

## Comparison of bacterial community structure in coastal and offshore waters of the Bay of Bengal, India

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### Abstract

Bacterial community structure was analysed from the coastal and offshore surface waters of the Bay of Bengal (BoB) using of Next Generation Sequencing (NGS) approach. Taxonomic richness was high in the offshore waters compared to the coastal waters. Analysis of sequence data revealed that both the offshore and coastal waters in our study were dominated by Proteobacteria (67.14% and 61.91%, respectively). The most dominant genera in the coastal waters were *Synechococcus*, *Erythrobacter*, *Psychrobacter*, while *Prochlorococcus*, and *Vibrio* were dominant in the offshore waters. Interestingly, the distribution of minor phyla was distinctly different in coastal and offshore environments. *Fusobacteria*, *Synergistetes*, *Fibrobacteres*, *Spirochaetes*, *Gemmatimonadetes*, *Chlorobi*, *Chlamydiae*, and *Armatimonadetes* were the minor phyla (6.84%) present in offshore waters. The metabolic predictions of the bacterial communities revealed distinctly different activities in the coastal and offshore water samples, which could be attributed to the differences in the physico-chemical conditions and substrate availability. Major functions included sulfate reduction, oxalic acid degradation, dehalogenation, nitrite reduction, ammonia oxidation, nitrogen fixation, xylan degradation, and aromatic hydrocarbons degradation.

**Keywords:** Bacterial diversity; Bay of Bengal; Next generation sequencing; Proteobacteria; Minor phyla

## Introduction

Marine bacteria play crucial roles in the marine ecosystem functioning and regulate global biogeochemical cycles of nitrogen, carbon, sulphur, silica, iron and many other trace elements in seas. They are responsible for the degradation of organic matter and thus play important roles in the regulation of the Earth's climate. They occur in vast numbers inhabiting various marine habitats and are taxonomically diverse (Parkes et al., 2000). The variation in the bacterial community composition is influenced by environmental conditions and hence their spatiotemporal changes and biogeographical distribution warrants special attention (Treusch et al., 2009). Coastal and marine environments differ in physicochemical parameters *viz.*, salinity, temperature, and nutrient concentrations. Additionally, factors like pH, dissolved oxygen, solar radiation, hydrostatic pressure, the presence of metals and toxic compounds could affect bacterial biodiversity and distribution (Bell and Callaghan, 2012). Bacterial communities exhibit an enormous range of metabolic functions and play important roles in the transformation of organic matter (Marzorati et al., 2008).

In recent years, several attempts have been made to study the bacterial diversity and distribution in many marine environments (Sala et al., 2008; Vieira et al., 2008). These studies have improved the overall understanding of the global patterns and spatial distribution of marine bacterial diversity. The bacterial community structure varies in different aquatic environments (Hamdan et al., 2013), but can be relatively distinct in similar aquatic environments separated spatially by long distances (Walsh et al., 2016). The spatial variations in marine environments are driven by environmental factors and such studies help us to comprehend the biogeochemical process (Church et al., 2009). Recent studies have revealed that the aquatic environment contains an unexploited pool of high genetic, functional, and metabolic diversity of microbial assemblages (Ininbergs et al., 2015; Wang et al., 2017). Our understanding of marine bacterial diversity is limited due to the inadequacy of culture-dependent methods. Thus far, only <5% of the aquatic environment has been explored in detail that corresponds to <0.001% of the biodiversity studied. The functional and ecological roles of the vast majority of microbes in the aquatic environment are not known (Danovaro et al., 2014).

Genomic analysis is extensively used in taxonomic studies as it is more reliable and easier for taxonomic identification and also for assessing the environmental status of marine waters. Metagenomic data could be very valuable in monitoring the health and pollution status of marine waters. Metagenomic approaches have been employed to study the response of microbes to specific environmental perturbations such as oil spills, nutrients, and heavy metal inputs (Liao et al., 2018; Zhang et al., 2018). *Alcanivorax* or *Marinobacter* is a hydrocarbon-degrading bacteria which is used as an indicator of oil pollution (Kostka et al., 2011). Similarly, sulphide-oxidizing and sulphate-

reducing bacteria are used as indicators of elevated nutrient concentrations in marine sediments (Aranda et al., 2015; Aylagas et al., 2017). Certain microorganisms such as neutrophilic iron-oxidizing Proteobacteria and acidophilic and sulphur-metabolizing bacteria are capable of incorporating pollutants into their metabolic pathways (Zhang et al., 2019). Although the advent of next-generation sequencing techniques has facilitated the large-scale analysis of bacterial sequence diversity, the transformation of this data into understanding the interactions of microbes with the environments and ecosystem functioning offers a formidable challenge as many microbes have never been cultured independently.

The Bay of Bengal is a northern extension of the Indian Ocean. This unique semi-enclosed basin is approximately 2090 km long, 1600 km wide with an average depth of about 2600 m. The Bay receives an enormous amount of riverine inputs from Krishna, Godavari, Ganges-Brahmaputra, Mahanadi, Ayeyarwady, and Kaveri. Due to the riverine influx and heavy monsoonal rains, the freshwater layer on the sea surface suppresses the circulation of nutrients from deeper waters and hence the central Bay has low productivity (Kay et al., 2018). However, the coastal areas are much more productive due to the inputs of nutrients from rivers and also due to the mixing of coastal currents (Kay et al., 2018). Though the Bay is studied extensively from physical and biological aspects, the microbiology of the region has received less attention. In the present study, an in-depth analysis of the microbial community in the offshore and coastal waters of BoB was performed using metagenomic analysis. It is hypothesized that variations in the physico-chemical conditions in these two ecosystems would reflect on the microbial communities prevailing in their respective environments. Few studies have attempted to explore the bacterial diversity in the Bay of Bengal. These studies include a comparison of bacterial diversity in the oxygen minimum zones (OMZ) and non-OMZ regions (Rajpathak et al., 2018), comparison of culturable diversity in the Andaman Sea and Orissa coasts (Gopalakrishnan et al., 2014; Das, 2007), bacterial diversity in coastal and deep-sea sediments (Parvathi et al., 2019), and influence of abiotic factors on culturable diversity in the BoB (Dash et al., 2016). However, a study on the bacterial diversity between coastal and offshore environments has not been attempted in the past using in-depth sequencing methods. This study aimed to understand the spatial variation in the bacterial community structures between a coastal and the deep-sea environment.

## **2. Materials and methods**

### **2.1 Sample collection**

Water samples were collected from two locations in the Bay of Bengal representing the coastal and offshore environments (Krishna –Godavari (KG) basin) in April 2014 (spring inter monsoon season) during an inter-disciplinary cruise in Research Vessel (RV) *Sindhu Sankalp* (Table 1). The site location SD1 was close to the shore (~30 km), whereas SD2 was in the offshore region (~3000 km). Surface water samples were collected in triplicates using Niskin water samplers attached to a Conductivity Temperature Depth profiler (CTD) rosette. The detailed description of each site is provided in **Table 1**. Samples were collected in clean, sterile bottles and processed onboard *RV Sindhu Sankalp* of the Council for Scientific and Industrial Research (CSIR)-National Institute of Oceanography (NIO), India.

### **2.2 Environmental parameters**

The physico-chemical parameters such as depth, temperature, dissolved oxygen, and salinity were measured using a CTD profiler. Water samples collected using Niskin samplers were used for the estimation of chemical parameters. Dissolved oxygen and dissolved inorganic nutrients (in  $\mu\text{M}$ ) such as silicate ( $\text{SiO}_4$ ), phosphate ( $\text{PO}_4$ ), ammonia ( $\text{NH}_4$ ), nitrite ( $\text{NO}_2$ ) and nitrate ( $\text{NO}_3$ ) were analysed spectrophotometrically using standard procedures (Grasshoff et al., 2009).

### **2.3 Determination of Prokaryotic Abundance (PA)**

Five ml samples were fixed with formaldehyde (final concentration of 2%), filtered through 0.2  $\mu\text{m}$  black nucleopore filter (25mm diameter, Whatman USA, Cat no: 110658) and stained using acridine orange (concentration of 0.1g/100 ml). The prokaryotic abundance was estimated after examining 10 randomly selected microscopic fields under blue excitation using an epifluorescence microscope (Olympus BX 41, Japan) (Cragg and Parkes, 2014).

### **2.4 DNA extraction and PCR analysis**

Triplicate water samples (5 L each) were collected from both the sites in sterile glass bottles to define the sampling variability at each sampling station. The samples were filtered through a 0.22  $\mu\text{m}$  membrane filters (Millipore, USA) and the filter papers were stored at  $-80^\circ\text{C}$  until DNA extraction. For DNA extraction, the filter papers from  $-80^\circ\text{C}$  were thawed at room temperature and cut into small pieces using sterilized scissors under aseptic conditions. The genomic DNA was extracted using the Power soil DNA Isolation Kit (Mol Bio Laboratories, CA). Three subsamples from each location were

used in the study. After DNA extraction, the individual DNA was combined (by pooling) representing a single sample.

The quantification of the genomic DNA was done using Nanodrop (Thermo Fisher Scientific, USA). Genomic DNA with an average concentration of 25 to 30 ng/μl of DNA was used for the PCR reaction. The variable regions (V3-V4) of the 16S rRNA gene targeted by V3- V4 specific primers were used for PCR amplification (16S Forward Primer TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Reverse Primer-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTCHVGGGT ATCTAATCC). The library construction was performed according to Illumina MiSeq metagenomic-library-prep-guidelines: [https://support.illumina.com/documents/documentation/chemistry\\_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf](https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). The PureLink PCR Purification kit (Invitrogen, CA, USA) was used to purify PCR products. The purified samples were diluted, denatured, neutralized, and pooled with other libraries according to next-generation sequencing guidelines of Illumina MiSeq (Illumina, CA, USA) at Genotypic Technologies, Bangalore, India (<https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/hiseq-3000-4000-specification-sheet-770-2014-057.pdf>). The automated Perl code was used to remove raw reads with adapter sequences. For analysis, reads with more than 70% bases having a high-quality and Phred score of >20 were used in the Illumina BaseSpace 16S Metagenomics App and Greengenes (v 13.5) database. The QIIME python script was used for calculating alpha diversity (alpha\_diversity.py). Hierarchical clustering and dendrogram were performed using the Illumina 16s App and heat map using R scripts. A Venn diagram was constructed to identify the shared and unique species in the coastal and offshore water samples.

## **2.5 Statistical analysis**

For analysing the comparative complexity of communities, different statistical tools such as Goods coverage (QIIME, alpha\_diversity.py), richness and diversity statistics (DOTUR, abundance-based coverage estimator ACE (SACE), bias-corrected Chao1 (Schao1) and the Shannon-Weaver and Simpson diversity index were used. The web-based analytical pipeline, METAGENassist were used for comparative metagenomic studies ([http://www.metagenassist.ca/METAGEN\\_assist/faces/Home.jsp](http://www.metagenassist.ca/METAGEN_assist/faces/Home.jsp)).

## **2.6 Nucleotide sequence accession number**

Data of the samples is available on the Sequence Read Archive of NCBI (<http://www.ncbi.nlm.nih.gov/sra/>) project reference PRJNA543554.

### 3. Results and Discussion

#### 3.1 Environmental parameters

The physico-chemical characteristics of the coastal and offshore waters are provided in Table 1. The water temperatures in the coastal and offshore surface waters were 32°C and 28°C, respectively. The salinity in the coastal water was low (30.24 psu) when compared to the offshore water (35.64 psu). Similarly, the pH of the coastal water sample was low (7.2) compared to offshore (8.32). But, the dissolved oxygen (DO) was higher in the offshore waters (183.30 µM) compared to coastal waters (134.30 µM). The dissolved inorganic nutrients such as NO<sub>3</sub>, PO<sub>4</sub>, and SiO<sub>4</sub> were higher in the coastal waters, whereas, NO<sub>2</sub> was higher in the offshore waters. (**Table 1**).

#### 3.2 Prokaryotic Abundance (PA)

The prokaryotic abundance was higher in coastal waters ( $2.18 \times 10^6$  Cells/mL) compared to offshore waters ( $1.13 \times 10^6$  Cells/mL) (**Table 1**). There are several reports suggesting high prokaryotic abundance in coastal waters (Parkes et al., 1994; Ramaiah et al., 1996) compared to the open ocean. This trend is more or less similar in all geographical regions of the oceans (Baltar et al., 2007).

#### 3.3 Taxonomic richness and diversity of the major prokaryotic communities

The valid reads were higher in offshore waters (0.27 million), compared to coastal waters (0.19 million) with a high Good's average value (0.99) (**Table 2**). The number of species identified was higher in the offshore waters (1059 species) with a higher species coverage ( $\geq 98.8\%$ ) compared to coastal waters (719 species) with a species coverage of  $\geq 97\%$ . However, based on Shannon diversity, evenness, and abundance of the species were high in the coastal waters compared to offshore waters (**Table 2**). The higher taxonomic diversity was observed in offshore water with more than 28 phyla and >100 classes of bacterial communities. Comparative sequence analysis showed that 28 major species (**Figure 2a**) and 22 minor species were shared between the samples (**Figure 2b**). Among the total species, 45 major species were unique to the coastal waters, while 366 species were unique to offshore waters. However, a vast difference was noticed in minor species, with 70 and 1070 unique species in the coastal and offshore waters, respectively. This indicated that minor species brought about more variations in diversity among coastal and offshore regions.

The relative abundances of different phyla in the two locations are shown in **Figure 3**. Total bacterial diversity was distributed among 35 different phyla. The five most dominant phyla, Proteobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Actinobacteria represented >95% of the total sequences in both the samples (**Figure 3**). In coastal waters, Proteobacteria was

the major phyla (67.15%), followed by Bacteroidetes (16.43%), Cyanobacteria (13.57%), Actinobacteria (1.03%), and Planctomycetes (1.01%). Similarly, in offshore waters, Proteobacteria (59.13%) was the major phyla followed by Cyanobacteria (13.75%), Firmicutes (12.47%), Actinobacteria (4.22%), and Bacteroidetes (3.55%). Proteobacteria contributed to >50% of the microbial biomass in the aquatic environment and thus are considered as a major phylogenetic lineage especially in surface waters (Bowman and McCuaig, 2003). Though similar taxonomic groups were identified, their alpha diversity values, relative abundances, and community composition varied between coastal and offshore waters. This is probably due to distinct physico-chemical characteristics in offshore and the coastal environments. In the coastal environment, riverine influx and high nutrient concentrations sustain a high primary production resulting in high bacterial abundance and diversity. The factors such as pH, salinity, temperature, and chemical composition, and their variation influence the bacterial diversity and distribution (Bendia et al., 2018). The high predominance of phylum Proteobacteria was reported from coastal and offshore waters (Cottrell Kirchman, 2000; Pommier et al., 2008). This observation is in concurrence with reports from Ushuaia, Argentina, Sub-Antarctica seawaters (Prabakaran et al., 2007), Southern oceans (Murray and Grzymiski, 2007) Antarctic, and sub-Antarctic seawaters (Bano and Hollibaugh, 2002). The abundance and distribution of bacterial communities vary according to the biogeochemistry of a particular environment (Edward et al., 2006). Microbes are involved in major biogeochemical cycles of carbon, nitrogen, and sulphur, leading to CO<sub>2</sub> fixation and respiration, nitrification, denitrification, N<sub>2</sub> fixation, sulphur assimilation, anaerobic sulphate respiration, and sulphide oxidation (Llorens-Marès et al., 2015). Phylum Proteobacteria constitute a population of highly active cells in the marine ecosystem that actively took up organic matter.

The bacterial structure in Proteobacteria was further analysed. In coastal waters, Alphaproteobacteria (61.75%) were the major class in Proteobacteria, followed by Gammaproteobacteria (38.18%) and Deltaproteobacteria (0.03%). On the contrary, Gammaproteobacteria (35.12%) was the most dominant class followed by Alphaproteobacteria (5.47%), Deltaproteobacteria (3.55%), Betaproteobacteria (1.2%) and Epsilonproteobacteria (0.42%) in the offshore waters. In general, Alphaproteobacteria and Gammaproteobacteria were the dominant classes in the majority of the ocean waters reported (Crump et al., 1999). Alphaproteobacteria are dominant in low saline waters with low nutrient concentrations. They are capable of degrading different organic compounds (Salka et al., 2014). On the contrary, Gammaproteobacteria are more abundant in oceanic environments or saline lakes than in freshwater environments (Wu et al., 2009). Dominance of Gammaproteobacteria in the Bay of Bengal was

comparable with diversity in the Mediterranean Sea (Polymenakou et al., 2006) and Pacific Ocean (Kouridaki et al., 2010). These studies suggest that different aquatic environments with varying environmental characteristics support different bacterial communities and diversity (Hamdan et al., 2013). Gammaproteobacteria is the most dominant (5-25%) bacteria in the polar waters and other marine environments (Wietz et al., 2010; Ghiglione et al., 2012). In the Mediterranean Sea, Gorgonians associated benthic microbial communities reveal the dominance of Alphaproteobacteria and Gammaproteobacteria (van de Water et al., 2017). The Alpha and Gammaproteobacteria are actively involved in carbon fixation, sulfur oxidation, and ammonia oxidation (Gilbert et al., 2012). Bacteroidetes are abundant phyla in the marine ecosystem including chemoorganotrophic bacteria and are major utilizers of complex polymers such as cellulose, chitin, and pectin in the aquatic environment, especially in eutrophic coastal waters (Lydell et al., 2004).

### **3.4 The diversity of the minor prokaryotic communities**

Diversity of *Fusobacteria*, *Synergistetes*, *Fibrobacteres*, *Spirochaetes*, *Gemmatimonadetes*, *Chlorobi*, *Chlamydiae*, *Armatimonadetes*, etc were the minor phyla (6.84%) present in offshore waters (**Figure 3**). These minor species have been reported from soil samples from Hangzhou, Zhejiang Province in China (Fu et al., 2014), Taihu Lake area, China (Wei et al., 2017), and Sossego copper mine, Brazil (Pereira et al., 2014). Globally abundant clades SAR406, TM7, OD1, SBR1093, and WS3 are present in coastal waters only (**Figure 3**). In this study, several unique bacterial assemblages have been identified, the majority of them deeply branched off within the bacterial hierarchy and some minor percentages were unbiased with available phyla of bacteria. These minor clades have been reported from Central Baltic Sea (Brettar et al., 2012), Continental shelf off central Chile (Aldunate et al., 2018), Southern Ocean waters (Milici et al., 2017), Lake Pavin-France (Borrel et al., 2010), Sakinaw Lake (Youssef et al., 2014), and East Pacific Rise (Santelli et al., 2009).

This study indicates that abundant phylum present in both environments was similar but minor phylum was distinctly dissimilar in both the waters. Several reports state that the major bacterial biomass in an environment is contributed by abundant taxa, but biodiversity is contributed by minor communities. This minor community contributes to the biodiversity in every ecosystem (Pedros-Alio, 2012). The bacterial assemblages have different growth and metabolic requirements, and a majority of them depend on the environmental parameters to be sustaining in a particular environment. Thus, the community composition and diversity of the microbes vary from one environment to another. Perhaps, the least abundant communities might be the most active and they play a crucial role in fluxes of dissolved nutrients (Cottrell and Kirchman, 2003). The minor bacterial phylum in the community has



unique distribution with low predation or low viral disintegration which in turn influence the stochastic immigration (Pedrós-Alió, 2006). Within the environment, the abundant community along with the minor community plays a significant role which in turn influences the functioning of the aquatic ecosystem.

### 3.5 Species diversity in coastal and offshore waters

The number of unclassified species were higher in coastal waters (71.82%) compared to offshore waters (43.28%). In the coastal waters, the most abundant species were *Synechococcus* spp. (12.85%) followed by *Erythrobacter nanhaisediminis* (12.78%), *Psychrobacter pacificensis* (5.30%), *Leeuwenhoekiella marinoflava* (4.21%), and *Nonlabens tegetincola* (4.01%) (**Figure 4**). Interestingly, these species were not present in offshore waters. The heat map analysis of coastal and offshore waters showed a distinct variation in the abundance of species (Supplementary figure 1). The *Synechococcus* spp were reported to be abundant in coastal waters and various estuarine ecosystems such as the Chesapeake Bay (Affronti and Marshall, 1993) Florida Bay (Phlips et al., 1999), coastal waters of Arabian Sea (Tarran, 1999), and coastal waters of Hong Kong (Xia et al., 2015). In coastal waters of the South China Sea (Xu et al., 2010), West Pacific Ocean and Taiwan Strait-China (Lu et al., 2018), *Erythrobacter* species belonging to the Alphaproteobacteria are frequently detected.

*Prochlorococcus marinus* (6.76%), *Vibrio owensii* (5.82%), *Vibrio rotiferianus* (5.78%), *Peptoniphilus coxii* (4.25%), ~~ete~~ were the abundant species in the offshore waters. (**Figure 4**). The *Prochlorococcus* are reported to be abundant in many oceanic environments such as North Atlantic (Li 2009), Tropical Pacific (Campbell, 1997), Tropical Atlantic (Partensky et al., 1999), Central and southern shelf waters of the Great Barrier (Crosbie and Furnas, 2001), and Bermuda Atlantic in the Sargasso Sea (DuRand et al., 2001). The high abundance of *Prochlorococcus marinus* has been reported from the Red Sea, Arabian Sea, Atlantic Ocean, Mediterranean Sea, and the Bay of Bengal (Reckermann and Veldhuis, 1997; West et al., 2001; Fuller et al., 2005; Johnson et al., 2006; Jyothibabu et al., 2013). There were reports of high *Prochlorococcus* abundances in deep -sea waters and *Synechococcus* abundances in coastal waters in the tropical central Mexican Pacific (Santana-Vega et al., 2018), East China Sea (Jiao et al., 2005), Gulf of Mexico (Linacre et al., 2015), the Gulf of California (Hernández-Becerril and Pastén-Miranda, 2015), and the South Atlantic (Brazil) (Bergo et al., 2017). The distribution and diversity of *Synechococcus* and *Prochlorococcus* were dependent on several environmental factors such as light, water temperature, nutrient levels, salinity, and biological parameters such as zooplankton grazing, the sinking of the particulate organic matter and sloppy feeding of the zooplankton. In a particular ecosystem, the microbial components vary both horizontally and vertically depending on the environmental parameters (Hirose et al., 2007). The

*Vibrio* spp were dominant in the offshore waters. They are ubiquitous in marine environments (Takemura et al., 2014) as free-living or in association with other aquatic organisms (Heidelberg et al., 2002) and found in warm waters (Vezzulli et al., 2017).

### **3.6 Prediction of metabolic activity of bacterial communities**

Based on the analysis using METAGENassist, the metabolic and functional properties of microbial components in the coastal and offshore waters displayed significantly different ecological functions (**Figure 5**). The major functional groups of microbes involved were those performing sulfate reduction, dehalogenation, nitrite reduction, ammonia oxidation, nitrogen fixation, xylan degradation, atrazine metabolism, chitin degradation, pollutant degradation, hydrogen production, carbon dioxide fixation, saccharolytic, methanogen, oxalic acid degradation, hydrocarbon degradation, and iron oxidation. Bacteria involved in hydrocarbon degradation, oxalic acid degradation, thiosulfate reduction, gramicidin production, syntrophic, saccharolytic, nocardicin A production, methane oxidation, and trichloroethylene (TCE) degradation, are prominent only in coastal waters compared to offshore waters. In both environments, the major activity was sulfate reduction and dehalogenation. In offshore waters, the concentration of sulfate is comparatively higher (28 mM at an ocean salinity of 35 psu). The sulfate-reducing microorganisms (SRM) use sulfate as an electron acceptor for their respiration (Jørgensen et al., 2019). The major activities of the bacterial communities in both environments were distinctly dissimilar. This variation in the functions could be attributed to significant differences in community structure and diversity and also due to distinctly different physicochemical conditions (Narayan et al., 2008). Hence, the results highlight the functioning of bacterial communities in the coastal and offshore waters in the BoB region.

### **Conclusion**

In conclusion, this study presents a detailed investigation of bacterial diversity and community structures in the coastal and offshore waters of the Bay of Bengal. The major phylum in our study was dominated by Proteobacteria both in coastal and offshore waters, whereas the distribution of minor phyla was different between them. Bacteria belonging to the genera *Synechococcus*, *Erythrobacter*, *Psychrobacter*, *Leeuwenhoekiella*, and *Nonlabens* were prominent only in coastal waters, whereas bacteria belonging to the genera *Prochlorococcus*, *Vibrio* were predominant only in offshore waters. The major functional characteristics of microbes involved were TCE degradation, sulfate reduction, pollutant degradation, hydrogen production, carbon dioxide fixation, saccharolytic, hydrocarbon degradation, xylan degradation, and iron oxidation. This preliminary data gives an opportunity to understand the bacterial diversity in two contrasting environments in the Bay of Bengal region.

## **Acknowledgements**

The authors are grateful to the Director, NIO, Goa and, Scientist- in- charge, NIO (RC), Cochin for their support and advice. JV is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, for financial support for senior research fellowship grant and NVK grateful to SERB-India. This is NIO contribution number XXXX.

## **Funding**

This study forms a part of Eastern Offshore Environmental Monitoring Program of Oil and Natural Gas Corporation Ltd., (ONGC).

## **Ethics Statement**

This study does not involve the use of human participants, specimens or tissue samples, or vertebrate animals, embryos or tissues.

## **Data Availability**

All data are fully available without restriction

## **Authors Contributions**

AP, NVK and VJ, have collected the samples, performed sample analysis, DNA extraction, PCR, data analysis and prepared the manuscript.

## **Conflict of Interest**

Authors declare no conflict of interest.

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## Legends

**Figure: 1.** Rarefaction analysis of the 16S rRNA gene sequences from the bacterial communities in the coastal and offshore water samples. The analysis was performed using the free online analytic rarefaction 1.3 software (Analytic Rarefaction 1.3. [www.uga.edu/~strata/software/](http://www.uga.edu/~strata/software/)).

**Figure 2** Venn diagram based on the percentages of shared OTUs among the coastal and offshore water samples.

**Figure 3 a)** The taxonomic classification of the major 16S rRNA gene sequences from the bacterial communities in the coastal and offshore water samples at the Phylum level. Each colour represents the percentage of the phylum in the total effective tags in the samples **b)** representing the taxonomic classification of the minor communities in the coastal and offshore water samples at the Phylum level.

**Figure 4 a)** The taxonomic classification of the major 16S rRNA gene sequences from the bacterial communities in the coastal and offshore water samples at species level. Each colour represents the percentage of the phylum in the total effective tags in the samples **b)** representing the taxonomic classification of the minor communities in the coastal and offshore water samples at species level.

**Figure 5** The prominent metabolic predictions of the bacterial communities in the coastal and offshore water samples

**Table 1. The mean value of biological and physicochemical parameters.** PA- prokaryotic abundance, Chl *a* -Chlorophyll *a*, Temp- Temperature, Sal-Salinity, DO - Dissolved Oxygen, NO<sub>2</sub><sup>-</sup> - Nitrite, NO<sub>3</sub><sup>-</sup> - Nitrate, NH<sub>4</sub><sup>-</sup>-Ammonia, PO<sub>4</sub><sup>-</sup> - Phosphate, and SiO<sub>4</sub><sup>-</sup> - Silicate. One-way Analysis of Variance (ANOVA) was performed to see if there is significant variation in physico-chemical parameters between coastal and offshore environments. \*Indicates significant values at \*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001 by using SPSS software (Version 23)

**Table 2. Summary of the operational taxonomic units (OTUs) of coastal (SD1) and offshore water samples (SD2).** The number of raw and processed reads using Illumina Miseq sequencing is indicated for all the samples. The OTU diversity and richness estimates of water samples are represented using Goods coverage and Shannon.

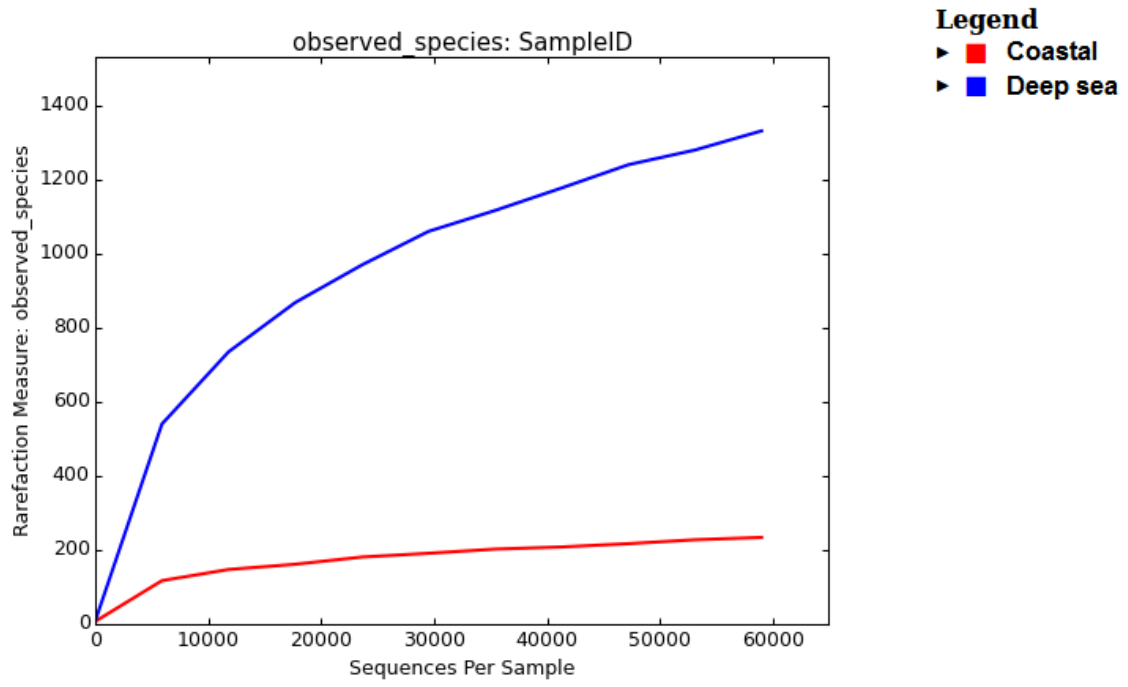
**Supplementary Figure: 1** Generalized heat map with dendrogram representing the 16S rRNA gene sequences from the coastal and offshore water samples. The colour key represents centered and scaled counts of each taxon.

**Table 1**

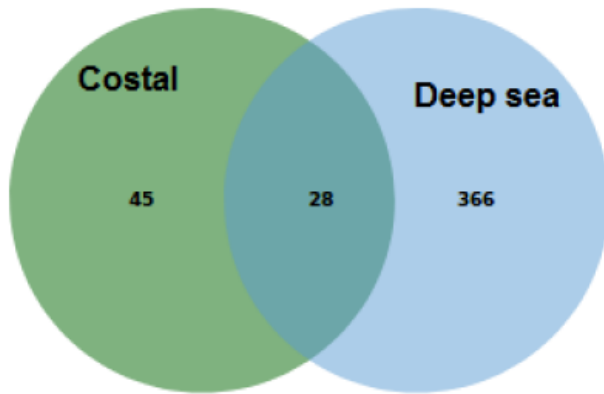
Parameters	Coastal (SD1)	Offshore (SD2)
Latitude	16.5683 N	13.6281 N
Longitude	82.3341 E	83.87963E
Depth (m)	~50 meter	~3274.5 meter
PA( $10^6$ Cells/mL) <sup>***</sup>	2.18	1.13
Chl <i>a</i> (mg/m <sup>3</sup> ) <sup>***</sup>	2.77	0.12
Salinity (psu) <sup>**</sup>	30.24	35.64
Temp (°C) <sup>**</sup>	32	28
pH <sup>***</sup>	8.32	7.2
DO ( $\mu$ M) <sup>***</sup>	134.30	183.30
NO <sub>2</sub> <sup>-</sup> ( $\mu$ M)	0.01	0.03
NO <sub>3</sub> <sup>-</sup> ( $\mu$ M) <sup>***</sup>	5.54	0.11
PO <sub>4</sub> <sup>-</sup> ( $\mu$ M) <sup>**</sup>	0.13	0.08
SiO <sub>4</sub> <sup>-</sup> ( $\mu$ M) <sup>***</sup>	2.445	1.9

**Table 2**

	Coastal (SD1)	Offshore (SD2)
Total raw reads	0.19 million	0.27 million
processed reads	173111	257,154
<b>OTU Richness and diversity</b>		
No. of species identified	719	1059
Shannon diversity index	1.941	1.312
Goods coverage	0.9969	0.9967



**Figure :1**



b) Rare Species

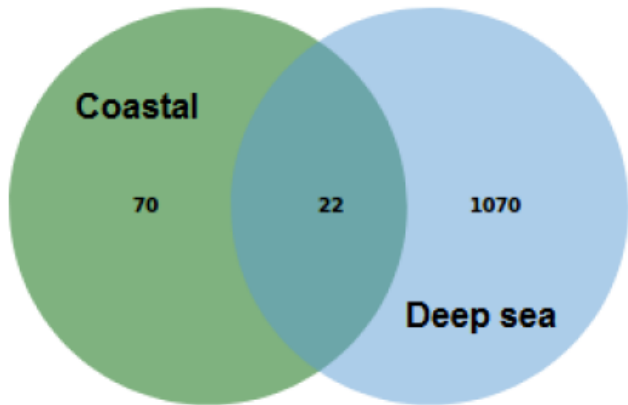


Figure: 2

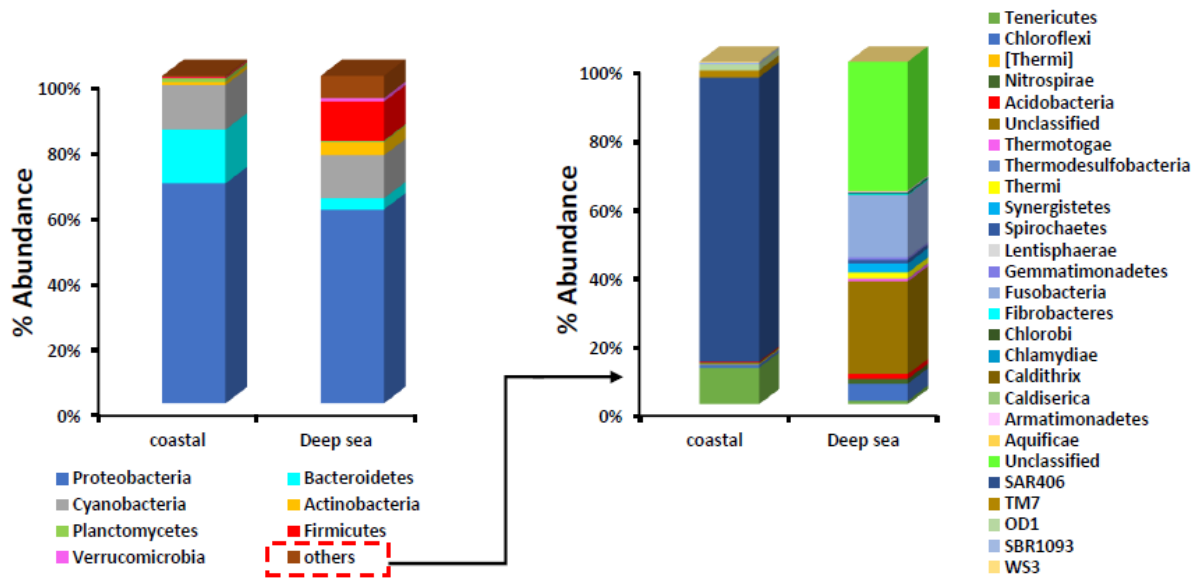


Figure 3



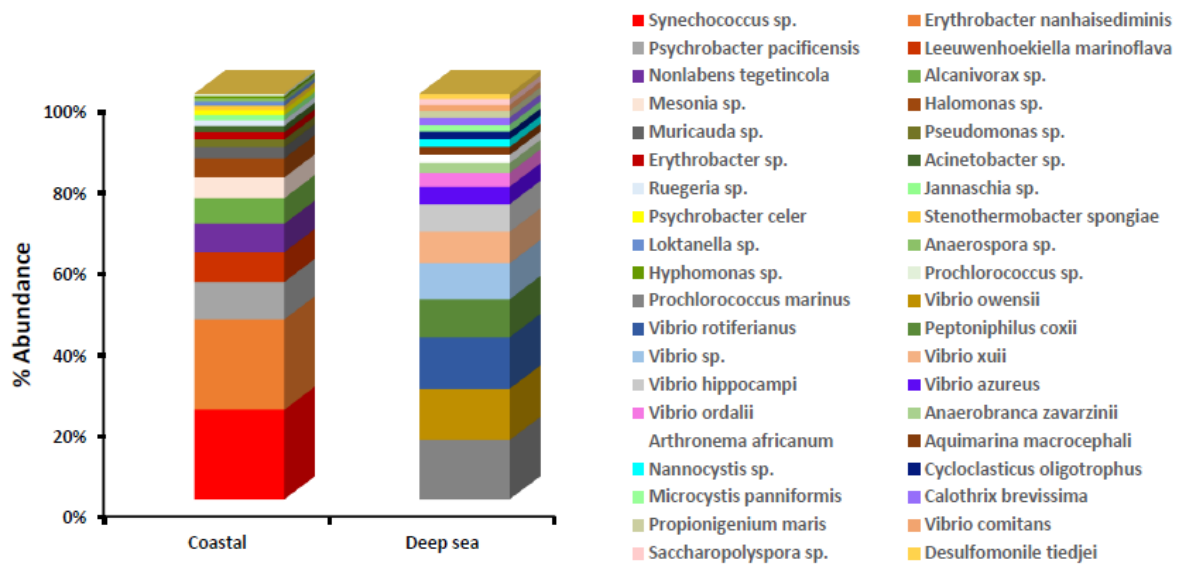


Figure 4

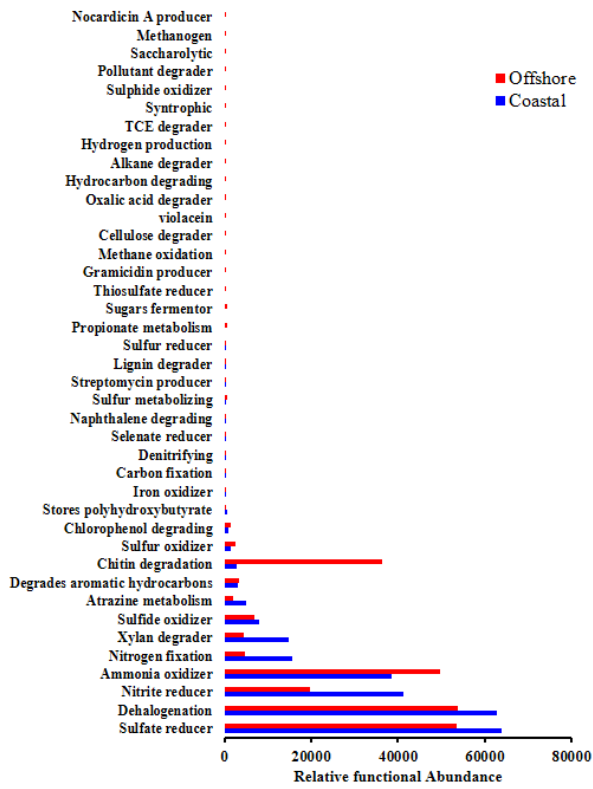
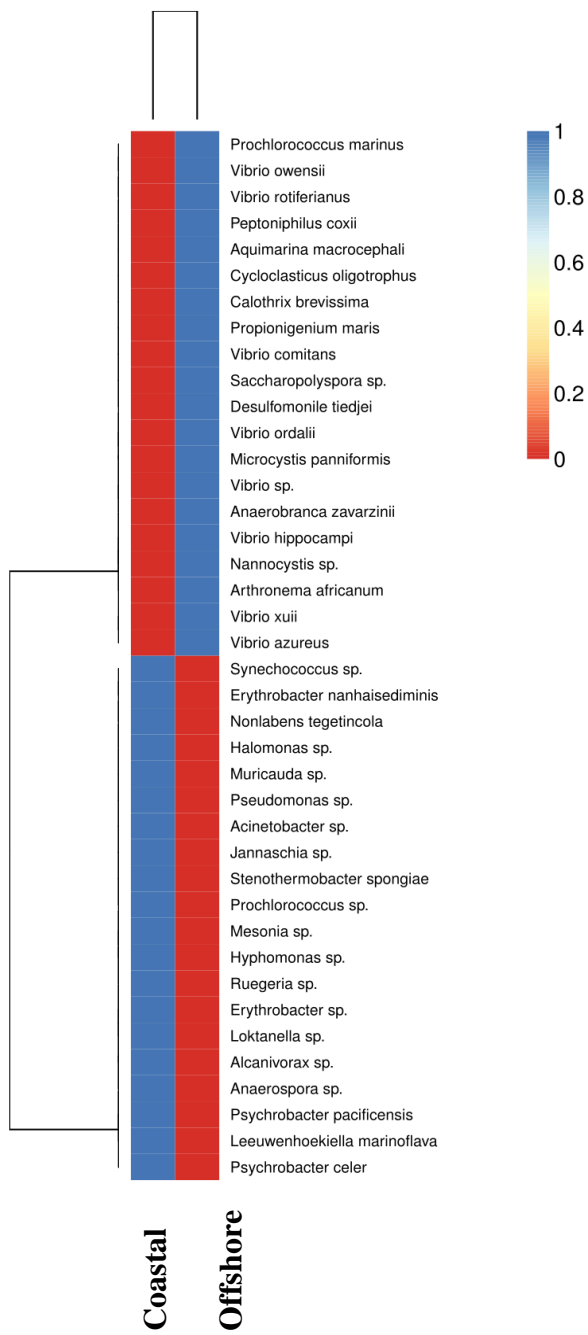


Figure 5



**Supplementary Figure 1**