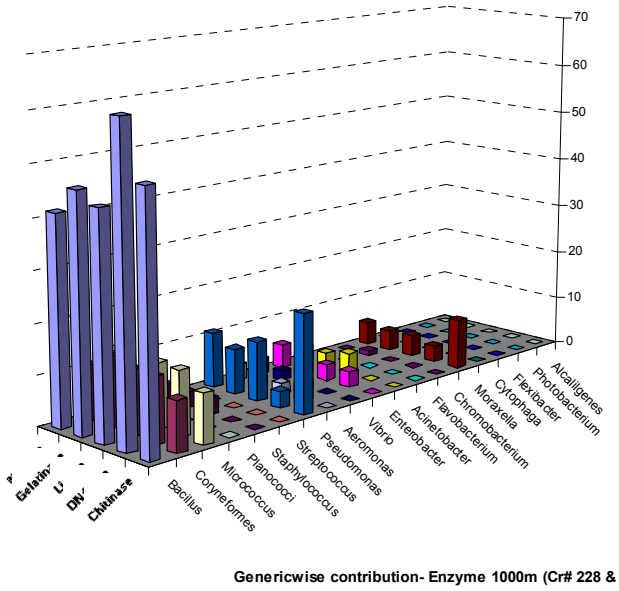


Figure 5.4. Contribution of different bacterial genera isolated from the slope sediments of Arabian Sea towards the production of various extracellular hydrolytic enzymes (Sampling I).

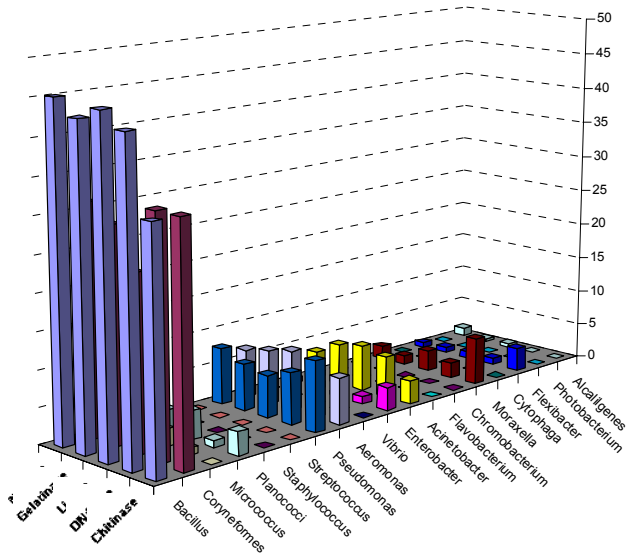
In the 1000m depth range also *Bacillus* was the predominant genus in terms of hydrolytic potential followed by *Pseudomonas, Micrococcus* and Coryneforms.

The ability of bacteria isolated during Sampling II (Cruise No. 254) to decompose macromolecular organic compounds revealed that in 200m depth ranges over 43-45% of amylase, gelatinase, lipase, and DNase were contributed by genus *Bacillus*. The percentage contribution of various bacterial genera to the extracellular hydrolytic enzyme production (Sampling II) is presented in Fig. 5.5.The second major contributor was Coryneforms contributing nearly 29-32% of the enzyme production (amylase, gelatinase, DNase and chitinase). However, their contribution to Amylase production accounted to 24%. The other major contributors were *Pseudomonas* and *Aeromonas* comprising 6-8% of the total producers for all the enzymes. The Genus *Acinetobacter* also contributed to nearly 4-6% of hydrolytic enzyme production. Chitin degradation was chiefly contributed by *Bacillus* and Coryneforms accounting

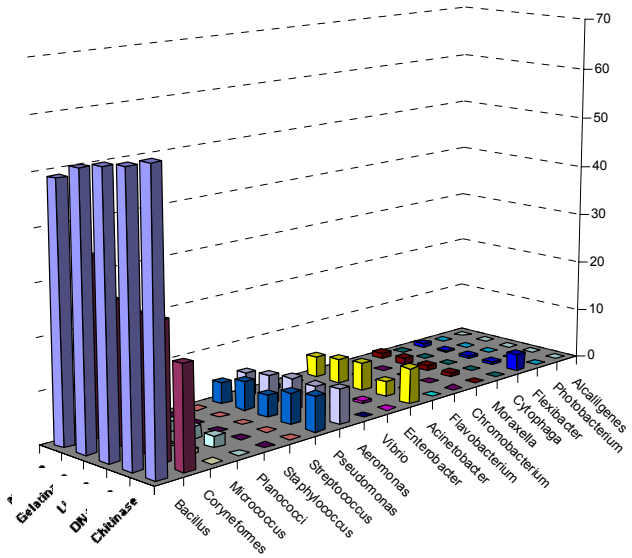
to 32% each. While *Pseudomonas* contributed to 10% of the chitinoclastic forms, *Aeromonas* and *Moraxella* recorded 6% each.

In the 500m depth range also group *Bacillus* predominated over the other forms in the production capability of the hydrolytic enzymes ranging between 49-55% for all the macromolecules. The second major group involved in the production capability of these hydrolytic enzymes were Coryneforms accounting to 34% of the total producers of amylase, and between 20-27% for the rest of the enzymes. *Pseudomonas* and *Aeromonas* were the other major enzyme producers at this depth. The lesser degree of contribution were also met from genus *Acinetobacter*. In the case of chitin degradation *Pseudomonas, Aeromonas, Acientobacter* and *Flexibacter* were the major genera involved.

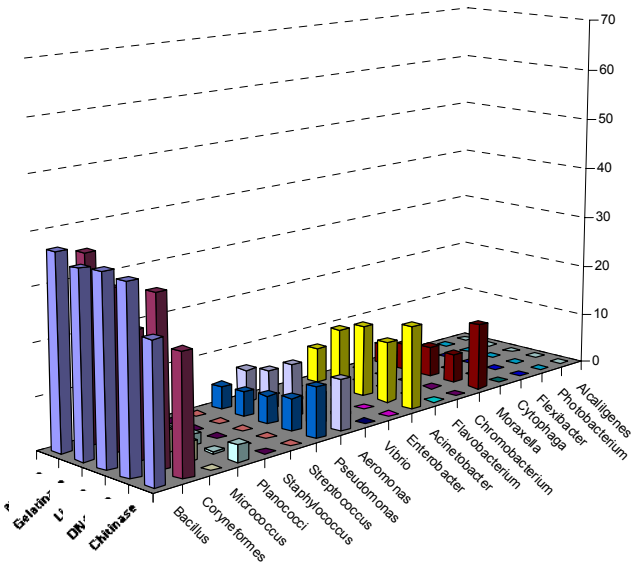
At 1000m depth range nearly 50-70% of the hydrolytic forms constituted *Bacillus* and Coryneform groups, the former being the predominant group. *Acinetobacter, Aeromonas Pseudomonas, Micrococcus* and *Moraxella* formed other major contributors for the extracellular enzyme production.



Genericwise contribution- Enzyme 200m (Cr# 254)



Genericwise contribution- Enzyme 500m (Cr# 254)



Genericwise contribution- Enzyme 1000m (Cr# 254)

Figure 5.5. Contribution of different bacterial genera isolated during Sampling II towards the production of various extracellular hydrolytic enzymes.

5.4. DISCUSSION

Deep sea sediments constitute the largest compartment of the global biosphere. Most of the oceanic sedimentary mineralization occurs over the continental margin, one of the most important boundaries on Earth (Walsh, 1991) where most of the organic matter breakdown is carried out by microorganisms, mainly by bacteria (Jedrezejczak, 1999). The production and secretion of extracellular hydrolytic enzymes in deep sea sedimentary environment may have important biogeochemical implications, especially in organic biopolymer compound degradation, nutrient recycling and biogeoelements mobilization. In the continental slope bacteria showing the capacity to decompose lipids, proteins and DNA were the most numerous. Large quantities of those physiological groups of bacteria in waters and sediment marine basins were also observed by Hashimoto *et al.* (1983) and Mudryk and Donderski (1997).

According to Boetius (1995), production and activity of bacterial hydrolytic enzymes depend on the availability, distribution and concentration of organic substrates. Therefore, activity of enzymes in horizontal profiles can be expected to mirror the distribution of organic matter in water basin sediments. Results of the present study do not indicate clear differences in the level of potential activity of bacterial enzymes among different parts of the study area. According to Martinez *et al.* (1996) this might indicate no horizontal shifts in both the availability and degradability of organic compounds in the deep sea sites. The potential enzymatic activity of bacterial strains may not be identical to bacterial activity in natural environments. The results obtained in the present study and their ecological interpretation can, however, be an important source of information on the potential activity of bacterial enzymes in the process of transformation of organic matter in marine ecosystems.

For heterotrophic bacteria lipids are very important source of carbon and energy (Arts *et al.,* 1992), which explains why bacteria capable of hydrolyzing

lipids constitute one of the most numerous physiological groups in aquatic ecosystems. Lipolytic bacteria have been shown to play a key role in the processes of modifying and transforming lipid compounds in water basins (Gajewski *et al.,* 1997). In aquatic ecosystems, lipid compounds constitute 3 to 55% of all organic matter (Marty *et al.,* 1996); considerable amounts (i.e. 2-45%) of lipids are accumulated by ciliates, zooplankton, phytoplankton, benthos and detritus (Albers *et al.,* 1996; Harvey *et al.,* 1997). Previous study conducted by Schulte *et al.* (2000) has shown that the sediments within the oxygen minimum zone in the Arabian Sea are enriched with bacterial and terrestrial fatty acids. Thus the production of lipase by the bacterial isolates reflect the high Lipase activity in the sediments (Middelboe *et al.,* 1995). Lipolytic bacteria accounted for >60 % of all the studied bacteria. High lipolytic activity were also recorded in the Baltic sea sediments by Bolter and Reinheimer (1987) and Mudryk (1998). In the Norwegian Sea, Adriatic Sea, Atlantic Ocean and Arctic Ocean, they constituted 60 to 100% of the total viable counts (Krstulovic and Solic, 1988).

Gelatin decomposing bacteria were the second most abundant group of the strains under investigation. Bacteria capable of hydrolyzing proteins predominated in the water basins as well, accounting to 70-100% of the total bacterial number (Hashimoto *et al.,* 1983; Krstulovic and Solic, 1988). According to Little *et al.* (1979), this can be explained by the fact that the main components of organic matter in water bodies are lipids, proteins, polypeptides, peptides and amino acids. Extracts and dead remains of the phytoplankton, zooplankton and benthos are their basic sources (Billen and Fontigny, 1987). Besides, microbial degradation of extracellular DNA in deep sea ecosystem may provide another suitable source of carbon and nitrogen for sediment prokaryote metabolism (Jorgensen *et al.,* 1993; Dell Anno and Danavaro, 2005). Nucleic acids are present in all living organisms and are released from all decaying plants and animals. Hence, they occur in aquatic basins in relatively high concentrations. Marine bacteria are able to synthesize

extracellular deoxyribonucleases, catalyzing the process of DNA decomposition (Paul *et al.,* 1988). Occurrence of this physiological group of bacteria in relatively high numbers in coastal marine ecosystems Off Florida was determined by Jorgensen *et al.* (1993) and also through the studies of Mudryk (1998) in the sub surface layers of Baltic Sea.

Mudryk (1991) have demonstrated that bacteria capable of chitin and cellulose hydrolysis were generally rare in the sand of the Sopot beach. This is probably related to a high content of very diverse food substances, many of which can be metabolized more easily and more readily than chitin or cellulose. Additionally, Munster and Chrost (1990) drew attention to the fact that microbial depolymerization of chitin and cellulose requires synergistic activity of many hydrolytic enzymes, which are produced mainly by fungi and actinomycetes. The intensity of decomposition of macromolecular organic compounds in water basins is usually determined not only by the number of microorganisms capable of carrying out the depolymerisation, but also by the activity of their extracellular enzymes (Fabiano and Donovaro, 1998; Mudryk and Skorczewski, 2000). Earlier studies (Lamy *et al.,* 1999; Patil *et al.,* 2001) indicate that heterotrophic bacteria inhabiting water basins are capable of synthesizing many hydrolytic enzymes, including lipases, proteases and phosphatases. Lipolytic enzymes (esterase, lipase), proteases (leucine acrylaminidase) and phosphatases (alkaline phosphatase) were most active.

Most of the oceanic sedimentary mineralization occurs over the continental margin, one of the most important boundaries on earth (Walsh, 1991), where bacteria are the major players for the organic matter mineralization process. Besides to be low nutrient and cold adaptive, the deep sea sediment strains represent diverse ecophysiology in culture and might play various ecological and geo microbiological roles *in-situ*. Microbial extracellular hydrolytic enzymes are the major biological mechanism for the decomposition of sedimentary particulate

organic carbon and nitrogen. Our study showed that diverse and abundant bacterial isolates could secrete at least one of the extracellular enzymes screened, indicating that the indigenous microbiota have developed the genetic and physiological adaptivity for utilizing the high content of particulate organic matter in deep sea sediments *via.* exoenzyme production. Some strains even produced all the extracellular hydrolytic enzymes screened. The major source of chitin in the deep sea sediments may be dead marine planktonic crustaceans exported from the water column. Lesser percentage of chitinolytic forms and the prevalence of other extracellular hydrolytic enzyme activities of our bacterial isolates indicate that the terrestrial export of the particulate organic matter may be the major source of the biopolymers buried in the deep sea sediments. Diverse extracellular hydrolytic enzymes, including chitinase have been isolated from the deep seafloor organics and methane rich sediments off Shimokita Peninsula (Kobayashi *et al.,* 2008). The microbial ecophysiology may present a good bioindicator of the terrestrial impact on the marine benthic microbial ecosystem in the Arabian Sea deep sea environment. The diverse extracellular enzymes detected in the current study might also provide a resource for novel biocatalysts discovery and application, especially for low temperature conditions. The metabolic potential rather than the *in situ* activity of the isolates were determined since *in situ* measurements are only representative of the conditions at the time of sampling. Assessment of the potential metabolic capacity, therefore provides a better picture of the ecological roles of the organisms.

Chapter 6

MOLECULAR AND PHYLOGENETIC CHARACTERIZATION OF *BACILLUS* SPP. ISOLATED FROM THE SLOPE SEDIMENTS OF ARABIAN SEA

Contents

6.1. INTRODUCTION

A long-standing challenge in the field of microbial ecology and evolution has been the matter of defining the diversity and distribution of natural microbial communities in marine sediments. About 95% of the organic matter produced photosynthetically in surface waters appears to be recycled in the upper 100-300m (Jannasch and Taylor, 1984), while only about 1% of the organic carbon reaches the deep-sea floor. Therefore, it is evident that the deep-sea environment is a microbial habitat with a relatively low input of organic carbon. However, deep-sea sediments may be unique habitats for microbial communities; the availability of nutrients is highly variable and these environments are subject to highly elevated pressures.

Marine bacterioplankton represent one of the most thoroughly studied environmental communities on the planet yet bacteria inhabiting marine sediments remain largely uncharacterized. One apparent yet relatively unexplored difference between seawater and sediment bacterial communities is the relative abundance of Gram-positive bacteria. While early research estimated that only 5% of the bacteria in the ocean are Gram-positive (ZoBell, 1946), more recent studies suggest that the

abundance and diversity of Gram-positive strains in sediments may be considerably greater (Priest, 1989; Jensen *et al.,* 2005; Stach *et al.,* 2005). While the most thoroughly studied Gram-positive bacteria include soil-derived antibiotic-producing actinomycetes (Berdy, 2005) relatively little is known about the diversity and distribution of Gram-positive bacteria in the marine environment. This lack of information persists despite the fact that Gram-positive bacteria have been cultured from the ocean for decades (Bonde, 1981; Jensen and Fenical, 1995; Maldonado *et al.,* 2005a) and consistently appear in culture-independent studies (Suzuki *et al.,* 2004; Venter *et al.,* 2004).

Gram-positive bacteria are likely to play important microbiological roles in the marine environment, yet without a fundamental understanding of their diversity and ecophysiology, it is difficult to assess the ecological significance of this relatively overlooked component of the marine bacterial community. Although Gram-positive bacteria have been cultivated from seawater, marine invertebrates, and other sample types (Han *et al.,* 2003; Hill, 2004; Montalvo *et al.,* 2005), marine sediments including deep-sea sediments are the primary oceanic habitat from which they have been recovered (Mincer *et al.,* 2002; Jensen *et al.,* 2005). While it is probable that some marine-derived Gram-positive bacteria are terrigenous microorganisms, washed or blown into the marine environment, species occurring exclusively in the sea have been described (Han *et al.,* 2003; Yi *et al.,* 2004). The recovery of Gram-positive bacteria that require seawater for growth, including several *Bacillus* species (Imada *et al.,* 1998; Ruger *et al.,* 2000; Gugliandolo *et al.,* 2003) and the recently described actinomycete genus *Salinospora* (Maldonado *et al.,* 2005b), suggests that additional, obligate marine taxa reside in marine sediments. These results indicate that considerably diverse Gram-positive microbial population can be cultured from marine sediments and reinforces the concept that relatively simple cultivation techniques can be used successfully to isolate many yet undescribed taxa (Connon and Giovannoni, 2002; Jansen *et al.,* 2002 and Maldonado *et al.,* 2005b).

Members of the genus *Bacillus* and related genera are ubiquitous in nature. However, *Bacillus* species isolated from marine sediments have attracted less interest compared to their terrestrial relatives. The genus *Bacillus* is one of the well-known genera of the Gram-positive *Firmicutes* low G+C phylum. It encompasses rod-shaped bacteria capable of aerobically forming resistant endospores which make them ubiquitous in the environment. They have been isolated from terrestrial and freshwater habitats and are widely distributed in the world oceans (Slepecky and Hemphill, 2006). *Bacillus* spp. isolated from marine environs are not, in reality, known to display specific traits. Indeed, the ubiquity of the genus members, might explain the fact that they could survive under diverse conditions such as seawater and sediment. Even halotolerance, a property thought to be characteristic of marine bacteria, is not exclusive and the majority of marine *Bacillus* do not depend on seawater containing media for their growth. More recent reports describing the isolation of *Bacillus* requiring seawater for their growth (Ruger *et al.,* 2000), readdress the question on the existence of obligate marine *Bacillus* species.

While the majority of the identified *Bacillus* species are rather terrestrial bacteria blown or deposited as spores into marine sediments, some *Bacillus* species such as those of the *B. firmus/ B. foraminis* and the halotolerant groups, without being obligate marine bacteria, seemed to be adapted in such environment which is in agreement with recent findings (Sass *et al.,* 2008). Indeed, they may be implicated, even as active spores as reported previously (Dick *et al.,* 2006), in the biogeochemical cycles and diverse degradation process. Moreover, a careful physiological, metabolic and enzymatic characterization should be carried out in order to assess the real biotechnological potential of these bacteria.

The genus *Bacillus* comprised a phylogenetically and phenotypically heterogeneous group. Recently, the systematics of the *Bacillus* group has been widely modified. On the basis of extensive studies of the small-subunit ribosomal

RNA sequences, the species of the genus *Bacillus* were split into five distinct clusters and several ungrouped species, such as: group 1 (*Bacillus* sensu stricto), which includes *B. subtilis*, the type species of the genus, and 27 other species (Ash *et al.,* 1991); group 2 includes the round-spore-forming bacilli, together with some asporogenous taxa (the genera *Caryophanon, Exiguobacterium* and *Kurthia*); the group constitutes a distinct cluster, only remotely related to *B. subtilis* (Farrow *et al.,* 1994); group 3, with ten representatives, comprises *B. polymyxa* and *B. macerans*, which have been reclassified in the new genus *Paenibacillus* (Ash *et al.,* 1993) and group 4, with strains classified into two newly created genera, *Aneurinibacillus* and *Brevibacillus* (Shida *et al.,* 1996). Besides, a new genus, *Virgibacillus,* was recently created to accommodate former *B. pantothenticus* (Heyndrickx *et al.,* 1998). Finally, several newly isolated *Bacillus* species have been described, including *B. mojavensis* and *B. vallismortis* (Roberts *et al.,* 1994; 1996), *B. ehimensis* and *B. chitinolyticus* (Kuroshima *et al.,* 1996), *B. infernos* (Boone *et al.,* 1995), *B. carboniphilus*, (Fujita *et al.,* 1996), and *B. horti* (Yumoto *et al.,* 1998).

Few works are devoted to the study of the *Bacillus* species isolated from the marine environment. Due to their ubiquity and capability to survive under adverse conditions, heterotrophic *Bacillus* strains are hardly considered to be species of certain habitats (Claus and Berkeley, 1986). A heterogeneous group of moderately halophilic bacteria, which comprises *B. salexigens,* and three species of the new genus *Halobacillus*, *H. halophilus, H. litoralis*, and *H. trueperi* (Garabito *et al.,* 1997) may be differentiated by their ability to grow at 10 to 20% of total salts and the possession of an unusual type of murein. Species of *B. marinus, B. badius B. subtilis, B. cereus, B. licheniformis, B. firmus,* and *B. lentus* were often isolated from marine habitats (Claus and Berkeley, 1986; Ortigosa *et al.,* 1997). Studies on marine *Bacillus* strains showed that strains of *B. marinus, B. subtilis, B. pumilus, B. licheniformis, B. cereus,* and *B. mycoides* are common inhabitants of the Pacific Ocean habitat (Ivanova *et al.,* 1992).

The systematics of the genus was subjected to many revisions and it was based on phenotypical approaches, mainly, morphological, physiological and biochemical properties (Smith *et al.,* 1952), spore shape and sporangium swelling (Gordon *et al.,* 1973), fatty acid composition and enzyme patterns (Baptist *et al.,* 1978). Molecular characterization mediating DNA-DNA reassociation (Priest *et. al.,* 1981) and DNA base composition (Fahmy *et al.,* 1985) have been also applied which led to the proliferation of the species number within the genus. The real reorganization of the *Bacillus* taxonomy has occurred when Ash *et al.* (1991) used the 16S small-subunit ribosomal RNA gene sequences allowing the definition of 51 species scattered in 5 distinct phylogenetic groups. rRNA group 1 represent the most homogeneous group and includes *B. subtilis*, the type strain of the genus, and *B. cereus*. Based on multiphasic approaches, the other rRNA groups have been then redefined as separate *Bacillus* derived genera such as *Paenibacillus*, *Halobacillus*, *Aneurinibacillus*, *Brevibacillus* (Shida *et al.,* 1996), *Virgibacillus* (*et al.,* 1998), *Gracilibacillus* and *Salibacillus* (Waino *et al.,* 1999).

In its original classification, the genus *Bacillus* contained a heterogeneous assembly of aerobic, or facultatively anaerobic, Gram-positive, rod-shaped, spore-forming bacteria widely distributed in the environment. Traditionally, *Bacillus* spp. are identified in the laboratory by biochemical tests and fatty acid methyl ester (FAME) profiling (Vaerewijck *et al.,* 2001). Alternatively, the API (Analytab Products, Inc.) system of identification has been shown to be more reproducible than classical methods (Logan and Berkeley, 1984) and is capable of speciating *Bacillus* strains using a combination of the 12 tests in the API 20E strip, and 49 tests in the API 50CHB strips. These phenotyping protocols are laborious and time-consuming to undertake and cannot provide a rapid screening system (Wattiau *et al.,* 2001). The shortcomings of phenotypically based identification methods have led to the development of molecular alternatives based on the microbial genotype or DNA sequence. This approach minimizes problems associated with typability

and reproducibility, and importantly, facilitates the assembly of large reference databases.

In recent years, the use of molecular techniques has greatly changed the original taxonomic classification of the *Bacillus* taxa. Comparisons of the 16S rRNA gene sequence is one of the most powerful tools for the classification of microorganisms (Woese, 1987; Wang *et al.,* 2003) and have provided sequence specific primers as good standards for the identification of pure cultures of *Bacillus* species such as *B. subtilis* (Wattiau *et al.,* 2001), *B. cereus* and *B. thuringiensis* (Hansen *et al.,* 2001) and *Paenibacillus alvei* (formerly *Bacillus alvei*) (Djordjevic *et al.,* 2000). However, environmental samples such as those from sewage, water, soil, faeces and even beehives, usually contain mixtures of *Bacillus* species. The presence of such combinations can be better detected with the use of a group-specific primer that distinguishes as many member species as possible within the genus. Individual species representative of different *Bacillus* species can then be characterized by subjecting the amplicons to restriction enzyme digestion. Although genus-specific primers have been successfully developed for Lactobacilli (Dubernet *et al.,* 2002), Mycoplasmas (Van Kuppeveld *et al.,* 1992), *Bifidobacterium* (Matsuki *et al.,* 1999), Pandoraea (Coenye *et al.,* 2001) and *Clostridium* (Van Dyke and McCarthy, 2002), a group-specific primer pair capable of amplifying a specific sequence of 16S rDNA from all *Bacillus* taxa has not been developed in accompaniment with restriction digest mapping.

The recent development of molecular biology techniques, which do not rely on cultivation methods, allows microbial ecologists to reveal inhabitants of natural microbial communities which have not yet been cultured (Pace, 1996; Hugenholtz *et al.,* 1998; Snaidr *et al.,* 1998). As a result, these techniques are now widely applied to characterize microbial community structures in different environments such as biological wastewater systems (Holben *et al.,* 1998, Snaidr *et al.,* 1998). Two of these techniques, cloning and sequencing, allow us to determine which

microorganisms are present in the community, but they are time-consuming. Hybridization and probing are faster, but require a sufficient knowledge of the community to choose the appropriate target sequences (Amann, 1995). In this study, another molecular biology technique, the Amplified Ribosomal DNA Restriction Analysis (ARDRA), is applied. Even faster than hybridization and probing, ARDRA has been used in the analysis of mixed bacterial populations from different environments (Martínez *et al.,* 1995; Acinas *et al.,* 1997). Although ARDRA gives little or no information about the type of microorganisms present in the sample, it can be used for a quick assessment of genotypic changes in the community over time, or to compare communities subject to different environmental conditions.

It is now widely accepted that the ribosomal RNA (rRNA) molecules and/or their respective genes can be used as molecular chronometers (Woese, 1987). Particularly 16S rRNA gene or rDNA sequences have been used in phylogenetic studies of prokaryotes. They contain highly conserved regions because of their crucial structural and functional constraints but also highly variable signatures. Conserved regions proximal to the 5' and 3' termini are found in all prokaryotic 16S rRNA gene sequences and are therefore used as primer sequences in polymerase chain reactions (PCR) to amplify almost the entire gene (Weisburg *et al.,* 1991). The most straight forward method to determine phylogenetic relationships between organisms concern the comparison of primary sequences of 16S rRNA or rDNA by numerical methods. An alternative approach to search for phylogenetic and taxonomic information enclosed in the SSU molecule comprises restriction enzyme studies, either by mapping the restriction sites in the 16S rRNA gene, or by estimating the proportion of shared DNA fragments in gel electrophoretic patterns obtained after restriction digestion of the 16S rDNA amplicons. The latter method is more appropriate for studying large sets of strains. The mathematical relationship between the number of shared restriction sites or of shared DNA fragments and genetic divergence between strains was

already discussed by Nei and Li (1979). However, it is only in the last few years that studies on amplified 16S rDNA restriction analysis have been performed on a variety of organisms such as *Clostridium* (Gurtler *et al.,* 1991), *Streptococcus* (Jayaro *et al.,* 1991), *Mycobacterium* (Vaneechoutte *et al.,* 1993), *Moraxella* (Jannes *et al.,* 1993), *Leptospira* (Ralph *et al.,* 1993), *Rhizobium* (Laguerre *et al.,* 1994), *Brevibacterium* (Carlotti and Funke, 1994), *Acinetobacter* (Vaneechoutte *et al.,* 1995), *Xanthomonas, Stenotrophomonas* (Nesme *et al.,* 1995) and *Capnocytophaga* (Wilson *et al.,* 1995). Most studies deal with rapid identification at the species level using a small number of enzymes and by a visual interpretation of the restriction patterns. In a few attempts, a so-called phylogenetic clustering of a (limited) set of strains with parsimony analysis (Gurtler *et al.,* 1991; Ralph *et al.,* 1993) or genetic distance analysis (Laguerre *et al.,* 1994; Wilson *et al.,* 1995) based on the visual comparison of the restriction patterns, was performed. In the latter three studies, as many as 9-10 different restriction enzymes were used to generate an equal number of profiles, rendering this approach again laborious and complex.

Amplified ribosomal DNA restriction analysis (ARDRA) is another simple method based on restriction endonuclease digestion of the amplified bacterial 16S rDNA. In that study, a universal bacterial 16S rDNA primer set was used to amplify a 1500-bp amplicon, followed by construction of ARDRA patterns using 5 restriction enzymes. The obtained ARDRA patterns for each species were combined to form a database for strain identification in each genus.

The suitability of ARDRA for clustering the strains as a first identification step for members of the genus *Bacillus* and related genera has been reported previously (Shaver, 2002; Cherif *et al.,* 2003). ARDRA technique based on the combination of five selected restriction enzymes is reliable and valuable for phylogenetic and taxonomic studies of large sets of strains. Heyndrickx and colleagues have developed an ARDRA method for identification of strains of the

genera *Alcaligenes*, *Bordetella*, *Bacillus* and *Paenibacillus* (Heyndrickx *et al.,* 1996). Heyndrickx also tested different combinations of restriction enzymes in the ARDRA assay and found that 3 enzymes yielded similar results in terms of species identification as did by 5 enzymes. Some authors have suggested that two restriction enzymes are adequate for ARDRA, especially in analyzing species composition changes in complex communities (Gich *et al.,* 2000) In the present study, the ARDRA assay using universal eubacterial primers and two restriction enzymes, *Alu*I and *Taq*I, were employed in differentiating most *Bacillus* strains.

Although culture dependent approaches also have well-known biases these methods may prove to be the most effective way to detect certain groups of marine bacteria. In addition, cultured strains can be subjected to taxonomic characterization, and their physiology, ecology, and biotechnological potential can be explored. Marine microorganisms have only recently become a target for natural product drug discovery, it has become increasingly clear that Gram-positive strains are a rich source of new structures that possess promising antimicrobial and anticancer activities (Bernan *et al.,* 2004) and that a better understanding of microbial diversity will provide important insight into how to devise intelligent strategies for natural product discovery (Bull, 2004). In the last decade, an increasing interest is observed for the Gram-positive spore-forming bacteria of the *Bacillales* order from marine environments. This is due to their biotechnological potential, with respect to their terrigenous relatives, for the production of new bioactive substances like enzymes and antibiotics.

The present study is aimed at understanding the diversity of *Bacillus* species in the marine environment and their phylogenetic relationship with other *Bacillus* species reported from marine as well as terrestrial systems.

6.2. MATERIALS AND METHODS

6.2.1. Bacterial Strains Used for the Study

Gram-positive spore forming bacilli (*Bacillus* spp.) isolated from the slope sediments of Arabian Sea (200-1000m depth) during the FORV *Sagar Sampada* cruises (Cruises 228, 233 and 254) were used for the study. Seventy two isolates were selected for the study. From each depth range the isolates were segregated to represent the various forms in terms of colony appearance (Colony shape, color and texture). Twenty eight (28) isolates were taken from 200m depth range, 21 isolates from 500m depth range and 23 isolates from 1000m depth stations. These isolates were streaked on nutrient agar plates for checking purity and transferred to nutrient agar vials for the study. Details of the bacterial strains used in the study are summarised in Table 6.1.

Table 6.1. Phenotypic characteristics of the *Bacillus* spp. used for the study

				Colony characterestics		Spore Morphology			
Sl. No.	Strain Code	Culture No	Depth	Shape	Size	Spore	Shape	Position	Sporangia
1	A1	607	200 m	Irregular	Large	+	Oval	ST	NS
2	A3	848	200 m	Circular	Small	+	Oval	ST	S
3	A5	970	200 m	Circular	Medium	+	Oval	ST	S
4	AF	856	200 m	Circular	Small	+	Oval	C	S
5	A10	849	200 m	Circular	Small	+	Oval	ST	S
6	A12	1040	200 m	Circular	Small	+	Oval	ST	S
7	A14	1130	200 m	Circular	Small	+	Oval	ST	S
8	A26	1553	200 m	Circular	Small	+	Oval	ST	S
9	A27	1549	200 m	Circular	Small	+	Oval	ST	S
10	A29	1564	200 m	Irregular	Large	+	Oval	C	NS
11	A31	1674	200 m	Circular	Medium	+	Oval	T	S
12	A32	1673	200 m	Irregular	Large	+	Oval	C	NS
13	A37	1890	200 m	Circular	Small	+	Oval	ST	S
14	A40	2324	200 m	Irregular	Large	+	Oval	ST	NS
15	A43	1624	200 m	Circular	Small	+	Oval	ST	S
16	A44	1635	200 m	Circular	Small	+	Oval	C	S
17	A46	2446	200 m	Circular	Small	+	Oval	C	S
18	A47	2326	200 m	Circular	Small	+	Oval	C	S
19	A38	2009	200 m	Irregular	Large	+	Oval	ST	S

				Colony characterestics		Spore Morphology			
Sl. No.	Strain Code	Culture No	Depth	Shape	Size	Spore	Shape	Sl. No.	Strain Code
20	A30	1569	200 m	Circular	Small	+	Oval	C	S
21	A34	1745	200 m	Circular	Small	+	Oval	C	S
22	A35	1747	200 m	Irregular	Large	+	Oval	C	NS
23	A39	2039	200 m	Circular	Small	+	Oval	ST	S
24	A42	2463	200 m	Circular	Medium	+	Oval	ST	NS
25	A48	2327	200 m	Irregular	Large	+	Oval	C	NS
26	A49	2332	200 m	Irregular	Large	+	Oval	C	NS
27	Ai	922	200 m	Circular	Small	+	Oval	C	S
28	A33	1740	200 m	Irregular	Large	+	Oval	C	S
29	A9	1031	200 m	Irregular	Medium	+	Oval	C	NS
30	B1	539	500 m	Circular	Small	+	Oval	ST	S
31	B2	647	500 m	Irregular	Large	+	Oval	ST	S
32	B4	807	500 m	Circular	Medium	+	Oval	ST	S
33	B5	814	500 m	Circular	Small	+	Oval	C	S
34	B9	822	500 m	Irregular	Large	+	Oval	C	NS
35	B10	823	500 m	Irregular	Large	+	Oval	ST	S
36	B14	868	500 m	Circular	Small	+	Oval	C	S
37	B15	869	500 m	Circular	Small	+	Oval	ST	S
38	B22	947	500 m	Circular	Medium	+	Oval	ST	NS
39	B17	875	500 m	Circular	Medium	+	Oval	ST	NS
40	B19	886	500 m	Irregular	Medium	+	Oval	C	NS
41	B20	938	500 m	Irregular	Medium	+	Oval	C	NS
42	B31	1656	500 m	Circular	Small	+	Oval	ST	S
43	B37	1923	500 m	Irregular	Medium	+	Oval	C	NS
44	B38	2054	500 m	Irregular	Large	+	Oval	ST	NS
45	B43	2148	500 m	Circular	Small	+	Oval	ST	S
46	B49	2159	500 m	Circular	Small	+	Oval	ST	S
47	B28	1573	500 m	Irregular	Large	+	Oval	ST	S
48	B44	2511	500 m	Circular	Small	+	Oval	ST	S
49	B35	2340	500 m	Irregular	Large	+	Oval	ST	NS
50	B39	2156	500 m	Irregular	Medium	+	Oval	C	NS
51	C1	687	1000m	Circular	Medium	+	Oval	ST	S
52	C3	891	1000m	Circular	Medium	+	Oval	ST	S
53	C4	892	1000m	Circular	Small	+	Oval	C	S
54	C10	895	1000m	Irregular	Large	+	Oval	C	NS
55	C21	1058	1000m	Irregular	Large	+	Oval	C	NS
56	C22	840	1000m	Irregular	Large	+	Oval	ST	S
57	C24	846	1000m	Circular	Small	+	Oval	ST	S
58	C28	1547	1000m	Irregular	Large	+	Oval	ST	S
59	C29	1597	1000m	Irregular	Large	+	Oval	ST	NS

				Colony characterestics		Spore Morphology			
Sl. No.	Strain Code	Culture No	Depth	Shape	Size	Spore	Shape	Sl. No.	Strain Code
60	C30	1606	1000m	Circular	Small	+	Oval	C	S
61	C31	1607	1000m	Circular	Medium	+	Oval	C	S
62	C34	1932	1000m	Irregular	Large	+	Oval	ST	S
63	C37	2178	1000m	Irregular	Large	+	Oval	ST	NS
64	C38	2181	1000m	Circular	Small	+	Oval	ST	S
65	C39	2184	1000m	Irregular	Large	+	Oval	ST	S
66	C41	2172	1000m	Circular	Small	+	Oval	ST	S
67	C42	2173	1000m	Circular	Medium	+	Oval	ST	S
68	C43	2176	1000m	Irregular	Large	+	Oval	ST	S
69	C44	2192	1000m	Irregular	Large	+	Oval	C	NS
70	C45	2194	1000m	Circular	Medium	+	Oval	ST	S
71	C47	2488	1000m	Irregular	Large	+	Oval	C	NS
72	C48	2489	1000m	Circular	Small	+	Oval	ST	S

Small=<0.5 cm; Medium=0.5-1 cm; *Large = >1 cm
Position: ST- sub terminal; C- central; T- terminal; Sporangia : S- swollen; NS- non swollen.

6.2.2. Identification of the Selected *Bacillus* Strains Using 16S rDNA Sequencing

i) Extraction of DNA from Bacterial Strains

Bacterial cultures at log phase grown in Marine ZoBell's Agar (2216E) medium (Peptone, 0.5g; Bacto yeast extract, 0.1g; Agar, 2.0g; Sea water: 35psu, 100ml) was washed and harvested at 7500 rpm for 5 min. The cell pellet thus obtained was resuspended in 500µl of TEN buffer (1000mM Tris HCL; 10mM EDTA and 250mM NaCI) and centrifuged at 10000 rpm for 5 min. The cell suspension was again resuspended in 500µl TE buffer (10mM Tris HCl-pH 7.5; 1mM EDTA), added 50µl 20% SDS and 20µl of proteinase K (20mg ml^{-1}) and incubated at 370 ˚C in a water bath overnight. Then extracted with 500µl of Tris equilibrated phenol and centrifuged at 10000 rpm for 15 min. The top layer was transferred to a new tube avoiding interface and the process was repeated two times. It was then extracted with equal volume of chloroform isoamyl alcohol (24:1) and centrifuged at 10000 rpm for 10 min. After transferring the aqueous layer to a new vial, 0.1 volume of 3M sodium acetate (pH 5.2) was added, mixed

gently and precipitated with 0.6 volume of isopropanol at -20 ˚C overnight. DNA pellets were washed twice with 70% ethanol and once with absolute ethanol, then suspended in sterile TE buffer and stored at -20 ˚C.

ii) Determination of the Quality of DNA

Quality and quantity of the isolated DNA was checked by measuring optical density in a UV spectrophotometer and visualizing DNA using gel electrophoresis. The ratio of absorbance at 260nm and 280nm is an indication of the DNA quality. The ratio ranges from 1.6 to 1.8 for pure DNA.

iii) PCR Amplification of 16S rDNA

Extracted DNA was diluted to a concentration of 100ng μl^{-1}. 0.8% agarose gel was prepared in 1x TBE (Tris base, 10.8g; 0.5 M EDTA, 4ml; Boric acid, 5.5g; Double Distilled water, 100ml; pH-8) Ethidium bromide (0.2 mg ml^{-1} stock stored in dark) was added to the melted agarose to a final concentration of 0.2 pg ml^{-1}. After cooling to about 45 ˚C, the agarose was poured on to gel tray and was allowed to solidify. The gel tray was transferred in to a buffer tank and was submerged in 1x TBE buffer. Appropriate quantity of DNA in a volume of 5µl was mixed with 1µl of 6x loading dye (Bromophenol Blue, O.125g; Xylene Cyanol, 0.125g; Glycerol, 15ml; Double Distilled water, 50ml) and loaded into the well. Electrophoresis was done at a voltage of 3-4 volt cm^{-1} till the bromophenol blue dye front migrated to the middle of the gel. The gel was visualized on a UV transilluminator (GelDoc, Bio-Rad, Richmond, California). A 100-bp DNA ladder was used as the marker (Promega, Australia).

To amplify the 16S rRNA gene from the *Bacillus* strains PCR was performed using 1µl of the DNA (100ng) as the template. Amplification was performed using universal eubacterial primers 27f and 1492r, which target universally conserved regions and permit the amplification of the 16S rDNA fragment of ca. 1500-bp (Weisburg *et al*., 1991; Cheneby *et al.,* 2000; Long and

Azam, 2001). Details of the PCR reaction mixture used in the study are summarised in Table 6.2.

Primer 1 (27f) 5'- AGAGTTTGATCMTGGCTCAG-3'

Primer 2 (1492 r) 5'- GGTTACCTTGTTACGACTT- 3'

Table 6.2: PCR Reaction Mixture

Sl.No	Item	Amount (µl)
1	10 X PCR Buffer	2.5
2	dNTP 2.5mM	2
3	Primer F (27f) 10pmol μl^{-1}	1
4	Primer R (1492r) 10pmol μl^{-1}	1
5	Template DNA 100ng μl^{-1}	1
6	Autoclaved MilliQ Water	16.5
7	Taq Polymerase 1U μl^{-1}	1
Total		25

The amplification was done according to the following protocol. Initial denaturation was carried out at 94 ˚C for 5min. The samples were placed in ice and 1µl of *Taq* polymerase was added. This was followed by 35 cycles of denaturation at 94 ˚C for 45 seconds, annealing at 59 ˚C for 45 seconds, extension at 72°C for 1min and a final extension at 72 ˚C for 10min. The PCR product (16S rRNA gene) was monitored by 1% agarose gel electrophoresis.

iv) Agarose Gel Electrophoresis

A 5µl sample of PCR product was mixed with 1µl of 6x gel loading dye and loaded onto a 1.0% agarose gel containing ethidium bromide (1 mg ml^{-1}) and electrophoresis was performed at 80V for 30min using 0.5x TBE buffer. DNA in the gel was visualized using a Gel documentation system (Gel Doc, Bio-Rad, Richmond, California) as shown in Fig. 6.1. The size of the amplicon was estimated using a 100-bp DNA ladder (Promega, Australia).

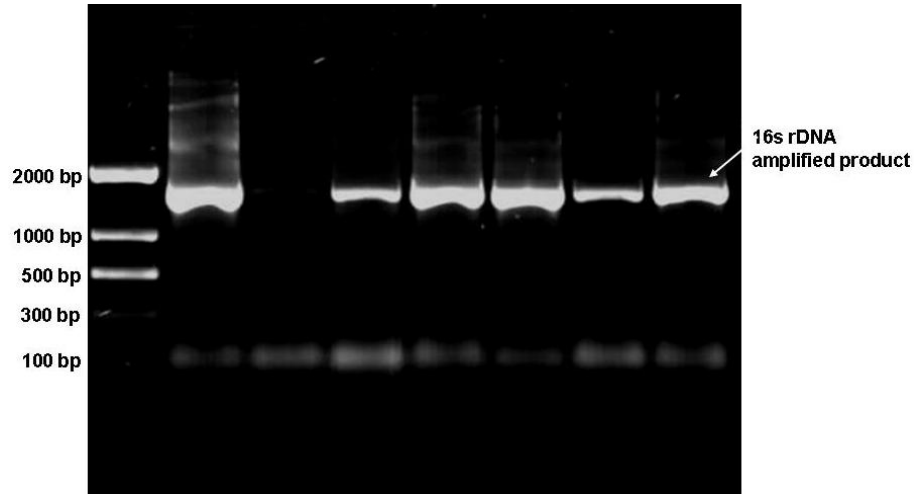


Fig. 6.1. Amplified 16S rDNA run on 1% agarose gel

v) ARDRA analysis of PCR amplicons

The PCR products were cleaned using Wizard SV gel PCR clean up System (Promega) and was used for Amplified Ribosomal DNA Restriction Analysis (ARDRA).

To screen the clones for grouping into similar clone types and subsequent sequence analysis, clones containing full-length 16S rDNA were subjected to ARDRA analysis using restriction enzymes which recognize a 4-bp restriction site. The restriction enzymes used in this study were *Alu*I (AG'CT) and *Taq*I (T'CGA). A 5µl of PCR amplicon was digested with 1 U of individual restriction enzyme in a 10µl reaction volume for 4h using the conditions recommended by the manufacturer for each enzyme. The incubation temperature for *Alu*I was 37 ˚C and for *Taq*I 65 ˚C. The restriction fragments were electrophoresed through a 2% agarose gel (containing ethidium bromide). A 100-bp ladder was used as the DNA marker (Fermentas). Digitized gel images were analysed to construct ARDRA profiles for *Bacillus* strains. Fig. 6.2. illustrates different ARDRA patterns developed on 2% agarose gel.

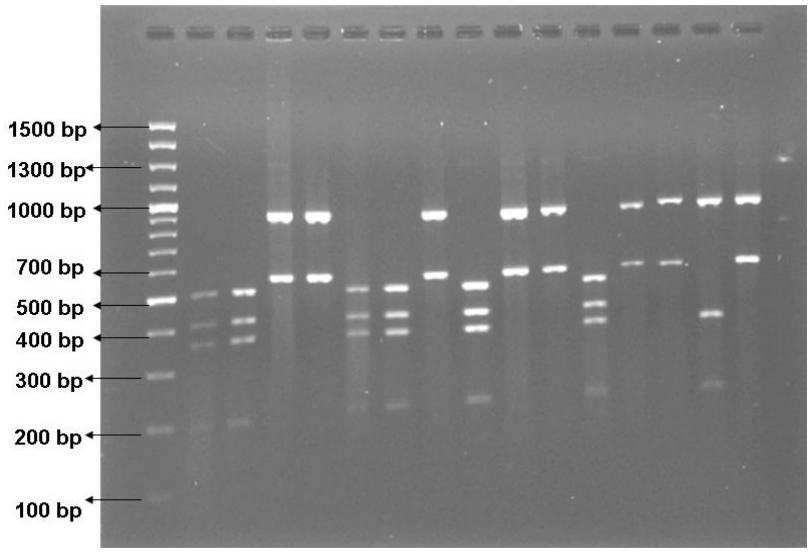


Fig 6.2. showing different ARDRA pattern developed on 2% agarose gel using *Taq*I restriction enzyme digestion.

vi) DNA Sequencing

The representatives of each ARDRA types were selected and the purified 1.5-kb amplicon (16S rDNA) was sequenced using primers 27f, 530f and 1492r. Sequencing was done by Sangar dideoxy chain teminator sequencing (automated fluorescent DNA sequencing) using ABI Prism model 3700 Big Dye Sequencer (Applied Biosystems, USA) at SciGenom, Kochi. The nucleotide sequences obtained were assembled using GeneTool software and were aligned to find regions of similarity between sequences in the GenBank database through BLAST (Basic Local Alignment Search Tool) search at National Centre for Biotechnology Information (NCBI) USA. (http://www.ncbi.nlm.nih.gov). Based on the percentage similarity with the GenBank sequences, the isolates were identified. Sequences were aligned and phylogenetic trees were constructed from a matrix of pairwise genetic distances by the neighbor-joining method using MEGA 4.0 (Tamura *et. al*.,

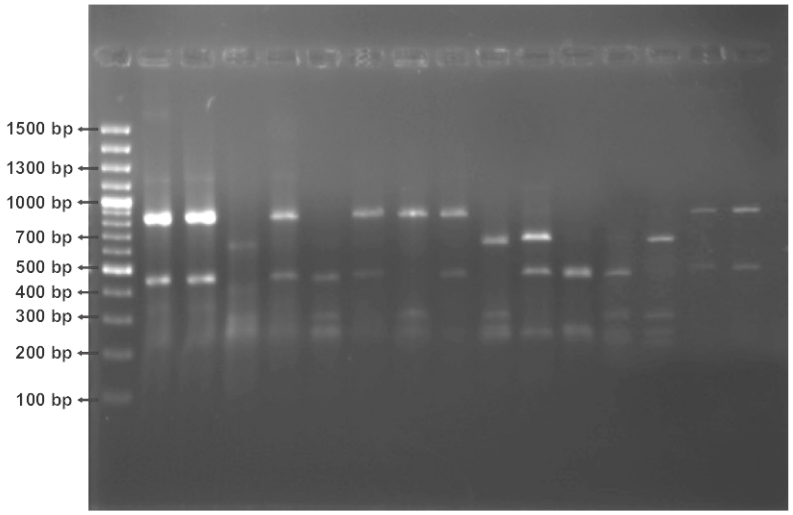


Fig 6.2b. showing different ARDRA pattern developed on 2% agarose gel using *Alu*I restriction enzyme digestion.

2007). Fig. 6.8. shows Clustal W multiple alignment of the *Bacillus* strains and the NCBI strains. The sequences of the *Bacillus* strains are deposited in GenBank under accession numbers JX080178 to JX080196.

6.3. RESULTS

In order to estimate the phylogenetic relationship of *Bacillus* strains from different depth zones a total of 72 *Bacillus* strains were subjected to molecular characterization based on amplification of 16S rDNA using eubacterial primers and subsequent digestion with the aid of restriction enzymes, a technique referred to as Amplified Ribosomal DNA Restriction Analysis (ARDRA). The technique allows the clustering of bacterial strains into groups whose taxonomical identity is revealed from sequence databases by partial 16S rRNA gene sequencing of representative strains. Stringent PCR conditions allowed the amplification of a single DNA fragment of 1500-bp. The restriction enzymes used for the study were *Alu*I and *Taq*I.

i) ARDRA patterns

The band pattern types developed using each restriction enzymes were variable and distinct. Each ARDRA-type was composed by 2 to 5 distinct bands with molecular weights (MW) ranging from 100 to 900-bp. ARDRA with *Alu*I digestion resulted in the highest number of pattern types. Using *Alu*I digestion 10 different band patterns were developed where as *Taq*I restriction digestion yielded 5 different band patterns. A summary of fragment patterns obtained from each enzyme is shown in Table 6.3.

Table 6.3. ARDRA pattern and DNA fragments generated with the digestion using restriction enzymes a) *Alu*I and b) *Taq*I on 16S rDNA amplicons

a)

Cluster types (*Alu*I)	No. of Fragments	Fragment Size (bp)*
1	3	850, 450 and 200-bp
2	2	850 and 450-bp
3	3	850, 250 and 200-bp
4	4	650, 450, 250 and 200-bp
5	3	650, 450 and 200-bp
6	4	650, 250, 200 and 100-bp
7	3	650, 250 and 200-bp
8	4	450, 250, 200 and 150-bp
9	3	450, 250 and 200-bp
10	2	450 and 200-bp

b)

Cluster types (*Taq*I)	No. of Fragments	Fragment Size (bp)
1	2	900, 550-bp
2	3	900, 350, 200-bp
3	3	550, 500, 400-bp
4	4	500, 400, 350, 200-bp
5	2	900, 450-bp

* Fragments less than 100-bp was not considered

The restriction pattern developed using single enzymes had its limitation in differentiating the strains to species level; hence a combination of the ARDRA pattern generated independently from restriction digestion with *Alu*I and *Taq*I was used in this study. Following digestion independently with two enzymes (*Alu*I and *Taq*I) a dendrogram was plotted to differentiate the strains based on the ARDRA patterns generated as shown in Fig. 6.3. The strains were grouped into 20 distinct ARDRA types on the basis of a combined banding pattern. The details of the ARDRA patterns of the bacterial strains used in this study is presented in Table 6.4.

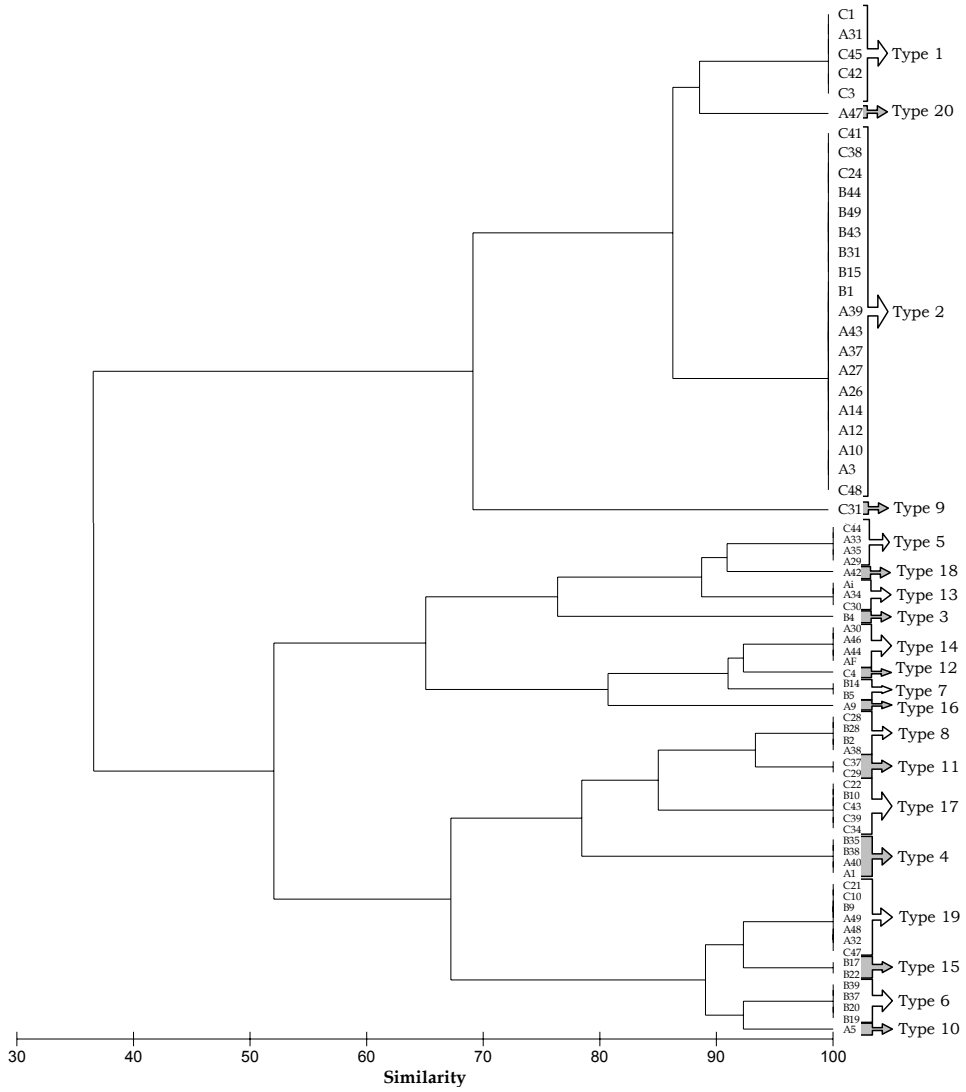


Fig. 6.3. Simplified dendrogram showing the clustering of the strains into various ARDRA types based on ARDRA patterns developed by *Alu*I and *Taq*I digest of 16S rDNA on 72 Gram-positive endospore-forming rods belonging to *Bacillus* species

Table 6.4. ARDRA patterns generated using restriction enzymes *Alu*I and *Taq*I in 16S rDNA amplicons from *Bacillus* strains isolated from different depths.

200m			500m			1000m			ARDRA types	Selected strains	Nucleotide Bp	Nearest Identity	% Similarity
Cultures	Category (*Alu*I)	*Taq*I digest	Cultures	Category (*Alu*I)	*Taq*I digest	Cultures	Category (*Alu*I)	*Taq*I digest					
1674 (A31)	I	I	539 (B1)	II	I	687 (C1)	I	I	1	A31	1392	*B. firmus*	99%
			869(B15)	II	I	891 (C3)	I	I					
848 (A3)			2148(B43)	II	I	2173 (C42)	I	I					
849 (A10)	II	I	2159 (B49)	II	I	2194 (C45)	I	I					
1040 (A12)	II	I	2511 (B44)	II	I	1607 (C31)	I	V	9	C31	1317	*B. infantis*	99%
1130 (A14)	II	I	1656 (B31)	II	I								
1553 (A26)	II	I				846 (C24)	II	I	2	A10	1332	*B.niabensis*	99%
1549 (A27)	II	I	807 (B4)	III	I	2181 (C38)	II	I				(licheniformis/litoralis)	
1890 (A37)	II	I	2054 (B38)	III	IV	2172(C41)	II	I	3	B 4	1353	*B. firmus*	99%
1624 (A43)	II	I	2340 (B35)	III	IV	2489 (C48)	II	I	4	A40	1330	*B. licheniformis*	99%
2039 (A39)	II	I							5	A29	1384	*B.megaterium*	99%
	II	I	886 (B19)	IV	II	2192 (C44)	IV	I					
607 (A1)			938 (B20)	IV	II								
2324 (A40)	III	IV	1923 (B37)	IV	II				6	B 39	1316	*B. aerophilus*	99%
	III	IV	2156 (B39)	IV	II	1547 (C28)	IV	IV	7	B 14	1330	*B. aquimaris*	99%
1564 (A29)			814 (B5)	IV	III								
1747 (A35)	IV	I	868 (B14)	IV	III				8	A38	1363	*B. subtilis*	100%
1740 (A33)	IV	I	647 (B2)	IV	IV	1597 (C29)	V	IV	10	A 5	852	*B. baekryungensis*	100%
	IV	I	1573(B28)	IV	IV	2178 (C37)	V	IV				(marine bacterium)	
2009 (A38)									11	C 29	630	*B. licheniformis*	94%
	IV	IV	947 (B22)	VII	II	892 (C4)	VI	III					
970 (A5)			875 (B17)	VII	II				12	C4	1317	*B. marisflavi*	99%
	V	II	823 (B10)	VII	IV	1606 (C30)	VII	I	13			(baekryungensis)	
										Ai	857	*B. aquimaris*	100%
922 (Ai)			822 (B9)	IX	II	1932 (C34)	VIII	IV					
1745 (A34)	VII	I				2184 (C39)	VIII	IV	14				
856 (AF)	VII	I				2176 (C43)	VIII	IV		A44	1362	*B. aquimaris*	100%
1635 (A44)	VII	III											
2446 (A46)	VII	III				840 (C22)	VIII	IV					
1569 (A30)	VII	III							15	B 22	1054	*B. pumilus*	97%
	VII	III				895 (C10)	IX	II					
1031 (A9)						1058 (C21)	IX	II	16	A9	1349	*B. aerophilus*	99%
	VIII]	III				2488 (C47)	IX	II	17	C22	1296	*B. subtilis*	100%
2463 (A42)									18	A42	1363	*B. pumilus*	100%
1673 (A32)	IX	I							19	A32	1322	*B.megaterium*	99%
2327 (A48)	IX	II							20	A47	1343	*B. cibi*	97%
2332 (A49)	IX	II											
	IX	II											
2326 (A47)	X	I											

As shown in the figure each group presented a specific banding pattern, and groups further known to be of different species were clearly differentiated. However, exceptions were found. Atleast one isolate from each of the 20 ARDRA types was selected for 16S rDNA sequencing. The following is a description of the types of band pattern obtained using both restriction enzymes.

i) ARDRA-Type 1

One of the major haplotypes encountered was ARDRA-type 1. This pattern was characterized by the presence of 3 bands of apparent size 850, 450 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of an apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus firmus.*

ii) ARDRA-Type 2

Another haplotype encountered was ARDRA-type 2. Using restriction enzyme *Alu*I this pattern was characterized by the presence of 2 bands of an apparent size 850 and 450-bp. Using *Taq*I it generated a pattern with 2 bands of apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus niabensis.*

iii) ARDRA-Type 3

Another major haplotype encountered was ARDRA-type 3. This pattern was characterized by the presence of 3 bands of an apparent size 850, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of an apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus firmus.*

iv) ARDRA-Type 4

Another major haplotype encountered was ARDRA-type 4. This pattern was characterized by the presence of 3 bands of an apparent size 850, 250 and 200-

bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of an apparent size 500, 400, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus licheniformis.*

v) ARDRA-Type 5

Another major haplotype encountered was ARDRA-type 5. This pattern was characterized by the presence of 4 bands of apparent size of 650, 450, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 4 bands of apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus megaterium.*

vi) ARDRA-Type 6

Another major haplotype encountered was ARDRA-type 6. This pattern was characterized by the presence of 4 bands of apparent size 650, 450, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 4 bands of apparent size 900, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus aerophilus.*

vii) ARDRA-Type 7

Another major haplotype encountered was ARDRA-type 7.This pattern was characterized by the presence of 4 bands of apparent size 650, 450, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 4 bands of an apparent size MW of 550, 500 and 400-bp. On sequencing the representative isolate was identified as *Bacillus aquimaris*

viii) ARDRA-Type 8

Another major haplotype encountered was ARDRA-type 8. This pattern was characterized by the presence of 4 bands of apparent size 650, 450, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 4

bands of apparent size 500, 400, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus subtilis.*

ix) ARDRA-Type 9

Other haplotype encountered was ARDRA-type 9. This pattern was characterized by the presence of 3 bands of an apparent size 850, 450 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of apparent size 900 and 450-bp. On sequencing the representative isolate was identified as *Bacillus infantis.*

x) ARDRA-Type 10

Another major haplotype encountered was ARDRA-type 10. This pattern was characterized by the presence of 4 bands of apparent size 650, 450 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 3 bands of apparent size 900, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus baekryungensis.*

xi) ARDRA-Type 11

Another major haplotype encountered was ARDRA-type 11. This pattern was characterized by the presence of 4 bands of apparent size 650, 450 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 3 bands of apparent size 500, 400, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus licheniformis.*

xii) ARDRA-Type 12

Another major haplotype encountered is ARDRA-type 12 characterized by the presence of 3 bands of apparent size 650, 250, 200 and 100-bp using Restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 3 bands of apparent size 550, 500 and 400-bp. On sequencing the representative isolate was identified as *Bacillus marisflavi.*

xiii) ARDRA-Type 13

Another major haplotype encountered was ARDRA-type 13 characterized by the presence of 3 bands of apparent size 650, 250 and 200-bp with restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus aquimaris.*

xiv) ARDRA-Type 14

Another major haplotypes encountered is ARDRA-type 14. This pattern was characterized by the presence of 3 bands of apparent size 650, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 3 bands of apparent size 550, 500 and 400-bp. On sequencing the representative isolate was identified as *Bacillus aquimaris.*

xv) ARDRA-Type 15

Another major haplotype encountered was ARDRA-type 15. This pattern was characterized by the presence of 4 bands of an apparent size (MW) of 450, 250, 200 and 150-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 4 bands of an apparent MW of 900, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus pumilus.*

xvi) ARDRA-Type 16

Another major haplotype encountered was ARDRA-type 16. This pattern was characterized by the presence of 4 bands of apparent size 450, 250, 200 and 150-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 3 bands of apparent size 550, 500 and 400-bp. On sequencing the representative isolate was identified as Partial sequencing of the representative isolate was identified as *Bacillus aerophilus.*

xvii) ARDRA-Type 17

Another major haplotype encountered was ARDRA-type 17. This pattern was characterized by the presence of 4 bands of apparent size 450, 250, 200 and 150-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 4 bands of apparent size 500, 400, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus subtilis.*

xviii) ARDRA-Type 18

Another major haplotype encountered was ARDRA-type 18. This pattern was characterized by the presence of 3 bands of apparent size 450, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus pumilus.*

xix) ARDRA-Type 19

Another major haplotype encountered was ARDRA-type 19. This pattern was characterized by the presence of 3 bands of an apparent size MW of 450, 250 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 3 bands of an apparent size MW of 900, 350 and 200-bp. On sequencing the representative isolate was identified as *Bacillus megaterium.*

xx) ARDRA-Type 20

Another major haplotypes encountered was ARDRA-type 20. This pattern was characterized by the presence of 2 bands of apparent size 450 and 200-bp using restriction enzyme *Alu*I. Using *Taq*I it generated a pattern with 2 bands of apparent size 900 and 550-bp. On sequencing the representative isolate was identified as *Bacillus cibi.*

The result of phylogenetic analysis revealed that the *Bacillus* strains belonged to 12 species. With *Alu*I and *Taq*I, most *Bacillus* species could be readily

speciated with certain exceptions. The utility of this procedure by digestion with a single restriction enzyme is restricted by its inability to distinguish properly. For eg., using restriction enzyme *Alu*I alone was incompetent to distinguish between certain members of the *B. subtilis* from *B. pumilus*, another group of *B. firmus* and *B. licheniformis* and three species each in the *B. aerohilus, B. megaterium* and *B. pumilus* cluster as well as between *B. baekryungensis* and *B. licheniformis* cluster. The most remarkable group was *Alu*I type 4 which represented *B. megaterium, B. aerophilus, B. aquimaris* and *B. subtilis*. However, none of the ARDRA types generated using *Taq*I digest was able to clearly differentiate the *Bacillus* strains into respective species level.

However certain strains of *Bacillus* species were represented in more than one ARDRA type. ARDRA types 7, 13 and 14 showed similarities to *B. aquimaris*. ARDRA types 1 and 3 showed similarities to *B. firmus*. Where as *B. licheniformis* were associated with ARDRA pattern 4 and 11. Strains related to *B. megaterium* also were represented in ARDRA types 5 and 19, ARDRA types 15 and 18 were identified as *B. pumilus*, another strain related to *B. subtilis* were represented in ARDRA types 8 and 17 also. *Bacillus aerophilus* fell into 2 other ARDRA types, type 6 and 16.

6.3.2. Phylogenies of the bacterial isolates

Partial 16S rRNA gene sequencing, approximately 1500-bp, was performed for 72 isolates representing distinct haplotypes. Phylogenetic analysis of the bacterial strains demonstrated that 20 ARDRA types falling into 12 species of bacteria. Most of the 16S rDNA sequences of our isolates had quite high sequence identity (usually >98%) to the nearest neighbouring GenBank sequences, usually determined from the culturable bacterial strains. Following BLAST analysis to search for homology, partial sequences obtained together with their closest relatives in GenBank were used for the construction of the Neighbor joining phylogenetic tree (Fig 6.4).

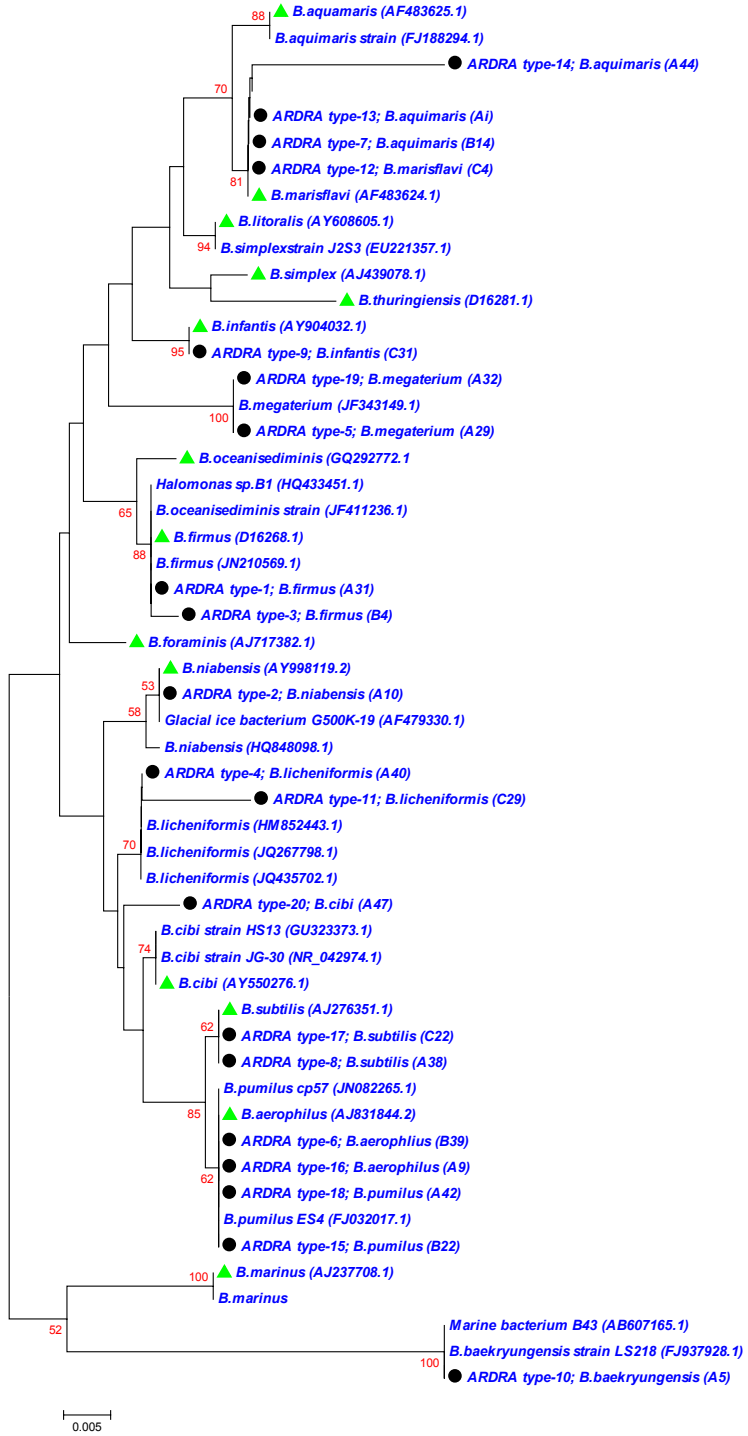


Fig. 6.4. Neighbour-joining phylogenetic tree, based on partial sequences of 16S rRNA genes, showing the relationships among the species of the genus *Bacillus* from the three depth regions. Bootstrap values are shown as percentages from 1000 replications at branch points. ARDRA category and species name are given. ● -Strains used for this study; ▲ - Type strain sequences obtained form NCBI.

Phylogenetic analysis of 16S rDNA sequences of *Bacillus* strains from sediments collected at different depths revealed a significant degree of *Bacillus* diversity. 12 phylogenetic groups related to distinct species or genera in the Bacillaceae family, could be distinguished. Strains identical to 16S rRNA genes of members of the *Bacillus niabensis* were observed in all of the sediments examined. The second most common group of sequences found in the present study were identical to the 16S rRNA gene of *Bacillus megaterium*. Common to three sediment samples, there were several 16S rDNA sequences related to the 16S rRNA gene of *Bacillus aquimaris*, *B. subtilis, B. firmus* and *B. licheniformis*. Phylogenetic sequence identity of *Bacillus aerophilus* and *Bacillus pumilus* were retrieved from sediment samples lying in 200m and 500m depth region. While *Bacillus baekryungensis* and *B. cibi* were confined to 200m depth region. *B. marisflavi* and *B. infantis* were retrieved from 1000m depth region only. No sequences exclusively confined to 500m depth region were isolated.

The most abundantly represented ARDRA type was type 2 which also formed the simplest pattern of ARDRA types, composed by two bands with a MW of 850 and 450-bp with *Alu*I, and 2 bands with a MW of 900 and 550-bp using *Taq*I digestion. The phylogenetic analysis revealed that the group is related to *Bacillus niabensis* which represented 19 strains forming 26% of the total strains. Sediments from all 3 depths were dominated by the populations of bacterial strains belonging to this group. The second most frequently represented ARDRA type was type 19 with three bands of MW 450, 250 and 200-bp with *Alu*I, and 3 bands with a MW of 900, 350 and 200-bp using *Taq*I digestion. The phylogenetic analysis revealed that the group is related to *Bacillus megaterium* which represented 7 strains. The species were also represented by ARDRA type 5 which represented 3 strains. In total, 10 of such strains comprising 14% of the total strains were isolated from sediment samples. The third commonly represented strain was related to *Bacillus subtilis* which encompassed 9 strains represented by two ARDRA types, type 8 (4 isolates) and type 17 (5 isolates). The phylogenetic group related to

Bacillus aquimaris also represented 9 isolates falling into 3 ARDRA types, type 7, 13 and 14 ,both the group contributing to 13% each to the total strains. The fourth commonly represented strain was more closely related to *Bacillus firmus* encompassing 6 strains forming 9% of the total *Bacillus* strains. The strain is represented by ARDRA type 1 and 3. The phylogenetic group related to *Bacillus licheniformis* also represented 6 isolates falling into ARDRA types 4 and 11. Sequences of ARDRA type 6 and 16 were closely related to the *Bacillus aerophilus*, forming the sixth largest group. Another important group encompassed 3 isolates related to *Bacillus pumilus* which is represented by ARDRA type 15 and 18. Phylogenetic analysis of other important ARDRA types revealed that type 9 was related to *Bacillus infantis*, type 10 to *Bacillus baekryungensis* and ARDRA type 12 represented as *Bacillus marisflavi* and ARDRA type 20 as *Bacillus cibi*.

In 200m depth range it showed that 12 ARDRA types falling into 9 phylotypes were represented. The most abundant being strains closely related to *B. niabensis* followed by *B. aquimaris, B. megaterium* and *B. licheniformis*. The other strains represented also include *B. firmus, B. subtilis, B. pumilus, B. baekryyungensis,* and *B. cibi*. In 500m depth range it showed that 9 ARDRA types falling into 8 phylotypes were represented. The most abundant being strains closely related to *B. niabensis, B. aerophilus* and *B. subtilis*. Strains closely related to *B. aquimaris, B. licheniformis, B. pumilus, B. firmus* and *B. megaterium* are also abundant in this depth range. In 1000m depth range it showed that 9 ARDRA types falling into 8 phylotypes were represented. The most abundant being strains closely related to *B. firmus* and *B. subtilis* followed by *B. niabensis, B. megaterium* and *B. licheniformis*. . The other strains represented also include *B. infantis, B. marisflavi* and *B. aquimaris*. A phylogenetic tree constructed using the neighbour-joining method showed a wide distribution of the members of the genus *Bacillus* in the marine sediments as shown in Figures 6.5. to Fig. 6.7.

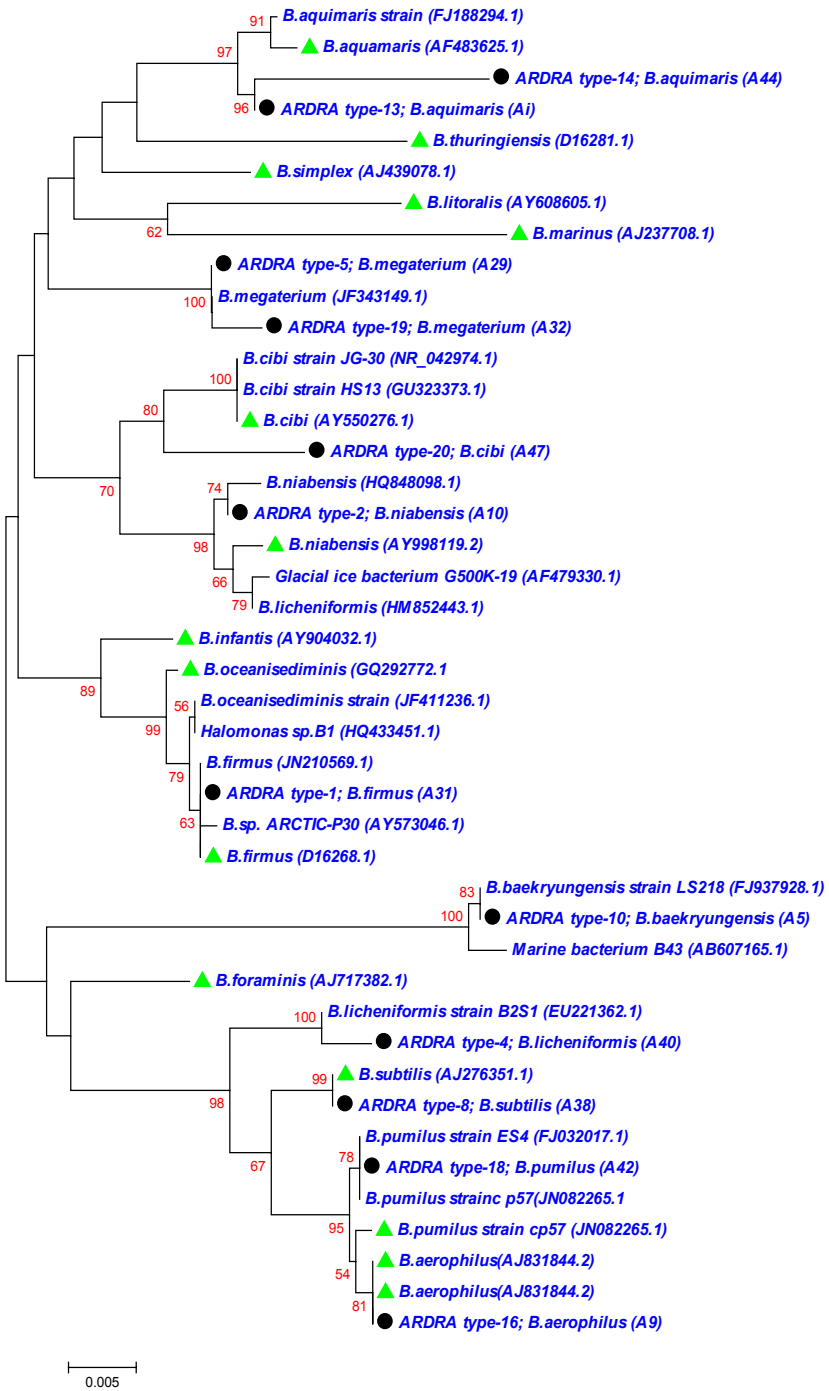


Fig. 6.5. Neighbour-joining phylogenetic tree, based on partial sequences of 16S rRNA genes, showing the relationships among the species of the genus *Bacillus* in 200m depth region. Bootstrap values are shown as percentages from 1000 replications at branch points. ARDRA category and species name are given. ● -Strains used for this study; ▲: Type strains sequences obtained form NCBI.

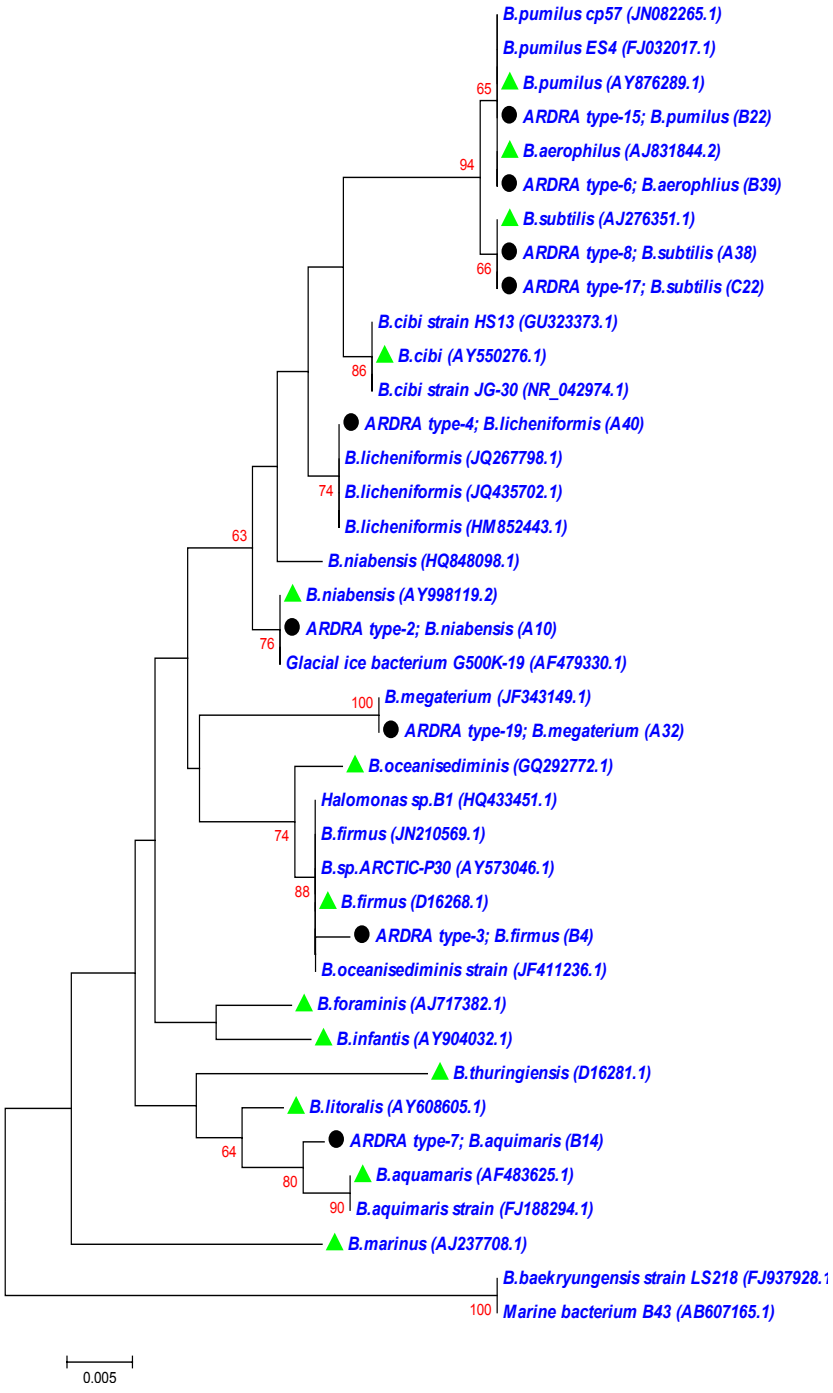


Fig. 6.6. Neighbour-joining phylogenetic tree, based on partial sequences of 16S rRNA genes, showing the relationships among the species of the genus *Bacillus* in 500m depth region. Bootstrap values are shown as percentages from 1000 replications at branch points. ARDRA category and species name are given. ● -Strains used for this study; ▲ - Type strains sequences obtained form NCBI.

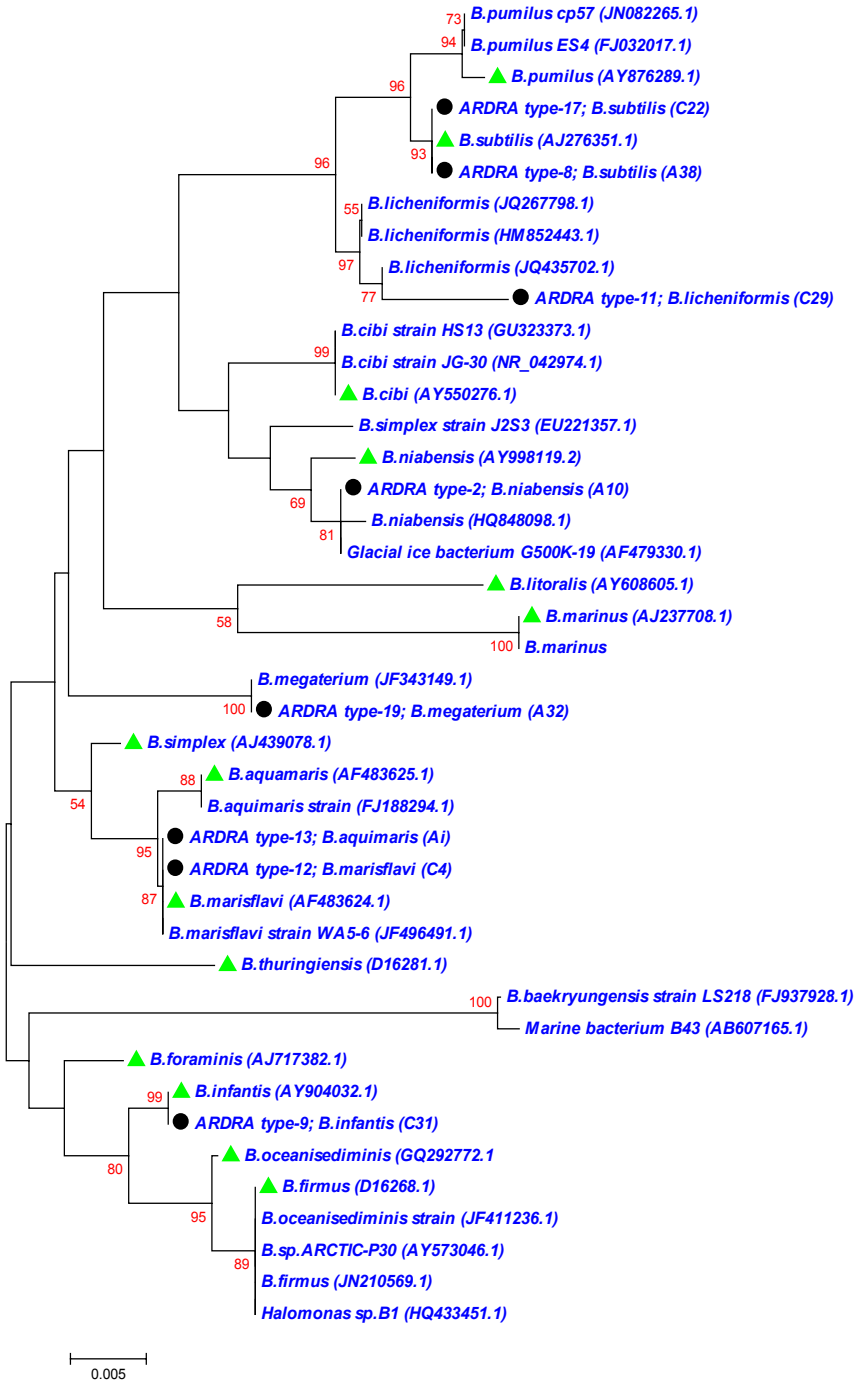
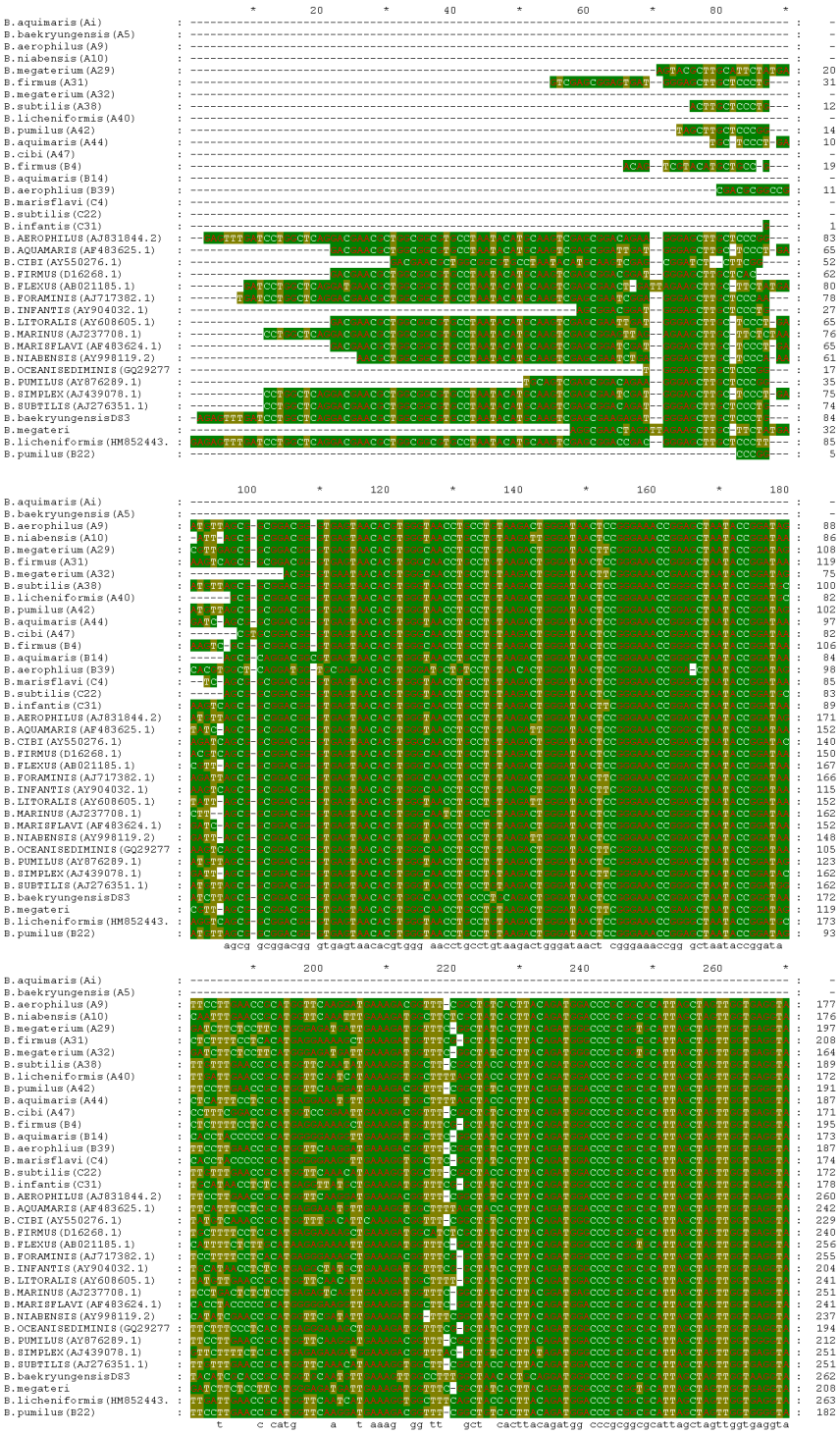
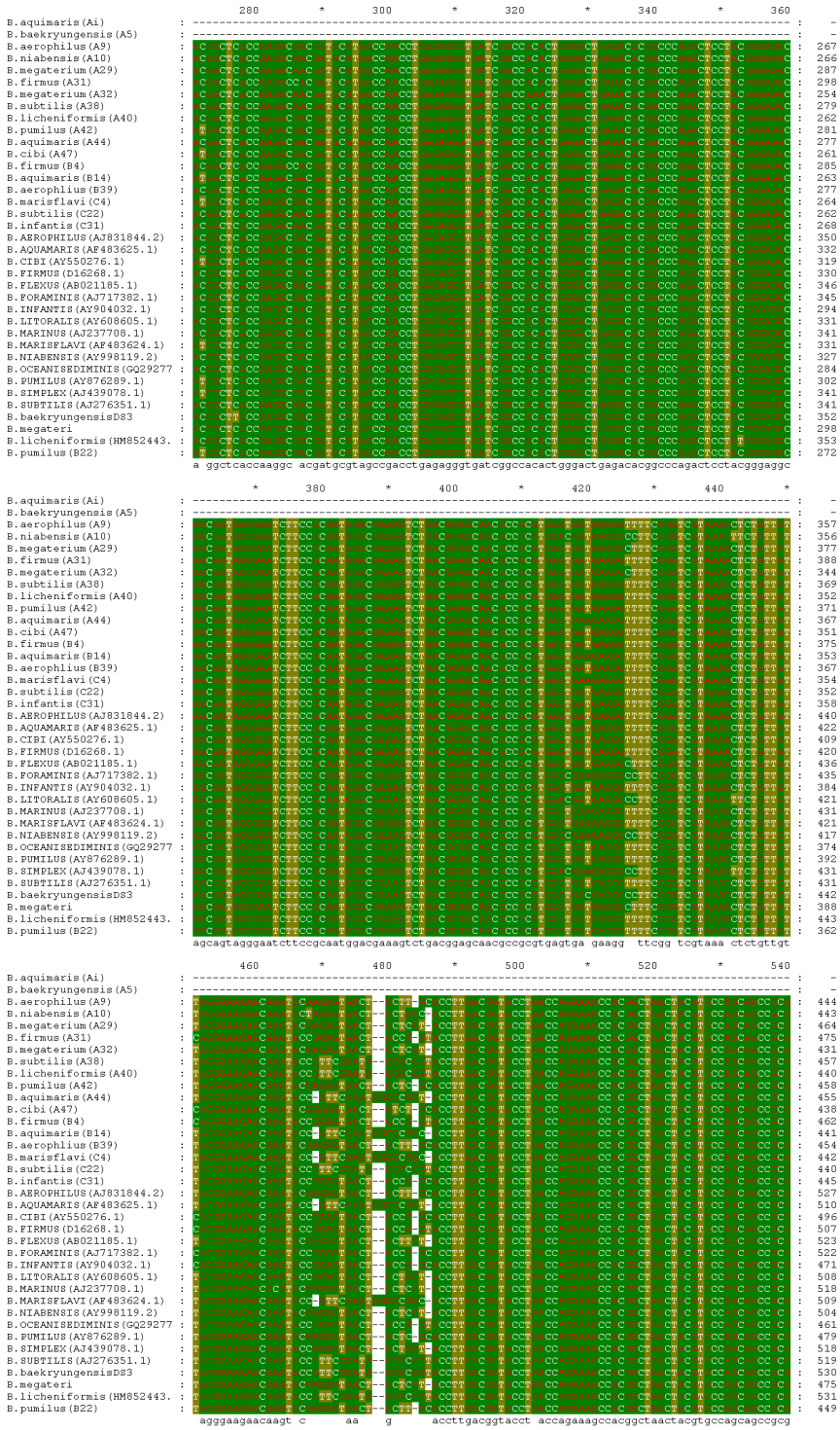
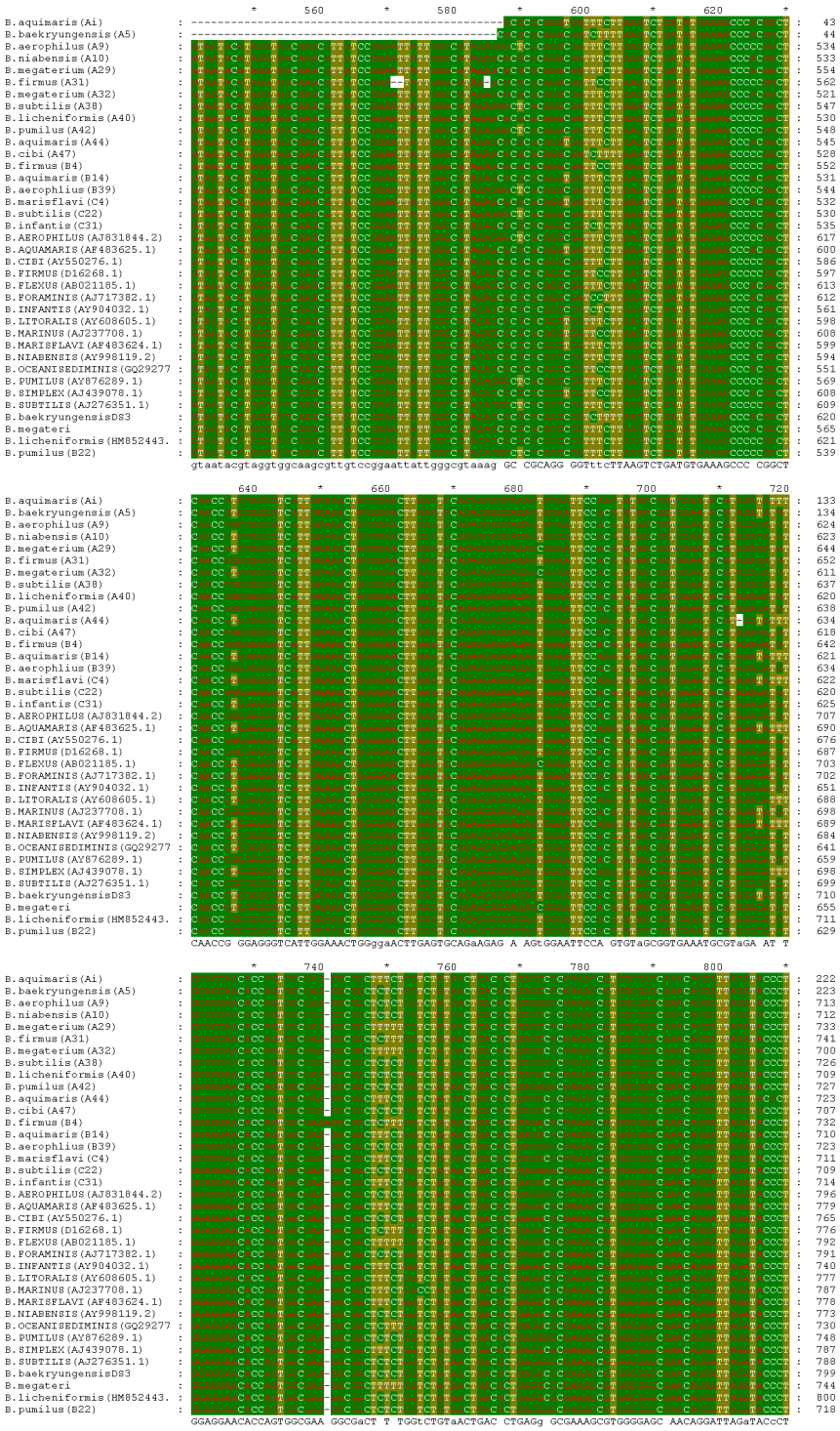
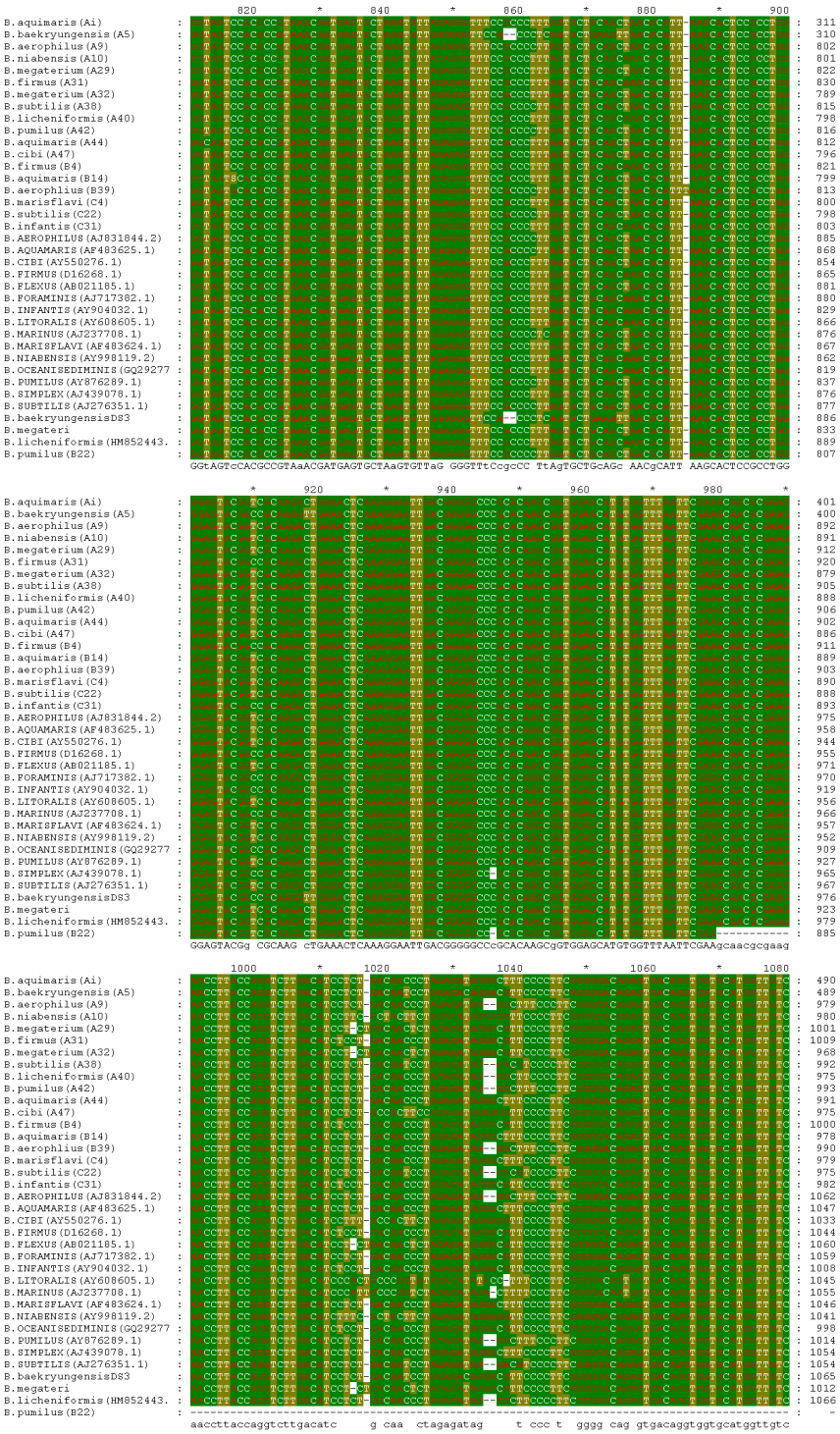


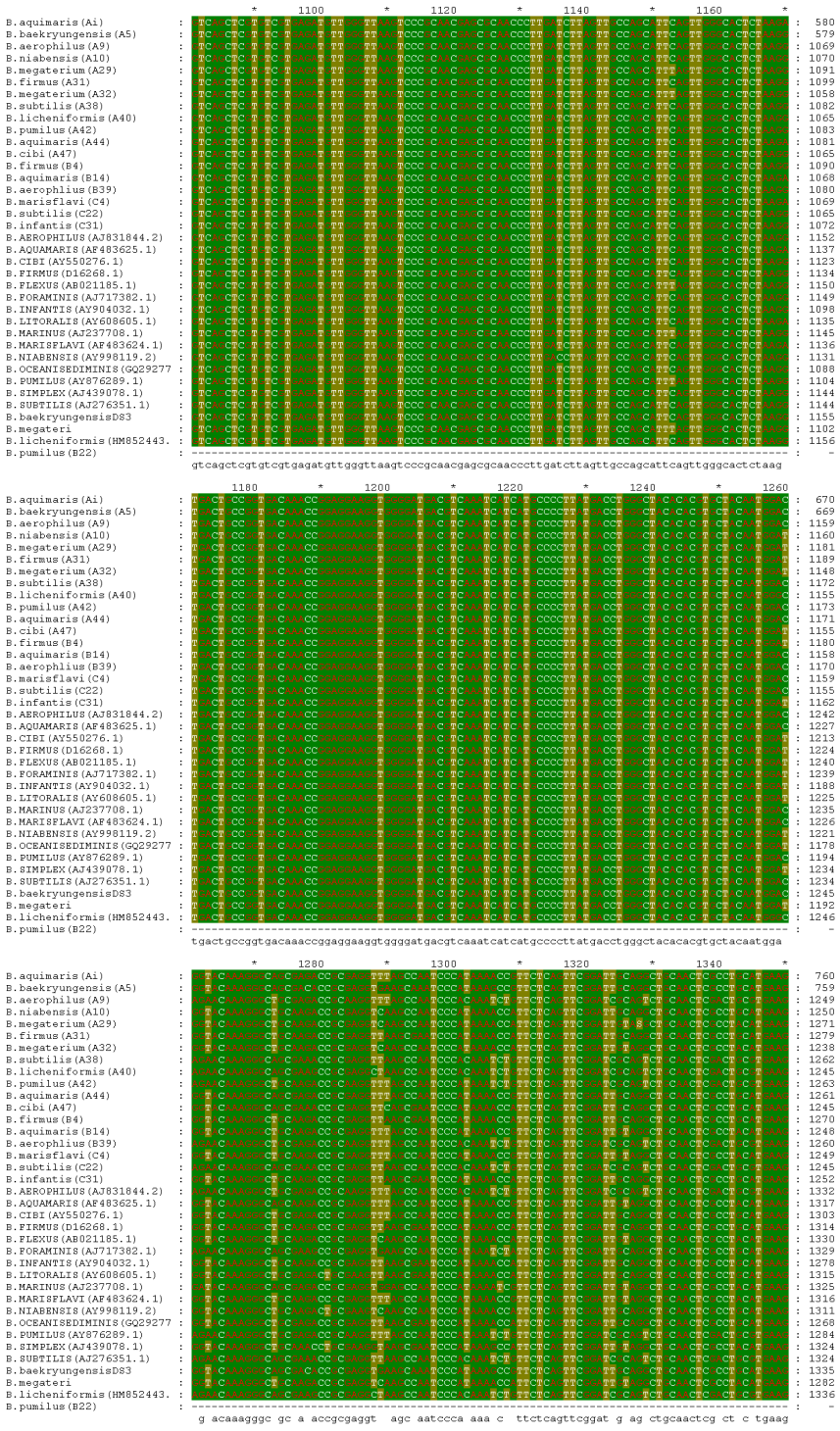
Fig.6.7. Neighbour-joining phylogenetic tree, based on partial sequences of 16S rRNA genes, showing the relationships among the species of the genus *Bacillus* in 1000m depth region. Bootstrap values are shown as percentages from 1000 replications at branch points. ARDRA category and species name are given. ● -Strains used for this study; ▲- Type strains sequences obtained form NCBI.


B.aquimaris(Ai)
B.baekryungensis(A5)
B.aerophilus(A9)
B.niabensis(A10)
B.megaterium(A29)
B.firmus(A31)
B.megaterium(A32)
B.subtilis(A38)
B.licheniformis(A40)
B.pumilus(A42)
B.aquimaris(A44)
B.cibi(A47)
B.firmus(B4)
B.aquimaris(B14)
B.aerophilus(B39)
B.marisflavi(C4)
B.subtilis(C22)
B.infantis(C31)
B.AEROPHILUS(AJ831844.2)
B.AQUAMARIS(AF483625.1)
B.CIBI(AY550276.1)
B.FIRMUS(D16268.1)
B.FLEXUS(AB021185.1)
B.FORAMINIS(AJ717382.1)
B.INFANTIS(AY904032.1)
B.LITORALIS(AY608605.1)
B.MARINUS(AJ237708.1)
B.MARISFLAVI(AF483624.1)
B.NIABENSIS(AY998119.2)
B.OCEANISEDIMINIS(GQ29277
B.PUMILUS(AY876289.1)
B.SIMPLEX(AJ439078.1)
B.SUBTILIS(AJ276351.1)
B.baekryungensisD83
B.megateri
B.licheniformis(HM852443.
B.pumilus(B22)
agcg gcggacgg gtgagtaacacgtggg aacctgcctgtaagactgggataact cgggaaaccgg gctaataccggata









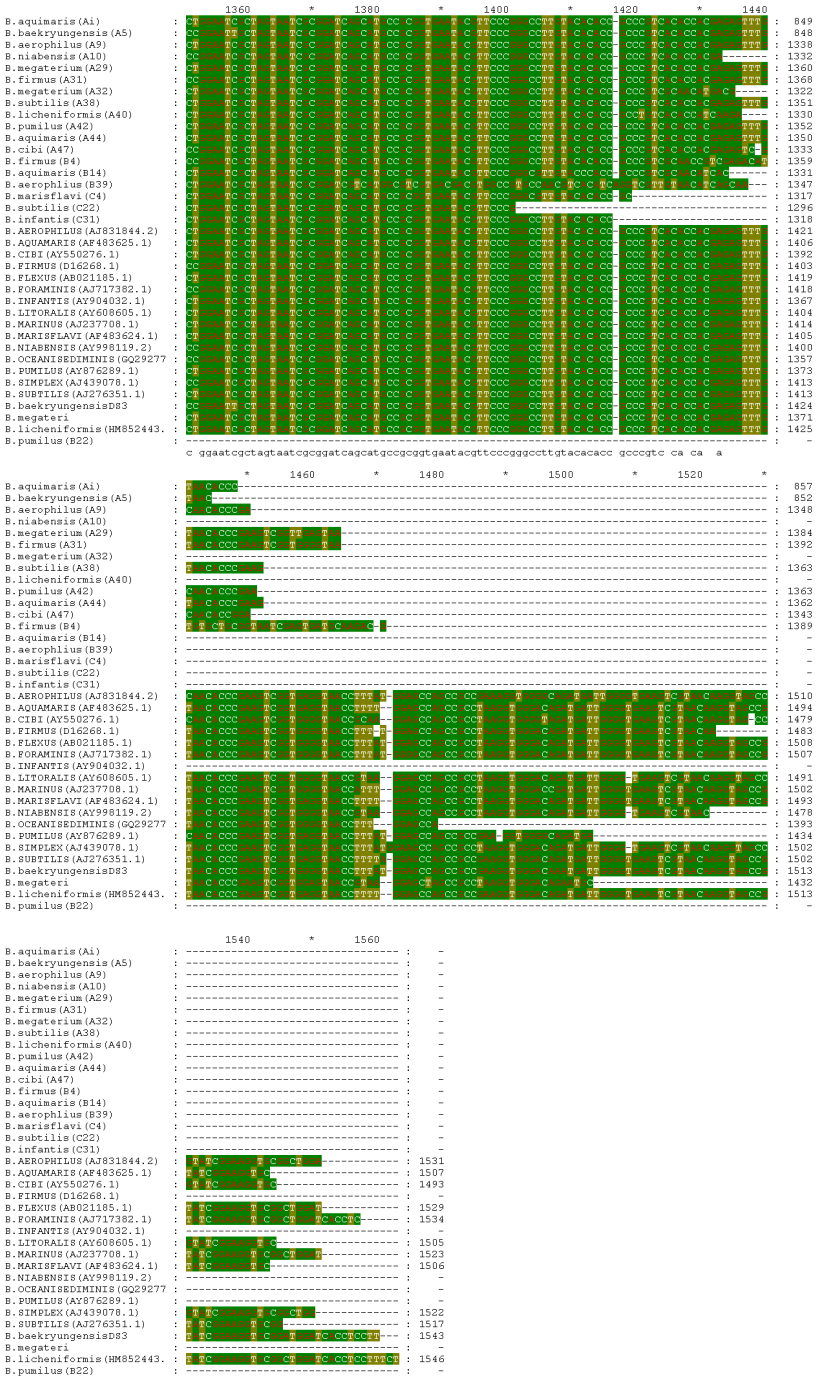


Fig. 6.8. Clustal W multiple alignment of the 16S rRNA gene from *Bacillus* strains isolated during the present study and phylogenetically related strain sequences from NCBI.

6.4. DISCUSSION

Marine bacterioplankton represent one of the most thoroughly studied environmental communities on the planet (Giovannoni and Stingl, 2005), yet bacteria inhabiting marine sediments remain largely uncharacterized. This lack of information hinders an effective assessment of marine bacterial diversity and limits our understanding of the fundamental differences between the bacterial population inhabiting two major ocean ecosystems. One apparent yet relatively unexplored difference between seawater and sediment bacterial communities is the relative abundance of Gram-positive bacteria. Microbial community structure analysis can be extended to give us an understanding of functional and biogeographical relationships and such data are vital for an improved understanding of benthic ecosystem processes and the role that the benthos play in overall oceanic processes. Deep sea benthic communities contain many ubiquitous, broadly distributed prokaryotic groups, since environmental conditions (temperature, nutrient availability and supply, and pressure) and processes (elemental reduction, and carbon mineralization, etc.) can be considered to be generally similar over wide tracts of the oceanic seabed.

While early research estimated that only 5% of the bacteria in the ocean are Gram-positive (ZoBell, 1946), more recent studies suggest that the abundance and diversity of Gram-positive strains in sediments may be considerably greater (Priest, 1989; Stach *et al.,* 2005; Jensen *et al.,* 2005). The previous reports indicate that considerably diverse Gram-positive microbial population can be cultured from marine sediments and reinforces the concept that relatively simple cultivation techniques can be used successfully to isolate many as-yet-undescribed taxa (Connon and Giovannoni, 2002; Maldonado *et al.,* 2005b). Members of the genus *Bacillus* and related genera are ubiquitous in nature. However, *Bacillus* spp. isolated from marine sediments have attracted less interest with respect to their terrestrial relatives. The widespread of *Bacillus* spp. and their high diversity level observed in this work point out the need of more extensive studies to understand

their distribution and ecology in deep-sea environments. Using 16S rDNA sequencing and phenotypic analyses, we conducted studies to understand the phylogenetic relationship of the members of the family *Bacillaceae*. The present study employed a combination of cultivation dependent and molecular identification methods to assess the diversity of Gram-positive spore forming *Bacillus* in marine sediments collected from Arabian Sea.

Application of ARDRA helped us to group the strains based on the different band pattern. Therefore, ARDRA at the outset of a taxonomic study is recommended to obtain indicative phylogenetic and taxonomic information, which can be used to select strains for detailed polyphasic taxonomic studies. As a 16S rDNA based molecular technique, ARDRA has been used for phylogenetic and taxonomic studies in *Mycobacterium* (De Baere *et al.,* 2002), *Brevibacillus* (Logan *et al.,* 2002), *Clostridium* (Gurtler *et al.,* 1991), *Streptococcus* (Jayaro *et al.,* 1991) and *Acinetobacter* species (Vaneechoutte *et al.,* 1995). Heyndrickx and colleagues developed an ARDRA method for identification of strains of the genera *Alcaligenes, Bordetella, Bacillus* and *Paenibacillus* (Heyndrickx *et al.,* 1996). ARDRA has also been used in the analysis of mixed bacterial populations from different environments such as activated sludge, compost samples, marine bacterioplancton and hypersaline environments (Acinas *et al.,* 1999; Gich *et al.,* 2000; Koschinsky *et al.,* 2000; Martinez-Murcia *et al.,* 1995). It has been proven to be sensitive for quick assessment of genotypic changes in the community over time, and successfully used to compare communities subject to different environmental conditions.

In these studies, a universal bacterial 16S rDNA primer set was used to amplify a 1500-bp amplicon, followed by construction of ARDRA patterns using 5 restriction enzymes. The obtained ARDRA patterns for each species were combined to form a database for strain identification in each genus. Theoretically the more restriction enzymes used in ARDRA, the more accurate result will be obtained. Heyndrickx also tested different combinations of restriction enzymes in

the ARDRA assay and found that 3 enzymes yielded similar results in terms of species identification as did 5 enzymes (Heyndrickx *et al.,* 1996). Some authors have suggested that two restriction enzymes are adequate for ARDRA, especially in analyzing species composition changes in complex communities (Gich *et al.,* 2000; Koschinsky *et al.,* 2000; Moyer *et al.,* 1996). In our study we have used universal eubacterial primers and two restriction enzymes *Alu*I and *Taq*I which was found to be adequate in differentiating most *Bacillus* strains. This was sufficient to allow ARDRA identification in our assay. In a study, conducted by XiYang *et al.* (2006) found that the ARDRA assay using *Bacillus*-specific primers and two restriction enzymes, *Alu*I and *Taq*I was quite adequate in differentiating most of the reference *Bacillus* strains.

The results indicate that *Bacillus* populations can be readily cultured from marine sediments. In the present study, ARDRA of the culturable bacterial isolates categorized them into various phylotypes, representatives of which were then sequenced for identification. Using a combination of ARDRA patterns generated on independent digestion of 16S rDNA amplicon of the strains by means of *Alu*I and *Taq*I restriction enzymes, we were able to attain 20 different ARDRA patterns representing 12 *Bacillus* species. Interestingly, most of the strains subsequently sequenced had high similarity (98%) with earlier reported culturable bacterial strains of the deep sea sediment (Dang *et al.,* 2009). The most abundant strains obtained from the sediments were related to *B. niabensis, B. megaterium, B. subtilis, B. aquimaris, B. firmus* and *B. licheniformis*.

The band pattern types developed using each restriction enzymes were variable and distinct. Each ARDRA-type was composed of 2 to 5 distinct bands with molecular weights (MW) ranging from 100 to 900-bp. The results are well in accordance with the results obtained with the work of XiYang *et al.* (2006) where the amplicons from different *Bacillus* strains digested with restriction enzymes *Alu*I and *Taq*I, generated different profiles of 2 to 5 fragments ranging in size from 76 to 804-bp. ARDRA with *Alu*I digestion resulted in the highest number of pattern

types. Using *Alu*I digestion 10 different band patterns were developed where as *Taq*I restriction digestion yielded 4 different band patterns. The most common DNA fragment for *Alu*I digest was 450, 200 and 250-bp and the common Taq1 generated fragments were in the range of 550 and 400-bp.

In our study it was observed that *B. subtilis* and *B. pumilus* produced 4 fragments in the size range 450 to 150-bp with *Alu*I, and with *Taq*I it produced 3 fragments in the range 500 to 200-bp for *B. subtilis,* and 2 fragments (750 and 350-bp) with *Taq*I digestion. Strains which showed close resemblance to *B. licheniformis* produced 2 fragments (800 and 250-bp) with *Alu*I and 3 fragments of size range between 500 and 200-bp with *Taq*I. These results were very similar to the ARDRA patterns observed for these strains in the study of XiYang *et al.* (2006). It was previously reported that ARDRA using universal primers could not separate *B. licheniformis* from *B. pumilus* and *B. subtilis* from *B. amyloliquefaciens* (Vaerewijck *et al.,* 2001). However, the ARDRA assay developed in this study was able to differentiate B. subtilis and *B. licheniformis* from the other species of the *B. subtilis* phylogenetic cluster (*B. pumilus, B. amyloliquefaciens* and *B. atrophaeus*).

Phylogenetic analysis of 16S rDNA sequences of *Bacillus* strains in this study revealed that sediments from all 3 depths were dominated by strains closely related to *Bacillus niabensis*. The presence of *B. niabensis* in the marine sediments of Palk Strait in the east coast of India was also reported in a study carried out by Chari Nithya and Pandiyan (2010). The study also indicated the remarkable occurrence of other forms of *Bacillus* including *B. aquimaris, B. subtilis, B. pumilus, B. cibi, B. marisflavi, B. megaterium* and *B. licheniformis* in Palk Strait. The present study also reported high abundance of *Bacillus megaterium. Bacillus aquimaris, B. subtilis, B. firmus* and *B. licheniformis* were other strains common to all three depth regions. While *Bacillus baekryungensis* and *B. cibi* were confined to 200m depth region, *B. marisflavi* and *B. infantis* were retrieved from 1000m depth region only. No strains exclusively confined to 500m depth region were isolated. Presence of *B. aerophilus* and *B. pumilus* confined to upper continental slope area

Among the numerous *Bacillus* species, only species of *B. subtilis, B. licheniformis, B. firmus, B. pumilus, B. mycoides,* and *B. lentus* were reported to have been detected from marine environments. Nevertheless, according to the observations of Ruger *et al.* (2000) and Besma *et al.* (2009), the strains of *B. subtilis* and *B. pumilus* were the most abundant among those associated with marine sponges, ascidians, soft corals, and they were present in seawater as well. The *B. firmus* strains similar to those reported by Siefert *et al.* (2000) isolated from the Gulf of Mexico, appeared to be phenotypically and phylogenetically diverse from the closely related *B. badius* and *B. lentus* reference strains and may represent strict marine group. This could also be supported by the fact that a *B. firmus* isolated from Hong Kong sediments showing high metal bioaccumulation capabilities (Keung *et al.,* 2008) confirming the widespread of Mn(II)-oxidizing *Bacillus* in these marine environments (Dick *et al.,* 2006).

All the strains were able to utilize a wide range of organic compounds which may reflect their great metabolic flexibility. Species such as *B. subtilis, B. amyloliquefaciens* and *B. licheniformis* are used industrially for the production of enzymes, antibiotics, solvents and other molecules (Gerhartz, 1990). *B. subtilis* is not only a renowned classical model organism for genetic research but also widely used in the production of enzymes, antibiotics, antagonistic substances or surfactants or, on the other hand, degradation of xenobiotics. Species of the *B. subtilis* group are closely related and thus not easily distinguishable. *B. pumilus* is starch negative and hippurate positive, and *B. licheniformis* is propionate positive, grows at temperatures up to 55 ˚C, and is facultatively anaerobic. All species can be differentiated at genetic level and it is to be expected that when genotypic analyses are applied to a wider range of strains additional species will be detected.

While majority of the identified *Bacillus* were rather terrestrial bacteria blown or deposited as spores into marine sediments, some *Bacillus* species such as those of the *B. firmus/ B. foraminis* and the halotolerant groups, without being obligate marine bacteria, seemed to be adapted in such environment which is in

agreement with recent findings (Sass *et al.,* 2008). Indeed, they may be implicated, even as active spores as reported previously (Dick *et al.,* 2006), in the biogeochemical cycles and diverse degradation process. To better understand the distribution and ecology of *Bacillus* species in deep-sea environments, more extensive studies would be needed. Moreover, a careful physiological, metabolic and enzymatic characterization should be carried out in order to assess the real biotechnological potential of these bacteria.

However, our finding may indicate a specific adaptation to marine environments of the *B. firmus* groups that could play an important role in oceanic biogeochemical cycles by oxidation, precipitation and bioaccumulation. Few publications are devoted to the study of the *Bacillus* species isolated from the marine environment. Due to their ubiquity and capability to survive under adverse conditions, heterotrophic *Bacillus* strains are hardly considered to be species of certain habitats (Claus and Berkeley, 1986). Results of the present phylogenetic study confirm the widespread of the *Bacillales* members in deep-sea environments and highlight their large diversity on culture-dependent approach. Unlike, high bacterial diversity reported through studies of Fuchs *et al.* (2005) for oxygen minimum zone of the Arabian Sea by culture independent methods, most of cultivable bacteria in the deep sea sediments were restricted to *Firmicutes* in spite of the fact that the sediment were rich in organic matter. Previous reports from marine sediments underlying Arabian Sea also show that it is dominated by *Firmicutes* (Divya *et al.,* 2010). This may be due to the competition and surveillance capability of Gram-positive forms for the limited labile substrates that reach the bottom of the sea from the overlying euphotic zone especially in the slope of the Arabian Sea.

Chapter 7

SUMMARY AND CONCLUSION

The Arabian Sea is one of the most biologically productive oceanic regions mainly due to the upwelling of nutrients during monsoon. Physical processes such as wind driven coastal upwelling, coastal runoff during monsoon and convective overturning of surface waters due to winter cooling, all add up nutrients into the region. Deep-sea sediments may be unique habitats for microbial communities; the availability of nutrients is geographically highly variable and these environments are subject to highly elevated pressures. They play a major role in most biogeochemical cycles maintaining the pristine nature of the environment by converting organic detritus into living biomass. Being largely consumed by heterotrophic nannoflagellates the organic matter might be channeled to higher trophic levels. A long-standing challenge in the field of microbial ecology and evolution has been the matter of determining the diversity and distribution of natural microbial communities in sea sediments. The microorganisms mediate between the dissolved, particulate and gaseous states of chemical compounds, thereby regulating the distribution of organic and inorganic nutrients throughout the ocean. Benthic biogeochemical processes, especially on the upper slope and within the oxygen minimum zone, including fluxes of nutrients and dissolved organic matter, may be of global significance but remain poorly quantified. Our knowledge about the culturable heterotrophic bacteria from Indian continental slope region is still insufficient. Hence we focused our study on heterotrophic bacteria in the continental slope sediments with the objective of evaluating their occurrence and diversity besides the hydrolytic potential in the decomposition of organic compounds.

The present work is focused on the latitudinal and depthwise distribution of total heterotrophic bacteria (THB) in the continental slope sediments of Arabian Sea. Sediment samples were collected from 48 stations (16 transects) at 200, 500 and 1000m depths along the continental slope of Arabian Sea. Hydrographical features and sediment characteristics were studied besides the enumeration of the total heterotrophic bacterial (THB) population through culture dependent studies. The isolates were identified based on morphological, physiological and biochemical characteristics. Hydrolytic enzyme production property of the isolates was also studied. On the basis of abundance, the Gram-positive spore forming bacilli were subjected to Amplified Ribosomal DNA Restriction Analysis (ARDRA) followed by identification based on 16S rDNA sequencing and phylogenetic analysis.

i). Salient finding of the present investigations are as follows.

- The study area was confined to the upper continental slope region from 200m to 1000m depths of eastern Arabian Sea along the west coast of India. Forty eight (48) stations distributed along 16 transects were covered in replica in two separate samplings. Each transect was situated one degree latitude apart which extended Off Cape Comorin (6°57'96" N and 77°21'96" E) to Off Porbandar (21°29'99" N and 67°46'96" E).
- Sample collection was done onboard FORV *Sagar Sampada* (MoES, Govt. of India). Cruise No. 228 (August-September, 2005) and 233 (March-April, 2006) were carried out to complete the first set of sampling (Sampling I). Second set of samples (Sampling II) were collected during Cruise No. 254 (May-June, 2007).
- During Sampling I, the bottom water temperature ranged from 14.5 to 16.1 ˚C at 200m stations, 9.9 to 13.2 ˚C at 500m stations and 6.9 to 10.1 ˚C at 1000m. During Sampling II the bottom water temperature varied between

12.8 to 17.3 ˚C at 200m, 10.6 to 13.1 ˚C at 500m and 7.2 to 10.2 ˚C at 1000m depth stations.

- The bottom water salinity ranged between 34.9 to 35.8 psu during Sampling I and 35.0 to 36.0 psu during Sampling II. The bottom water salinity showed a gradual increase towards the northern region in all depth ranges.
- The hydrographical recording have shown that the bottom water properties for temperature and salinity indicated the presence of low salinity water masses occupying the southern region which gradually is replaced by water masses of high salinity in the northern region. The incursion of water masses of Persian Gulf, Red Sea and Bay of Bengal origin occupying higher depths were also noticeable.
- During both the sampling periods in the region, the bottom water was found to be highly deficient of dissolved oxygen. The distribution shows that dissolved oxygen of the bottom water during Sampling I varied between 0.23 and 1.40ml l^{-1}. The dissolved oxygen of the bottom water during Sampling II varied between 0.10 and 1.09ml l^{-1}. In northern region the values were lesser indicating a hypoxic zone occupying the depth range 200-1000m.
- The characteristics of sediment in the area were generally silty clay in nature. It was noticed that region along 200m depth zone was more coarser in nature; subsequently changing to a finer sediment texture in the 500 and 1000m depth zones of the continental slope area in the Arabian Sea. A regional variation could be noticed as the southern region were more coarser than the northern region.
- Organic matter in the sediment recorded was in the range of 2-18% which was relatively high compared to most other parts of the global oceans. The

organic matter was found to be more in higher depths than at stations in the 200m depth region. An increase in the mean organic matter content of sediment was observed from 200 to 1000m depth i.e. 2.56 to 7.14% during Sampling I and 4.72 to 8.84% during Sampling II.

- Generally the organic matter (OM) was comparatively higher in northern region with the highest value being recorded Off Mumbai II. An increasing trend in organic matter (OM) was also noticed towards the northern region.
- Biochemical constituents of the organic matter stated that protein and lipid constituted high fraction of the organic matter than carbohydrate in the area under study.
- A culture dependent technique was adopted to study the total heterotrophic bacteria. The total heterotrophic bacterial counts (THB) showed a progressive increase from southern to northern transects with region of anecdotal distribution pattern found amid different depth zones and a decrease with increase in depth. However, a clear zonation or gradation of the bacterial abundance was not observed in this study.
- The culturable heterotrophic bacterial densities were usually higher at 200m depth ranges than at deeper depths, however *one-way ANOVA* results showed that the THB densities were not statistically significant between depths ($F_{sampling\ I} = 1.9$; $p=0.162$; $F_{sampling\ II} = 3.15$; $p=0.053$) and between samplings (Student's *t*-test; $p>0.05$).
- As revealed by a post-hoc Tukey analysis, bacterial population during Sampling I was found to be maximum at 200m depth stations ($104.42 \pm 248.34 \times 10^3$ cfu g^{-1} dry sediment weight) followed by 1000m ($20.36 \pm 24.11 \times 10^3$ cfu g^{-1} dry sediment weight) and 500m ($16.48 \pm 21.5 \times 10^3$ cfu g^{-1} dry sediment weight) depth. In Sampling II the higher bacterial population was observed at 200m depth stations ($7.23 \pm 6.97 \times 10^3$ cfu g^{-1} dry

sediment weight) followed by 1000m (4.6±4.94 x 10^3 cfu g^{-1} dry sediment weight) and 500m (2.72±2.98 x 10^3 cfu g^{-1} dry sediment weight) stations.

- Heterotrophic bacterial abundance has shown that the southern and northern regions of the eastern Arabian Sea harboured more heterotrophic bacteria than the central region.
- The differentiation of the bacterial strains based on Gram's reaction revealed that the sediment harbour considerable proportion of Gram-positive forms in the continental slope area of the western Indian Peninsula. However a clear gradation of the Gram-positives on a depthwise scale was lacking and found to be not statistically different between depths ($F_{sampling\ I}$ = 1.34; p=0.271; $F_{sampling\ II}$ = 0.82; p=0.45). Distribution of Gram-negatives was parallel with Gram-positives. During both the sampling, the proportion of Gram-positive and Gram-negative bacteria were about 70 and 30% respectively.
- The relation between biotic components with the environmental variables were investigated by BIOENV analysis In the 200m and 500m depth region the highest rank correlation with THB data (R=0.185 and 0.221 respectively) were obtained with salinity and DO exerting equally with additional variables Clay and PRT-C in 200m and CHO-C and LPD-C in 500m depth region. In 1000m depth region the results relied on four variables to obtain the highest rank correlation (R=0.284).
- In the case of Gram-positives DO was detrimental for all the depth zones, while additionally PRT-C, LPD-C and salinity contributed in each depth region to obtain the highest rank correlation. For Gram-negatives also DO was the major variable and LPD-C, CHO-C and BPC contributed to obtain the highest rank correlation. These results point to the key role of the geographical differences of these three regions, combined with the

complex local hydrological and substratum properties, may explain the observed variability in bacterial community composition.

- Bacterial diversity exposed about 15 genera/group of heterotrophic bacteria identified from the study area. Genus *Bacillus* was the dominant form followed by Coryneform group and *Pseudomonas*. Other important genera/groups represented in the sediments were *Micrococcus*, *Acinetobacter*, *Aeromonas*, *Staphylococcus*, *Vibrio*, Enterobacteriaceae, *Moraxella*, *Flexibacter*, *Flavobacterium, Cytophaga, Planococcus, Photobacterium* and *Chromobacterium*.

- The hydrolytic properties of the bacterial isolates were determined for extracellular enzymes including amylase, gelatinase, lipase, DNase and chitinase. Majority of the isolates from the sediments showed great capability of gelatinolytic and lipolytic hydrolytic enzymes production potential. Percentage of DNase positive forms were also remarkable. The contribution of amylase producers were comparatively lesser. Chitin degradation potential amongst the isolates were found to be the least.

- The ability of the bacteria to decompose macromolecular organic compounds revealed that the chief contribution of the enzymes *viz*., amylase, gelatinase, lipase, chitinase and DNase was from the genus *Bacillus*. The second major contributor was Coryneforms. The other major contributors were *Pseudomonas*, *Aeromonas* and *Acinetobacter*.

- *Bacillus* strains from different depth zones were grouped based on Amplified Ribosomal DNA Restriction Analysis (ARDRA) pattern. Restriction digestion independently with two restriction enzymes *Alu*I and *Taq*I, grouped the strains into 20 distinct ARDRA types based on the banding pattern.

- Based on 16S rRNA gene sequencing and BLAST analysis, the *Bacillus* strains could be identified upto species level. Totally 12 species could be identified.
- *Bacillus niabensis* was found to be the most dominant form in sediments at all three depth regions. The second most common group found in the present study was *B. megaterium*.
- *Bacillus aquimaris, B. subtilis, B. firmus* and *B. licheniformis* were found to be common to the three depth zones in the sudy area. *B. aerophilus* and *B. pumilus* were also retrieved from sediment samples at 200m and 500m depth region. While *B. baekryungensis* and *B. cibi* were confined to 200m depth region, *B. marisflavi* and *B. simplex* were retrieved from 1000m depth region only.

The present study provides an account of the occurrence and diversity of culturable heterotrophic bacteria in the slope sediments of Arabian Sea. Gram-positive forms were found to be dominant in the slope sediments. Organic matter was found to increase as the depth increased and lipid formed the major fraction of labile organic matter. Like their terrestrial relatives, marine Gram-positive bacteria were found to play a significant role in the breakdown of organic matter and therefore in the ocean's biogeochemical cycle. A remarkable observation in the present study was the dominance of Gram-positive bacteria of the genus *Bacillus*. Both the number and phylogeny of the strains belonging to genus *Bacillus* was intriguing as they were ubiquitous in distribution throughout the study area. This genus has been generally recognized to be among the most heterogeneous within the bacterial domain. The present study recovered strains belonging to 12 *Bacillus* species. The study indicated that considerably diverse *Bacillus* spp. could be cultured from marine sediments.

ii) Conclusion

Benthic biogeochemical processes, especially on the upper slope and within the oxygen minimum zone, including fluxes of nutrients and dissolved organic matter, may be of global significance. Deep-sea sediments harbour a vast majority of microbial populations hitherto unexplored. Our knowledge about the culturable heterotrophic bacteria from Indian continental slope particularly in a high productive system like Arabian Sea region are still insufficient. The study focused on culturable heterotrophic bacteria from the continental slope sediments along west coast of India with an objective of evaluating their occurrence and diversity besides the hydrolytic potential in the decomposition of organic compounds. The total heterotrophic bacteria in the sediments revealed that their distribution was effected both on a latitudinal and depthwise scale. The results showed that distribution followed regional trends in productivity in the shallow regions indicating high bacterial abundance in the high productive regions of Northern West region. Spatial distribution showed trend on a depthwise scale revealing lower bacterial densities in deeper depths on contrary to high organic matter present in the region. Extracellular hydrolytic enzyme production by bacterial forms indicated that the majority of the isolates were proteolytic and lipolytic followed by amylolyitc and DNA degrading forms. Chitin degradation capabilities in the heterotrophic bacteria were least recorded. Bacterial diversity revealed that there were a considerable high proportion of Gram-positives types in the sediments which is predominated by spore forming types. The most common and predominant group belonged to genus *Bacillus* which was recovered from all the station locations. Phylogenetic analysis carried out by molecular investigations also revealed a considerable high diversity of genus *Bacillus* from the sediments of Arabian Sea. It is globally appreciated that Gram-positive bacteria, especially members of the order *Bacillales* and *Actinobacteria*, are well-known producers of important secondary metabolites. Exploring hitherto pristine marine environment

near Indian subcontinent with the aid of modern cultivation-dependent and cultivation independent techniques will undoubtedly lead to the discovery of more strains representing diverse microbial flora and provide a direct means to learn more about their ecophysiology and applications in biotechnology.

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Table 2.A1. Hydrography parameters recorded at various depths (200, 500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

	Temperature (°C)						Salinity (psu)						D oxygen (ml l^{-1})					
	Sampling 1			Sampling II			Sampling 1			Sampling II			Sampling 1			Sampling II		
	200 m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m
Cape comorin	16.06	13.23	7.49	14.07	10.67	8.30	34.97	35.09	34.97	35.03	35.09	35.03	0.53	0.65	0.87	0.24	0.63	0.77
Trivandrum	14.67	9.87	7.33	15.45	10.88	8.01	35.03	35.09	34.98	35.05	35.13	35.05	1.29	0.49	0.82	0.35	0.65	0.83
Kollam	15.79	10.67	6.86	13.99	11.32	7.26	35.11	35.14	34.99	35.14	35.11	35.02	0.47	0.56	1.00	0.62	0.73	1.09
Kochi	14.58	11.05	7.73	12.84	11.15	8.49	35.15	35.17	35.07	35.15	35.16	35.10	0.32	0.47	0.65	0.54	0.58	0.54
Ponnani	14.79	10.94	7.13	13.50	10.62	8.82	35.17	35.18	35.03	35.17	35.20	35.15	0.37	0.57	0.29	0.43	0.53	0.65
Kannur	14.96	11.38	8.83	14.81	10.83	8.16	35.18	35.23	35.17	35.18	35.21	35.11	0.23	0.61	0.32	1.07	0.36	0.47
Mangalore	15.39	11.36	7.52	15.27	11.38	8.99	35.23	35.32	35.10	35.20	35.22	35.19	0.39	0.25	0.79	0.21	0.36	0.40
Coondapore	14.67	11.26	7.09	14.99	11.65	8.64	35.24	35.36	35.07	35.29	35.32	35.18	1.40	0.88	1.27	0.49	0.31	0.50
Karwar	14.46	11.35	8.36	15.23	11.14	9.09	35.33	35.40	35.20	35.31	35.41	35.22	0.86	0.49	0.58	0.15	0.25	0.74
Goa	15.03	11.63	7.82	15.14	11.93	9.57	35.33	35.45	35.20	35.35	35.45	35.31	0.55	0.41	0.67	0.16	0.11	0.34
Ratnagiri	14.62	12.66	8.52	15.53	11.97	9.74	35.40	35.48	35.25	35.39	35.51	35.38	1.05	0.47	0.77	0.13	0.18	0.38
Dabhol	15.25	12.10	8.62	16.76	12.49	9.64	35.49	35.53	35.31	35.62	35.56	35.40	0.76	0.50	0.78	0.12	0.08	0.18
Mumbai 1	14.60	11.95	9.67	15.29	12.38	10.05	35.57	35.58	35.43	35.70	35.61	35.45	0.69	0.72	1.27	0.29	0.09	0.31
Mumbai 2	15.81	12.59	9.76	16.48	12.68	10.11	35.62	35.68	35.46	35.80	35.64	35.49	0.63	0.81	0.63	0.12	0.22	0.26
Veraval	15.86	12.14	10.20	17.02	12.90	10.17	35.64	35.66	35.51	36.02	35.68	35.50	0.48	0.16	0.62	0.16	0.18	0.30
Porbandar	16.13	ND	10.13	17.33	13.09	10.21	35.84	ND	35.50	36.03	35.77	35.52	0.48	ND	0.47	0.10	0.07	0.12
Min	14.46	9.87	6.86	12.84	10.62	7.26	34.97	35.09	34.97	35.03	35.09	35.02	0.23	0.16	0.29	0.10	0.07	0.12
Max	16.13	13.23	10.20	17.33	13.09	10.21	35.84	35.68	35.51	36.03	35.77	35.52	1.40	0.88	1.27	1.07	0.73	1.09
Mean	15.17	11.61	8.32	15.23	11.69	9.08	35.33	35.36	35.20	35.40	35.38	35.26	0.66	0.54	0.74	0.32	0.33	0.49
SD	0.59	0.86	1.13	1.25	0.82	0.89	0.24	0.20	0.19	0.33	0.23	0.18	0.34	0.19	0.28	0.26	0.22	0.26

ND- not determined

Table 2.A2. Texture of slope sediments at various depths (200, 500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

	%SAND						%SILT						%CLAY					
	Sampling 1			Sampling II			Sampling 1			Sampling II			Sampling 1			Sampling II		
	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000 m
Cape comorin	66.66	71.30	35.56	72.53	52.77	30.91	16.99	14.63	43.79	20.24	35.95	53.64	16.36	14.07	20.65	7.22	11.28	15.45
Trivandrum	61.00	35.97	25.81	94.16	13.60	12.19	29.12	44.09	52.43	3.68	68.93	70.33	9.88	19.94	21.76	2.16	17.47	17.48
Kollam	75.96	83.63	18.22	88.84	90.46	ND	7.52	7.73	57.37	5.00	5.40	ND	16.52	8.63	24.41	6.15	4.15	ND
Kochi	81.35	10.91	6.53	79.43	5.87	2.56	1.46	62.23	67.05	9.61	73.21	72.80	17.18	26.86	26.43	10.97	20.92	24.64
Ponnani	59.48	10.85	6.86	66.46	5.81	4.59	18.21	65.88	55.20	18.75	71.30	74.77	22.31	23.27	37.95	14.79	22.89	20.64
Kannur	48.58	9.20	6.34	54.21	13.10	2.96	28.07	66.29	65.32	28.52	69.76	71.63	23.35	24.51	28.34	17.27	17.14	25.41
Mangalore	50.42	7.21	3.39	81.36	3.04	4.25	34.50	65.49	56.81	6.98	74.77	72.72	15.07	27.30	39.80	11.66	22.19	23.04
Coondapore	63.03	9.76	21.83	47.29	8.94	4.98	11.38	63.10	57.79	38.41	71.08	71.71	25.60	27.14	20.38	14.30	19.98	23.31
Karwar	52.12	74.76	8.76	58.43	3.73	4.82	30.21	18.57	65.08	28.25	74.23	70.46	17.68	6.67	26.16	13.33	22.04	24.72
Goa	60.24	13.39	15.04	90.07	6.95	11.44	26.59	61.65	57.91	5.20	72.50	65.72	13.17	24.96	27.05	4.72	20.55	22.84
Ratnagiri	ND	23.99	79.91	78.09	7.10	7.48	ND	52.80	3.55	15.30	73.31	74.13	ND	23.21	16.54	6.61	19.59	18.39
Dabhol	75.02	63.84	4.89	20.73	5.02	14.61	2.89	7.63	75.04	59.02	73.78	61.86	22.09	28.54	20.08	20.26	21.20	23.53
Mumbai 1	ND	ND	18.64	92.77	7.80	12.00	ND	ND	66.61	4.60	71.08	66.93	ND	ND	14.75	2.62	21.13	21.07
Mumbai 2	61.92	17.37	30.08	90.34	4.49	0.74	21.90	58.48	41.50	6.41	76.07	75.23	16.18	24.15	28.43	3.24	19.44	24.03
Veraval	24.25	7.90	2.26	7.61	13.44	1.74	55.04	60.58	65.49	70.94	70.27	74.61	20.71	31.53	32.25	21.45	16.29	23.65
Porbandar	51.68	51.88	18.10	4.59	46.88	4.92	28.76	28.31	56.49	68.13	37.81	68.68	19.57	19.82	25.41	27.28	15.30	26.40
Min	24.25	7.21	2.26	4.59	3.04	0.74	1.46	7.63	3.55	3.68	5.40	53.64	9.88	6.67	14.75	2.16	4.15	15.45
Max	81.35	83.63	79.91	94.16	90.46	30.91	55.04	66.29	75.04	70.94	76.07	75.23	25.60	31.53	39.80	27.28	22.89	26.40
Mean	59.41	32.80	18.89	64.18	18.06	8.01	22.33	45.16	55.46	24.32	63.72	69.68	18.26	22.04	25.65	11.50	18.22	22.31
SD	14.20	28.26	19.10	30.04	24.30	7.63	14.19	22.96	16.30	23.16	19.77	5.80	4.27	7.17	6.91	7.45	4.82	3.11

ND- not determined

Table 2.A3. Organic matter, Organic carbon and Biopolymeric carbon (BPC) of slope sediments at various depths (200, 500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

	% OM						% OC						BPC (mg g^{-1} dry wt)					
	Sampling I			Sampling II			Sampling I			Sampling II			Sampling I			Sampling II		
	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m
Cape comorin	3.17	3.86	5.70	0.95	6.88	5.82	1.85	2.24	3.31	0.55	4.00	3.38	4.77	6.28	8.08	4.52	4.23	4.74
Trivandrum	0.89	6.26	6.74	1.07	10.44	11.87	0.52	3.64	3.92	0.62	6.07	6.90	2.04	8.05	10.22	4.09	6.35	7.83
Kollam	2.73	1.72	6.29	2.61	4.51	ND	1.59	1.00	3.66	1.52	2.62	ND	4.58	2.50	8.54	3.95	4.29	ND
Kochi	0.83	8.07	8.19	3.09	11.04	11.63	0.48	4.69	4.76	1.79	6.42	6.76	1.97	9.97	10.28	2.68	6.65	5.70
Ponnani	1.78	9.49	7.36	5.70	12.22	11.87	1.04	5.52	4.28	3.31	7.11	6.90	3.31	9.65	9.74	5.01	5.08	6.90
Kannur	2.08	6.35	5.99	1.54	8.90	11.63	1.21	3.69	3.48	0.90	5.18	6.76	2.73	7.72	7.09	2.72	5.05	3.41
Mangalore	0.83	4.87	6.76	3.32	9.85	11.87	0.48	2.83	3.93	1.93	5.73	6.90	1.94	7.52	6.16	3.23	5.65	5.30
Coondapore	2.67	6.44	7.48	6.29	11.04	8.07	1.55	3.74	4.35	3.66	6.42	4.69	3.52	7.12	7.75	3.88	4.34	5.79
Karwar	3.32	6.65	6.59	7.71	11.39	9.61	1.93	3.86	3.83	4.49	6.62	5.59	4.40	9.68	6.53	5.86	6.46	6.31
Goa	2.67	10.92	7.00	3.32	11.39	8.66	1.55	6.35	4.07	1.93	6.62	5.04	3.49	15.36	6.52	3.97	7.76	4.87
Dabhol	ND	10.56	3.03	3.92	11.87	8.07	ND	6.14	1.76	2.28	6.90	4.69	0.00	13.69	4.30	3.27	5.04	3.69
Ratnagiri	2.79	6.41	10.62	11.63	16.02	9.14	1.62	3.73	6.18	6.76	9.32	5.31	4.55	11.09	11.96	3.68	8.27	3.28
Mumbai 1	ND	0.95	10.03	2.73	14.36	9.85	ND	0.55	5.83	1.59	8.35	5.73	0.00	10.63	2.74	3.98	6.12	3.98
Mumbai 2	2.91	12.82	12.88	3.68	17.56	6.17	1.69	7.45	7.49	2.14	10.21	3.59	3.12	14.37	13.86	3.57	7.13	1.42
Veraval	3.62	3.56	7.42	8.31	2.02	4.98	2.10	2.07	4.31	4.83	1.17	2.90	4.60	9.70	8.25	5.93	3.09	4.90
Porbandar	5.52	1.90	2.20	9.61	3.92	3.32	3.21	1.10	1.28	5.59	2.28	1.93	6.08	2.23	9.76	5.82	3.82	7.22
Min	0.83	0.95	2.20	0.95	2.02	3.32	0.48	0.55	1.28	0.55	1.17	1.93	0.00	2.23	2.74	2.68	3.09	1.42
Max	5.52	12.82	12.88	11.63	17.56	11.87	3.21	7.45	7.49	6.76	10.21	6.90	6.08	15.36	13.86	5.93	8.27	7.83
Mean	2.56	6.30	7.14	4.72	10.21	8.84	1.49	3.66	4.15	2.74	5.94	5.14	3.19	9.10	8.24	4.14	5.58	5.02
SD	1.26	3.46	2.59	3.18	4.23	2.77	0.73	2.01	1.51	1.85	2.46	1.61	1.70	3.69	2.78	1.04	1.47	1.70

ND- not determined

Table 2.A4. Labile Organic matter (Protein, Carbohydrate and Lipids) of slope sediments at various depths (200, 500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

	Protein (PRT) (mg g^{-1} dry wt)						Carbohydrate (CHO) (mg g^{-1} dry wt)						Lipid (LPD) (mg g^{-1} dry wt)					
	Sampling I			Sampling II			Sampling I			Sampling II			Sampling I			Sampling II		
	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m
Cape ccmorin	2.36	4.67	6.36	2.06	2.90	2.01	0.20	0.32	0.49	0.26	0.33	0.15	4.71	5.15	6.36	4.54	3.57	4.93
Trivandrum	0.68	5.71	7.84	0.13	3.28	7.43	0.06	0.45	0.66	0.18	0.67	0.82	2.25	6.76	8.15	5.27	5.97	5.15
Kollam	2.88	1.24	5.74	0.20	1.28	ND	0.38	0.11	0.54	0.08	0.15	ND	4.02	2.46	7.34	5.09	4.80	ND
Kochi	0.82	6.99	6.43	0.66	6.31	5.23	0.06	0.69	0.73	0.11	0.60	0.76	2.06	8.36	9.12	3.09	4.43	3.78
Ponnani	1.91	7.04	7.58	2.23	4.45	6.48	0.17	0.79	0.76	0.32	0.62	0.57	3.08	7.85	7.63	5.05	3.53	4.66
Kannur	1.36	5.05	6.12	0.74	2.78	1.57	0.12	0.51	0.55	0.12	0.57	0.62	2.69	6.72	5.17	3.08	4.61	3.19
Mangalore	0.91	4.76	3.84	0.98	3.80	2.46	0.05	0.58	0.65	0.15	0.59	0.75	1.97	6.60	5.36	3.59	4.73	5.06
Coondapore	2.09	4.53	7.44	0.68	3.54	2.38	0.20	0.51	0.53	0.40	0.73	0.54	3.22	6.26	5.19	4.52	3.08	5.88
Karwar	3.38	7.35	5.68	2.58	4.58	3.39	0.19	0.73	0.46	0.38	0.90	0.67	3.56	7.71	4.75	5.93	5.14	5.84
Goa	2.43	10.53	5.62	0.85	4.81	2.06	0.23	1.02	0.48	0.19	1.03	0.48	2.95	13.06	4.77	4.63	6.66	4.89
Dabhol	ND	9.40	2.56	0.66	3.82	1.99	ND	0.86	0.26	0.16	0.52	0.44	ND	11.65	3.92	3.84	3.95	3.39
Ratnagiri	2.99	8.24	7.97	0.61	4.13	2.03	0.26	0.76	0.75	0.83	0.46	0.39	3.98	9.00	10.34	4.06	8.08	2.84
Mumbai 1	ND	7.94	5.22	0.15	2.77	1.46	ND	0.89	0.45	0.06	0.87	0.45	ND	8.51	ND	5.17	5.89	4.11
Mumbai 2	1.95	9.67	9.94	0.57	5.76	0.72	0.19	0.89	0.83	0.12	1.03	0.22	2.79	12.36	11.54	4.32	5.19	1.30
Veraval	3.70	7.46	6.39	1.94	0.19	3.52	0.28	0.60	0.60	0.43	0.19	0.28	3.57	7.74	6.50	6.41	3.90	4.08
Porbandar	4.12	0.99	7.85	2.79	0.81	3.44	0.33	0.10	0.72	0.69	0.15	0.83	5.24	2.28	7.50	5.57	4.49	6.94
Min	0.68	0.99	2.56	0.13	0.19	0.72	0.05	0.10	0.26	0.06	0.15	0.15	1.97	2.28	3.92	3.08	3.08	1.30
Max	4.12	10.53	9.94	2.79	6.31	7.43	0.38	1.02	0.83	0.83	1.03	0.83	5.24	13.06	11.54	6.41	8.08	6.94
Mean	2.26	6.35	6.41	1.11	3.45	3.08	0.19	0.61	0.59	0.28	0.59	0.53	3.29	7.65	6.91	4.64	4.88	4.40
SD	1.08	2.74	1.75	0.89	1.67	1.92	0.10	0.27	0.15	0.22	0.29	0.21	0.96	3.03	2.20	0.96	1.29	1.40

ND- not determined

Table 3.A1. Microbial parameters from the slope sediments at various depths (200, 500 and 1000m) during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254).

	THB (x 10^3 cfu/g dry wt.)						% Gram Positive						% Gram Negative					
	Sampling I			Sampling II			Sampling I			Sampling II			Sampling I			Sampling II		
	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m	200m	500m	1000m
Cape comorin	36.00	0.73	1.57	12.30	1.54	1.10	84.21	42.11	94.74	90.48	85.00	76.19	15.79	57.89	5.26	9.52	15.00	23.81
Trivandrum	40.45	0.23	1.41	3.28	2.82	3.78	33.33	87.50	90.91	50.00	68.00	100	66.67	12.50	9.09	50.00	32.00	0.00
Kollam	5.47	0.54	1.72	1.94	0.67	ND	58.82	81.82	90.00	60.00	78.95	ND	41.18	18.18	10.00	40.00	21.05	ND
Kochi	2.83	1.47	1.83	3.09	0.47	16.09	43.75	73.33	80.00	90.00	95.24	86.96	56.25	26.67	20.00	10.00	4.76	13.04
Ponnani	143.35	11.72	54.61	8.61	1.07	1.56	68.42	69.23	88.89	57.14	60.00	80.95	31.58	30.77	11.11	42.86	40.00	19.05
Kannur	955.70	75.38	22.64	26.72	3.74	6.32	100	87.50	90.00	90.00	95.24	26.09	0.00	12.50	10.00	10.00	4.76	73.91
Mangalore	5.79	45.13	25.31	2.10	2.34	1.47	84.21	90.00	70.59	86.36	82.61	76.19	15.79	10.00	29.41	13.64	17.39	23.81
Coondapore	9.14	23.41	42.34	4.36	0.85	2.19	31.25	50.00	37.50	68.42	100	59.09	68.75	50.00	62.50	31.58	0.00	40.91
Karwar	18.62	9.49	0.83	1.47	0.58	0.33	33.33	60.00	55.56	91.30	100	78.95	66.67	40.00	44.44	8.70	0.00	21.05
Goa	4.59	6.81	9.34	1.96	0.92	0.76	90.91	50.00	50.00	66.67	82.35	34.78	9.09	50.00	50.00	33.33	17.65	65.22
Dabhol	ND	3.37	29.37	2.39	1.67	1.61	ND	33.33	69.23	60.00	66.67	50.00	ND	66.67	30.77	40.00	33.33	50.00
Ratnagiri	97.35	46.70	1.01	6.84	10.49	2.24	47.06	76.92	70.00	77.27	45.00	66.67	52.94	23.08	30.00	22.73	55.00	33.33
Mumbai 1	ND	3.72	87.06	13.88	2.32	4.62	ND	77.78	100	72.73	37.50	85.71	ND	22.22	0.00	27.27	62.50	14.29
Mumbai 2	28.09	13.19	24.49	1.70	1.20	4.64	21.43	85.71	40.00	76.92	78.95	72.73	78.57	14.29	60.00	23.08	21.05	27.27
Veraval	67.63	3.24	15.53	ND	9.23	6.30	28.57	44.44	28.57	ND	93.33	76.92	71.43	55.56	71.43	ND	6.67	23.08
Porbandar	46.83	18.56	6.71	14.73	3.61	1.94	75.00	100	73.33	78.57	73.68	72.73	25.00	0.00	26.67	21.43	26.32	27.27
Min	2.83	0.23	0.83	1.47	0.47	0.33	21.43	33.33	28.57	50.00	37.50	26.09	0.00	0.00	0.00	8.70	0.00	0.00
Max	955.70	75.38	87.06	26.72	10.49	16.09	100	100	100	91.30	100	100	78.57	66.67	71.43	50.00	62.50	73.91
Mean	104.42	16.48	20.36	7.02	2.72	3.66	57.16	69.36	70.58	74.39	77.66	69.60	42.84	30.64	29.42	25.61	22.34	30.40
SD	248.34	21.50	24.11	7.16	2.98	3.94	26.41	20.15	22.35	13.66	18.60	19.77	26.41	20.15	22.35	13.66	18.60	19.77

ND- not determined

Table 3.A2. Correlation co-efficient (Pearson) matrix (n=46) for physico-chemical parameters recorded during Sampling I (Cruise No. 228&233)

	Depth	Temp	Salinity	DO	Sand	Silt	Clay	Protein	Carbohydrate	Lipid	Biopolymeric-C	OC (%)	OM (%)	THB (cfu)	Gram + %	Gram - %
Depth	1.00															
Temp	**-0.94*****	1.00														
Salinity	-0.25	**0.37***	1.00													
DO	0.22	-0.20	-0.21	1.00												
Sand	**-0.59*****	**0.58*****	-0.06	0.00	1.00											
Silt	**0.56*****	**-0.56*****	0.06	0.05	**-0.98*****	1.00										
Clay	**0.46****	**-0.43****	0.05	-0.17	**-0.69*****	**0.52*****	1.00									
Protein	**0.55*****	**-0.53*****	0.21	0.00	**-0.63*****	**0.61*****	**0.43****	1.00								
Carbohydrate	**0.53*****	**-0.54*****	0.19	-0.04	**-0.67*****	**0.65*****	**0.51*****	**0.95*****	1.00							
Lipid	**0.43****	**-0.43****	0.23	-0.01	**-0.57*****	**0.56*****	**0.39****	**0.93*****	**0.94*****	1.00						
Biopolymeric-C	**0.49*****	**-0.48****	0.23	0.00	**-0.61*****	**0.59*****	**0.41****	**0.98*****	**0.96*****	**0.99*****	1.00					
OC (%)	**0.53*****	**-0.5*****	0.10	0.15	**-0.58*****	**0.57*****	**0.38***	**0.79*****	**0.76*****	**0.83*****	**0.82*****	1.00				
OM (%)	**0.53*****	**-0.51*****	0.10	0.15	**-0.58*****	**0.57*****	**0.38***	**0.79*****	**0.76*****	**0.83*****	**0.82*****	1.00	1.00			
THB (cfu)	-0.22	0.22	-0.05	-0.19	0.09	-0.12	0.04	-0.25	-0.25	-0.22	-0.23	-0.17	-0.17	1.00		
Gram + ve (%)	0.19	-0.25	-0.23	-0.22	-0.11	0.14	-0.03	-0.02	0.05	0.06	0.03	0.01	0.01	0.22	1.00	
Gram – ve (%)	-0.21	0.28	0.26	0.24	0.14	-0.15	-0.02	0.00	-0.07	-0.07	-0.05	-0.02	-0.02	-0.22	**-1.00*****	1.00

Level of significance ***P<0.001; **P<0.01; *P<0.05

Table 3.A3. Correlation co-efficient (Pearson) matrix (n=46) for physico-chemical parameters recorded during Sampling II (Cruise No. 254)

	Depth	Temp	Salinity	DO	Sand	Silt	Clay	Protein	Carbohydrate	Lipid	Biopolymeric-C	OC (%)	OM (%)	THB (cfu)	Gram + %	Gram - %
Depth	1.00															
Temp	**-0.90*****	1.00														
Salinity	-0.18	**0.43****	1.00													
DO	0.23	**-0.41****	**-0.71*****	1.00												
Sand	**-0.67*****	**0.64*****	-0.14	0.03	1.00											
Silt	**0.67*****	**-0.64*****	0.14	-0.03	**-1.00*****	1.00										
Clay	**0.64*****	**-0.57*****	0.14	-0.02	**-0.94*****	**0.90*****	1.00									
Protein	**0.39****	**-0.51*****	-0.16	0.18	**-0.62*****	**0.64*****	**0.52*****	1.00								
Carbohydrate	**0.33***	**-0.35***	0.09	-0.12	**-0.74*****	**0.74*****	**0.68*****	**0.71*****	1.00							
Lipid	-0.03	0.06	0.12	-0.16	-0.07	0.08	0.03	0.29	**0.35***	1.00						
Biopolymeric-C	0.24	**-0.30***	-0.01	0.00	**-0.47****	**0.49****	**0.39****	**0.82*****	**0.71*****	**0.78*****	1.00					
OC (%)	**0.36***	**-0.41****	0.10	-0.07	**-0.74*****	**0.75*****	**0.65*****	**0.71*****	**0.81*****	0.23	**0.62*****	1.00				
OM (%)	**0.36***	**-0.41****	0.10	-0.07	**-0.74*****	**0.75*****	**0.65*****	**0.71*****	**0.81*****	0.23	**0.62*****	1.00	1.00			
THB (cfu)	-0.20	**0.30***	0.14	0.15	0.09	-0.12	0.02	-0.18	-0.20	-0.10	-0.18	-0.23	-0.23	1.00		
Gram + ve (%)	-0.14	0.05	-0.06	0.17	0.00	0.01	-0.05	0.27	0.07	-0.15	0.08	-0.10	-0.10	0.09	1.00	
Gram – ve (%)	0.14	-0.05	0.06	-0.17	0.00	-0.01	0.05	-0.27	-0.07	0.15	-0.08	0.10	0.10	-0.09	**-1.00*****	1.00

Level of significance ***P<0.001; **P<0.01; *P<0.05

Table 4.A1. Generic composition of bacteria in 200m depth range during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254)

STN	TRANSECT	No of isolates	No. of Genera	*Bacillus*	Coryneforms	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling I (Cruise # 228&233)																					
0	**Cape comorin**	19	4	52.63	26.32	5.26	0.00	0.00	0.00	15.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	**Trivandrum**	18	5	16.67	16.67	0.00	0.00	0.00	0.00	27.78	11.11	0.00	0.00	0.00	0.00	0.00	27.78	0.00	0.00	0.00	0.00
6	**Kollam**	17	6	35.29	23.53	0.00	0.00	0.00	0.00	17.65	0.00	0.00	0.00	0.00	11.76	0.00	5.88	5.88	0.00	0.00	0.00
9	**Kochi**	16	6	25.00	12.50	0.00	6.25	0.00	0.00	43.75	0.00	0.00	0.00	0.00	6.25	0.00	0.00	0.00	0.00	6.25	0.00
12	**Ponnani**	19	3	0.00	57.89	10.53	0.00	0.00	0.00	31.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	**Kannur**	19	3	31.58	26.32	42.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	**Mangalore**	19	6	42.11	31.58	0.00	0.00	10.53	0.00	0.00	5.26	0.00	5.26	0.00	0.00	0.00	0.00	0.00	5.26	0.00	0.00
21	**Coondapor**	16	6	6.25	6.25	18.75	0.00	0.00	0.00	6.25	0.00	31.25	0.00	31.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	**Karwar**	6	3	0.00	0.00	33.33	0.00	0.00	0.00	50.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	**Goa**	11	4	18.18	0.00	45.45	0.00	27.27	0.00	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	**Dabhol**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
33	**Ratnagiri**	17	7	5.88	5.88	35.29	0.00	0.00	0.00	29.41	5.88	0.00	5.88	11.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36	**Mumbai 1**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
39	**Mumbai 2**	14	7	7.14	0.00	14.29	0.00	0.00	0.00	50.00	7.14	7.14	7.14	7.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42	**Veraval**	21	7	0.00	14.29	14.29	0.00	0.00	0.00	38.10	4.76	4.76	14.29	9.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45	**Porbandar**	8	5	12.50	37.50	25.00	0.00	0.00	0.00	0.00	12.50	0.00	0.00	0.00	12.50	0.00	0.00	0.00	0.00	0.00	0.00

Table continued

STN	TRANSECT	No of isolates	No. of Genera	*Bacillus*	Coryneforms	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling II (Cruise # 254)																					
0	**Cape comorin**	21	6	52.38	23.81	9.52	4.76	0.00	0.00	0.00	4.76	0.00	0.00	4.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	**Trivandrum**	20	5	35.00	15.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	15.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00
6	**Kollam**	20	6	35.00	25.00	0.00	0.00	0.00	0.00	15.00	15.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00
9	**Kochi**	20	4	50.00	30.00	10.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	**Ponnani**	14	5	28.57	28.57	0.00	0.00	0.00	0.00	0.00	21.43	0.00	7.14	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	**Kannur**	20	6	50.00	15.00	10.00	15.00	0.00	0.00	5.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	**Mangalore**	22	3	86.36	0.00	0.00	0.00	0.00	0.00	4.55	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	**Coondapor**	19	6	42.11	26.32	0.00	0.00	0.00	0.00	0.00	5.26	0.00	10.53	10.53	0.00	0.00	0.00	0.00	5.26	0.00	0.00
24	**Karwar**	23	5	47.83	26.09	0.00	17.39	0.00	0.00	0.00	4.35	0.00	0.00	4.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	**Goa**	15	6	33.33	33.33	0.00	0.00	0.00	0.00	6.67	13.33	0.00	0.00	6.67	0.00	0.00	6.67	0.00	0.00	0.00	0.00
30	**Dabhol**	20	6	30.00	25.00	5.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	25.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
33	**Ratnagiri**	22	5	22.73	54.55	0.00	0.00	0.00	0.00	0.00	4.55	0.00	0.00	0.00	0.00	0.00	9.09	0.00	0.00	0.00	9.09
36	**Mumbai 1**	11	5	18.18	36.36	18.18	0.00	0.00	0.00	0.00	18.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.09	0.00	0.00
39	**Mumbai 2**	13	4	7.69	69.23	0.00	0.00	0.00	0.00	0.00	15.38	0.00	0.00	7.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42	**Veraval**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
45	**Porbandar**	14	4	28.57	50.00	0.00	0.00	0.00	0.00	7.14	0.00	0.00	0.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.A2. Generic composition of bacteria in 500m depth range during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254)

STN	TRANSECT	No of isolates	No. of Genera	*Bacillus*	Coryneforms	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling I (Cruise # 228&233)																					
1	**Cape comorin**	19	8	15.79	10.53	10.53	0.00	5.26	0.00	26.32	0.00	0.00	0.00	5.26	0.00	0.00	0.00	15.79	10.53	0.00	0.00
4	**Trivandrum**	24	6	79.17	0.00	4.17	0.00	4.17	0.00	4.17	0.00	0.00	0.00	4.17	0.00	0.00	0.00	0.00	4.17	0.00	0.00
7	**Kollam**	22	6	63.64	9.09	0.00	9.09	0.00	0.00	4.55	0.00	4.55	0.00	0.00	0.00	0.00	0.00	0.00	9.09	0.00	0.00
10	**Kochi**	15	4	66.67	6.67	0.00	0.00	0.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	0.00	0.00
13	**Ponnani**	13	3	15.38	53.85	0.00	0.00	0.00	0.00	30.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	**Kannur**	16	4	68.75	12.50	6.25	0.00	0.00	0.00	0.00	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	**Mangalore**	20	4	70.00	20.00	0.00	0.00	0.00	0.00	0.00	5.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	**Coondapore**	4	4	0.00	25.00	25.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	**Karwar**	5	4	0.00	20.00	40.00	0.00	0.00	0.00	20.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	**Goa**	12	6	8.33	25.00	16.67	0.00	0.00	0.00	33.33	8.33	8.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31	**Dabhol**	6	6	16.67	16.67	0.00	0.00	0.00	0.00	16.67	16.67	16.67	0.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34	**Ratnagiri**	13	5	7.69	7.69	61.54	0.00	0.00	0.00	7.69	0.00	0.00	0.00	15.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	**Mumbai 1**	9	5	11.11	44.44	22.22	0.00	0.00	0.00	0.00	11.11	11.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	**Mumbai 2**	7	4	28.57	0.00	42.86	0.00	14.29	0.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
43	**Veraval**	9	5	22.22	0.00	22.22	0.00	0.00	0.00	22.22	22.22	0.00	11.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	**Porbandar**	8	2	0.00	50.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table continued

STN	TRANSECT	No of isolates	No. of Genera	*Bacillus*	Coryneforms	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling II (Cruise # 254)																					
1	**Cape comorin**	20	4	10.00	75.00	0.00	0.00	0.00	0.00	5.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	**Trivandrum**	25	7	36.00	32.00	0.00	0.00	0.00	0.00	12.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.00	0.00	4.00
7	**Kollam**	19	5	57.89	21.05	0.00	0.00	0.00	0.00	5.26	10.53	0.00	0.00	5.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	**Kochi**	21	5	61.90	19.05	4.76	9.52	0.00	0.00	0.00	4.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	**Ponnani**	15	5	40.00	20.00	0.00	0.00	0.00	0.00	13.33	13.33	0.00	0.00	13.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	**Kannur**	21	4	71.43	19.05	0.00	4.76	0.00	0.00	0.00	0.00	0.00	0.00	4.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	**Mangalore**	23	5	52.17	30.43	0.00	0.00	0.00	0.00	8.70	0.00	4.35	0.00	4.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	**Coondapore**	19	3	47.37	42.11	0.00	10.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	**Karwar**	22	2	68.18	31.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	**Goa**	17	5	35.29	41.18	0.00	5.88	0.00	0.00	0.00	0.00	0.00	0.00	5.88	0.00	0.00	11.76	0.00	0.00	0.00	0.00
31	**Dabhol**	15	5	53.33	13.33	0.00	0.00	0.00	0.00	20.00	6.67	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	0.00
34	**Ratnagiri**	20	7	5.00	35.00	5.00	0.00	0.00	0.00	15.00	20.00	0.00	5.00	15.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	**Mumbai 1**	16	6	18.75	12.50	6.25	0.00	0.00	0.00	0.00	12.50	0.00	0.00	43.75	0.00	0.00	6.25	0.00	0.00	0.00	0.00
40	**Mumbai 2**	19	3	78.95	0.00	0.00	0.00	0.00	0.00	5.26	15.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
43	**Veraval**	15	3	53.33	40.00	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	**Porbandar**	19	5	52.63	21.05	0.00	0.00	0.00	0.00	0.00	5.26	0.00	0.00	15.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.A3. Generic composition of bacteria in 1000m depth range during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254)

STN	TRANSECT	No of isolates	No. of Genera	*Bacillus*	*Coryneforms*	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling I (Cruise # 228&233)																					
2	**Cape comorin**	19	3	73.68	21.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.26	0.00	0.00	0.00	0.00	0.00
5	**Trivandrum**	11	3	81.82	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	**Kollam**	20	5	75.00	10.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	5.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
11	**Kochi**	15	5	66.67	6.67	6.67	0.00	0.00	0.00	6.67	0.00	0.00	0.00	0.00	0.00	0.00	13.33	0.00	0.00	0.00	0.00
14	**Ponnani**	9	3	55.56	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00
17	**Kannur**	10	4	60.00	20.00	0.00	10.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	**Mangalore**	17	6	41.18	0.00	23.53	0.00	5.88	0.00	17.65	5.88	0.00	5.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	**Coondapore**	8	4	0.00	0.00	37.50	0.00	0.00	0.00	37.50	12.50	0.00	0.00	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	**Karwar**	9	4	33.33	0.00	0.00	0.00	22.22	0.00	33.33	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29	**Goa**	6	5	16.67	0.00	33.33	0.00	0.00	0.00	16.67	16.67	0.00	0.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32	**Dabhol**	13	4	30.77	7.69	30.77	0.00	0.00	0.00	30.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35	**Ratnagiri**	10	5	20.00	0.00	30.00	0.00	20.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00
38	**Mumbai 1**	7	3	28.57	0.00	42.86	0.00	28.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41	**Mumbai 2**	10	7	0.00	0.00	30.00	0.00	10.00	0.00	10.00	10.00	10.00	20.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
44	**Veraval**	7	4	0.00	0.00	28.57	0.00	0.00	0.00	0.00	28.57	14.29	0.00	28.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	**Porbandar**	15	4	0.00	26.67	46.67	0.00	0.00	0.00	13.33	0.00	0.00	0.00	13.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table continued

STN	TRANSECT	No of isolates	No. of Genera	*Bacillus*	Coryneforms	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling II (Cruise # 254)																					
2	**Cape comorin**	21	5	28.57	47.62	0.00	0.00	0.00	0.00	9.52	4.76	0.00	0.00	0.00	0.00	0.00	9.52	0.00	0.00	0.00	0.00
5	**Trivandrum**	15	2	53.33	46.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	**Kollam**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	**Kochi**	23	4	60.87	26.09	0.00	0.00	0.00	0.00	8.70	4.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	**Ponnani**	21	5	28.57	52.38	0.00	0.00	0.00	0.00	0.00	9.52	0.00	0.00	4.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	**Kannur**	23	8	8.70	4.35	8.70	4.35	0.00	0.00	17.39	30.43	21.74	0.00	0.00	0.00	0.00	4.35	0.00	0.00	0.00	0.00
20	**Mangalore**	21	5	9.52	28.57	38.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.05	0.00	0.00	4.76	0.00	0.00	0.00	0.00
23	**Coondapore**	22	7	27.27	22.73	9.09	0.00	0.00	0.00	4.55	0.00	0.00	0.00	27.27	0.00	0.00	4.55	0.00	0.00	0.00	0.00
26	**Karwar**	19	5	57.89	15.79	0.00	5.26	0.00	0.00	0.00	15.79	0.00	0.00	5.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29	**Goa**	23	6	17.39	17.39	0.00	0.00	0.00	0.00	4.35	8.70	0.00	0.00	39.13	0.00	0.00	13.04	0.00	0.00	0.00	0.00
32	**Dabhol**	8	4	25.00	25.00	0.00	0.00	0.00	0.00	12.50	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35	**Ratnagiri**	21	8	33.33	9.52	9.52	14.29	0.00	0.00	4.76	19.05	0.00	0.00	4.76	0.00	0.00	4.76	0.00	0.00	0.00	0.00
38	**Mumbai 1**	21	6	19.05	57.14	4.76	4.76	0.00	0.00	4.76	0.00	0.00	0.00	9.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41	**Mumbai 2**	11	5	18.18	45.45	9.09	0.00	0.00	0.00	18.18	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
44	**Veraval**	13	5	46.15	30.77	0.00	0.00	0.00	0.00	7.69	0.00	7.69	0.00	7.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	**Porbandar**	22	5	63.64	9.09	0.00	0.00	0.00	0.00	4.55	0.00	0.00	0.00	13.64	0.00	0.00	9.09	0.00	0.00	0.00	0.00

Table 4.A4. Mean percentage of the various bacterial genera from different depth ranges during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254)

	No of Strains	*Bacillus*	Coryneformes	*Micrococcus*	*Planococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Aeromonas*	*Vibrio*	Enterobacteriaceae	*Acinetobacter*	*Flavobacterium*	*Chromobacterium*	*Moraxella*	*Cytophaga*	*Flexibacter*	*Photobacterium*	*Alcaligenes*
Sampling I																			
200m	220	19.5	20.0	15.5	0.5	2.3	0.0	22.3	3.6	3.2	2.7	4.5	1.8	0.0	2.7	0.5	0.5	0.5	0.0
500m	202	40.1	16.3	13.9	1.0	1.5	0.0	12.4	4.0	3.0	0.5	3.0	0.0	0.5	0.0	1.5	2.5	0.0	0.0
1000m	186	41.9	9.7	17.2	0.5	4.8	0.0	11.3	3.2	1.1	2.2	4.8	0.0	0.5	2.2	0.0	0.0	0.5	0.0
Overall	608	33.2	15.6	15.5	0.7	2.8	0.0	15.6	3.6	2.5	1.8	4.1	0.7	0.3	1.6	0.7	1.0	0.3	0.0
Sampling II																			
200m	274	40.1	28.8	3.3	2.9	0.0	0.0	5.1	7.7	0.0	1.1	6.9	0.0	0.0	2.2	0.0	0.7	0.0	1.1
500m	306	46.7	28.8	1.0	2.0	0.0	0.0	5.2	6.9	0.3	0.3	6.2	0.0	0.0	1.3	0.0	0.3	0.0	0.3
1000m	284	33.1	28.2	5.6	2.1	0.0	0.0	6.0	8.5	2.1	0.0	9.9	0.0	0.0	3.9	0.0	0.0	0.0	0.0
Overall	864	40.2	28.6	3.2	2.3	0.0	0.0	5.4	7.6	0.8	0.5	7.6	0.0	0.0	2.4	0.0	0.3	0.0	0.5

Table 5.A1. Percentage of hydrolytic enzyme producing bacteria at different depth ranges during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254)

Sampling I (Cruise # 228&233)	200m					500m					1000m				
	Amylase	Gelatinase	Lipase	DNase	Chitinase	Amylase	Gelatinase	Lipase	DNase	Chitinase	Amylase	Gelatinase	Lipase	DNase	Chitinase
Cape comorin	32	53	47	--	--	21	16	37	11	--	68	95	63	--	--
Trivandrum	28	67	61	--	--	46	79	29	67	17	73	91	64	73	--
Kollam	35	47	35	41	--	27	41	48	27	23	20	55	70	30	25
Kochi	19	56	69	31	25	27	53	47	53	13	27	40	40	40	13
Ponnani	53	32	68	11	11	38	46	27	15	--	67	56	44	67	11
Kannur	16	37	37	--	--	38	81	66	--	19	20	50	30	--	--
Mangalore	21	74	63	58	--	50	70	53	20	15	41	82	82	--	12
Coondapore	38	44	50	13	19	25	25	50	--	--	67	56	44	67	--
Karwar	33	50	33	17	--	40	20	60	20	--	20	50	30	--	--
Goa	33	50	33	17	--	58	75	67	17	25	41	82	82	--	--
Dabhol	ND	ND	ND	ND	ND	33	50	50	--	--	54	62	77	--	--
Ratnagiri	35	59	59	--	6	31	46	54	--	--	10	57	10	--	--
Mumbai 1	ND	ND	ND	ND	ND	56	67	56	44	--	43	57	57	43	--
Mumbai 2	29	57	50	7	14	56	67	56	44	--	20	50	60	--	--
Veraval	52	62	52	33	--	67	78	44	--	--	71	86	71	29	--
Porbandar	38	63	88	50	--	67	78	63	38	--	7	47	47	--	--
Mean Value	33	53	53	19	5	41	57	48	25	10	39	64	56	20	5

Table continued

	200m					500m					1000m				
Sampling II (Cruise # 254)	**Amylase**	**Gelatinase**	**Lipase**	**DNase**	**Chitinase**	**Amylase**	**Gelatinase**	**Lipase**	**DNase**	**Chitinase**	**Amylase**	**Gelatinase**	**Lipase**	**DNase**	**Chitinase**
Cape comorin	62	71	76	67	10	75	95	60	70	5	57	57	57	48	19
Trivandrum	40	40	65	70	20	72	84	72	84	12	87	100	100	100	--
Kollam	55	75	50	60	--	68	89	74	89	5	ND	ND	ND	ND	ND
Kochi	55	80	75	70	15	52	86	71	110	--	70	83	78	70	--
Ponnani	43	93	100	79	21	33	73	67	67	13	76	81	57	71	14
Kannur	45	75	70	70	--	38	81	76	67	14	39	61	78	61	9
Mangalore	91	91	86	23	--	74	87	74	87	--	43	38	33	24	5
Coondapore	58	74	63	68	11	63	89	63	79	21	50	68	50	64	--
Karwar	39	96	78	74	4	68	82	64	82	5	37	63	63	47	5
Goa	73	93	100	93	27	71	100	88	94	18	39	100	96	91	35
Dabhol	50	85	60	70	15	40	73	47	53	20	38	88	63	50	--
Ratnagiri	68	86	55	86	9	55	75	65	65	10	43	81	76	67	10
Mumbai 1	64	73	82	45	18	19	56	50	13	13	76	67	52	67	5
Mumbai 2	69	85	77	62	15	42	74	74	89	5	91	100	45	100	9
Veraval	ND	ND	ND	ND	ND	53	93	73	73	--	46	62	54	77	--
Porbandar	64	100	71	86	7	53	95	84	79	16	64	100	91	100	5
Mean Value	58	81	73	68	11	56	84	69	76	9	56	75	67	68	8

Table 5.A2. Percentage contribution of various bacterial genera to the hydrolytic enzymes production from different depth ranges during Sampling I (Cruise No. 228&233) and Sampling II (Cruise No. 254)

Sampling I (Cruise # 228&233)	200m					500m					1000m				
	Amylase	Gelatinase	Lipase	DNase	Chitinase	Amylase	Gelatinase	Lipase	DNase	Chitinase	Amylase	Gelatinase	Lipase	DNase	Chitinase
Bacillus	17	30	28	35	25	44	54	52	67	70	41	46	44	61	50
Coryneforms	30	21	26	30	17	18	14	16	19	15	11	14	13	13	10
Micrococcus	4	6	7	3	0	3	6	10	4	0	13	10	13	13	10
Planococcus	1	1	1	0	0	3	0	0	0	0	2	1	0	0	0
Staphylococcus	0	0	0	0	0	1	1	1	2	0	3	3	3	0	0
Streptococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas	16	24	22	15	33	12	10	9	4	10	11	9	12	3	20
Aeromonas	6	6	5	5	0	7	4	5	0	5	3	2	2	3	0
Vibrio	9	4	5	3	17	4	6	5	2	0	3	2	1	0	0
Enterobacteriaceae	6	3	2	0	0	1	1	0	0	0	5	4	3	3	0
Acinetobacter	3	3	1	3	8	1	2	0	2	0	3	3	4	0	0
Flavobacterium	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Chromobacterium	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Moraxella	4	0	2	3	0	0	0	0	0	0	5	4	4	3	10
Cytophaga	0	0	0	0	0	3	1	1	0	0	0	0	0	0	0
Flexibacter	0	1	0	3	0	3	1	2	0	0	0	0	0	0	0
Photobacterium	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Alcaligens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table continued

	200m					500m					1000m				
Sampling II (Cruise # 254)	**Amylase**	**Gelatinase**	**Lipase**	**DNase**	**Chitinase**	**Amylase**	**Gelatinase**	**Lipase**	**DNase**	**Chitinase**	**Amylase**	**Gelatinase**	**Lipase**	**DNase**	**Chitinase**
Bacillus	45	43	45	43	32	49	52	53	54	55	37	35	35	35	26
Coryneforms	31	29	24	32	32	34	27	26	26	20	35	30	24	32	23
Micrococcus	1	2	2	1	0	1	1	0	1	0	5	4	4	4	0
Planococcus	1	3	4	1	3	1	2	2	2	0	1	1	2	1	3
Staphylococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas	8	7	6	7	10	4	6	4	6	7	4	5	5	6	10
Aeromonas	7	7	8	6	6	5	6	6	6	7	6	8	10	6	10
Vibrio	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Enterobacteriaceae	1	1	0	1	3	0	0	0	0	0	0	0	0	0	0
Acinetobacter	4	6	6	6	3	4	5	5	3	7	7	12	14	12	16
Flavobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chromobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Moraxella	2	1	3	2	6	1	1	1	1	0	4	5	6	6	13
Cytophaga	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Flexibacter	1	1	1	1	3	1	0	0	0	3	0	0	0	0	0
Photobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alcaligens	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

GenBank: JX080178.1

Bacillus niabensis strain N849-A10 16S ribosomal RNA gene, partial sequence

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VERSION     JX080178.1  GI:394773498
SOURCE      Bacillus niabensis
  ORGANISM  Bacillus niabensis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1332)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1332)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080179.1

Bacillus megaterium strain N1673-A32 16S ribosomal RNA gene, partial sequence

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ACCESSION   JX080179
VERSION     JX080179.1  GI:394773499
SOURCE      Bacillus megaterium
  ORGANISM  Bacillus megaterium
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1322)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1322)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080180.1

Bacillus licheniformis strain N2324-A40 16S ribosomal RNA gene, partial sequence

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ACCESSION   JX080180
VERSION     JX080180.1  GI:394773500
SOURCE      Bacillus licheniformis
  ORGANISM  Bacillus licheniformis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1330)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1330)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080181.1

Bacillus aquimaris strain N1635-A44 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus aquimaris strain N1635-A44 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080181
VERSION     JX080181.1  GI:394773501
SOURCE      Bacillus aquimaris
  ORGANISM  Bacillus aquimaris
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1362)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1362)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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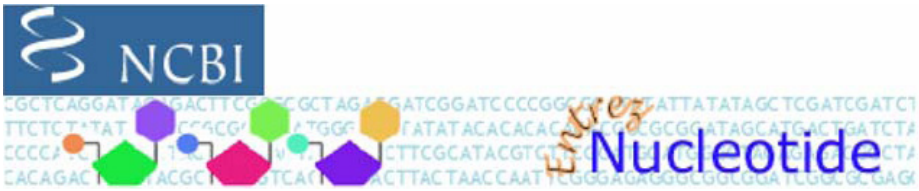

GenBank: JX080182.1

Bacillus megaterium strain N1564-A29 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus megaterium strain N1564-A29 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   JX080182
VERSION     JX080182.1  GI:394773502
SOURCE      Bacillus megaterium
  ORGANISM  Bacillus megaterium
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1384)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1384)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080183.1

Bacillus firmus strain N1674-A31 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus firmus strain N1674-A31 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   JX080183
VERSION     JX080183.1  GI:394773503
SOURCE      Bacillus firmus
  ORGANISM  Bacillus firmus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1392)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1392)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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GenBank: JX080184.1

Bacillus subtilis strain N2009-A38 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus subtilis strain N2009-A38 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080184
VERSION     JX080184.1  GI:394773504
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1363)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1363)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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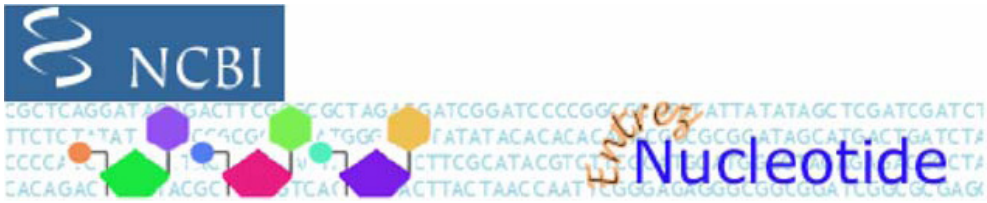

GenBank: JX080185.1

Bacillus pumilus strain N2463-A42 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus pumilus strain N2463-A42 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080185
VERSION     JX080185.1  GI:394773505
SOURCE      Bacillus pumilus
  ORGANISM  Bacillus pumilus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1363)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1363)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080186.1

Bacillus cibi strain N2326-A47 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus cibi strain N2326-A47 16S ribosomal RNA gene, partial
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VERSION     JX080186.1  GI:394773506
SOURCE      Bacillus cibi
  ORGANISM  Bacillus cibi
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1343)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1343)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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       61 aaccggagct aataccggat aacctttcgg accgcatggt ccggaattga aagacggttt
      121 cggctgtcac ttacagatgg gcccgcggcg cattagctag ttggtgaggt aatggctcac
      181 caaggcgacg atgcgtagcc gacctgagag ggtgatcggc cacactggga ctgagacacg
      241 gcccagactc ctacgggagg cagcagtagg gaatcttccg caatggacga aagtctgacg
      301 gagcaacgcc gcgtgagtga tgaaggtttt cggatcgtaa agctctgttg tcagggaaga
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      421 tacgtgccag cagccgcggt aatacgtagg tggcaagcgt tgtccggaat tattgggcgt
      481 aaagcgcgcg caggcggtct tttaagtctg atgtgaaagc ccccggctca accggggagg
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GenBank: JX080187.1

Bacillus aquimaris strain N922-Ai 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus aquimaris strain N922-Ai 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080187
VERSION     JX080187.1  GI:394773507
SOURCE      Bacillus aquimaris
  ORGANISM  Bacillus aquimaris
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 857)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 857)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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     rRNA            <1..>857
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ORIGIN
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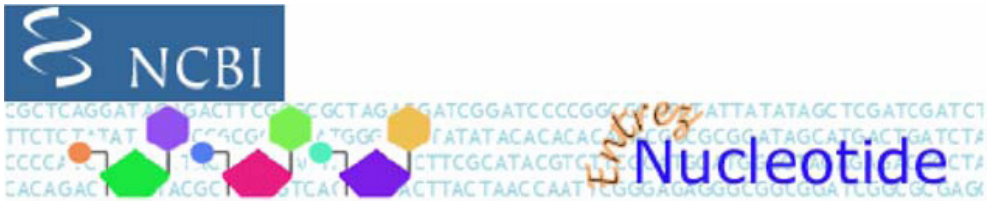

GenBank: JX080188.1

Bacillus baekryungensis strain N970-A5 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus baekryungensis strain N970-A5 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   JX080188
VERSION     JX080188.1  GI:394773508
SOURCE      Bacillus baekryungensis
  ORGANISM  Bacillus baekryungensis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 852)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 852)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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      601 aggaaggtgg ggatgacgtc aaatcatcat gccccttatg acctgggcta cacacgtgct
      661 acaatggacg gtacaaaggg cagcgacacc gcgaggtgaa gcaaatccca taaagccgtt
      721 ctcagttcgg attgcaggct gcaactcgcc tgcatgaagc cggaattgct agtaatcgcg
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GenBank: JX080189.1

Bacillus aerophilus strain N1031-A9 16S ribosomal RNA gene, partial sequence

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LOCUS       JX080189            1348 bp    DNA     linear   BCT 14-JUL-2012
DEFINITION  Bacillus aerophilus strain N1031-A9 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080189
VERSION     JX080189.1  GI:394773509
SOURCE      Bacillus aerophilus
  ORGANISM  Bacillus aerophilus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1348)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1348)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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      181 gctcaccaag gcgacgatgc gtagccgacc tgagagggtg atcggccaca ctgggactga
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GenBank: JX080190.1

Bacillus aquimaris strain N868-B14 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus aquimaris strain N868-B14 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080190
VERSION     JX080190.1  GI:394773510
SOURCE      Bacillus aquimaris
  ORGANISM  Bacillus aquimaris
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1331)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1331)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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GenBank: JX080191.1

Bacillus aerophilus strain N2156-B39 16S ribosomal RNA gene, partial sequence

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LOCUS       JX080191            1347 bp    DNA     linear   BCT 14-JUL-2012
DEFINITION  Bacillus aerophilus strain N2156-B39 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   JX080191
VERSION     JX080191.1  GI:394773511
SOURCE      Bacillus aerophilus
  ORGANISM  Bacillus aerophilus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1347)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1347)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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GenBank: JX080192.1

Bacillus firmus strain N807-B4 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus firmus strain N807-B4 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   JX080192
VERSION     JX080192.1  GI:394773512
SOURCE      Bacillus firmus
  ORGANISM  Bacillus firmus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1389)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1389)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080193.1

Bacillus pumilus strain N947-B22 16S ribosomal RNA gene, partial sequence

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LOCUS       JX080193             880 bp    DNA     linear   BCT 14-JUL-2012
DEFINITION  Bacillus pumilus strain N947-B22 16S ribosomal RNA gene, partial
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ACCESSION   JX080193
VERSION     JX080193.1  GI:394773513
SOURCE      Bacillus pumilus
  ORGANISM  Bacillus pumilus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 880)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 880)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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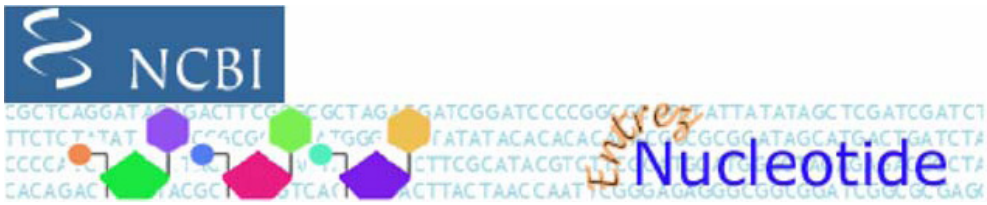

GenBank: JX080194.1

Bacillus marisflavi strain N892-C4 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus marisflavi strain N892-C4 16S ribosomal RNA gene, partial sequence.
ACCESSION   JX080194
VERSION     JX080194.1  GI:394773514
SOURCE      Bacillus marisflavi
ORGANISM    Bacillus marisflavi
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1317)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1317)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
FEATURES             Location/Qualifiers
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GenBank: JX080195.1

Bacillus subtilis strain N840-C22 16S ribosomal RNA gene, partial sequence

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LOCUS       JX080195            1296 bp    DNA     linear   BCT 14-JUL-2012
DEFINITION  Bacillus subtilis strain N840-C22 16S ribosomal RNA gene, partial  sequence.
ACCESSION   JX080195
VERSION     JX080195.1  GI:394773515
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1296)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1296)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2012) Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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GenBank: JX080196.1

Bacillus infantis strain N1607-C31 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus infantis strain N1607-C31 16S ribosomal RNA gene,   partial sequence.
ACCESSION   JX080196
VERSION     JX080196.1  GI:394773516
SOURCE      Bacillus infantis
ORGANISM    Bacillus infantis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1318)
  AUTHORS   Correya,N.S.
  TITLE     Microbenthos from the continental slope sediments of the Arabian
            Sea
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1318)
  AUTHORS   Correya,N.S.
  TITLE     Direct Submission
JOURNAL     Submitted (16-MAY-2012) Department of Marine Biology, Microbiology     and Biochemistry,
Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682016, India
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