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Biogeochemistry of the shelf sediments of south eastern Arabian sea: Effect on benthic bacterial heterotrophs

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ABSTRACT

The composition and variability of heterotrophic bacteria along the shelf sediments of south west coast of India and its relationship with the sediment biogeochemistry was investigated. The bacterial abundance ranged from 1.12 x 10^3 – 1.88 x 10^6 CFU g^{-1} dry wt. of sediment. The population showed significant positive correlation with silt (r = 0.529, p< 0.05), organic carbon (OC) (r = 0.679, p< 0.05), total nitrogen (TN) (r = 0.638, p< 0.05), total protein (TPRT) (r = 0.615, p< 0.05) and total carbohydrate (TCHO) (r = 0.675, p< 0.05) and significant negative correlation with sand (r = -0.488, p< 0.05). Community was mainly composed of Bacillus, Alteromonas, Vibrio, Coryneforms, Micrococcus, Planococcus, Staphylococcus, Moraxella, Alcaligenes, Enterobacteriaceae, Pseudomonas, Acinetobacter, Flavobacterium and Aeromonas. BIOENV analysis explained the best possible environmental parameters i.e., carbohydrate, total nitrogen, temperature, pH and sand at 50m depth and organic matter, BPC, protein, lipid and temperature at 200m depth controlling the distribution pattern of heterotrophic bacterial population in shelf sediments. The Principal Component Analysis (PCA) of the environmental variables showed that the first and second principal component accounted for 65% and 30.6% of the data variance respectively. Canonical Correspondence Analysis (CCA) revealed a strong correspondence between bacterial distribution and environmental variables in the study area. Moreover, non-metric MDS (Multidimensional Scaling) analysis demarcated the northern and southern latitudes of the study area based on the bioavailable organic matter.

Keywords: Arabian Sea, Shelf sediments, Heterotrophic bacteria, Organic matter, BPC, PCA

INTRODUCTION

Marine sediment overlay two-thirds of the earth's surface representing one of the largest microbial habitats on Earth [1]. It is complex in nature and acts as the final storage ground for organic input from water column processes [2]. Bacteria that dwell on these sediments account for most of the benthic biomass and play a decisive role in the decomposition as well as production of organic matter [3-4] and thereby contribute greatly to overall biochemical cycles [5]. They carry half of the primary production on the planet [6] and play a major role in the structure and functioning of the benthic food web [4].

Marine sediment harbour remarkably high density of approximately 10^9 bacterial cells cm^{-3}, irrespective of ocean depth [7]. Their distribution, structure and activity are strongly affected by a great variety of physical factors such as temperature [8], sediment type [9-10], chemical factors such as organic matter content [11], quality of organic matter [12-13] and biological factors such as community structure [14], level of predation or grazing [15]. Besides these, the composition of benthic microbial communities is strongly correlated with latitude, demonstrating that biogeographic factors are significant determinants of the microbial diversity [16].

Exploration of microbial diversity is undoubtedly a topic of great importance. Microbial community structure analysis can provide a better understanding of functional and biogeographical relationships [17]. Microbial diversity can be studied by a combination of cultivation-dependent and independent approaches. Though culture–independent techniques provide good taxonomic classification [18] they do not elaborate their metabolic potentials and roles in maintaining various ecosystem processes and provide new and potentially active species for further application. Moreover, Ettoumi et al [19] noticed almost same proportions of bacteria in both culture- dependent and independent approaches. In the present study we followed culture-dependent approach to study the bacterial diversity present in the shelf sediments of Arabian Sea. To date several studies have been carried out to assess the diversity of bacteria in marine sediments [20-26] but little has been carried out in the continental shelf sediments of south west coast of India. In this study we investigated the spatial and vertical distribution of benthic bacteria, and their diversity in relation to sediment biogeochemistry of the shelf sediments of south west coast of Arabian Sea.

MATERIALS AND METHODS

Sampling site and sample collection

The sampling site was the continental shelf region of south west coast of India. Sediment samples were collected from 50 and 200m depth using Smith McIntyre grab (0.2m^2) during Cruise No.258 (October 2008) of FORV *Sagar Sampada*. The study area spanned between latitudes 07^{0}47'84"N to 17^{0}00'40"N and longitudes 77^{0}29'89"E to 73^{0}00'32"E (Fig.1) covering 20 stations over 10 transect (Cape Comorin, Trivandrum, Kollam, Kochi, Calicut, Kannur, Mangalore, Bhatkal, Goa and Ratnagiri). Samples were transferred aseptically into sterile polythene bags and immediately subjected to microbiological analysis. The remaining portion was frozen at -20°C for organic matter and texture analysis. Details of the sampling stations are given in Table 1.

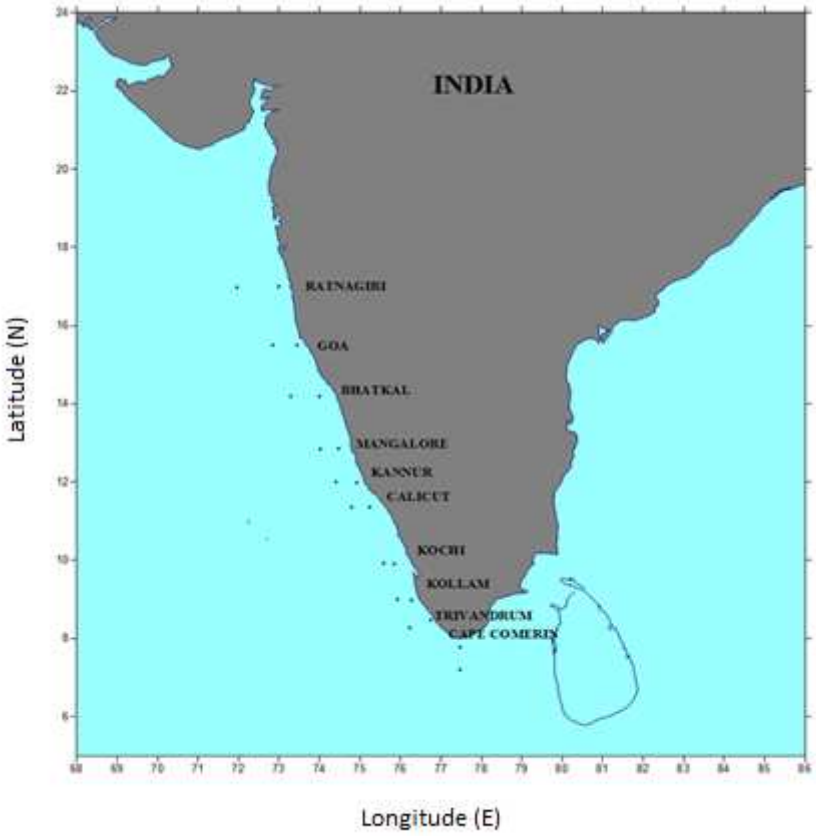


Fig 1: Location of sampling stations in the study area

Abiotic parameters

Water temperature, salinity and dissolved oxygen of bottom water overlying the sediment were recorded from each station using on board Sea Bird CTD system (USA). Sediment pH was determined using a portable pH meter.

Sediment characterization

Grain size analysis

The sediment samples were dried overnight in a hot air oven at 60^0 C. 10g each of dried sample was accurately weighed and dispersed using sodium hexametaphosphate (10%) and kept overnight. Grain size analysis was performed using a Laser Diffraction Particle Size Analyzer (SYMPA TECH, Germany) and was characterized following the textural classification by Shepard and Moore [27].

Table 1: Details of the sampling stations along the shelf sediments of south west coast of India

TRANSECT	STATION	DATE OF SAMPLING	DEPTH (M)	LATITUDE (°N)	LONGITUDE(°E)
CAPE COMERIN (CAPE)	2	16-10-08	52	07^0 47'. 84	77^0 29'. 89
	4	16-10-08	207	07^0 13'.95	77^0 29'. 09
TRIVANDRUM (TVM)	7	15-10-08	53	08^0 28'.06	76^0 45'. 72
	9	15-10-08	200	08^0 30'.09	76^0 46'. 76
KOLLAM (KLM)	12	14-10-08	51	08^0 59'.98	76^0 17'. 22
	14	14-10-08	202	09^0 00'.18	75^0 56'. 87
KOCHI (CHN)	17	13-10-08	51	09^0 55'.99	75^0 51'. 02
	19	13-10-08	203	09^0 56' .52	75^0 35'. 70
CALICUT (KZD)	22	12-10-08	54	11^0 21'.40	75^0 15'. 74
	24	12-10-08	203	11^0 22'.14	74^0 48'. 87
KANNUR (KNR)	27	11-10-08	54	11^0 59'.53	74^0 55'. 71
	29	11-10-08	220	12^0 00'.12	74^0 24'. 99
MANGALORE (MGLRE)	32	8-10-08	51	12^0 51'.91	74^0 29'. 86
	34	8-10-08	215	12^0 50'.68	74^0 01'. 80
BHATKAL (BTKL)	37	7-10-08	53	14^0 11'.70	74^0 00'. 41
	39	6-10-08	212	14^0 11'.27	73^0 17'. 16
GOA	42	6-10-08	54	15^0 30'.06	73^0 27'. 04
	44	5-10-08	215	15^0 30'.08	72^0 50'. 94
RATNAGIRI (RTNGRI)	47	4-10-08	52	17^0 00'.40	73^0 00'. 32
	49	4-10-08	214	16^0 59'.98	71^0 58'. 25

Sediment organic matter content

Sediment samples were powdered well after drying in hot air oven at 60^0 C for 48 hrs and the organic carbon was determined by wet oxidation method [28]. The amount of organic matter was determined by multiplying the organic carbon concentrations by the factor 1.724 [29].

Biochemical composition of sediment organic matter

Protein present in the sediment was estimated as per Lowry et al. [30] with bovine serum albumin as standard. Lipid was determined by Sulphophosphovanillin method [31] using cholesterol as standard and carbohydrate by phenol sulphuric acid method [32] with glucose as standard. All analysis was carried out on three replicates. Carbohydrate, protein and lipid concentrations were converted to carbon equivalents by using the conversion factors 0.40, 0.49 and 0.75, respectively [2] and biopolymeric carbon concentration (BPC) was calculated as the sum of carbohydrate-C, protein-C and lipid-C [33]. The total carbon and total nitrogen content of the samples were determined using an automatic CHN Analyser.

Microbiological analysis

Estimation and identification of total heterotrophic bacteria (THB)

Sediment samples were subjected to serial dilution in sterile seawater and spread plated on to ZoBell's 2216e agar medium in duplicates. The plates were incubated at 28 ± 2°C for 5-7 days and all colonies developed were counted and expressed as colony forming units (CFU) per gram dry weight sediment. Morphologically different bacterial colonies were isolated, purified and identified by gram staining, spore staining and biochemical tests. The isolates were identified up to generic level following Bergey's Manual of Systematic Bacteriology [34] and taxonomic scheme of Oliver [35].

Statistical analyses and Graphics

All data were analysed statistically by various software's XLSTAT v.2012.6.01 (Addinsoft), ORIGIN v.6.0, and PRIMER-6. Pearson correlation analysis was performed to test correlation between the investigated variables. A t-test analysis was carried out to test differences in variables between different depths (50 and 200m). Diversity indices like Shannon–Wiener diversity index, H'(log 2); Margalef's index, d; and Pielou's evenness index, J' were calculated to describe the bacterial community. To check for similarity among stations, all stations were ordinated by a non-metric MDS (Multidimensional Scaling) and classified with CLUSTER analysis. In the cluster analysis, hierarchical agglomerative clustering (Bray-Curtis similarity) was used. Non metric multidimensional scaling (MDS) was also employed to assess the organometric differences between stations using carbohydrate, protein, lipid and BPC as input variables. BIOENV analysis was carried out (Spearman rank correlation method) to select the best

environmental variables to explain distribution pattern of heterotrophic bacteria in the sediment. Principal component analysis (PCA, normalised data) was used to reveal the relationship between environmental variables. The canonical correspondence analysis (CCA) was performed to identify relationships among the distribution patterns of heterotrophic bacteria with selected environmental variables.

The distribution maps of environmental parameters (temperature, salinity, dissolved oxygen and organic matter) and Total Heterotrophic Bacteria were generated using Surfer 8 (Golden Software Inc., USA).

RESULTS

Abiotic parameters

The important physico-chemical parameters such as temperature, salinity, pH and dissolved oxygen recorded on-board. Temperature was significantly higher at 50m depth compared to 200m depth (t-test, $p < 0.05$) and ranged from 21.61°C to 28.15°C (mean = 24.68 ± 2.34°C) at 50 m depth and 14.44°C to 16.11 °C (15.16 ±0.64°C) at 200m depth (Fig.2). Salinity was also significantly higher at 50m depth (35.46 ±0.15) compared to 200m depth (35.18 ± 0.08; t-test, $p < 0.05$) (Fig.3). Dissolved oxygen (1.19 ±1.47 ml/l at 50m; 0.18 ± 0.09 ml/l at 200m) (Fig.4) and pH (7.86 ±0.13 at 50m; 7.83 ± 0.12 at 200 m) did not show any significant variation between different depth regions.

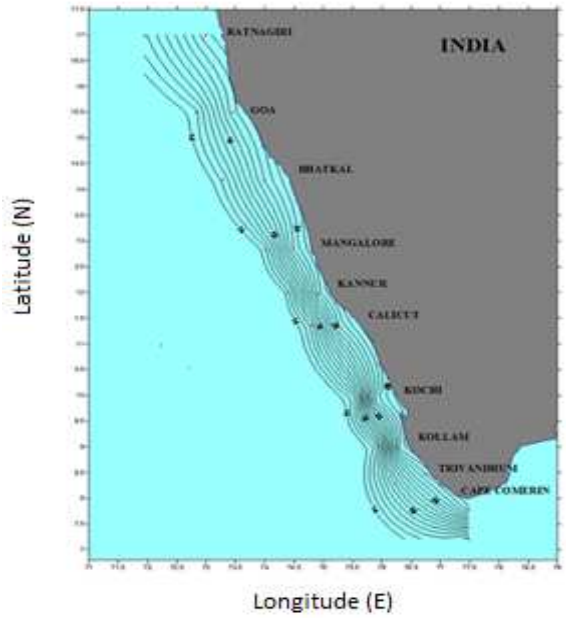


Fig.2.Distribution of temperature along the shelf sediments of south west coast of India

Sediment characteristics

Sediment texture

Sediment was olive green in colour in the study area. The granulometric analysis showed that sediment was silty sand in nature at 50m depth in almost all stations except Cape Comerin (sandy) and Ratnagiri (clayey silt) and at 200m depth it was sandy or silty sand in nature. Towards the northern region the proportion of sand decreased and silt content increased (Fig.5).

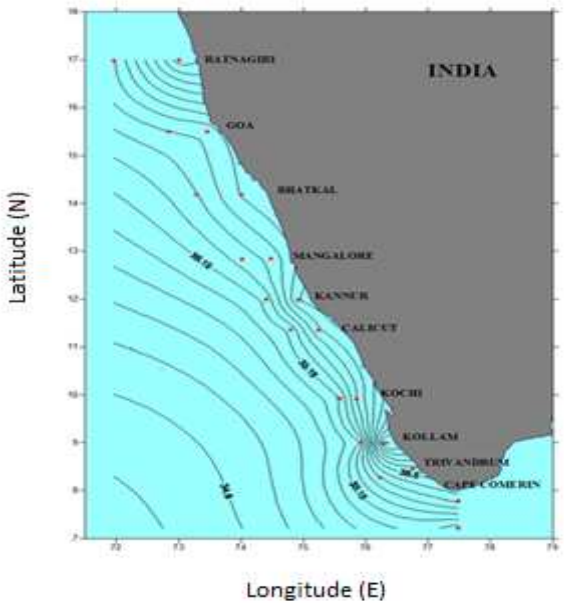


Fig.3.Distribution of salinity along the shelf sediments of south west coast of India

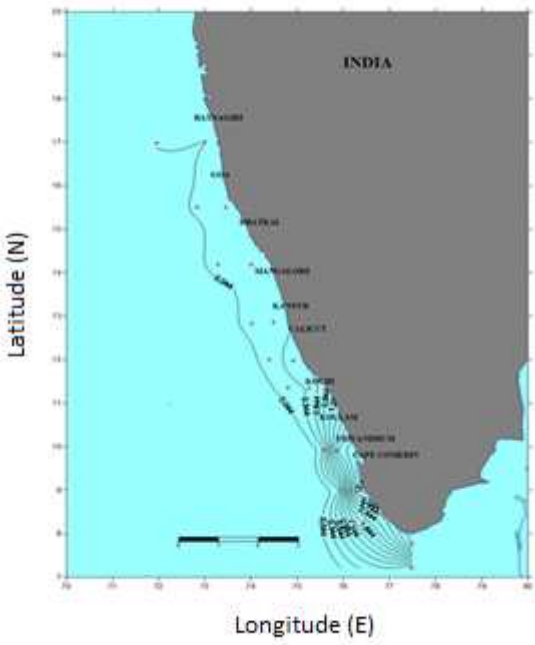
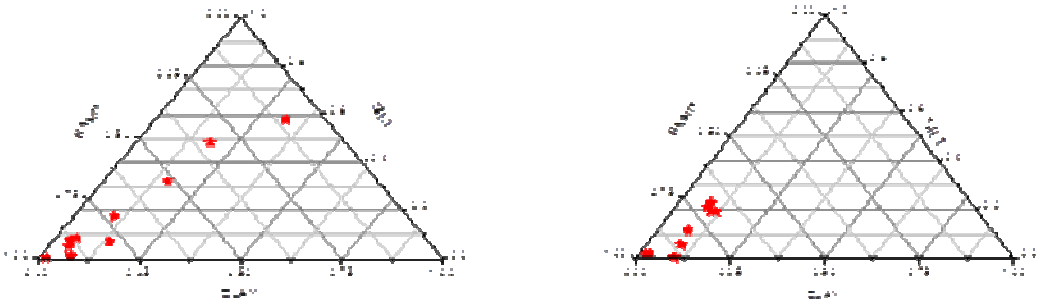


Fig.4. Distribution of dissolved oxygen along the shelf sediments of south west coast of India

Sediment organic matter

Organic matter in the shelf sediments of south west coast of India ranged from 0.95- 6.29% (mean, 2.67 ± 1.57%). Significant depth wise variation was observed in the northern part of the study area (t-test, $p < 0.05$). Organic matter was found to be more towards northern latitudes in the study area. Highest amount of organic matter was observed at 50m depth off Ratnagiri where sediment was clayey silt in nature (Fig.6). Total carbon (TC) showed significant depth wise variation and was found to be higher at 200m depth regions (8.85 ±1.29 %) compared to 50m depth (3.89 ± 2.24 %). But total organic carbon (TOC; 1.54 ± 0.92%) and total nitrogen (TN; 0.12 ± 0.08%) concentration did not show significant depth wise variation. Variation in nitrogen followed the same trend as organic carbon. C/N ratio in the sediment varied from 9.49 to 36.91 with a mean value 14.83 ± 6.23.



50m depth **200m depth**

Fig. 5: Distribution of silt, sand and clay in the shelf sediments of south west coast of India

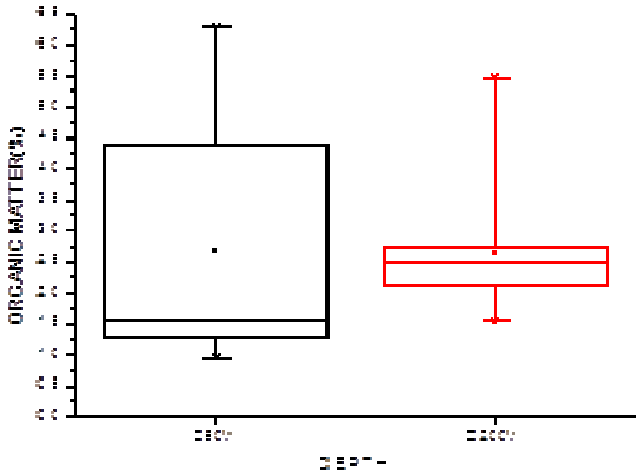


Fig.6. Percentage of organic matter found at 50 and 200m depth regions along the shelf sediments of south west coast of India

Biochemical composition of sediment organic matter

Concentrations of total carbohydrates (TCHO), proteins (TPRT) and lipids (TLIP) in the shelf sediments of south west coast of India were calculated. Lipids were the major class among labile organic compounds (range: 0.9- 5.1 mg/g; mean= 3.0 ± 1.2 mg/g) followed by protein (range: 0.1- 7.3 mg/g; mean= 1.5± 2.0 mg/g) and carbohydrate (range: 0.4- 3.5mg/g; mean=1.2 ± 0.9 mg/g).These three variables did not display significant depth wise variation. MDS analysis showed that the northern and southern latitudes of southwest coast of India clearly differentiate each other based on biochemical composition of the sediment (Fig.7).

Biopolymeric carbon ranged between 1.08 – 8.5 mg/g dry wt. sediment (mean = 3.42 ± 1.9 8mg/g) and did not show any significant depth wise variation in the shelf sediments .About 71% of BPC was constituted by lipid (2.26 ± 0.89 mg/g) followed by protein (15%; 0.68 ± 0.99 mg/g) and carbohydrates (14%; 0.48 ± 0.33 mg/g). BPC was found to be higher towards the northern regions of the study area.

Total heterotrophic bacterial population

Total heterotrophic (culturable) bacterial population did not show significant depth wise and latitudinal variation. Population ranged from 1.12 x 10^3 – 1.88 x 10^6 CFU g^{-1} dry wt. with its maximum at 50m depth off Ratnagiri and minimum at 200m depth off Ratnagiri. The sediment was clayey silt in nature at 50m depth off Ratnagiri resulting in higher bacterial population whereas at 200m depth it was silty sand in nature containing numerous dead shells. Comparatively higher bacterial population was noticed at 200m depth off Bhatkal (9.5 x 10^5) and 50m depth off Goa (5.9 x 10^5)(Fig.8).

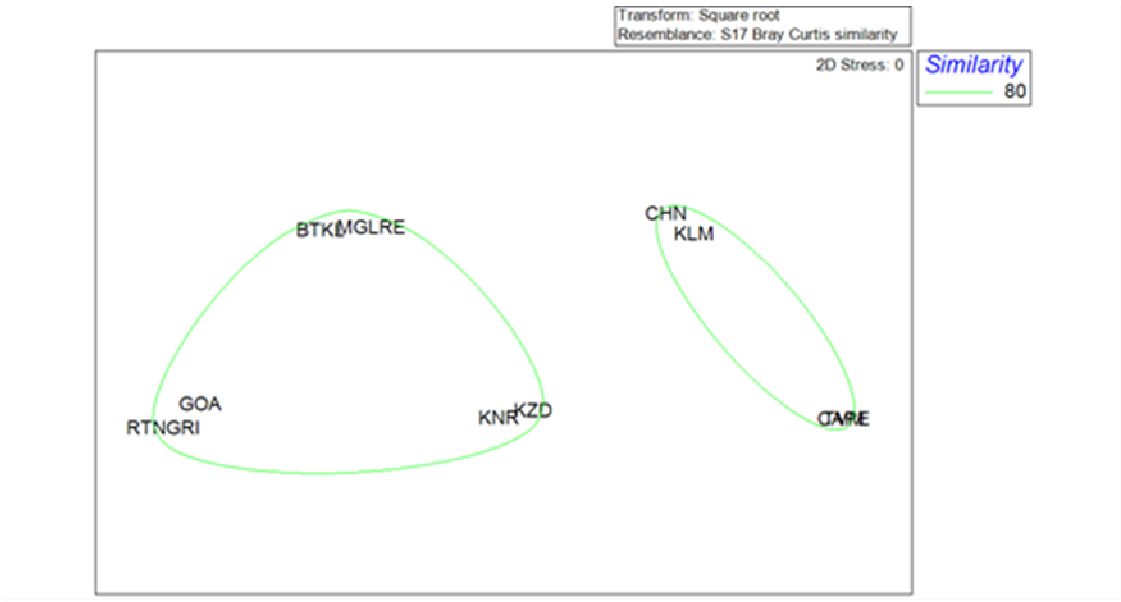


Fig 7: MDS plot based on biochemical composition of the sediment

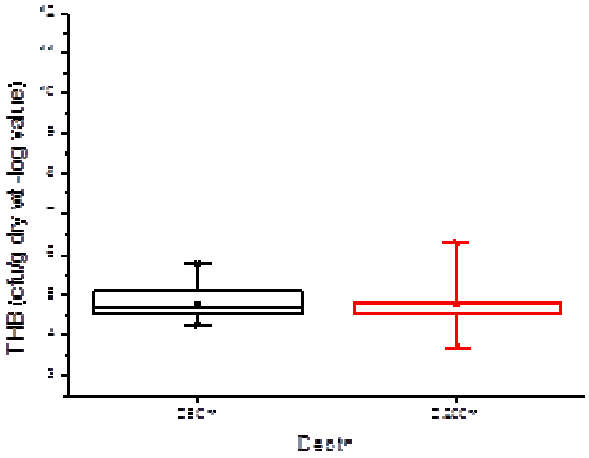


Fig.8.Distribution of total heterotrophic bacterial population at 50 and 200m depth regions along the shelf sediments of south west coast of India

Generic composition of heterotrophic bacteria

Altogether 342 cultures were isolated from the shelf sediments of south west coast of India. Out of 342 cultures 45% were gram positive and 55% gram negative. Depth wise variation was meagre in the distribution of gram positive and gram negative bacteria. In the sediment *Bacillus* (17 %) was found to be the dominant genus followed by *Alteromonas* (12%) and *Vibrio* (11%). Coryneforms, *Micrococcus*, *Planococcus*, *Staphylococcus*, *Moraxella*, *Alcaligenes*, Enterobacteriaceae, *Pseudomonas*, *Acinetobacter*, *Flavobacterium* and *Aeromonas* were the other identified genera/groups from the shelf region (Fig.9). *Bacillus*, *Alteromonas*, *Vibrio* and *Micrococcus* showed a cosmopolitan distribution along the shelf sediments. Coryneforms was dominant off Goa, Kozhikode and Cochin. *Planococcus*, *Flavobacterium* and *Pseudomonas* dominated the southern transects. *Alcaligenes*, Enterobateriaceae, *Acinetobacter* and *Aeromonas* were found to be dominant along Ratnagiri coast. *Flexibacter* showed their representation only along Mangalore and Cochin coast. At 50m depth *Bacillus* (20%) was the dominant genus followed by *Alteromonas* (14%), *Vibrio* (11%), *Micrococcus* (10%) and *Acinectobacter* (7%) (Fig.10). At 200m depth also *Bacillus* (14%) was the dominant genera followed by *Vibrio* (11%), *Alteromonas* (9%), *Acinetobacter* (9%) and *Micrococcus* (10%) (Fig.11).

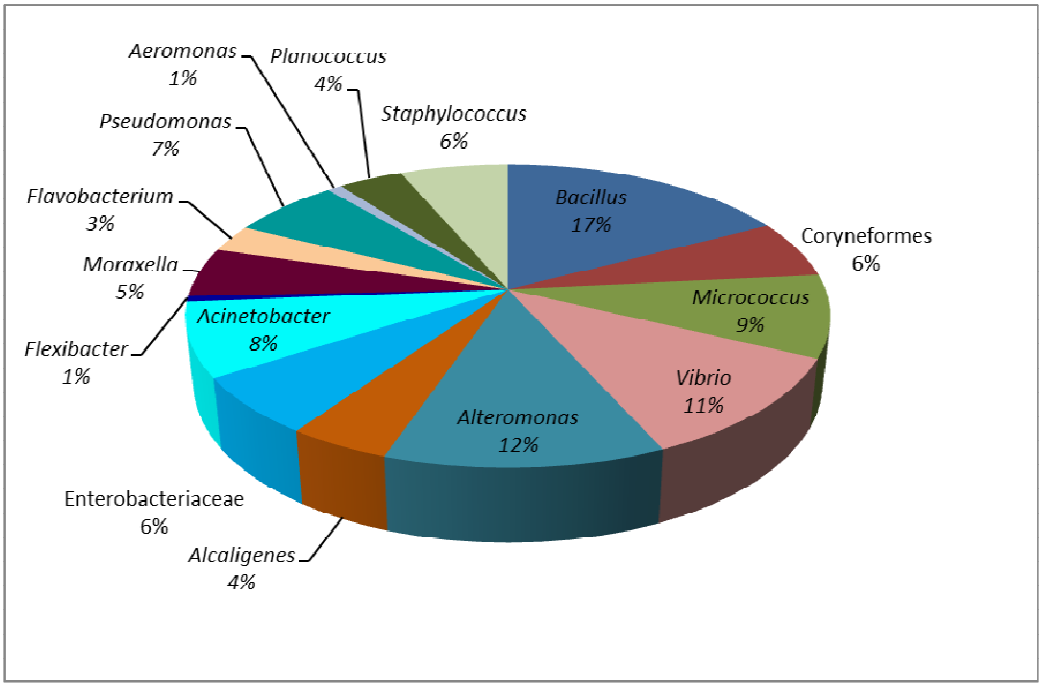


Fig 9: Percentage contribution of different genera in the shelf sediments of south west coast of India

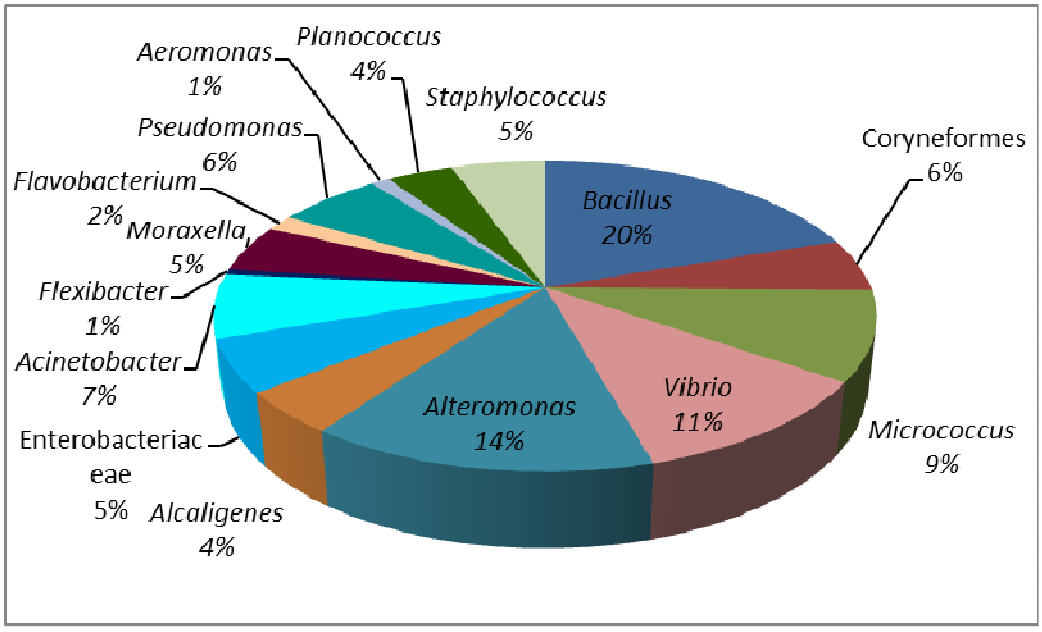


Fig 10: Percentage contribution of different genera in the shelf sediments of south west coast of India at 50m depth

Statistical analysis

The Shannon-Wiener diversity (H'(log 2)) ranged from 2.5 to 3.5, clearly showing the diverse nature of heterotrophic bacteria along south west coast of India (Table 2). With the number of genera ranging from 6 -12, the study area showed considerable depth wise variation. Species richness ranged between 2.44 to 4.27.

The Pearson correlation coefficients (Table 3) showed significant positive correlation of total heterotrophic bacteria with silt ($r = 0.529$, $p< 0.05$), organic carbon (OC) ($r = 0.679$, $p< 0.05$), organic matter (OM) ($r = 0.665$, $p< 0.05$), total nitrogen (TN) ($r = 0.638$, $p< 0.05$), total protein (TPRT) ($r = 0.615$, $p< 0.05$) and carbohydrate (TCHO) ($r = 0.675$, $p< 0.05$). Significant negative correlation could be observed with sand ($r = -0.488$, $p< 0.05$). Total carbon ($r = 0.815$, $p< 0.05$) showed a significant positive correlation with depth. Organic matter showed significant positive correlation with silt ($r = 0.883$, $p< 0.05$) and clay ($r = 0.785$, $p< 0.05$) and significant negative correlation with sand ($r = -0.878$, $p< 0.05$).

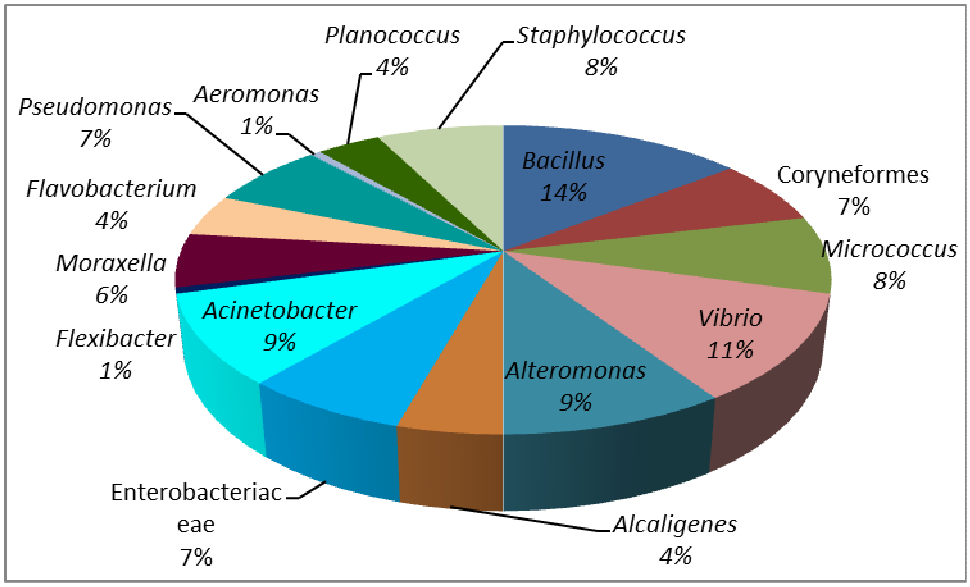


Fig 11: Percentage contribution of different genera in the shelf sediments of south west coast of India at 200m depth

BIOENV analysis revealed that the combination of environmental parameters which best explained the distribution patterns of heterotrophic bacteria at 50m depth was Carbohydrate ,total nitrogen, temperature, pH and Sand (q = 0.733; Table 4) and at 200m, organic matter, BPC, protein, lipid and temperature (q = 0.602; Table 4)

Table 2: Diversity indices of bacterial genera present in the shelf sediments of south west coast of India (S- number of genera, N- total number, d- species richness, J'- species evenness, H'(log2) - species diversity, 1-λ'- species dominance)

TRANSECT	DEPTH	DIVERSITY INDICES					
		S	N	d	J'	H'(log2)	1-Lambda'
CAPE COMERIN	52	10	12	3.662	0.9973	3.313	0.9829
	207	9	11	3.392	0.9981	3.164	0.9807
TRIVANDRUM	53	8	10	3.084	0.998	2.994	0.9747
KOLLAM	51	9	11	3.401	0.9952	3.155	0.9797
	202	11	12	4.066	0.9979	3.452	0.9931
KOCHIN	51	6	8	2.442	0.9959	2.574	0.9541
	203	10	12	3.644	0.9956	3.307	0.9809
CALICUT	54	12	13	4.279	0.998	3.578	0.9917
	203	9	10	3.502	0.9967	3.159	0.9878
KANNUR	54	8	10	3.108	0.9951	2.985	0.975
	220	11	12	4.01	0.9965	3.447	0.9892
MANGALORE	51	12	13	4.24	0.9976	3.576	0.9896
	215	10	11	3.717	0.9979	3.315	0.9867
BHATKAL	53	10	11	3.712	0.9965	3.31	0.9856
	212	9	11	3.345	0.9971	3.161	0.9769
GOA	54	8	9	3.116	0.9989	2.997	0.9779
	215	7	8	2.812	0.9944	2.792	0.9686
RATNAGIRI	52	8	9	3.127	0.9954	2.986	0.9767
	214	11	12	4.024	0.9957	3.444	0.9895

Cluster analysis of complete linkage indicated that there are two main clusters. Cluster I mainly consisted of 50m depth regions of Ratnagiri, Mangalore and Goa. Cluster II divided into two groups i.e., 50m depth regions of Cape Comorin, Trivandrum, Kollam, Cochin, Calicut, Kannur, Mangalore, Bhatkal and the 200m depth regions (Fig.12). Principal component I explained 65% of the total variability, whereas component II explained only 30.6% variability (Fig.13). Temperature, dissolved oxygen, pH, silt, clay, organic matter, total protein, total lipid, total carbohydrate and bioploymeric carbon showed a positive correlation with PC I, whereas sand and total carbon showed a negative correlation. With PC II, temperature, dissolved oxygen and pH showed a positive correlation, whereas all other factors showed a negative correlation. Canonical Correspondence Analysis (CCA) was carried out using environmental variables and the distribution of 5 dominant bacterial genera (*Bacillus*, *Vibrio*, *Alteromonas*, *Micrococcus*, *Pseudomonas*, *Acinetobacter*). CCA biplot showed the eigen values of the first two axes as 0.029 and 0.0180 respectively. The first two CCA axes explained 73.2% variability of environment parameters (Fig.14).

Table.3 Pearson correlation matrix of the environmental variables and THB in the shelf sediments of south west coast of India

	Depth (m)	Temperature (°C)	Dissolved oxygen (ml/l)	Salinity (ppt)	pH	Silt(%)	Clay (%)	Sand (%)	OC (%)	OM(%)	TC (% /dryw t)	TN(%/dryw t)	TIC (%)	C/N (%)	PRT	CHO	LIP	BPC	THB (log)
Depth (m)	1																		
Temperature (°C)	-0.946	1																	
Dissolved oxygen (ml/l)	-0.488	0.719	1																
Salinity (ppt)	-0.734	0.804	0.736	1															
pH	-0.196	0.165	-0.045	0.113	1														
Silt (%)	-0.208	0.175	0.170	0.065	-0.535	1													
Clay (%)	-0.248	0.234	0.190	0.074	-0.188	0.870	1												
Sand (%)	0.227	-0.196	-0.161	-0.064	0.298	-0.968	-0.986	1											
OC (%)	0.008	-0.072	-0.047	-0.121	-0.575	0.883	0.765	-0.878	1										
OM(%)	0.052	-0.068	-0.016	-0.155	-0.548	0.891	0.743	-0.870	0.980	1									
TC (%/dryw t)	0.815	-0.865	-0.602	-0.767	-0.071	-0.056	-0.162	0.097	0.141	0.178	1								
TN(%/dryw t)	0.057	-0.055	-0.007	-0.095	-0.541	0.892	0.792	-0.867	0.960	0.986	0.156	1							
TIC (%)	0.811	-0.842	-0.588	-0.730	0.059	-0.515	-0.412	0.355	-0.152	-0.110	0.997	-0.126	1						
C/N (%)	-0.288	0.134	-0.221	0.019	0.134	-0.284	-0.060	0.222	-0.166	-0.334	-0.095	-0.415	-0.047	1					
PRT	-0.060	0.129	0.257	0.048	-0.187	0.649	0.684	-0.669	0.769	0.781	0.052	0.755	-0.173	-0.234	1				
CHO	-0.077	0.067	0.154	0.053	-0.536	0.896	0.758	-0.878	0.998	0.965	0.051	0.959	-0.224	-0.365	0.799	1			
LIP	-0.034	0.054	-0.065	-0.052	0.057	0.509	0.446	-0.504	0.547	0.603	-0.026	0.627	-0.157	-0.454	0.598	0.637	1		
BPC	-0.054	0.091	0.112	0.019	-0.124	0.710	0.661	-0.715	0.796	0.832	0.025	0.828	-0.211	-0.388	0.910	0.862	0.867	1	
THB (log)	-0.239	0.195	0.075	0.116	-0.124	0.529	0.551	-0.468	0.679	0.665	0.037	0.638	-0.165	0.002	0.615	0.675	0.457	0.629	1

Table 4: BIO-ENV analyses indicating the environmental parameters which best explain the community pattern in shelf sediments

No. Variables	Correlation	Selections
50m depth		
5	0.733	Carbohydrate, Temperature, pH, Sand, Total Nitrogen
4	0.719	Carbohydrate, Temperature, Sand, Total Nitrogen
5	0.724	Carbohydrate, Temperature, Sand, Total Nitrogen, Depth
200m depth		
5	0.602	Protein, Lipid, BPC, Temperature, Organic Matter
5	0.596	Protein, Lipid, BPC, pH , Organic Matter
4	0.596	Protein, Lipid, BPC, Organic Matter

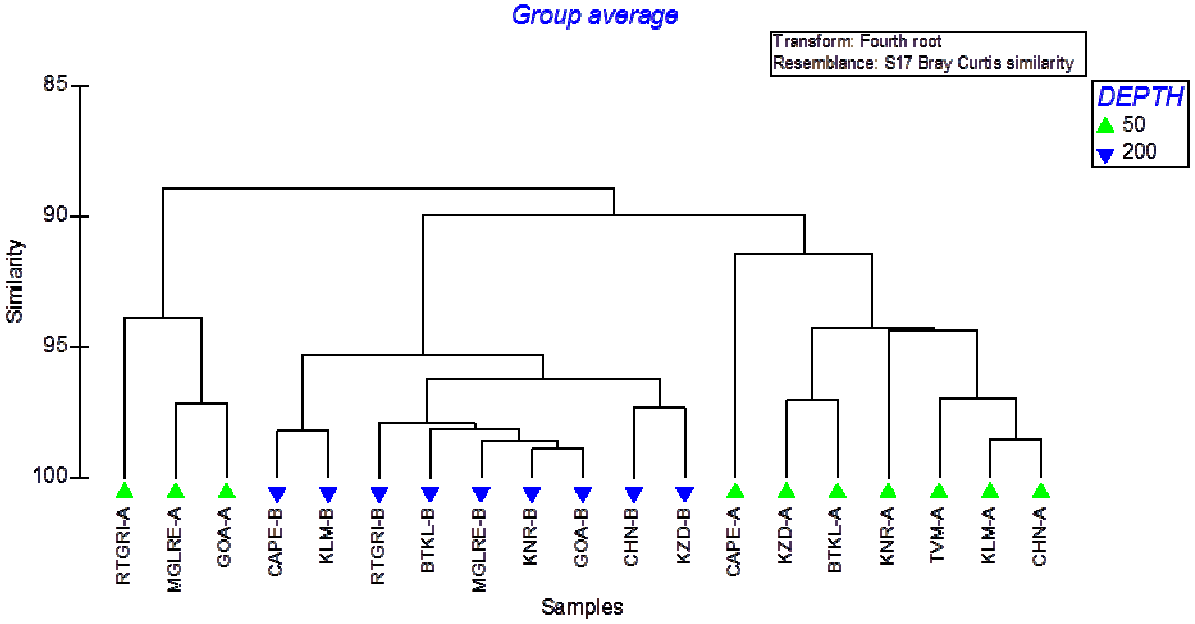


Fig 12: Dendrogram based on sediment biogeochemical characteristics recorded at various stations in the south west coast of India

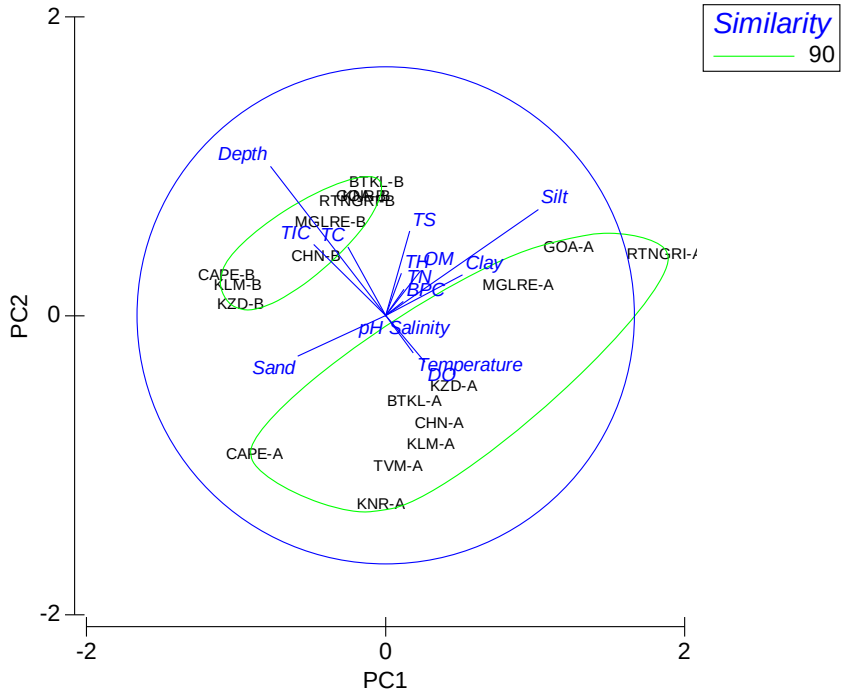


Fig 13: PCA with habitat data showing the variables that better explain the distribution pattern of bacteria in the shelf sediments of south west coast of India.

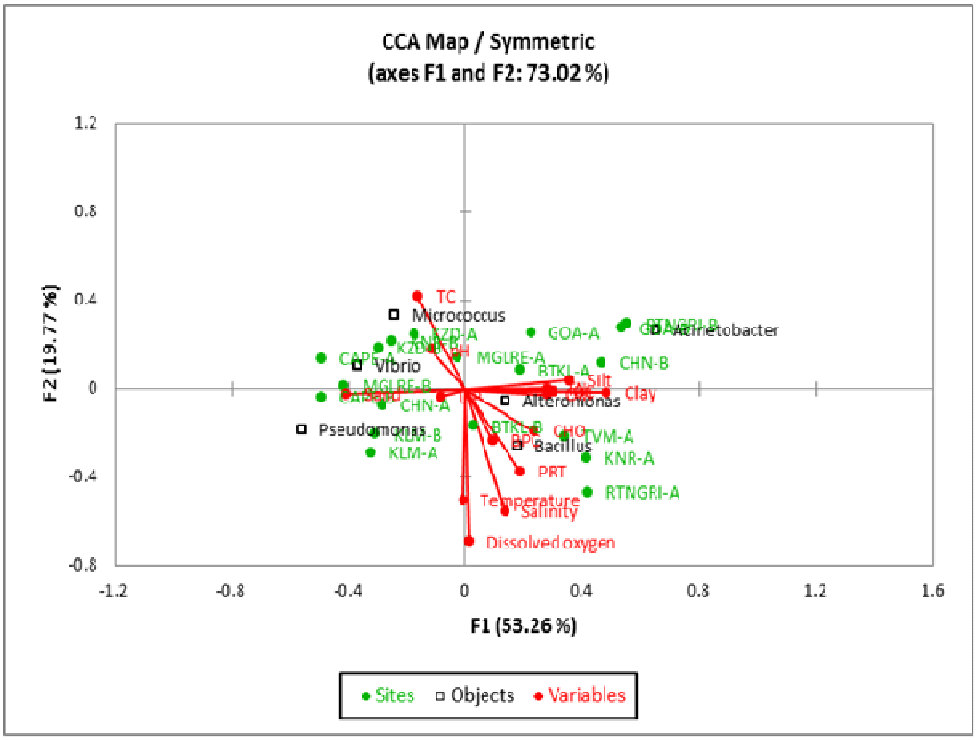


Fig 14: Result of CCA showing the variables that better explain the distribution pattern of dominant bacteria in the shelf sediments of south west coast of India.

DISCUSSION

Sediment bacteria are one of the most important components in benthic microbial food webs and they play significant roles in the ecosystem as degraders and foragers of organic matter [36]. Explanations for the distribution pattern of microbial communities and their associated activity often focus on resource availability and abiotic conditions [37]. It is well documented that physico-chemical parameters such as temperature, salinity, total organic carbon, pH, and the sedimentary process can modify the abundance of microorganisms in sediments [4].

In the present study, distribution of bacteria lacks any depth wise variations. This was observed earlier by Danovaro et al. [38] in Aegean Sea and Bianchi et al. [39] in Western Mediterranean Sea. Bacterial population in the shelf sediments showed an uneven distribution and were found to be in a range of $1.12 \times 10^3 - 1.88 \times 10^6$ CFU g^{-1} dry wt. of sediment. This uneven distribution of bacteria in the shelf sediments could be attributed mainly to the differences in the nature of the sediment and availability of organic matter. The grain size of sediment determines to great extent the number of species within a community and therefore, it functions as an environmental influence on biological diversity [40]. The influence of sediment properties on distribution of bacteria becomes evident from the correlation matrix. In the present study a significant positive correlation was observed between bacterial population with silt ($r = 0.529$, $p< 0.05$) and negative correlation with sand ($r = -0.488$, $p< 0.05$). This positive correlation between silt content and bacterial population is in agreement with other reports of greater biomass in more fine grained sediments [3].The variation seen in the population in different types of sediment is attributed mainly to the difference in the organic matter content. Organic matter in marine sediments is a complex mixture of detrital material from water column transport and in situ bacterial synthesis [41]. Sandy sediments have organic contents that are 1–2 orders of magnitude lower than those of muddy sediments [42] and large grain size of sand results in less surface area for bacterial attachment compared to muddy sediments [43]. In the present study organic matter showed a significant positive correlation with clay ($r = 0.785$, $p< 0.05$) and silt content ($r = 0.883$, $p< 0.05$) of the sediment and negative correlation with sand content ($r = -0.878$, $p< 0.05$). High bacterial population with high organic matter was found in clayey silt (50m depth off Ratnagiri and 50m depth off Goa) or silty sand (200m depth off Bhatkal) sediments of Arabian Sea.

Besides being affected by the quantity of organic matter, the distribution and density of the microorganisms seem to be controlled by the availability and nutritional quality of degradable organic matter [12]. The lability or bioavailability of the organic matter may be a limiting factor for bacterial growth and for most of the benthic organisms [44]. The determination of carbohydrate, lipid and protein carbon could be suitable to estimate the fraction potentially available to sediment ingesting organisms [45]. In the present study lipids formed the main component among labile organic pool followed by protein and carbohydrates. A significant positive correlation was observed between bacterial population and the concentration of protein and carbohydrate in the study area. These results are in agreement with those reported by Danovaro et al. [38] for a wider sector of the Eastern Mediterranean Sea.

The high bacterial density coincided with high concentrations of BPC in 50m depth off Ratnagiri and low bacterial density coincided with low concentration of BPC in 200m depth off Ratnagiri suggesting that biopolymeric carbon is the main factor that control distribution of heterotrophic bacteria in the sediment.

Nitrogen is a major nutrient element influencing the cycling of organic matter in the biosphere. Both its organic and inorganic forms are closely related to biological productivity and take part in a series of interconnected reactions which form the nitrogen cycle [46]. A significant positive correlation was found between nitrogen and silt ($r = 0.892$, $p< 0.05$) and clay ($r = 0.732$, $p< 0.05$) content of the sediment and negative correlation with sand content ($r = -0.867$, $p< 0.05$). High nitrogen content was recorded in sediments containing higher amount of clay and silt content whereas sandy sediments showed minimum content. Similar relationship between nitrogen content and particle size were found in studies by Trask [47] in marine sediments.

In addition to biopolymeric cabon, the C/N ratio was also used to highlight the nutritional quality of organic matter. Low values of C/N represent easily degradable organic matter of high nutritional quality, whilst high C/N ratio represents more refractory material [48]. The analysis of the C/N ratio at continental shelf sediments of Arabian Sea showed values higher than 10, except for two stations, 50m depth off Kozhikode (9.49) and off Goa (9.9).The presence of high C/N ratios in all other stations suggest the presence of more refractory organic matter in the study area.

The results of BIOENVI analysis in the present study define possible environmental constraints controlling the distribution pattern of heterotrophic bacterial populations in shelf sediments and found that distribution of bacteria is not controlled simply by a single parameter but is influenced by various factors, such as temperature, pH, nitrogen content, organic matter, biopolymeric carbon, lipid, protein and carbohydrate concentration and sediment texture.

Heterotrophic microorganisms are the major agents shaping the organic composition of the marine environment [49]. About 90% of heterotrophic bacteria in this environment constitute gram negative bacteria because the gram negative cell wall is adapted better for survival in the marine environment [50]. In the present study, gram negatives (about 55%) were dominant which is in agreement with other investigations [21]. The present study also observed reasonably high abundance of gram positive bacteria (45%) in the shelf sediments which, however, is a common trend observed in tropical and temperate marine environments [21].

Major groups that contain culturable members include the gamma proteobacteria (*Vibrio* spp., *Pseudoalteromonas* spp. and *Pseudomonas* spp.), the *Flexibacter*, *Bacterioides* and Cytophaga phylum [51]. In our study representatives of these groups (*Vibrio, Alteromonas, Pseudomonas* and *Flexibacter*) were identified.

Bacillus represented the predominant genus among the Gram positive bacteria. This is in accordance with several studies [52-53] which have reported *Bacillus* as the important gram-positive bacteria in marine environment occurring ubiquitously in waters and sediment samples from polar to tropics [54]. Vibrios which constitute a significant component of the autochthonous bacteria of various marine environments [26] were also quite abundant along the shelf sediments. The abundance of *Vibrio* (11%) observed in the present study is in well agreement with the reports of high percentage isolation of vibrios off Madras, India [55]. Genus *Micrococcus* constituted a significant fraction of the culturable bacterial communities of Arabian Sea shelf which was reported previously by Wood [56] in Australian waters. *Pseudomonas* form a considerable fraction among gram negatives characterized by high metabolic versatility, and it is known for its capacity to degrade a considerable amount of synthetic compounds [57].

Distribution of these dominant groups depends on the prevailing biogeochemistry of the shelf sediment. The CCA analysis identified TC, silt, clay, sand and BPC as the best explanatory variables in the distribution of dominant genera like *Bacillus*, *Vibrio*, *Alteromonas*, *Micrococcus*, *Pseudomonas* and *Acinetobacter* present in the shelf sediments. Distribution of *Bacillus* was influenced by a combination of sediment variables such as carbohydrate, protein and BPC concentration.

CONCLUSION

Our understanding of the relationship between biogeochemical parameters of sediment and bacterial community in the shelf sediments of south west coast of India is limited. The study was carried out to assess the heterotrophic bacteria and their relation to associated environmental variables of the region. One of the major revelations of the study was that, of all the tested parameters, sediment texture and quantity/ quality of organic matter were determining the bacterial diversity in the area. Gram negative forms were found to dominate the shelf sediments of Arabian Sea. Bacterial communities in the shelf sediments represent a dynamic functional component in the marine ecosystem contributing to the degradation of organic matter in sediments and an understanding of their correlation with the environmental variables highlight their role in remineralization.

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