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Bathymetric and latitudinal shift in sedimentary parameters along the shelf sediments of Bay of Bengal: Influence on heterotrophic bacteria

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ABSTRACT

Bathymetric and spatial alterations of sedimentary parameters and its influence on heterotrophic bacterial population in the shelf sediments of south east coast of India (Bay of Bengal) were analysed. The hydrographical parameters did not show significant ($p<0.05$) spatial variation though it varied with depth. Sediment was fine sand at 50 -100m depth and clayey silt at 200m depth. Organic matter in the sediment ranged from 0.95 - 3.76% showing significant depth wise variation ($p<0.05$). Total heterotrophic bacterial population ranged from $4.87 \times 10^3 – 2.32 \times 10^5$ CFU g^{-1} dry wt. and the abundance was greater towards the northern latitudes. Bacillus, Vibrio and Alteromonas were the dominant genera in the shelf sediments followed by Alcaligenes, Enterobacteriaceae, Acinetobacter, Flexibacter and Moraxella. Significant positive correlation of bacterial abundance with sediment texture and organic matter was evident from the spearman rank correlation analysis. BIOENV identified dissolved oxygen, clay, silt and total nitrogen as a combination of environmental parameters that best explained the distribution patterns of heterotrophic bacteria ($\rho = 0.644$). The Principal component Analysis (PCA) displayed higher similarity of environmental characteristics within respective depth regions. First two components together explain 90% of the data variance between stations. The canonical correspondence analysis (CCA) further substantiated that major environmental parameters (such as temperature, dissolved oxygen, silt, sand and organic matter), had significant effects on the spatial distribution of culturable heterotrophic bacteria in the shelf sediments of Bay of Bengal.

Keywords: Bay of Bengal, Shelf sediments, Heterotrophic bacteria, TOC, C/N ratio, CCA

INTRODUCTION

The continental shelves which make up 8% of the global ocean surface area are episodic components of the marine ecosystem. The mean water depth of continental shelves is less than that in open sea, thereby implying the shelf floor a significantly more important role in the biogeochemistry and ecology [1]. Animal–sediment relationships in marine sediments are important for the studies on the distribution and abundance of benthic communities [2]. Large fractions of the total benthic biomass are dominated by sediment bacteria [3] which play a vital role in benthic ecosystems [4]. They are a critical component of marine ecosystem processing more than one half of the total primary production, promoting organic degradation, decomposition and mineralization processes, regenerating nutrients, and interacting widely with other organisms [5]. Moreover, bacteria are basic components of the benthic food chain and it represents an important food resource for benthic fauna [6] thereby facilitating the carbon transfer in the food chain.

Like other benthic inhabitants, bacteria in shelf sediments are related to the sediment properties. Major factors which control horizontal and vertical distribution of bacterial population include physical characteristics such as

temperature, light, salinity, dissolved oxygen, pH, hydrostatic pressure, water movements and sediment type [4, 7-10], chemical characteristics such as organic matter content [11] and concentration of labile compounds [12]. Sensitivity to each factor may vary among taxonomically different bacterial groups, reflecting differences in their community structures [13]. Besides these physicochemical parameters, the composition of benthic bacteria can also be correlated with latitude, indicating that biogeographic factors are important determinants of the microbial diversity [14]. A number of authors have emphasized the distribution and characterization of bacterial communities in marine sediments [3, 15-20]. This is essential for understanding the functional and biogeographical relationships of sediment bacteria in oceanic processes [21] and to know the flow and transfer of organic matter and energy in an ecosystem [14].

In the last few decades several studies have shown that the role of bacteria in benthic ecosystem is far more important than previously thought [22]. Some studies have characterized bacterial communities from the coastal waters along the east coast of India [23-25]. But no work has been done on the distribution and abundance of bacterial population in the shelf sediments of Bay of Bengal mainly because such environments have been so rarely sampled. Accordingly our principal aim was (1) to determine the prevailing sedimentary environmental parameters in the shelf sediment, (2) to investigate the numerical abundance, and spatial distribution patterns of culturable heterotrophic bacteria and (3) to corroborate the probable sediment properties that determine the bacterial distribution and diversity in the shelf sediments.

MATERIALS AND METHODS

Study Area

The study area was the continental shelf region of south east coast of India (Bay of Bengal), extending between latitude 10º 36' 00" N to 15º 14' 82" N and longitude 80º 07' 06" E to 81º 35' 09" E (Fig.1), covering 18 stations over 6 transects. Across the transects, 3 stations each at a depth of 50 m, 100 m and 200 m were sampled. Details of the stations are given in Table 1.

Table 1.Details of sample collection onboard FORV *Sagar Sampada* (Cruise No. 266)

TRANSECTS	STATIONS	DATE OF SAMPLING	DEPTH (M)	LATITUDE (°N)	LONGITUDE (°E)
Karaikal (KRKL)	1	5/5/2009	48	10º 36' 00	080º 07' 06
	2	5/5/2009	96	10º 35' 32	080º 11' 79
	3	5/5/2009	200	10º 35' 38	080º 21'34
Cuddalore (CDLR)	6	6/5/2009	49	11º 34' 52	079º 54' 94
	7	6/5/2009	123	11º 32'93	079º 57' 99
	8	6/5/2009	210	11º 32'14	079º 58'84
Cheyyur (CHYR)	11	7/5/2009	55	12º 31'35	080º 23' 68
	12	7/5/2009	90	12º 30'63	080º 35' 21
	13	7/5/2009	198	12º 29'44	080º 35'64
Chennai (CHNI)	16	9/5/2009	56	13º 06'46	080º 26' 80
	17	9/5/2009	108	13º 06'69	080º 31'92
	18	8/5/2009	205	13º 02'71	080º 37'18
Thamminapatnam (TPTM)	21	13/5/2009	53	14º 09'46	080º 21'89
	22	13/5/2009	179	14º 09'41	080º24'41
	23	12/5/2009	208	14º 09'43	080º 24'66
Singarayakonda (SKDA)	26	10/5/2009	50	15º 14'82	080º 24' 35
	27	10/5/2009	105	15º 15' 05	080º 29' 02
	28	10/5/2009	190	15º 14' 82	081º 35'09

Sample collection

Sediment samples for the present study were collected onboard Fisheries and Oceanographic Research Vessel (FORV) *Sagar Sampada* (Cruise No.266), Ministry of Earth Sciences, Govt. of India, during May 2009. Hydrographical data for temperature, salinity and dissolved oxygen were collected from each station using onboard CTD (Sea bird, USA). Sediment samples were collected using Smith McIntyre grab (0.2 m^2) from desired depths. Sediment from the surface layer (top 5 cm) was aseptically transferred into sterile polythene bags and immediately subjected to microbiological analysis. Sediment samples were preserved at -20°C in a deep freezer for organic matter and texture analysis.

Sediment analysis

Grain size analysis

The sediment sample was dried in a hot air oven at 60^0 C for 48hrs. 10g each of dried sample was accurately weighed and dispersed using sodium hexametaphosphate (10%) and kept overnight. Grain size of the sediment was measured by separating the fine fraction (<180µm) by wet sieving. The fine fraction of the sediment was determined

using Particle Size Analyzer (SYMPA TECH, Germany). The fraction of the sample >180µm was dried and weighed separately.

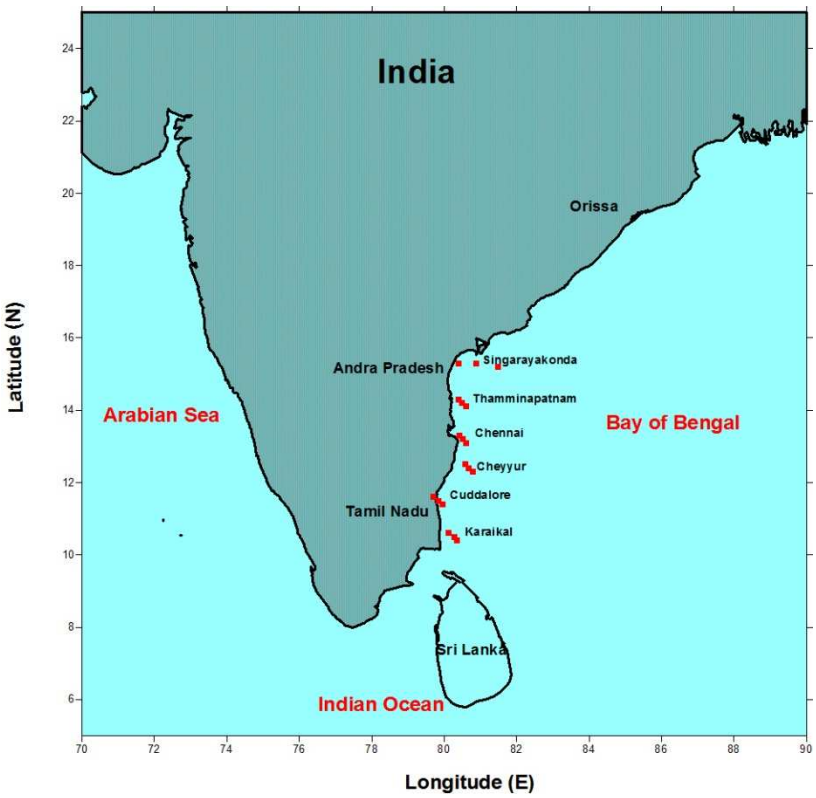


Figure 1: Location of sampling stations in the study area.

Biochemical and elemental analysis

Sediment samples from each station were subjected to chemical analysis to determine the total organic carbon (TOC) and organic matter (OM) content. Samples were powdered well after drying in hot air oven at 60^0C for 48 hrs. The organic carbon was determined using a titration method according to El Wakeel and Riley [26] and organic matter contents were determined by multiplying the organic carbon concentrations by the factor 1.724 [27]. Total carbon (TC) and total nitrogen (TN) analyses were performed using a Vario EL III CHNS Analyzer.

Microbiological analysis

Estimation and identification of total heterotrophic bacteria (THB)

Sediment samples collected from the shelf regions were serially diluted with sterile seawater. ZoBell's 2216e agar was used for the isolation of total heterotrophic bacteria employing the conventional spread plate method.The plates in duplicate were incubated at 28 ± 2°C for 5-7 days. Colonies were counted and expressed as colony forming units (CFU) per gram dry weight of sediment. Well isolated bacterial colonies were randomly isolated from plates. The cultures were repeatedly streaked on nutrient agar plates for purity and preserved in nutrient agar vials overlaid with sterile liquid paraffin. By gram staining, spore staining and biochemical tests, the isolates were identified up to generic level following Bergey's Manual of Systematic Bacteriology [28] and taxonomic scheme of Oliver [29].

Statistical analysis

Univariate and multivariate community analyses were carried out using statistical softwares XLSTAT v.2012.6.01 (Addinsoft), ORIGIN v.6.0, SPSS v.19.0 and PRIMER v6. A Spearman-rank correlation analysis was performed to test relationships between environmental parameters and heterotrophic bacteria. Analysis of variance (ANOVA) was used to find out the differences in environmental variables and bacterial population between the three depth regions and transects. A *post-hoc* Tukey test was adopted to determine if there were significant differences among the depths. Probabilities (p) of <0.05 were considered to be significant. Similarity between stations with respect to the generic composition and diversity indices were analysed using statistical software PRIMER v6. To check the similarity between stations the heterotrophic bacterial community at different depths were analysed by hierarchical agglomerative cluster analysis based on Bray-Curtis similarities and the results were plotted into ordination graphs.To select the variables that best explain the distribution of heterotrophic bacterial communities in the sediment, the BIOENV analysis was carried out (Bray-Curtis similarity coefficient, Spearman rank correlation method). The Principal Component Analysis (PCA, normalized data) was done for ordination of the sample

locations in relation to sediment factors after suitable transformation of data. The canonical correspondence analysis (CCA) was performed to identify relationships among the spatial distribution patterns of heterotrophic bacteria and environmental gradients. CCA analyses included population data for 6 dominant bacteria and major environmental variables. CCA included a Monte Carlo permutation test (with 999 unrestricted permutations) to determine the significance of heterotrophic bacteria - environment relationships.

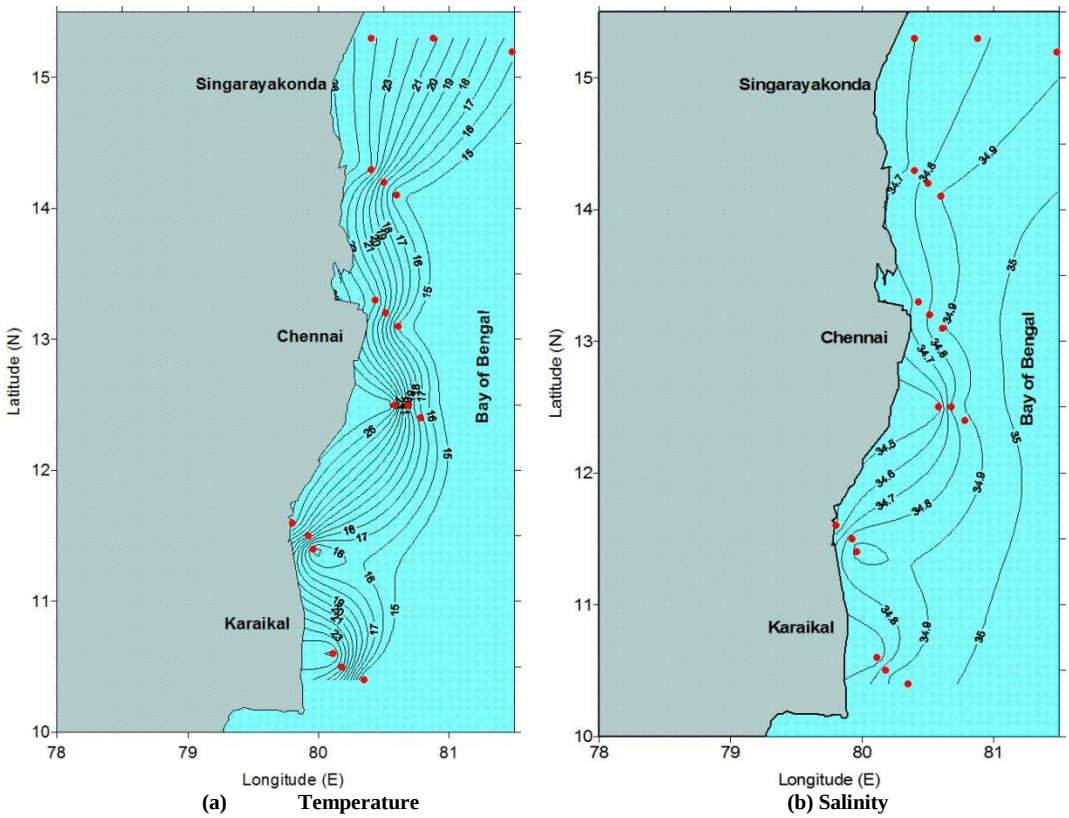
RESULTS

Abiotic factors

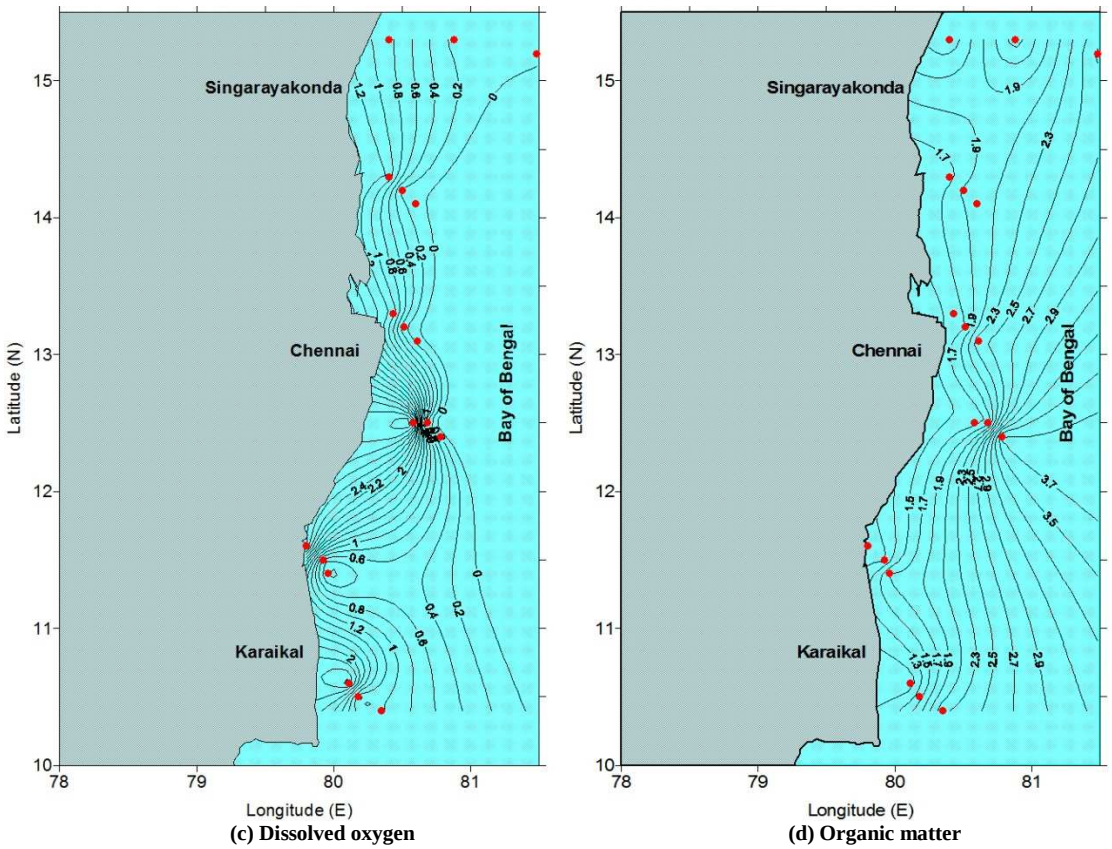
The key hydrographical parameters (Fig.2) such as temperature, salinity, dissolved oxygen and pH did not vary significantly ($p<0.05$) between transects. Temperature showed significant variation (13.10 - 27.52°C) with depth i.e., 50, 100 and 200m ($p<0.05$). Mean bottom water salinity was 34.78 ± 0.14 psu (range: 34.41 - 34.95 psu). Dissolved oxygen ranged from 0.07 - 3.63 ml/l (mean: 0.89 ± 1.05 ml/l) and was considerably low at 200 m (0.07 - 1.02 ml/l). Mean pH of the sediment was 7.71 ± 0.36 (range: 7.3 - 8.7).

Sediment characteristics

Sediment was olive green to grey in colour and the sediment types ranged from fine clay silt to fine sand (Fig.3). Organic matter present (Fig.2d) in the shelf sediments did not show any significant ($p<0.05$) spatial variation between transects, though it was slightly greater towards northern latitudes of the study area. Organic matter ranged from 0.95 - 3.76% of dry wt. and was found to be maximum at 200m depth (mean ± SD: 1.53 ± 0.53) followed by 100m (mean ± SD: 1.53 ± 0.29) and 50m (mean ± SD: 2.49 ± 0.65) (Fig. 4). Total Carbon (TC) and Total Nitrogen (TN) in the shelf sediments were 4.86 ± 2.83 and 0.08 ± 0.04 respectively. Organic matter quality as measured by molar Carbon/Nitrogen (C/N) ratio was found to be 14.28 ± 5.47 (range: 8.44 – 28.38). Significantly low values ($p<0.05$) were sited at 200m depth regions (9.64 ± 0.92). Total organic carbon (TOC) constituted only 36.22 ± 29.34% of total carbon (TC) indicating the dominance of inorganic carbon fractions in the shelf sediments. However deeper shelf sediments (200m) had an average of 59.87 ± 30.81% TOC.

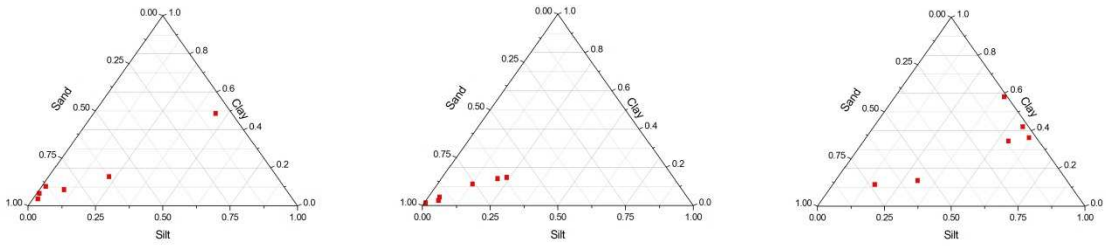


(a) Temperature **(b) Salinity**



(c) Dissolved oxygen **(d) Organic matter**

Figure 2: Distribution of (a) Temperature, (b) Salinity, (c) Dissolved oxygen and (d) Organic matter in the shelf sediments of south east coast of India



(a) 50m **(b) 100m** **(c) 200m**

Figure 3: Ternary plots showing the distribution of silt clay and sand at 50, 100 and 200m depth regions in the shelf sediments of south east coast of India.

Heterotrophic bacterial abundance

Total heterotrophic (culturable) bacterial population ranged from 4.87 x 10^3 – 2.32x 10^5 CFU g^{-1}dry wt. Significant ($p<0.05$) differences between the northern and southern latitudes was reflected in the bacterial abundance. The culturable bacterial population was higher towards northern latitudes. Though not statistically significant, bacterial density was higher in the deeper regions of the shelf sediment. Mean bacterial density at 50m was 4.88 ± 2.3 x 10^4 CFU/g dry wt., at 100m 4.28 ± 2.7 x 10^4 CFU/g dry wt. and at 200m, it was 2.11 ± 1 x 10^5 CFU/g dry wt. (Fig.5). Bacterial abundance was generally higher in clayey and clayey silt sediments.

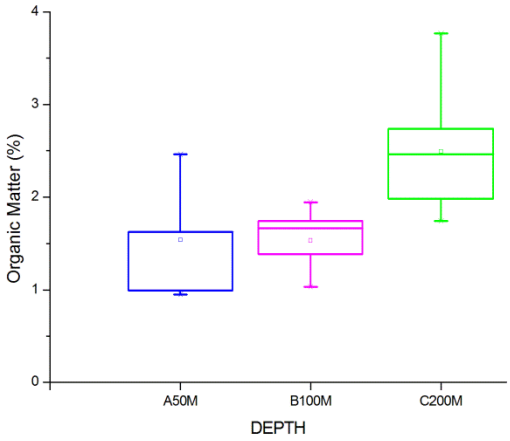


Figure 4: Box plot showing the concentration of organic matter at 50m, 100m and 200m depth regions along the shelf sediments of south east coast of India.

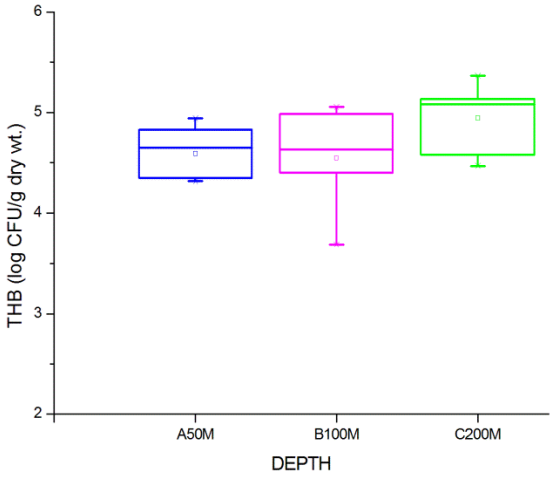
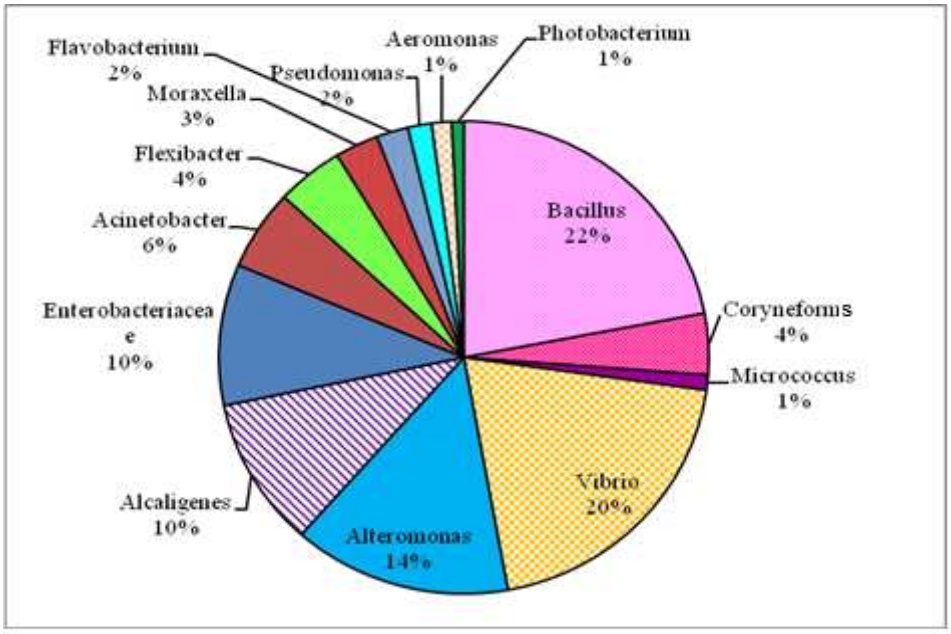


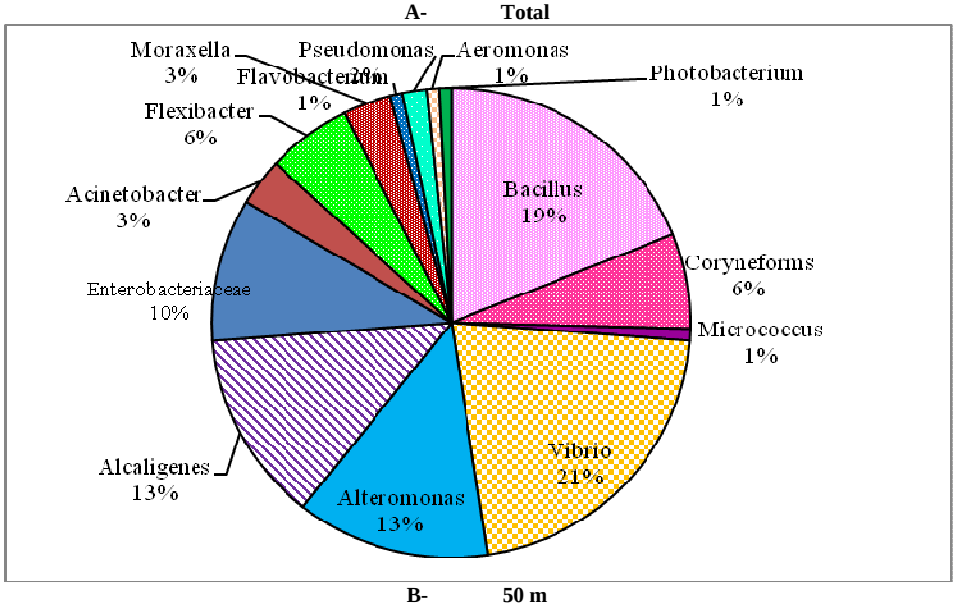
Figure 5: Box plot showing total heterotrophic bacterial population present in the shelf sediments of south east coast of India.

Generic composition of heterotrophic bacteria

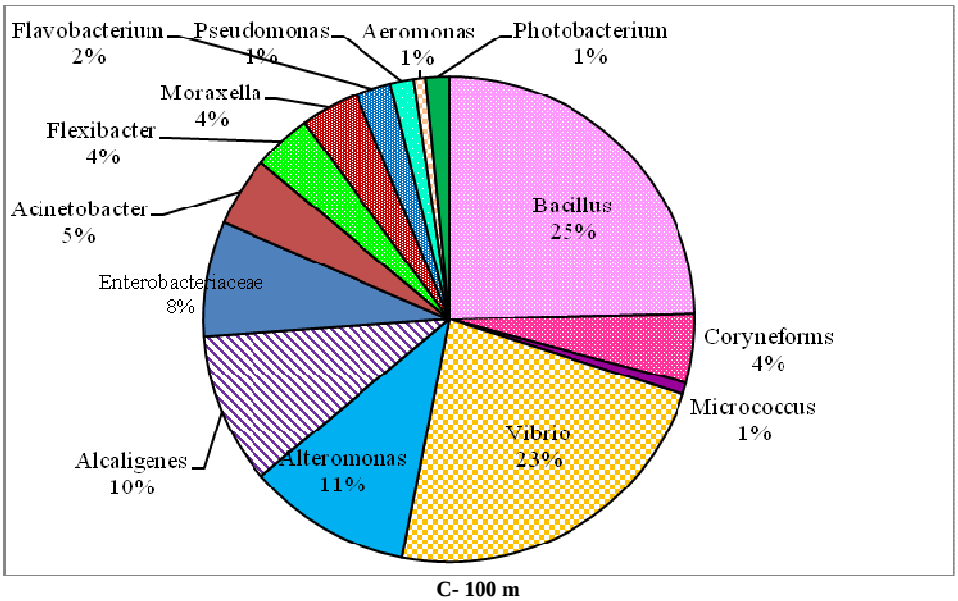
About 382 bacterial strains were isolated from the shelf sediments of south east coast of India. They were identified based on morphological and biochemical tests and classified into 14 genera. Among the 382 cultures, 27% were gram positive and 73% gram negative. Majority of the isolates were white or off white in colour and the pigmented (yellow and orange) forms were scanty. Most of the isolates exhibited considerable biochemical versatility. *Bacillus* (22%) was found to be the predominant genus along the shelf sediments followed by *Vibrio* (20%), *Alteromonas* (14%), *Alcaligenes* (10%), Enterobacteriaceae (10%), *Acinetobacter* (5%) and *Flexibacter* (4%) (Fig.6A). Gram positive cocci were comparatively lesser. At 50m depth (Fig.6B) *Vibrio* (21%) was the dominant genus followed by *Bacillus* (19%), and *Alteromonas* (13%). *Bacillus* was the abundant genus at 100m (25%) (Fig.6C) followed by *Vibrio* (23%). At 200m depth also *Bacillus* (23%) dominated followed by *Alteromonas* (17%) (Fig.6D). Bacterial communities in the shallower regions (50 and 100m) were more or less similar but at deeper regions (200m) they showed considerable differences in their occurrence. *Vibrio*, *Alcaligenes*, *Pseudomonas* and *Moraxella* which were dominant in the shallow sediments were lesser in the deeper regions. Incidence of Enterobacteriaceae and absence of *Photobacterium* at 200m was remarkable. *Bacillus* was prevalent off Karaikkal and Cuddalore; *Vibrio* off Thamminapatnam and Chennai and *Alteromonas* off Chennai and Cheyyur.



A- Total



B- 50 m



C- 100 m

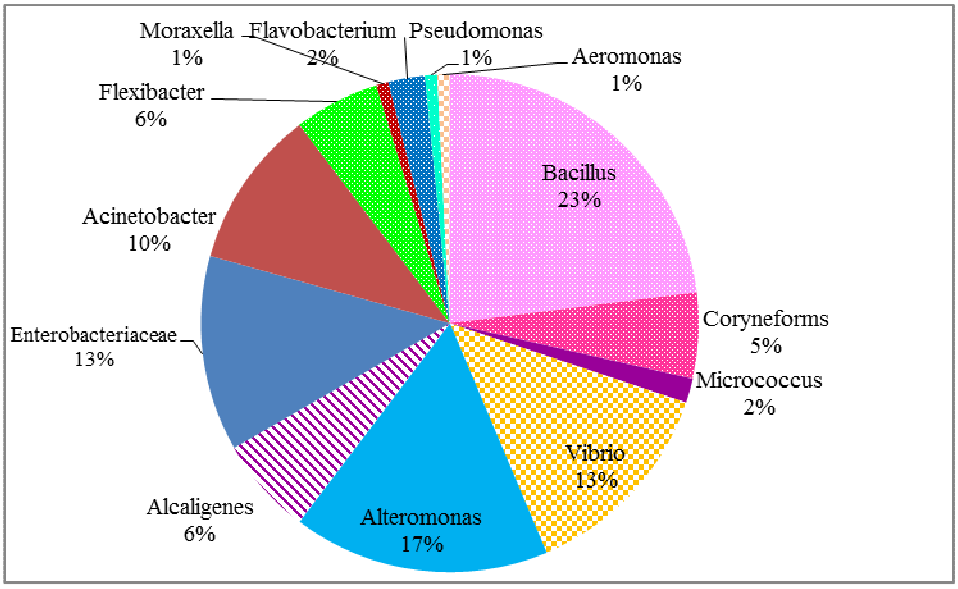


Figure 6: Percentage contribution of different genera present in the shelf sediments (A- Total, B- 50m, C- 100m and D- 200m depth) of south east coast of India.

Statistical analysis

The Shannon-Wiener diversity (*H'*) ranged from 2.48 to 3.11, clearly showing the diverse nature of heterotrophic bacteria at different stations (Table 2). With the number of species ranging from 6-9, *H'* values recorded were on the higher side and it attributed to the higher evenness (0.952 to 0.9973). *H'* values also implies a marginal depth wise variation in the study area. Species richness ranged between 1.56 to 2.39.

Spearman rank correlation coefficients were computed for all interrelationships between study variable. Except for pH, all variables were significantly correlated with each other (Table 3). Statistical analysis showed that bacterial abundance showed significant positive correlation with percentage of silt ($r = 0.849$, $p< 0.001$), clay ($r = 0.862$, $p< 0.001$), organic carbon ($r = 0.723$, $p< 0.001$) total nitrogen ($r = 0.833$, $p< 0.001$) and sediment depth ($r = 0.496$, $p< 0.05$). Correlation between these parameters is presented in figure 7. Organic carbon (OC) was strongly correlated to the fine fraction of sediment ($r = 0.827$, $p< 0.001$). BIOENV analysis for matrices showed the sets of variables with highest influence on bacterial distribution in the shelf sediments. The combination of environmental parameters which best explained the distribution patterns was dissolved oxygen, clay, silt and total nitrogen (highest ρ value = 0.644; Table 4).

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

Stations*	S	N	d	J'	H'(log2)	1-λ'
KRKL-A	9	29	2.378	0.9829	3.116	0.9122
KRKL-B	8	26	2.148	0.9595	2.879	0.8864
KRKL-C	9	28	2.39	0.9746	3.089	0.9081
CDLR-A	9	29	2.384	0.9778	3.1	0.91
CDLR-B	6	23	1.596	0.9611	2.484	0.8467
CDLR-C	6	23	1.596	0.9611	2.484	0.8467
CHYR-A	8	27	2.128	0.9741	2.922	0.8944
CHYR-B	9	28	2.39	0.9758	3.093	0.9082
CHYR-C	8	26	2.144	0.9634	2.89	0.8881
CHNI- A	7	25	1.87	0.9639	2.706	0.8717
CHNI- B	9	28	2.395	0.9737	3.087	0.9066
CHNI- C	8	27	2.122	0.9781	2.934	0.8968
TPTM-A	8	27	2.127	0.9753	2.926	0.8949
TPTM-B	6	22	1.607	0.952	2.461	0.839
TPTM-C	6	24	1.565	0.9973	2.578	0.8673
SKDA-A	8	27	2.118	0.9814	2.944	0.8983
SKDA-B	8	27	2.124	0.9762	2.929	0.8958
SKDA-C	7	26	1.838	0.9945	2.792	0.888

**(A-50 m, B-100 m, C-200 m depth)*

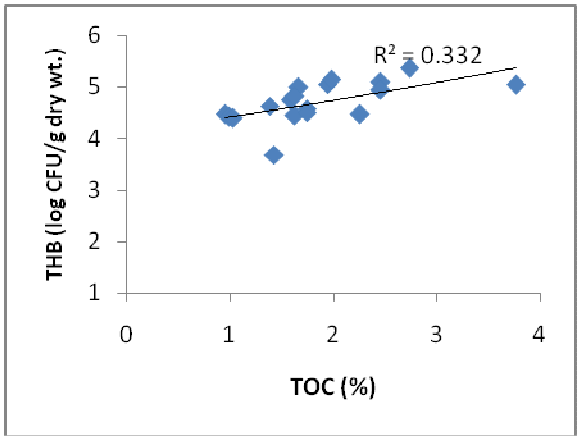
Table 3. Spearman correlation analysis between environmental variables and bacterial population. Significant values at the level of significance p <0.05 are given in bold

Variables	Depth (m)	Temperature (°C)	Dissolved oxygen (ml/l)	Salinity (psu)	pH	Silt (%)	Clay (%)	Sand (%)	OC (%)	OM (%)	TC (%)	TN (%)	C/N (%)	THB (log)
Depth (m)	**1**													
Temperature (°C)	**-0.930**	**1**												
Dissolved oxygen (ml/l)	**-0.787**	**0.792**	**1**											
Salinity (psu)	**0.891**	**-0.891**	**-0.672**	**1**										
pH	-0.154	0.231	-0.017	-0.181	**1**									
Silt (%)	**0.595**	**-0.676**	**-0.631**	0.434	-0.063	**1**								
Clay (%)	0.430	**-0.490**	**-0.558**	0.236	0.110	**0.905**	**1**							
Sand (%)	**-0.554**	**0.622**	**0.610**	-0.387	-0.023	**-0.948**	**-0.973**	**1**						
OC (%)	**0.596**	**-0.662**	**-0.604**	**0.480**	-0.077	**0.827**	**0.739**	**-0.795**	**1**					
OM (%)	**0.609**	**-0.672**	**-0.612**	**0.491**	-0.081	**0.828**	**0.735**	**-0.794**	**0.999**	**1**				
TC (%)	0.098	-0.073	-0.018	0.253	-0.257	-0.467	**-0.585**	**0.511**	-0.281	-0.279	**1**			
TN (%)	**0.711**	**-0.738**	**-0.688**	**0.527**	-0.100	**0.940**	**0.886**	**-0.944**	**0.836**	**0.839**	-0.335	**1**		
C/N (%)	**-0.633**	**0.593**	**0.521**	**-0.507**	0.418	-0.470	-0.428	**0.490**	-0.206	-0.217	0.024	**-0.614**	**1**	
THB (log)	**0.496**	**-0.552**	**-0.738**	0.286	0.204	**0.849**	**0.862**	**-0.866**	**0.723**	**0.727**	-0.395	**0.833**	-0.404	**1**

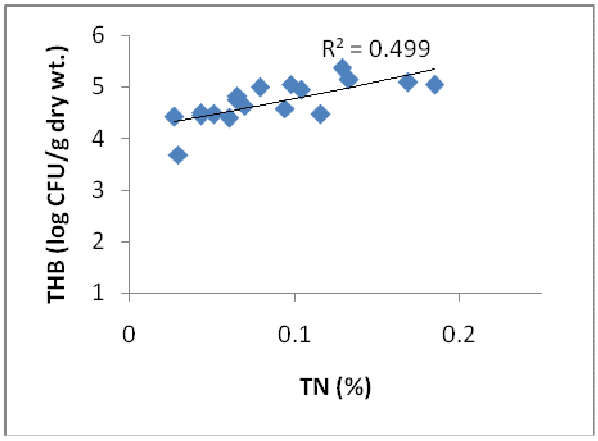
Table 4. BIO-ENV analyses indicating which of the measured environmental parameters best explain the community pattern in shelf sediments

Sl.No	Spearman rank correlation	Best matching variables
1	0.644	Dissolved Oxygen, Clay, Silt, Total Nitrogen
2	0.643	Dissolved Oxygen, Clay, Silt
3	0.636	Clay, Silt
4	0.635	Dissolved Oxygen, Clay, Silt, Total Organic Carbon
5	0.634	Dissolved Oxygen, Clay, pH

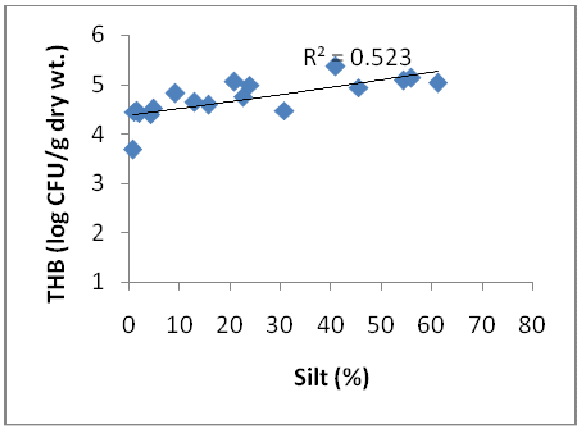
Hierarchical clustering analysis delineates the bacterial communities of the sampling stations into two main groups (Fig. 8). The dichotomy includes the one that groups 50m, 100m and 200m depth regions of Cuddalore forming 79% similarity and another cluster that incorporates all other stations (50% similarity). In the second group, all the representative depths at Karaikal and 100m depth of Cheyyur form a cluster with 65% similarity. Similarly 50 and 200m depth regions off Cheyyur and 200m depth off Chennai form another cluster with 70% similarity. The Principal component Analysis (PCA) showed higher similarity of environmental characteristics within respective depth regions (Fig.9). The PCA results shows clusters of sampling sites that correspond relatively well to their spatial distribution. The variability of temperature, dissolved oxygen, pH, organic carbon, silt, clay, sand, TOC, TC, TN, total inorganic carbon, and depth were evaluated. Principal component I explained 75.2% of the total variability, whereas component II explained 14.8% variability. First two components together explain 90% of the data variance between stations. Temperature, dissolved oxygen, pH, sand, total carbon and total inorganic carbon showed a positive correlation with PC I, while, dissolved oxygen, silt, clay, total organic carbon, total nitrogen and depth showed a negative correlation. With PC II, temperature, dissolved oxygen, pH, and clay showed a positive correlation, whereas all other factors were negatively correlated. The PCA analysis demonstrated that the sediment characteristics differ between the three depth regions in the shelf sediment. Based on the results of PCA, parameters such as dissolved oxygen, temperature, sand, and TOC were only included for the canonical correspondence analysis (CCA) as these parameters did showed significant ($p<0.05$) bathymetric variation. The CCA revealed that the selected environmental parameters had promising influence on the spatial distribution of culturable bacteria (Fig.10). The first two CCA axes could explain 74.71% variability of environment parameters (eigen value: axis 1 = 0.059 and for axis 2 = 0.043).



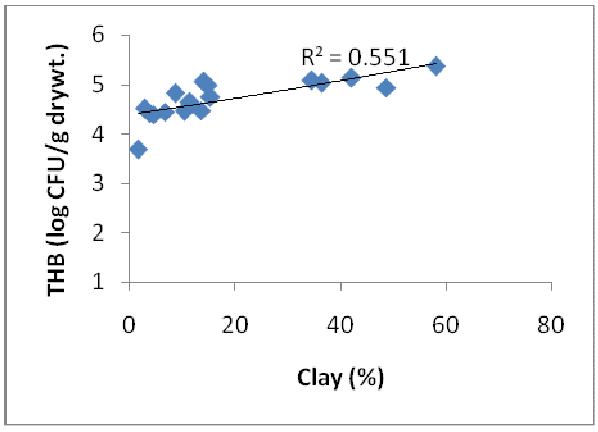
A



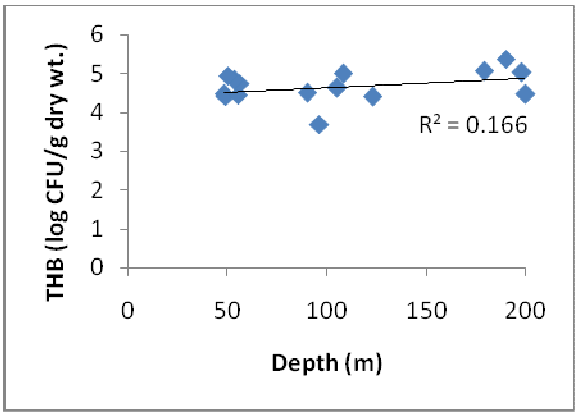
B



C



D



E

Figure 7: Relationship of bacterial population to: A- Total Organic Carbon (TOC); B- Total Nitrogen (TN); Sediment texture: C- Silt and D- Clay content; E- Depth.

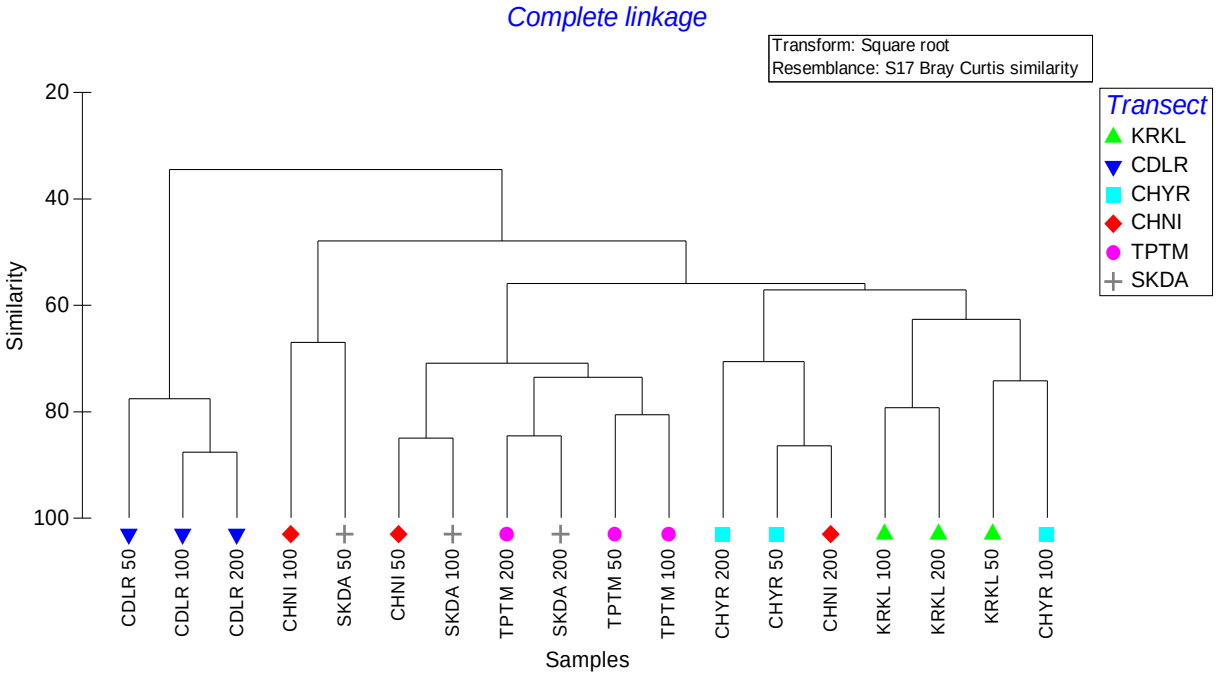


Figure 8: Dendrogram based on bacterial community recorded at various stations

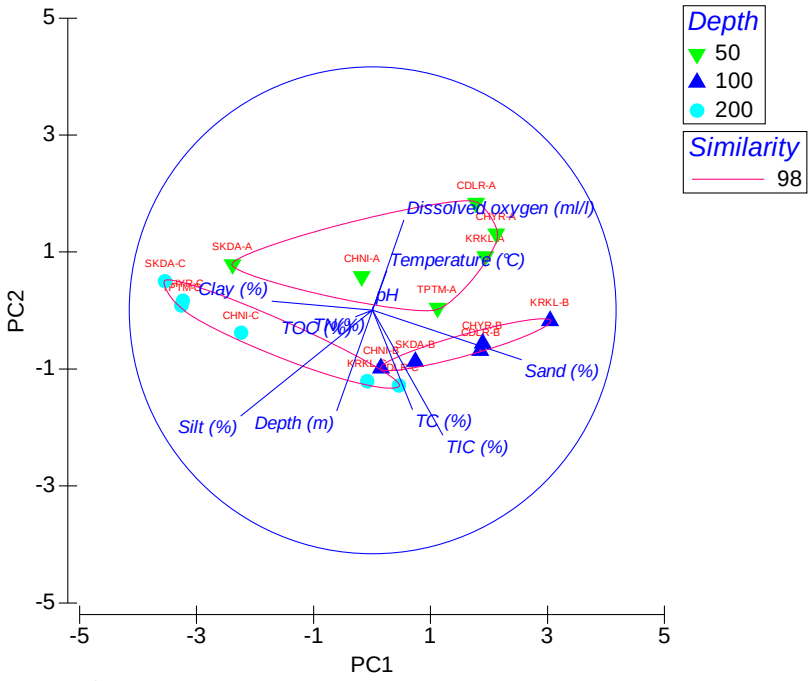


Figure 9: PCA with habitat data from several sites using the variables that better explain the distribution patterns of bacteria in the shelf sediments

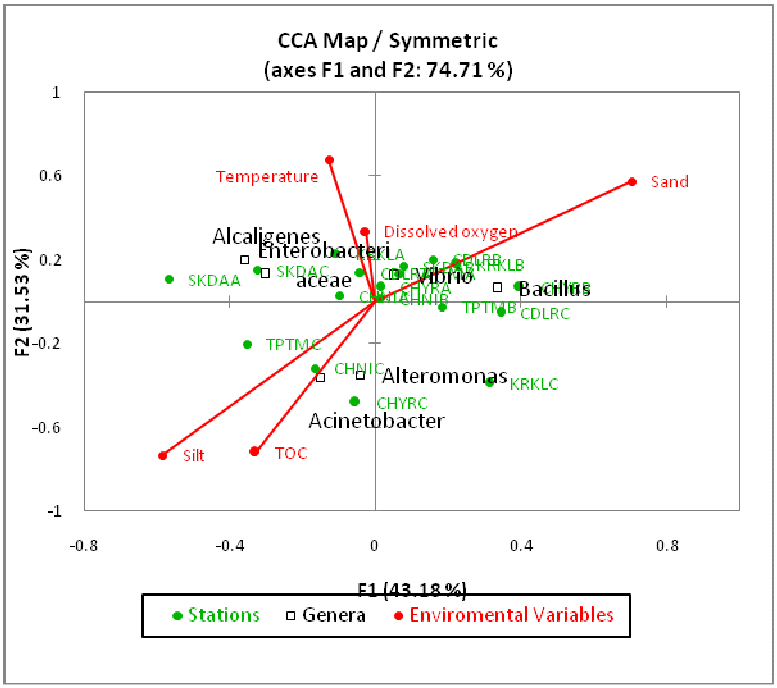


Figure 10: Canonical Correspondence Analysis (CCA) showing 6 most important bacterial genera, environmental variables, and sampling sites. Vector lines represent the relationship of significant environmental variables to the ordination axes; their length is proportional to their relative significance.

DISCUSSION

In the present study, bottom water temperature showed a significant ($p<0.001$) relation with heterotrophic bacterial density which is in agreement with other systems [9]. Duyl and Kop [30] suggested that temperature is not a single factor but it acts simultaneously with other factors (organic nutrient quality and quantity, phytoplankton activity, flagellate grazing) in controlling bacterial dynamics. Dissolved oxygen concentrations also showed a significant negative correlation with THB and depth. Dissolved oxygen values tend to decrease below 0.5 ml/l at 200 m depth which can be due to the existence of oxygen minimum layer (O_2 concentrations < 0.5 ml/l) at that region. Bacteria identified from this zone are of great ecological importance as they take part in decomposing, mineralizing and

subsequent recycling of organic matter [31]. Salinity showed least variation, latitudinal as well as depth-wise; Bay of Bengal has relatively low salinity due to high runoff [32] and precipitation [33].The pH of bottom water ranged from 7.3 to 8.7 similar to that reported for tropical Ocean waters [16].

The main parameter that limits the density, distribution and diversity of benthic community in sediment is the grain-size and organic matter [3]. Factors such as the tectonic setting, river input of sediment and transport by waves and currents governs the sediment type of the continental shelf [34]. Several studies have shown an inverse relationship between the particle size of marine sediments and various measures of 'nutritional quality' such as organic matter concentration or bacterial abundance [35]. Our study also presented a highly significant ($p<0.001$) and negative correlation between bacterial abundance and mean grain size. Fine grained sediments carried high organic matter content and supported a higher bacterial number as compared to coarse sediments. Percentage of fine grain sediments significantly correlated with depth ($r = 0.595$, $p <0.01$). In the shelf sediments of south east coast of India at 50 m and 100 m depth, the sediment was sandy and at 200 m depth it was clayey silt. This is in agreement with the report that sandy sediments dominate the continental shelves [36]. The organic contents in the sandy sediments were 1–2 orders of magnitude lower than those of muddy sediments [37] which support less surface area for bacterial attachment compared to clayey sediments [38]. Benthic bacteria which signify a key step in the benthic food web play a predominant role in the early diagenesis of organic material in marine environments [4]. But the benthic responses vary from place to place depending on the quantity and quality of the sedimenting particulate organic matter [39] which have attributed to the difference in total heterotrophic bacterial population in different stations. In the present study, THB showed a significant positive correlation to organic matter ($r = 0.727$, $p< 0.001$) and the organic matter present in the shelf sediments varied significantly with depth ($r = 0.609$, $p< 0.01$). Mean concentration of organic matter present in the eastern shelf sediment was $1.85 \pm 0.67\%$ which was lower than its western counterpart, Arabian Sea [40]. But the values were comparable to that of north western Black Sea shelf [41] Great Barrier Reef shelf [42], South Atlantic Bight continental shelf sediments [43], and the shelf sediments of China Sea [44]. In shelf sediments, concentration of organic matter reflects the productivity in overlying waters and shallow depth. The primary productivity in Bay of Bengal is relatively low due to narrow continental shelf and heavy cloud cover combined with high quantity of terrigenous organic matter which affects light penetration [45]. Due to high terrigenous input the organic carbon flux to this Bay is much reduced [46] which leads to the lower concentration of organic matter along the shelf sediments. Though the riverine flux may bring in nutrients, they are thought to be lost to the deep because of its narrow shelf [47].

The carbon/nitrogen ratio (C/N) has been used to highlight the quality of organic matter and influence of terrestrial environment in marine sediments [48]. Low values of C/N (< 10) indicate presence of relatively fresh and easily degradable organic matter of high nutritional quality, whilst high C/N ratio (> 10) indicates the presence of more refractory organic matter of continental origin [49]. In the present study average C/N ratio at 200m depth regions were 9.64 ± 0.92 denoting the presence of high nutritional quality organic matter in the deeper fractions of the sediment.Organic matter at 50 and 100m was more refractory in nature, with C/N ratio greater than 10. This observation was substantiated from the results of ANOVA that there was a significant variation in the concentration of organic matter between the upper and deeper sediment fractions. C/N ratio obtained in this study was comparable with that of north western Black Sea shelf (8.79 – 15.36) [41] and Pereque Beach, Brazil (6.08 – 22.6) [14]. Total Nitrogen (TN) in the marine sediments plays an important role as a source of nutrients [50] and was found to be more at 200m depth.

Bacteria accountable for most of the benthic biomass [3] in marine ecosystems can contribute significantly to the heterotrophic activity in the system. The traditional way of assessing the number of living bacteria is based on their ability to grow in culture media. Studies conducted by Pinhassi et al. [51] have shown that the culturable colony-forming units (CFU) on agar plates can form a large fraction from the marine environment. The numerical abundance of culturable heterotrophic bacteria in the present work (range: 4.87×10^3 to 2.32×10^5 CFU g^{-1} dry wt.) is within the ranges reported earlier from the coastal waters [23-25] and continental slope sediments [18] of south east coast of India. When compared to 50m depth, bacterial population was marginally higher in 200m depth. Remarkable variation in abundance was noticed between the northern and southern latitudes. Factors influencing the vertical and horizontal distribution of heterotrophic bacteria in the shelf sediments were evident from the statistical analysis. BIOENV analysis identified a set of environmental parameters which best explained the distribution patterns as dissolved oxygen, clay, silt and total nitrogen (ρ value = 0.644). The importance of temperature, dissolved oxygen, pH, sand and total carbon on bacterial distribution was validated from the Principal Component Analysis (PCA).

Among the 382 cultures isolated from the shelf sediments 27% were gram positive and 73% gram negative. Based on the assessments of culturable microbes, 80 to 95% of marine bacteria are gram negative rather than gram positive [52]. The Shannon –Wiener diversity, which is extensively used for comparing diversity between various habitats,

obviously showed the diverse nature of heterotrophic bacteria at different stations. Bacterial generic composition obtained in this study was comparable to those already reported from the coastal areas of Bay of Bengal [23-24, 53] and Central Indian Ocean Basin [54]. *Bacillus* (22%) was found to be the dominant genus followed by *Vibrio* (20%) *Alteromonas* (14%) and Enterobacteriaceae (10%). This predominant distribution of *Bacillus* in the marine environment is probably due to their ability to resist unfavourable conditions by the production of endospores [53]. *Pseudomonas* and *Vibrio* are the native inhabitants of marine environment and the dominant bacterial flora [55]. Furthermore *Pseudomonas*, *Vibrio* and *Moraxella* play an important role in nitrogen cycling and in the degradation of several synthetic organic compounds [16, 56-57]. Accordingly, the generic composition was conducive to suggest that the existing bacterial communities are likely to play a very active role in the rapid *in situ* degradation processes.
Canonical correspondence analysis (CCA) reveals the importance of dissolved oxygen, temperature, silt, sand and TOC in the spatial distribution of dominant genera/groups *i.e., Bacillus, Vibrio, Alteromonas, Alcaligenes,* Enterobacteriaceae and *Acinetobacter*. BIOENV analysis supplemented a significant correlation of dissolved oxygen to the biotic parameters. The decline of diversity on the continental margins (200m) may be due to the deleterious effects of hypoxia impacted by an OMZ. From the data it was clear that oxygen was a crucial factor controlling biodiversity in the regions of OMZ where D.O was lowest. Cluster analysis based on the different genera was supportive in finding natural groupings of the stations, such that stations within a group were found to be similar to each other.

CONCLUSION

Importance of sedimentary environmental parameters in controlling the benthic bacterial community along the south eastern shelf sediments of India was apparent from this study. Multivariate analysis revealed that organic matter concentration and sediment grain size are highly corroborating factors influencing the abundance, vertical/horizontal distribution and generic composition of culturable heterotrophic bacteria inhabiting the shelf sediments. Bacterial population in the south eastern shelf increases towards northern latitude and was greater at deeper regions characterised by clayey silt devouring higher organic matter. C/N ratio endorses the nutritional quality of the organic matter classifying it to be mainly labile.These factors promote the proliferation of THB at deeper segments and *Bacillus* was the single dominant genus in sediment.CCA results revealed that different bacterial genera have different relationships with sedimentary environments which allowed a shift in the community composition between the surface and deeper stratum of the sediment. Identified bacterial genera suggest that the continental shelf of Bay of Bengal sustains copious bacterial population with high metabolic versatility. These different bacterial groups could act synergistically to achieve the degradation of most of the autochthonous or allochthonous organic matter in the shelf sediments.

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