

Bacterial community composition markedly altered by coastal hypoxia

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Abstract

Monsoonal upwelling along south and central west coast of India leads to intense biological productivity. As a consequence of excess organic matter production following upwelling during June-October and low dissolved oxygen in the upwelled waters, denitrification occurs in the near shore waters. Implicitly, these processes ought to bring alterations in microbial communities. Therefore, diversity and community structure of bacteria from subsurface layers of a tropical region experiencing intense seasonal lows of oxygen were analyzed through sequencing of 16S rRNA gene clones. The overall diversity was more during hypoxic period of Fall intermonsoon (FIM, October) compared either to Spring intermonsoon (SIM, March) or summer monsoon (SuM, June) months. As many as 14 lineages of bacterial domains: *Gammaproteobacteria* (37%), *Alphaproteobacteria* (21%), *Cyanobacteria* (20%), *Deltaproteobacteria* (3%), *Firmicutes* (3%), *Betaproteobacteria* (2%), *Acidobacteria* (2%), *Actinobacteria* (7%), *Marinimicrobia* (2%), *Bacteroidetes* (1%), *Verrucomicrobia* (1%), *Planctomycetes* (0.4%), *Chloroflexi* (0.2%) and *Omnitrophica bacterium* (0.2%), were recognized from our coastal location. Notably, sequences of *Planctomycetes*, *Chloroflexi* and *Omnitrophica bacterium* were found exclusively during FIM. A generally higher representation of sequences of *Betaproteobacteria* during SuM and of *Actinobacteria* and *Firmicutes* during SIM was discernible. This study is thus useful to recognize that microbial community might undergo strong temporal shifts in the monsoon affected tropical coastal ecosystems.

Keywords: Tropical Coastal Ecosystem, Goa, Upwelling, Hypoxia, Bacterial Community, 16S rDNA Clones, Phylogenetic Composition

Introduction

Tropical coastal regions influenced by seasonal and intense rain fall experience land run off impacts besides receiving allochthonous bacteria and excessive nutrients from terrestrial sources. Typically, in these ecosystems there is higher primary production and generally higher microbial diversity. While many inorganic (e.g. nitrogenous, phosphatic and silicic acid) nutrients promote autotrophic processes, it is the heterotrophic microbial community which is largely responsible for the fertility of these ecosystems. By catalyzing the breakdown of organic substrates these heterotrophs provide inorganic nutrients for the rich growth and productivity of autotrophs [1]. Heterotrophic remineralization following excessive primary primary/biological production may also result in exhaustion of available oxygen subsequently leading to usurp nitrate as electron acceptor and eventually production of certain greenhouse gases. As such, being a formidable proportion of marine planktonic community, heterotrophic bacteria through their metabolic activities modify and breakdown organic matter. .

Upwelling-influenced coasts are among the highly biologically productive systems which contribute significantly to the downward flux of organic matter. Along the west coast of India, seasonal hypoxia occurs following elevated biological production soon after the southwest monsoon when there is intensive upwelling [2]. Due to high primary production, there is increased oxygen demand for increased oxidant supply to sustain the bacterial respiration. Observations of [2, 3] implicitly highlight the occurrence of depleted oxygen combined with hyposaline-cap during September-October months leading to considerable subsurface-hypoxia and denitrification events.

Seasonal hypoxia is acknowledged to be harmful and is known to be detrimental to fish, benthic fauna, crustaceans through altered trophic energy transfer. Responding through metabolic adjustments the bacterial communities, however, continue to function in hypoxic waters and, contribute to organic matter oxidation and transformation of many biologically essential elements. Denitrification-led loss of fixed nitrogen along many parts of the Eastern Arabian Sea [2, 3, 4] consequently bring about adverse impacts on fisheries [4]. In these coastal regions, since the predominant microflora extant may have vital roles in nitrogen chemistry and other bio-geochemical processes, it is crucial to find out which component of bacteria are pivotal in such ecosystems.

In a recent study culture-dependent phylogenetic diversity of denitrifying bacterial assemblage in the neritic waters off Goa is very high (unpublished data). These findings suggested that a wide range of bacterial species denitrify albeit only a few cultures were rapid denitrifiers. As such, it is bacterial community amenable to plate culture is a tiny fraction. Therefore, only a

miniscule of the total bacterial types are isolated from natural communities. Further, the flexibility to discern the seasonal variability, if any, cannot be easily made out from culture-based analyses that require not only elaborate, time taking analyses but also may not yield statistically reliable results to ascertain seasonal variability. In coastal areas experiencing seasonal hypoxia, such as the central west coast of India, it becomes essential to recognize what communities are extant because of hypoxia. Therefore, we resorted to culture-independent 16S rDNA sequencing to record that fraction of microbial community to provide an overview of possible temporal differences among communities off Goa experiencing seasonal hypoxia.

Materials and Method

Study Area

The central west coast of India undergoes intense upwelling during southwest monsoon season (June-October). Owing to shoreward movement of nutrient-rich but lowly oxic subsurface waters from the outer-shelf during May-June, the near-bottom waters are enriched in particular with nitrate. The upwelling brings nitrate-rich waters into the photic layers, promoting very high primary production. But the oxidation of organic matter following such production leads to decreased oxygen concentration that in turn would lead to denitrifying conditions in the subsurface waters. [4] report that the nitrate concentration progressively decreases from July and, in August there is quite a high accumulation of NO_2^- in the subsurface and near bottom waters. By October, denitrification exhausts both nitrate and nitrite, and builds up the accumulation of ammonium when the conditions are almost anoxic [4, 5]. With receding upwelling by late October, surface currents are northward and the dissolved oxygen gradually increases leading to sufficient oxygenation by December-January. By this period, as reported by [5, 6], the water column nutrient concentrations are generally low.

Sampling

The sampling was done under the program Sustained Indian Ocean Biogeochemistry and Ecological Research (SIBER). Samples were collected Off Goa during Fall intermonsoon (FIM; October 2013), Summer monsoon (SuM; June 2014) and Spring intermonsoon (SIM; March 2015) from three different depths (surface, mid-depth and bottom) at two locations: G5-0, 7 and 25m ($15^{\circ}51'N$, $73^{\circ}51'E$) and G9-0, 35 and 66m ($15^{\circ}40'N$, $73^{\circ}28'E$). Sampling was carried out from surface, mid-depth and bottom at two stations shown in Figure 1. Water samples were collected during the cruise SSK 056, SSD Tr-008 and SSK 079 of *R V Sindhu Sankalp* and *Sindhu Sadhana*. Pre-cleaned (ethanol rinsed and deionised water-washed and air dried) Niskin samplers were used for collecting water samples.

Concentrations of dissolved oxygen and nutrients

Dissolved oxygen (DO) was measured following the classical Winkler titration from each depth [7]. Concentrations of nitrate, nitrite, ammonia following standard methods [8] by using an autoanalyser (Skalar Analytical). The estimation was carried out immediately after the collection of water sample onboard.

Enumeration of total bacterial counts (TBC)

Volumes of 50 ml samples from each depth were fixed with buffered formaldehyde (2% final concentration). Following the method of [9], 2 ml sub samples were incubated for 20 minutes with 4', 6-diamidino-2-phenylindole (DAPI; 20 μ l of 1 mg ml⁻¹ working solution ml⁻¹) and filtered onto black 0.22 μ m pore-size polycarbonate membrane filters (Millipore). As many as 20 microscopic fields were chosen to obtain a reliable mean. Epifluorescence microscopic counts were made using Olympus (BX-51 Japan) microscope.

Extraction of Metagenomic DNA and PCR amplification of 16S rRNA gene

From each depth 2.5 L seawater were passed peristaltically through Sterivex cartridge fitted with 0.22 μ m pore size membrane filter (Millipore, USA), filled with lysis buffer (composition: Tris pH 8.3, 40mM EDTA and 0.75M Sucrose). These cartridges were sealed and stored at -80⁰C until taken up for DNA extraction by following method described by [10].

Bacterial 16S rRNA gene from the extracted DNA samples was amplified by following standard PCR method [11]. The PCR products obtained thus were checked by agarose gel (1.5%) electrophoresis.

Construction of clone libraries, sequencing and statistical analysis

All these steps are described in our previous publication [12]. Canonical Correspondence analysis to analyze the effect of environmental parameters on the bacterial community composition and Hierarchical Cluster Analysis (HCA) to identify the similarities in the selected stations was performed using PAST- 3 software (<https://folk.uio.no/ohammer/past/>).

Nucleotide sequence accession numbers

The GenBank accession numbers assigned to our submitted sequences are KX179919 to KX179927, KP076438 to KP076559, KT361318 to KT361395, KU498049 to KU498248, KX179928 to KX180027, and KX238959 to KX260224, MF480560 to MF480649, MF540159 to MF540248 and MF588563 to MF588652.

Results

Hydrographic features of the study location

Temperature variations between the seasons were minimum, ranging from 28°C at the surface layer to 22-25°C in the mid-depth and 19-22°C in the near-bottom water layers during SuM and FIM and a constant temperature of 28°C all through the water column during SIM. Dissolved oxygen (DO) was the poorest during FIM in the mid and near-bottom water samples. It was < 6µM to barely discernible levels in close to bottom samples. Notably, during SIM, the water column was well-oxygenated. Nitrate concentrations were quite high in the samples from mid-depth and close to bottom during FIM. It was 25µM in the bottom waters, 29µM to untraceable levels in the mid-depth and negligible in the surface. In contrast of higher DO throughout the water column, the nitrate was undetectable during SIM. Nitrite, ranging from negligible to 3µM, was higher only in the bottom waters; and undetectable in the surface and mid depths during FIM. It was not detected during SuM. During SIM its concentrations ranged from 0.03 µM at the surface to 0.05 µM near the bottom. The concentration of ammonia varied widely during the three seasons and was negligible in the surface, <4µM in the mid-depth and bottom during FIM. During SuM, its concentrations were 0.33-0.72 µM in the surface, 0.02-0.5 µM in the mid-depth and <0.3 in the near-bottom samples. While during SIM the surface values was 0.6 µM and <1 µM in the mid-depth and bottom. The TBC ranged respectively from 5.9 to 11.3 X (10⁹) L⁻¹, from 4.2 to 10.8 X (10⁹) L⁻¹ during and 4.9 to 11.8 X (10⁹) L⁻¹ during FIM, SuM and SIM. Higher counts were observed in the surface and were quite low in the near-bottom samples during all three seasons (Supplementary Material- Table 1).

Bacterial community composition

In all, 870 non-chimeric 16S rRNA gene sequences from the two stations G5 and G9 (145 clones each from the two stations for each season) were available for characterizing the community. They aligned into the domains of *Gammaproteobacteria* (37%), *Alphaproteobacteria* (21%), *Cyanobacteria* (20%), *Deltaproteobacteria* (3%), *Firmicutes* (3%), *Betaproteobacteria* (2%), *Acidobacteria* (2%), *Actinobacteria* (7%), *Marinimicrobia* (2%), *Bacteroidetes* (1%), *Verrucomicrobia* (1%), *Planctomycetes* (0.4%), *Chloroflexi* (0.2%) and *Omnitrophica bacterium* (0.2%).

Major Phylogenetic groups during SIM

During the oxic SIM, ten different phylogenetic groups were obtained from both the sampling stations. *Gammaproteobacteria* was the dominant group observed during SIM, followed by *Cyanobacteria*. Pronounced vertical differences in the community structure of bacteria was

evidenced. In that, *Cyanobacteria* and *Betaproteobacteria* were found at the oxygenated surface, while *Gammaproteobacteria*, *Alphaproteobacteria* and *Actinobacteria* were observed throughout the water column. Minor groups of bacteria like *Firmicutes*, *Bacteroidetes*, *Marinimicrobia*, *Acidobacteria* and *Verrucomicrobia* were observed at sub-surface waters (Fig. 2). The bacterial groups increased vertically from surface to bottom depth.

Major Phylogenetic groups during SuM

Seven Phylogenetic groups were detected at G5 and nine were detected at G9. As seen in SIM, the major dominant groups during SuM were *Gammaproteobacteria* and *Cyanobacteria*. *Gammaproteobacteria* was found to be increasing towards the bottom. *Alphaproteobacteria* was more prominent at the mid layer. The relative abundance of *Alphaproteobacteria* substantially decreased towards the bottom with the increase of *Gammaproteobacteria* with water depth. Minor groups of bacteria like *Deltaproteobacteria*, *Marinimicrobia*, *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* were observed at the low oxygenated subsurface layers (Fig. 2)

Major Phylogenetic groups during FIM

Compared to SIM and SuM, there were more number of bacterial groups during FIM. As many as 14 different Phylogenetic groups were obtained. *Planctomycetes*, *Chloroflexi* and *Omnitrophica bacterium* were evidenced in this suboxic season. *Gammaproteobacteria* was the dominant group, followed by *Alphaproteobacteria*. Unlike SuM, the clones of *Deltaproteobacteria* were more near-bottom. Also, minor groups of bacteria like *Acidobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Marinimicrobia* were found all through the water column. While *Chloroflexi* and *Firmicutes* were found at the mid depths (Fig. 2).

Phylogenetic analysis of bacterial 16SrRNA genes across seasons

There were as many as 128 OTUs formed from the 870 clones analyzed in this study. Among these 38 OTUs were of *Alphaproteobacteria*, 34 of *Gammaproteobacteria*, 13 of *Deltaproteobacteria*, eight of *Marinimicrobia*, seven of *Acidobacteria*, six each of *Firmicutes* and *Cyanobacteria*, five of *Actinobacteria*, three each of *Betaproteobacteria* and *Bacteroidetes*, two of *Verrucomicrobia* and, one OTU each were of *Chloroflexi*, *Planctomycetes* and *Omnitrophica bacterium* (Supplementary Material-Fig 3, 4, 5 and 6). The phylogenetic trees constructed show relationships between the dominant OTUs in each season and their closest neighbors. For constructing these trees, only representative phylotype from each OTU were taken after grouping with MOTHUR at 97% identity.

Statistical analyses of 16S rDNA clone libraries

Shannon and Simpson diversity indices were the highest during FIM (4.2 and 0.01, respectively) followed by SIM (3.84 and 0.06, respectively) and the lowest during SuM (3.22 and 0.10, respectively). The estimated coverage values ranged from 84% to 90% (Supplementary Material- Table 2). The diversity indices were also estimated from each depth across the seasons along the vertical profiles, similar patterns of richness (Chao1) and diversity indices (Shannon and Simpson) were discernible during all the three seasons (Supplementary Material- Table 3). The rarefaction analysis indicated that the sampling effort was adequate enough to cover the possible phylotypes extant in the study area (Fig 7).

Canonical correspondence analysis (CCA) was performed to discern the relationship between the environmental parameter and bacterial community structure (Fig 8). Five significant environmental variables: temperature, DO, nitrate, nitrite and ammonia were selected in the CCA biplot. The length of an environmental parameter arrow in the ordination plot indicates the strength of the relationship of that parameter to community composition. As such, temperature, DO, nitrate, nitrite and ammonia appeared to be the most important environmental parameters. CCA indicated that among the five different parameters, DO and temperature seemed to separate the bacterial communities into oxygenated surface and low oxygen mid and bottom depths. Surface depth positively correlated with DO and temperature during all three seasons whereas the mid and the bottom depths interconnected with nutrients (ammonia, nitrate, and nitrite).

Beta diversity of bacterial communities by Bray-Curtis based hierarchical clustering of bacterial clone libraries revealed clear patterns of vertical as well as temporal partitioning (Fig 9). The first cluster consisted of bacterial communities residing in the mid and bottom layers, while the second cluster consisted of bacterial communities in the surface layers from all the three seasons.

Discussion

Most organic matter in the marine ecosystem is oxidized and transformed via aerobic respiration by microbial heterotrophs. When the oxygen concentrations are low, many facultative aerobic, heterotrophic bacterial genera begin resorting to nitrate (NO_3^-) and sulphate (SO_4^{2-}) as electron acceptors for organic matter oxidation) [3, 9]. This process seems to be strongly influenced by reduced oxygen in particular during SuM and FIM in the study area [6, 12, 13]. Post-upwelling, adequate availability of nitrate and organic carbon under reduced DO concentrations lead to denitrification [3, 4]. As a consequence of such denitrification, the nitrite and nitrous oxide concentrations are reported to be $>15 \mu\text{M}$ and $>500 \text{ nM}$ respectively [3] from the coastal waters of

eastern Arabian Sea. It is acknowledged [4] that, it is this paradox which results in sizable loss of fixed nitrogen via effluxing of nitrous oxide a potent greenhouse gas.

Bacterial communities off Goa apparently exhibit distinct seasonal patterns. These patterns in abundance and distribution are seemingly influenced different environmental variables. In the sampling locations off Goa the circulation is clockwise during the southwest monsoon season) (southerly along central west coast) with surface flow equator ward and the subsurface flow of upwelling water northward [6]. The up-welled water is nitrate rich and oxygen poor and these nutrient-rich waters support very high primary production. Consequently, leading to higher consumption of oxygen during organic matter decomposition [4], which leads to subsequent intensification of denitrification [12, 13] as well as sulfate reduction.

Very low concentrations of dissolved oxygen were observed during FIM and SuM at mid depth and near the bottom. But the concentrations of nitrate, nitrite and ammonia were high. The DO was depleted owing to degradation of organic matter that follows strong primary/biological productivity. Many authors [4, 5, 12] have reported that along India's west coast, upwelling during the SWM (June to October) nitrate rich waters are entrained into photic layers ultimately leading to denitrification. Observations since 1997 [4] have also shown that high concentrations ($>1 \mu\text{M}$) of nitrite invariably persist when denitrification is at its peak and, later on ammonia builds up in the water column turning near/totally anoxic. Water column gets sufficiently oxygenated with the reversal of coastal circulation during SIM, especially during and after November which continues as [5] emphasize, to be so until June.

The 16S rDNA sequences from this study bring to fore that the bacterial community in the coastal waters off Goa is similar to the ones reported by [14] from the coastal and open oceans OMZs, semi-enclosed or enclosed euxinic basins, viz., the NESP, the ESTP, the Namibian upwelling region, and Saanich Inlet. The bacterial community composition is also similar to the one recently reported by [15] from a seasonally hypoxic estuary. In this study, a large proportion of the ribosomal sequences were found to belong to *Proteobacteria*. It is known that *Proteobacteria* exhibit a large phylogenetic diversity that ought to encompass nutritionally versatile species those that aid in the colonization of many types of ecological niches. The other phyla observed in our study were *Cyanobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia* and *Planctomycetes*. The following account of taxa-wise sequences is to provide a functional overview of bacterial community in the study area. For instance, the sequences of *Cyanobacteria* constituted 20% of the total sequences. These oxygen-evolving prokaryotes are known to play a fundamental role in cycling of nitrogen, sulphur and carbon, affecting bio-

geochemical cycles. SAR 11 cluster and Rhodobacteraceae were the dominant groups of the class *Alphaproteobacteria* as also reported from the ETSP OMZ. The SAR11 clade and the Rhodobacteraceae of *Alphaproteobacteria* account for as much as 30%–50% of all bacteria in the ocean surface waters. In the study area they accounted for 20%–32% between seasons.

The SAR 11 Group, *Roseobacter* sp. are known to control sulfur flux by converting the precursor dimethylsulfoniopropionate (DMSP) to dimethylsulfide (DMS) that are climatically inert [16]. Flux of DMS from surface of the ocean is the main source of sulfur to the atmosphere which influences climate by formation of aerosols. Also, SAR11 group is known to contribute to amino acid assimilation, may compete with other prokaryotes. This might imply that SAR11 Group in the study area may play a major role in cycling C, N, and S.

Gammaproteobacteria is a well known group in the marine waters [17]. Within this domain, *Alteromonas* sp. are copiotrophs known to play a notable role in the processing of dissolved organic carbon pools in the marine environment. A study by [18] highlighted the involvement of *Idiomarina* sp. and *Alteromonas macleodii* in metabolizing semilabile dissolved organic matter to methanol, formaldehyde or CO₂. These metabolites could be further oxidized and integrated by *Methylophaga* sp. In fact, *Methylophaga* sp. was found exclusively in low oxygen subsurface waters off Goa.

Vibrio sp. was among the predominant residents which are recognised to play pivotal role in biodegradation, nutrient regeneration and biogeochemical cycling. Efficient denitrifying ability by *Marinobacter* sp. [13], likewise, *Pseudomonas* sp. is one of the most active denitrifying heterotrophs and, a model species for assaying the denitrification process [13]. Uncultured *Gammaproteobacteria* accounted for (25%) reflecting the presence of large numbers of bacteria yet to be characterized.

The other minor groups contributing to about 10% or less of the total sequences were:

(a) *Actinobacteria* (7%) are reported as the significant fraction of bacterial community in the coastal waters of the Arabian Sea [17].

(b) *Deltaproteobacteria* (3%) they are reported to be abundant in the OMZs by several researchers [19]. They are understood to play important roles in linking the sulfur and nitrogen cycles.

(c) *Firmicutes* (3%) encompassing the genus *Bacillus* are also reported from OMZ sediments by [20]. Many different species of *Bacillus* [13] are reported to be efficient denitrifiers.

(d) *Betaproteobacteria* (2%) are reported to be predominant in the freshwater systems. Sequences related to *Burkholderia* sp. were found in the samples from off Goa. In the eastern Arabian Sea they were reported [20] from organic-rich sediments below the oxygen deficient waters.

(e) *Acidobacteria* (2%) are reported mainly from the soils and are reported to be involved in nitrate reduction [21].

(f) *Bacteroidetes* (1%) are known to contribute to <10% of the total bacterial community in marine environments and the family members of *Flavobacteraceae* are reported from the oceans. [22] reported that they are preponderant in nutrient-rich waters .

(g) *Verrucomicrobia* (1%) is another minor group seen during this study. In the marine environment they are known to play significant ecophysiological and biogeochemical roles [23].

(h) *Planctomycetes* (0.4%) are reported from hypoxic regions and are known to breakdown of sulfated heteropolysaccharides [24].

(i) *Chloroflexi* (0.2%) are involved denitrification and carbon fixation [25].

The present study is useful to recognize that microbial community undergoes seasonal shifts. Species/clones of *Deltaproteobacteria* and *Acidobacteria* were dominant during FIM, *Alphaproteobacteria* and *Betaproteobacteria* during SuM; *Firmicutes*, *Cyanobacteria* and *Actinobacteria* during SIM. There was clear difference in vertical profiles of bacterial community structure. Strong vertical stratification in community composition noted by [14] and, observations from this study corroborate with these. A combination of temporal variations in concentrations of dissolved oxygen, nitrate, nitrite and ammonia seems to influence bacterial community structure. All these environmental variables is known to be affected by stratification.

Statistical analysis showed that the number of OTUs during FIM was quite high compared to SIM and SuM. when suboxia prevailed. As such, diverse bacterial groups of may multiply with the higher availability of organic matter [17] as result of increased primary production due to higher nitrate concentrations in particular during FIM, followed by SIM and SuM. In this regard, substantial amounts of nutrient supply seem to support highly diverse bacterial community in the sampling region. Hierarchical clustering showed that seasonal variation in the bacterial communities mostly occurs in the surface layers despite the significant seasonal changes. A common observation was that the bacterial communities in the bottom waters were quite diverse from those at the surface, implying that the bacterial populations were affected by the prevailing physicochemical conditions in the water column.

In conclusion, in the monsoon affected tropical coastal waters off Goa, the bacterial communities respond rather strongly to changing seasonal environmental conditions with physiological adaptation or by shifts in the community composition. In all likelihood, the seasonally varying hydrographic conditions -in particular reduced oxygen- and their influence on bacteria, contribute significantly to the shifts both in diversity, type and predominance as observed in this study. The phylogenetic composition of bacterial communities found in this study matches those found in a variety of pelagic marine environments.

By using culture-independent methods several studies [14, 15, 17] report the dominance of *Alpha-* and *Gamma-proteobacteria*. These observations on abundance and distribution of bacterioplankton in the seasonally hypoxic coastal waters would serve useful to recognize their importance in temporally altering environmental conditions including those experiencing deoxygenation, acidification and/or eutrophication.

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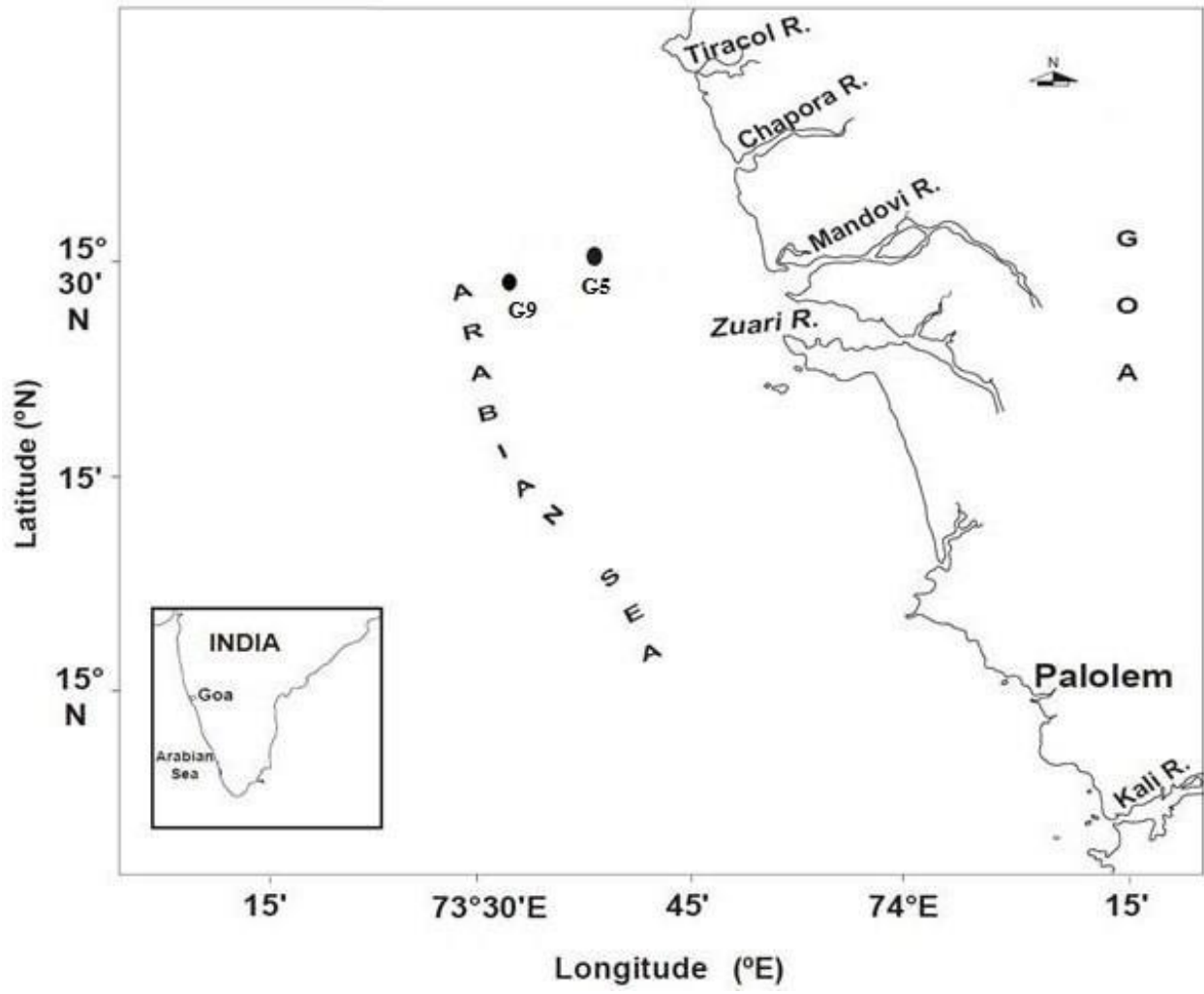


Fig.1 Sampling locations (G5 and G9) in the coastal waters off Goa, west coast of India.

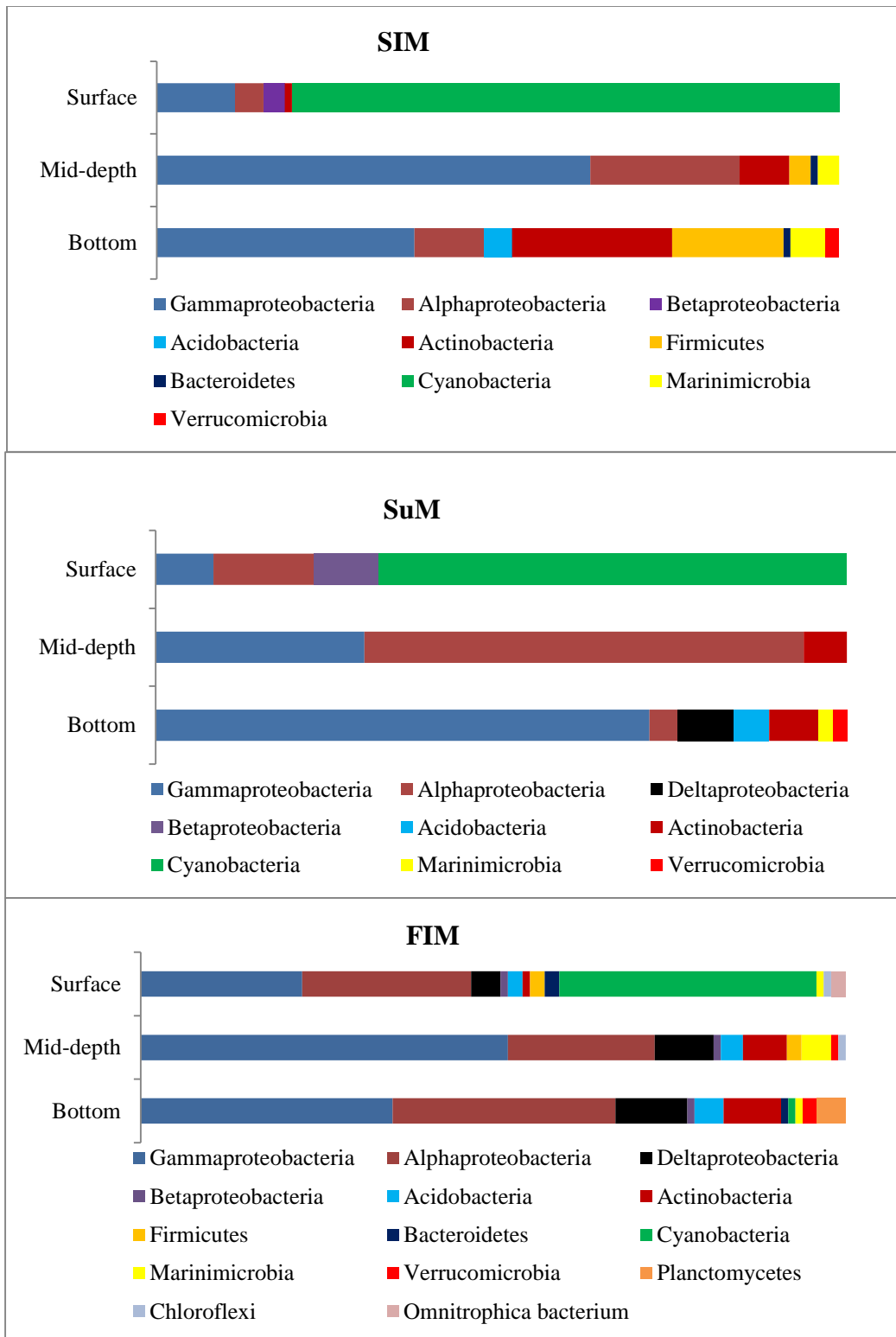


Fig. 2 Bacterial community structure during different seasons in the coastal waters off Goa, west coast of India

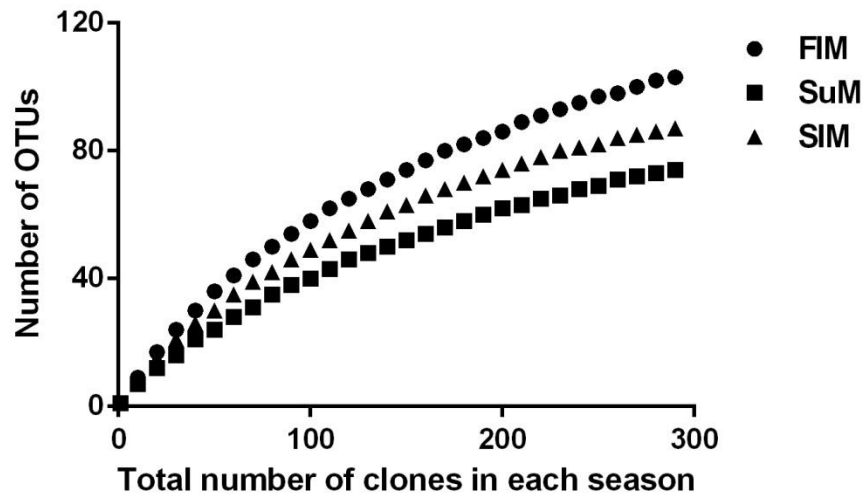


Fig. 7 Rarefaction curves of operational taxonomic units (OTU) of 16SrDNA sequences obtained in each season.

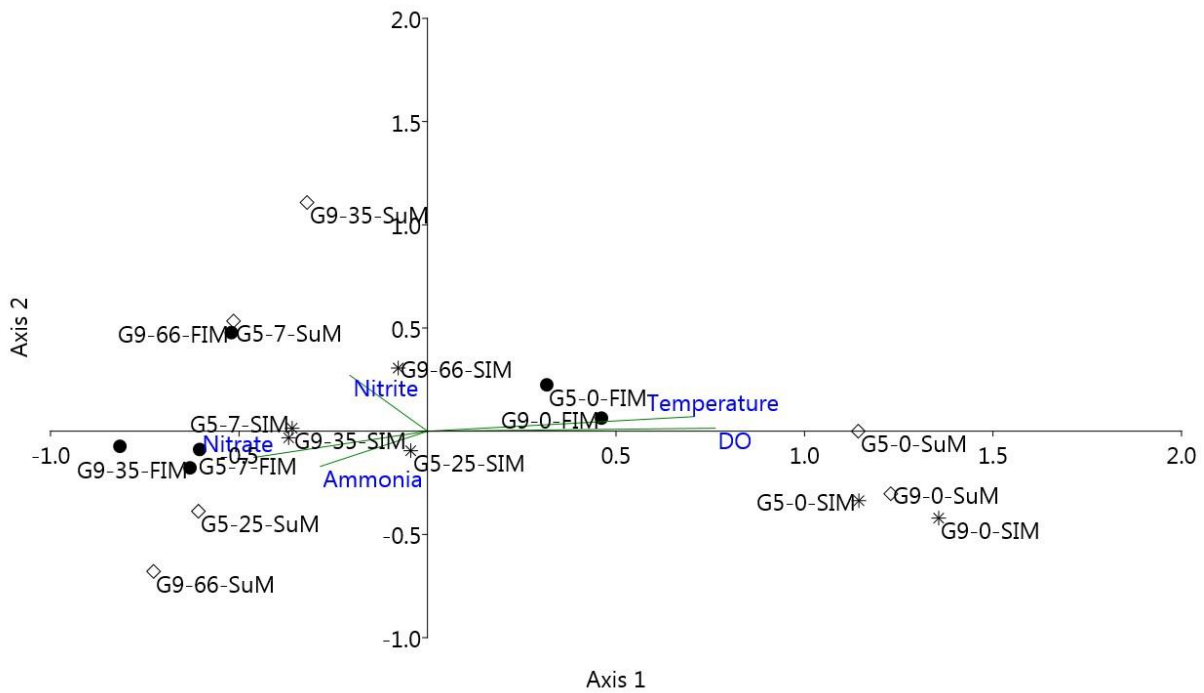


Fig. 8 Canonical correspondence analysis (CCA) ordination diagram of bacterial communities associated with environmental parameters.

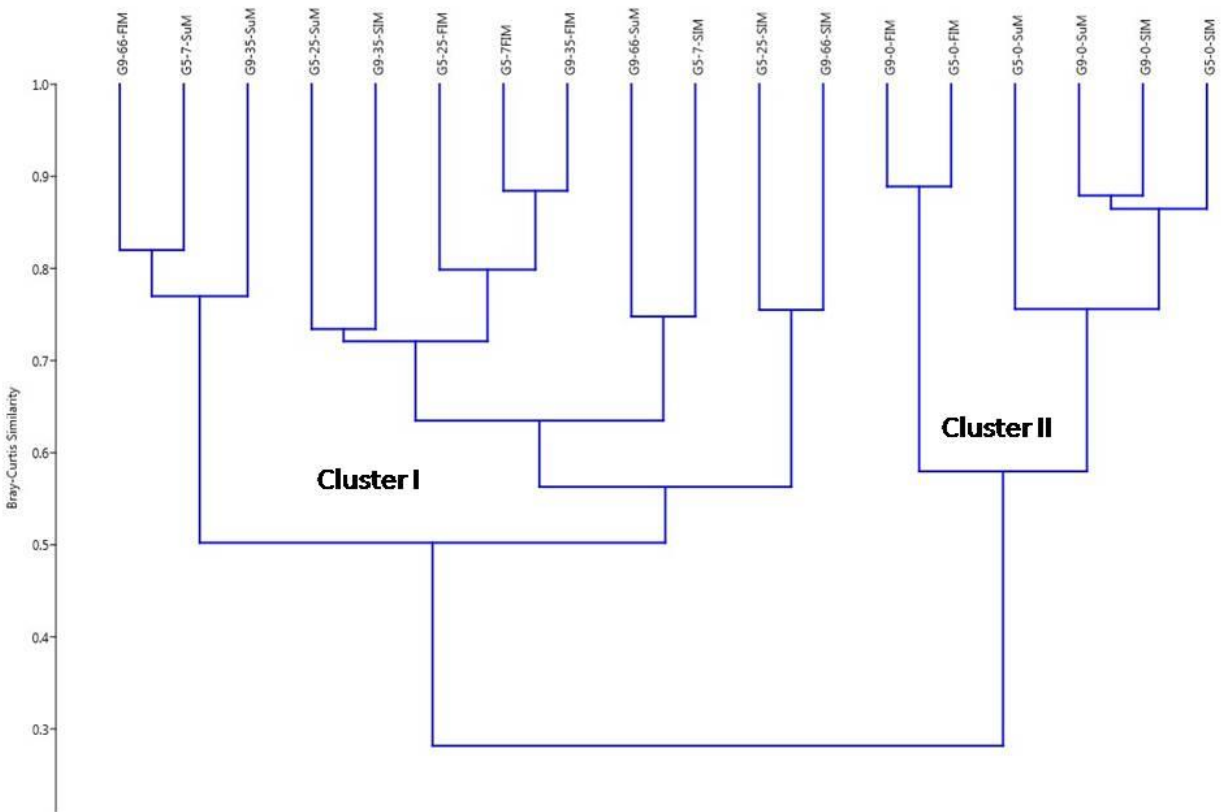


Fig. 9 Dendrogram of a hierarchical cluster analysis based on Bray-Curtis similarity index.