

Dominance of *Wolbachia* sp. in the deep-sea sediment bacterial metataxonomic sequencing analysis in the Bay of Bengal, Indian Ocean

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Abstract

The Bay of Bengal, located in the north-eastern part of the Indian Ocean is world's largest bay occupying an area of ~8,39,000 square miles. The variability in bacterial community structure and function in sediment ecosystems of the Bay of Bengal is examined by Illumina high-throughput metagenomic sequencing. Of five metataxonomics data sets presented, two (SD1 and SD2) were from stations close to the shore and three (SD4, SD5, and SD6) were from the deep-sea (~3000 m depth). Phylum Proteobacteria (90.27 to 92.52%) dominated the deep-sea samples, whereas phylum Firmicutes (65.35 to 90.98 %) dominated the coastal samples. Comparative analysis showed that coastal and deep-sea sediments showed distinct microbial communities. *Wolbachia* species, belonging to class Alphaproteobacteria was the most dominant species in the deep-sea sediments. The gene functions of bacterial communities were predicted for deep-sea and coastal sediment ecosystems. The results indicated that deep-sea sediment bacterial communities were involved in metabolic activities like dehalogenation and sulphide oxidation.

Keywords: 16S rDNA, microbial diversity, *Wolbachia*, Deep sea sediment, Bay of Bengal, metataxonomics

1. Introduction

Marine sediments harbour an extraordinary range of diverse and complex microbial communities [1]. Marine microbial communities are known to play pertinent roles in the structure and dynamics of trophic food webs, global biogeochemical cycles and in the remineralization of organic matter [2, 3]. The microbial processes occurring along the seafloor are essential in sustaining primary and secondary production in the water column [4]. Multidisciplinary efforts across the globe are undertaken to characterize the microbial communities in marine sediments [5, 6]. However, the deep-sea sediment environments form one of the largest and most understudied ecosystems on earth [7]. This shortcoming is mainly due to the incapability of traditional culture techniques (the great plate anomaly) to recover the marine microbial diversity [8]. However, recent developments in molecular fingerprinting techniques have been instrumental in providing new insights into the bacterial diversity and their response to environmental heterogeneity.

The Bay of Bengal, the largest bay in the world, is about 2090 km long and 1610 km wide, bordered by Myanmar and Thailand on the east, by Sri Lanka and India on the west, and by Bangladesh on the north. This region is strongly influenced by the southwest monsoon (June–September) driven river discharge from the major rivers, Godavari, Krishna, Ganga, Brahmaputra, and Mahanadi [9, 10]. This unique semi-enclosed basin experiences seasonally reversing monsoons, depressions, severe cyclonic storms and receives a large amount of rainfall and river run-off resulting in strong stratification [11, 12]. Though microbial communities are known to play pivotal roles in the decomposition of organic matter and regeneration of nutrients [13], information on microbial populations inhabiting the coastal and deep-sea sediments in the Bay of Bengal are limited. To the best of our knowledge, there is only a single report on the diversity of bacteria from coastal environments of the Bay of Bengal and a volcanic Barren Island in Andamans based on next-generation sequencing [14]. However, the microbial ecology in the deep-sea environments of this region remains one of the most poorly studied environments. We hypothesize that the microbial assemblages in the coastal and deep-sea sediments and the metabolic pathways in which they are involved in would be distinct due to their different trophic conditions. In order to elucidate the bacterial diversity and to understand how bacterial communities are distributed in different oceanic sediments, we studied and compared the microbial diversity in the deep-sea and coastal sediments in the Krishna-Godavari (KG) basin of Bay of Bengal, Indian Ocean, using the high-throughput sequencing technology. This study provides a comparative insight into the microbial diversity within coastal and deep-sea sediments of the Indian Ocean.

2. Materials and methods

2.1 Site description and sampling

Sediment samples were collected from five locations in the KG basin (Figure 1). Two stations (SD1 and SD2) were located in the coastal region with an overlying water column depth of 50 m and 80 m respectively, whereas the other three sites (SD4, SD5, and SD6) were located in the deep sea with ~3000 m water column depth above it. Samples were collected using a Van Veen grab (0.2 m²) and spade corer (30 cm × 15 cm × 50 cm) from coastal and deep-sea stations respectively, during spring inter monsoon (April 2014). The geographic locations and depth of the stations are indicated in Table 1. The sediment samples (top 2 cm from the corer and grab) were collected using sterile spatula and stored immediately at -20°C for metataxonomics analysis. Sediments subsamples were collected from four corners of the core to reduce the variability at deep sea locations as suggested by Darnley et al. [15] and triplicate grabs for coastal locations.

2.2 Environmental parameters

In situ measurements of the physicochemical parameters of seawater (temperature, dissolved oxygen, depth and salinity) were measured using a Conductivity Temperature Depth (CTD) profiler. The texture of the sediments was determined by measuring the percentage of sand, silt and clay content using conventional sedimentation techniques in the laboratory. Sediment total carbon, total nitrogen and total phosphorus were determined on dried sediment using an elemental analyser (Flash 2000 CHN elemental analyser, Thermo Fisher Scientific, USA) [16].

2.3 DNA isolation and amplicon library construction

Upon reaching the laboratory, sediment DNA extraction was performed using Ultraclean Soil DNA Kit, (MoBio lab, Geneworks, Australia). DNA was simultaneously extracted from 0.5 g of sediment subsamples from each location using bead beating and column purification according to the manufacturer's instructions. DNA extracted from the subsamples/triplicates were pooled and were considered as a single sample representing a particular location. DNA was stored at -80°C until further downstream processing. The sample DNA was quantified using a Nanodrop (Thermo Fisher Scientific, USA), and the average concentration was 25 ng/μl was used for PCR reaction. Library construction involved two PCR reactions. The possible lab and other contamination were checked by running the negative PCR reaction. The first PCR amplified V3-V4 region of 16S region with specific primers (16S Forward Primer TCGTCGGCAGCG TCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Reverse Primer GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAAT CC) [17]

containing a 'tag' sequence that is complementary to Illumina sequence adapter and index primers from the Nextera XT Index kit V2 with an initial DNA concentration of 25 ng/μl. The library was validated for quality by running an aliquot (1:10 Dilution) on High Sensitivity Bioanalyzer Chip (Agilent). https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

2.4 Illumina sequencing

PCR products were purified using PureLink PCR Purification kit (Invitrogen, CA, USA). The purified, pooled sample was diluted to 4 nM final concentration using resuspension buffer (Illumina, CA, USA). The sample was denatured for 5 minutes and neutralized using 0.2 N NaOH and HT1 Buffer, respectively (Illumina, CA, USA). It was then pooled with other libraries prepared for NGS in a ratio dependent on amplicon size/total panel size, desired sequencing depth, and the number of samples pooled in each sub-library. Pooled libraries were further diluted down to a final 15 pM library and spiked with 5% phiX (Illumina, CA, USA) as a control and diversity enhancer. Samples were then loaded into an Illumina MiSeq v3 cartridge (Illumina, CA, USA) and run in 2*250 mode on an Illumina MiSeq next generation sequencer (Illumina, CA, USA) at Genotypic Technology Pvt. Ltd (Bangalore, India) [18]. (<https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/hiseq-3000-4000-specification-sheet-770-2014-057.pdf>).

2.5 Initial processing of sequence reads

Demultiplexing was completed using bcl2fastq Conversion Software that was embedded in the MiSeq. The automated FASTQC (QIIME) Tool Kit application on Illumina BaseSpace Labs was used to filter out quality reads, in which quality reads above Q30 were kept for downstream analysis. Within the application, the TagCleaner software (0.16) was used to remove the adapter sequences. (https://www.illumina.com/documents/products/technotes/technote_Q-Scores.pdf)

2.6 Data analysis

The raw reads with adapter sequences and low-quality bases were removed using an automated Perl code. The processed high-quality reads with more than 70% bases having Phred score greater than 20 were considered significant for further downstream analysis. The data was uploaded to Illumina BaseSpace 16S Metagenomics App. The dataset was analyzed using Greengene database v 13.5 at the backend. The Basespace 16s Metagenome App uses a modified version of Classify Reads 32-base words data structures instead of 8-base words which provided more specificity for each species [19]. The illumina curated version of the May 2013 release of the Greengenes Consortium database was used.

The alpha diversity was calculated using QIIME python script (alpha_diversity.py). Sample-wise pie-charts were generated based on the absolute count percentage with a % cut off of 0.5%. Heat map and stacked bar chart were generated at phylum level using the relative abundance values with a percentage cut off 0.1% using R scripts. We generated phylogenetic trees in Newick format for deep sea and coastal sediment samples based on abundance count values using R-Ape package and these trees were further visualized using Dendroscope v3 to generate the tanglegram images. PCoA was generated using classical MDS on a Pearson covariance distance matrix. Hierarchical clustering dendrogram was generated based on genus-level classifications using the Illumina 16s App. A tanglegram images was constructed that identified the common and unique species between the coastal and deep sea samples by interlinking the two dendrograms. The distance and clusters were generated based on the height calculated during hierarchical clustering using complete agglomeration method, where height gives the distance between the two sets that are being agglomerated (hierarchical clustering algorithm, hclust, R “ape” package). The read classification was performed based on ClassifyReads (RDP classifier algorithm) using a 32-base words data structure in conjunction with Greengenes database [19].

2.7 Statistical analysis

Goods coverage, an alpha diversity metric, was calculated from the biome file (QIIME, alpha_diversity.py). Coverage indices provide information on what fraction of OTUs has been picked up more than once. Richness and diversity statistics were calculated using DOTUR, including the abundance-based coverage estimator ACE (S_{ACE}) [20] the bias-corrected Chao1 (S_{chao1}) [21] and the Shannon-Weaver and Simpson diversity index. These diversity indexes and richness estimators are useful statistical tools for comparing the relative complexity of communities and for estimating the completeness of sampling (calculated at a genetic distance level of 0.03).

Similarity analysis was conducted using Principal coordinate analysis (PCoA) using Base Space (Illumina, USA) and unweighted pair group method with arithmetic averages (UPMA) clustering using the online *UNIFRAC* program (<http://bmf.colorado.edu/unifrac/index.psp>) [1]. These analyses are particularly suitable for sequence data analysis which measures the molecular evolutionary distances of the sequences and compare the evolutionary relationships among microbial communities in deep-sea and coastal sediments. Comparative metataxonomic studies was performed using a user friendly, web-based analytical pipeline, METAGENassist (<http://www.metagenassist.ca/METAGENassist/faces/Home.jsp>) [22].

2.8 Confirmation of *Wolbachia* sp. in nematodes

The nematodes were hand-picked and fixed using a cold mixture of formalin and glutaraldehyde for 24-48 hrs at 4°C. The samples were washed thrice with sodium cacodylate buffer, The samples were fixed with cold osmium tetroxide for 4-8 hrs, rinsed with buffer and dehydrated with serial ethanol treatments (30, 50, 70, 90, and 95%, and twice in 100% ethanol) (23). The dehydrated samples analysis were smeared on a small piece of adhesive carbon tape fixed on a brass stub for scanning electron microscopy (SEM, Model: Jeol, JSM 6390LA). The samples were subjected to gold coating using sputtering unit (model: JFC1600) for 10 second at 10 mA of current. The gold coated samples were placed inside the SEM chamber (Jeol, JSM 6390LA) and secondary electron/back scattered electron images were recorded. The elemental analysis carried out in the scanned area /point /line using EDAX detector (Model EDAX Make OXFORD XMX N) and observed for the presence of *Wolbachia*-like bacilli on the nematode surface [24]. Polymerase chain reaction was performed using 16S rDNA to test for the presence of *Wolbachia* in homogenized nematode tissues. The PCR amplification reactions were carried out in a thermocycler (Dyad[®] Dual-Bay, Biorad, Hong Kong) using the 16S rDNA primers specific for *Wolbachia* species, 16SWsp F (5'-CATACCTATTCGAAGGGATAG) and 16SWsp R (5'-AGCTTCGAGTGAAACCAATTC) yielding a PCR product of 438 bp [25].

2.9 Nucleotide sequence accession number

The Illumina sequencing data were deposited into the NCBI Sequence Read Archive (SRA, <https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP106765>) under the bioproject number, PRJNA383283, and SRA study number, SRP106765.

3. Results and Discussion

3.1 Environmental parameters and sediment characteristics

The physicochemical conditions in the coastal and deep-sea stations were significantly different. Water temperature of the overlying water ranged from 17 to 20°C in the coastal waters, whereas it was as low as 1.46 to 1.56°C the deep-sea stations (Table 1). The salinity of the overlying water ranged from 34.72 to 34.88 in coastal and deep-sea stations, respectively. The sediments were classified on the basis of texture after determining the sand, silt, and clay content using conventional sedimentation techniques. The sediment texture was generally dominated by silt (61.54–72.96%) (Table 1). The coastal sediments were composed of sandy-silt (sand 18.44–20.61%) with lowest organic carbon (0.49 – 0.87%). The deep-sea sediments were, in contrast, clayey-silt (clay 20.31 – 28.17%) with high organic carbon content (1.34 – 2.07 %). The sediment organic carbon (SOC)

content was similar to those reported for surface sediments off the Krishna-Godavari (KG) basin, east coast of India [15].

In general, the Bay of Bengal coastal sediments were poor in TC ($1.04\pm 0.30\%$), TN ($0.10 - 1.00$ mg/g) and TP ($0.21 - 0.34$ mg/g) concentrations compared to the deep-sea sediments [TN ($1.10 - 1.20$ mg/g), TP ($0.50 - 0.90$ mg/g) and TC ($1.40\pm 0.27\%$)]. This is in accordance with an earlier observation that terrestrial runoff draining through the vast agricultural field has a greater contribution of organic matter than the *in-situ* production in the coastal sediments [26]. However, there was an organic enrichment towards the deeper regions, probably due to a relatively faster settling of particles through a weak oxygen minimum layer without getting completely oxidised [27]. Likewise, a preferential removal of N-rich (protein) compounds from the shelf compared to that from the slope region, could be the reason for an organic enrichment in the deep-sea sediments [27]. The variations in the sediment organic carbon along the Washington margin [28] and in the Gulf of Mexico [29] were also due to the above process.

3.2 Taxonomic richness and diversity of the prokaryotic community

Sequence tags (based on a BLASTN search in RefHVR_V3 database) were clustered into groups by defining the variation from unique sequences to 10% differences. The number of processed reads were significantly higher in deep-sea sediments (2,57,154 in SD4, 1,053,569 in SD5, and 6,29,859 in SD6) when compared to coastal sediments (91,183 in SD1 and 94,968 in SD2) (Table 2). The number of species identified was also high in deep-sea sediments (949-1325 species) when compared to coastal sediments (514-776 species). The overall species coverage of the samples was $\geq 97\%$ for coastal sediments and 98% for deep-sea sediments. The Good's coverage value (0.99) indicated that most of the prokaryotic diversity was detected in the samples. However, Shannon and Simpson diversity indices which represent abundance and evenness of the species were higher for the coastal sediments when compared to deep-sea sediments (Table 2). This suggests that the bacterial community diversity in coastal sediments were richer than the deep-sea sediments.

Rarefaction analysis also indicated that the abundance in each bacterial community were significantly higher in coastal sediments than the deep-sea sediments. In general, bacterial diversity is low in stressed environments, such as regions with high hydrostatic pressure or hydrocarbon [30, 31]. The rarefaction curves showed the distribution of the OTUs in the samples and strongly indicated that the majority of the OTUs in the samples were detected and only a marginal increase in richness would result with additional sequencing (Supplementary figure 1). Principal coordinate analysis (PCoA) showed that the deep-sea sediments clustered together, but were separated from coastal sediments

which formed another cluster (Figure 2a). Similarly, UPGMA clustering results showed that the bacterial communities in the deep-sea sediments were quite similar to each other, whereas the coastal sediments formed another cluster (Figure 2b). Comparative sequence analysis showed shared OTUs between different sediment samples, of which, 256 sequences were shared by all the five samples (Figure 2c).

3.3 Taxonomic composition of the prokaryotic community

Bacteria were the most abundant prokaryotic domain, constituting 99.95%, whereas archaeal reads showed a relative paucity (0.05%). The phylum level phylogenetic classification of the prokaryotic sequences from the deep-sea sediments is shown in Figure 3. Abundance analysis showed that phylum Proteobacteria accounted for 90.27- 92.52% of the total amplicons. Other phyla included Firmicutes (2.87 ± 0.44 %), Cyanobacteria ($2.66 \pm 0.48\%$), Bacteroidetes ($1.74 \pm 0.24\%$), Actinobacteria ($0.93 \pm 0.15\%$), Chloroflexi ($0.24 \pm 0.03\%$), Planctomycetes ($0.054 \pm 0.02\%$), etc. Among Proteobacteria, Alphaproteobacteria (84.68-86.95%) was the most abundant class followed by Gammaproteobacteria ($4.06 \pm 0.11\%$) and Deltaproteobacteria ($1.14 \pm 0.05\%$). Betaproteobacteria was also reported but in lower numbers (Figure 4). Rickettsiales were the prominent order in the deep-sea sediments accounting for about 81.04-83.49% of the class Alphaproteobacteria.

Proteobacteria has been reported as most dominant community in many marine environments of the Arctic Ocean [32], North China Sea [33] and the benthic North Sea [34]. However, a recent study on bacterial diversity in deep-sea sediments in the Bay of Bengal reported dominance of Actinobacteria followed by Firmicutes [14]. The study was conducted at two stations on the southern side of the present study sites towards Chennai ($13^{\circ}14.192'N$ Lat and $80^{\circ}49.061'E$ Long, 2000 m depth) and Cuddalore ($11^{\circ}45.681'N$ Lat and $80^{\circ}03.718'E$ Long, 1400 m depth). Study sites covered by Verma et al. [14] were sandy clay with a higher TN (0.64-1.54 mg/kg) and TP (504.2- 651.8 mg/kg), which were located away from the riverine inputs. In the marine environments, water masses with different environmental properties could harbour distinct microbial communities [35, 36]. Similarly, sub seafloor sedimentary environments with different properties separated by a few kilometres also could have distinct communities [37-39].

The dominant communities in SD1 were Firmicutes (90.98%), followed by Proteobacteria (7.60%) and Actinobacteria (0.63%) contributing to 99.22% of the total phyla. In SD2, there were variations in the relative abundance of the dominant phyla with Firmicutes (65.35%), Proteobacteria (16.59%), Actinobacteria (2.80%) and Bacteroidetes (12.02%) contributing to 96.76% of the total phyla (Figure 3). Among Firmicutes, Bacillales were the dominant order (55.42% in SD1 and 78.38% in SD2),

followed by Clostridiales (2.82% in SD1 and 7.69% in SD2) and Sphingomonadales (3.17% in SD1 and 5.08% in SD2) (Figure 4). Firmicutes are reported to be high in near-shore polluted environment environments with relative high NH_4^+ -N concentrations [39]. Among Bacteroidetes, Flavobacteriia (90.27%) dominated SD2, followed by Sphingobacteriia (7.52%). Representatives of the phylum Bacteroidetes, such as class Sphingobacteria and Flavobacteria, are important in the degradation of biopolymers in the sedimentary organic matter [41 -44] (Supplementary figure 2). The high abundance of Firmicutes suggested that the coastal environment was highly polluted [41]. Several reports have pointed out on the pollution in the coastal regions due to considerable anthropogenic pressure from sewage and industrial waste [44-46]. The high abundance of Firmicutes in the coastal waters is in concurrence with reports from coastal areas of the East China Sea (ECS) [47], and the North Sea [48]. The Firmicutes reported from the Northern Adriatic Sea and the Ionian Sea were hydrocarbon-degrading species and have been already reported in similar environments worldwide [49, 50]. Among Proteobacteria (7.61% in SD1 and 16.59% in SD2), percentage occurrence of dominant classes varied with depth (50 m at SD1 and 80 m at SD2). Alphaproteobacteria were dominant at SD1 (78.44%), followed by Gammaproteobacteria (19.33%), whereas Gammaproteobacteria (54.60%) were dominant at SD2 followed by Alphaproteobacteria (37.11%) (Figure 4). Alphaproteobacteria has been reported to be dominant in coastal waters [51]. Gammaproteobacteria has been reported previously in the Eastern Mediterranean Sea [52] and Northeastern Pacific Ocean [53].

3.4 Distribution and abundance of bacterial species in coastal and deep-sea sediments

Bacterial species in the coastal and deep-sea sediments were distinctly different at the species level (Figure 5). *Bacillus* sp., belonging to phylum Firmicutes and class Bacilli, was the most dominant species (53.92-69.24%), followed by *Bacillus cohnii* (11.89%) at SD1, whereas *Alkaliphilus peptidifermentans*, belonging to phylum Firmicutes and class Clostridia, was the most dominant species at SD2 (Figure 5). Based on Shannon diversity index, higher species diversity and richness were observed at the coastal stations (SD1-1.8 and SD2-2.8) than at the deep-sea stations (SD4-1.3, SD5-1.3 and SD6-1.2). The Krishna and Godavari rivers supply the bulk of the organic matter (OM) to the basin sediments which probably supports high taxonomic and metabolic diversity [54].

Interestingly, *Wolbachia* sp. belonging to phylum Proteobacteria and class Alpha proteobacteria, was the most dominant species in the deep-sea sediments obtained in our study. *Wolbachia* sp. (unclassified at the species level) contributed to 60.04% in SD4, 59.57% in SD5 and 63.84% in SD6. *Wolbachia pipientis* contributed to 23.86 % in SD4, 23.56% in SD5 and 24.55% in SD6 (Figure 5). *Wolbachia* is reported to have evolved from an ancient clade of Alpha proteobacteria that comprises other obligatory intracellular organisms, such as *Rickettsia*, *Ehrlichia*, *Anaplasma*,

Midichloria [55]. This forms the first report on dominance of *Wolbachia* species in the deep-sea environments. They are widely distributed in many invertebrates including nematodes as parasites and are estimated to infect more than 65% of all insect species [56]. The dominance of *Wolbachia* species prompted us to look into the major benthos in the deep-sea sediment. In the present study, nematodes were the dominant benthos inhabiting the deep-sea sediments. Previous studies report on the presence of nematodes in the study region [57-60]. However, characterisation of bacteria-associated with nematodes from deep-sea environments is not done in the past. We have demonstrated the presence of *Wolbachia* like- bacilli under scanning electron microscope which suggest the possible association of *Wolbachia* sp. with nematodes (Figure 6). Further, 16S rDNA primers specific for *Wolbachia* species was used to amplify DNA from nematode tissue to amplify *Wolbachia* DNA. The PCR yielded a 438 bp product indicating the presence of *Wolbachia* DNA in nematodes (Supplementary figure 3). *Wolbachia* was present in large numbers in the deep-sea sediments, possibly present in a symbiotic relationship with the dominant benthos, nematodes. However, the reason for dominance of this species in the deep-sea sediment and its nature of association with nematode hosts could not be proved in the present study.

Wolbachia possibly confer certain advantages to their nematode host in the deep-sea environments. In addition to providing essential nutrients to the hosts, they manipulate the reproduction of their hosts to increase their own transmission, via mechanisms such as cytoplasmic incompatibility, male killing, thelytokous parthenogenesis and feminization of genetic males [61, 62]. Alternatively, *Wolbachia* is able to survive in extracellular environments for extended time periods with the ability to invade cells and establish stable infections [62]. This ability would enhance their chance to colonize cells from an extracellular environment. The ectosymbiosis between marine nematodes and cuticle-associated sulfur-oxidizing bacteria and nematode-bacteria endosymbioses are well-established [64, 65]. There are reports on the dominance of bacterial deposit and epistrate feeders in the nematode assemblages of the deep-sea in the North Atlantic and Mediterranean Sea [66]. Symbiotic relationship between sediment agglutinating nematodes and bacteria for ‘enzyme sharing’ in detrital habitats have been reported [67]. Bacterivory might be a feeding strategy of nematodes in deep sea sediments [68].

3.5 Ecological roles of bacterial populations

The prediction of metabolic and functional roles of bacterial populations studied using METAGENassist (<http://www.metagenassist.ca/METAGENassist/faces/Home.jsp>) [69] provided accurate metabolic insights from the metagenomic sequences. The results indicated that bacterial populations in coastal and deep-sea environments were involved in various ecological activities

(Figure 7). Coastal sediment microbes were mainly involved in xylan degradation, chitin degradation, and streptomycin production apart from nitrite reduction, sulphate reduction, and ammonia oxidation. Chitin is one of the frequently occurring biopolymers in the shells of crustaceans in the sea. The fate of chitinous materials through microbial degradation is of great importance in the recycling of nutrients. The significance of chitin as a critical link between the carbon and nitrogen cycles has led to a revived interest in the quantitative importance of chitin turnover in marine systems [70]. Xylan degradation activity was a dominant activity in coastal sediments probably due to deposition of plant and animal debris from the surface waters. Consequently, these polysaccharide-degrading bacteria are copious and demonstrate important roles in the carbon cycle in these environments. Ammonia-oxidizing Bacteria (AOB) oxidize ammonia to nitrite, and therefore play essential roles in nitrification and global nitrogen cycling [71]. Detection of these metabolic activities, and streptomycin production indicate the potential of these communities to produce valuable bioactive compounds which could pave way for potential bioprospecting studies in these environments.

In the present study, the dehalogenation activity was very high in deep-sea sediments dominated by *Wolbachia* sp. indicating that they might be involved in halo-respiration and play important ecological roles in the biogeochemical cycles of chlorine, iodine and bromine as well as in halogenated carbon substrates. Halogenated organic compounds buried in the marine sediments serve as terminal electron acceptors for anaerobic respiration in a diverse range of microorganisms *via* their key enzymes reductive dehalogenases (Rdh) and has been reported from the sediments of Pacific Ocean [72]. Dehalogenation is considered as an important energy-yielding pathway in the subseafloor microbial ecosystem, implying the role for dissimilatory dehalorespiration in deep-sea environments [72, 73]. Another important function of deep-sea bacterial communities was sulphide oxidation. Nematodes are found in high abundance in the presence of reduced chemical compounds (H_2S and hydrocarbonates) in hypoxic or even anoxia waters and deep cold seeps with concomitantly high abundance and metabolic activity of chemoautotrophic bacteria [74].

4. Conclusion

To conclude, this study augmented our understanding on the bacterial community composition in sediment samples from coastal and deep-sea environments in the KG basin of the Bay of Bengal. The results showed that the bacterial communities are complex and distinct in these two environments. Statistical analysis showed that the bacterial diversity and richness were higher in coastal sediments when compared to the deep-sea sediments. Firmicutes were the dominant phyla in the coastal environments, whereas Proteobacteria dominated the deep-sea sediments. *Bacillus* sp. were the most dominant species in the coastal environments, whereas the deep-sea environments were dominated by

symbiotic *Wolbachia* sp. This study forms the first report on the presence and dominance of *Wolbachia* sp. in deep-sea environments. The results indicated that the deep-sea bacterial populations were involved in metabolic activities such as dehalogenation and sulphide oxidation. However, detailed insights into the factors that favour the dominance of symbiotic forms in deep sea environments and their potential biological impacts are warranted. Our observations emphasize the need to understand the dynamics of *Wolbachia* in deep-sea sediments in this study area and their ecological roles.”

Abbreviations

CTD-conductivity temperature depth profiler, BoB- Bay of Bengal, KG basin- Krishna-Godavari basin, SOC - Sediment Organic Carbon, PCoA- Principal Coordinate Analysis, OTUs- Operational Taxonomic Units, UPMA-Unweighted pair group method with arithmetic averages, OM-organic matter, AOA-Ammonia-oxidizing Archaea and AOB-ammonia-oxidizing Bacteria, Total Phosphate (TP), total Nitrogen (TN),

Declarations

Ethics approval

No need of ethics approval, this work doesn't include research on identifiable human material and data.

Consent to publish

The manuscript has written consent from all the authors and from the institution

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declares that they have no competing interests.

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Author Contributions

Conceived and designed the experiments: PA, JV, AS, and AVK. Performed the experiments: JV, PA, AVK, NVK and AS. Analyzed the data: JV, PA, AS, AVK, JBR, NVK. Contributed reagents/materials/analysis tools: PA and JBR. Wrote the paper: PA, AS, JV, AVK, VKN and JBR.

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Figure Legends

Figure 1 Map indicating the five sampling locations (Red colour) in the Bay of Bengal (BoB) India. Two stations were located in the coastal region (SD1 and SD2 with an overlying water column depth of 50 m and 80 m respectively) and three were located in the deep-sea (SD4, SD5 and SD6, with ~3000 m water column depth above). The figure 1 (station map) was created with the tool QGIS (version 2.18, Quantum GIS Development Team, 2017. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. "<http://qgis.osgeo.org>"). The legends included in the map such as rivers (Made with Natural Earth. Free vector and raster map data @ naturalearthdata.com,) and the Indian sub-continent (<http://www.diva-gis.org/>) [75] were retrieved as shape files. The bathymetry data (ETOPO1: [doi:10.7289/V5C8276M](https://doi.org/10.7289/V5C8276M)) [76] was downloaded from APDRC live access server (<http://apdrc.soest.hawaii.edu>) and the contour lines were generated as shape files using the Ocean Data View software package (version 4.7.10) [77]. All the three layers were arranged in QGIS tool. The blue colour indicates the previous reports from BoB.

Figure 2 The relationships among the bacterial communities by (a) Principal component analysis of metataxonomics based on taxonomic affiliation of reads in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples. The percentages of variance explained in each axis are indicated. (b) Similarity index of station location are compared based on Bray Curtis by group average (c) Venn diagram based on the percentages of shared OTUs among the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples

Figure 3 Figure representing the taxonomic classification of the 16S rRNA gene sequences from the bacterial communities in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples at the Phylum level. Each colour represents the percentage of the phylum in the total effective tags in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples.

Figure 4 Figure representing the taxonomic classification of the 16S rRNA gene sequences from the bacterial communities in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples at the class level for a) Proteobacteria (b) Firmicutes (c) Bacteroidetes (d) Cyanobacteria (e) Actinobacteria (f) Archaea. Each colour represent the percentage of the class in the total effective tags of sample (tags in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples.

Figure 5 Figure representing the taxonomic classification of the 16S rRNA gene sequences from the bacterial communities in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples at the species level. The phylogenetic tree of the five sediment samples is represented.

Figure 6 Association of *Wobalchia* sp. with the deep-sea nematodes. a) bacteria adhering on the nematode surface b) enlarged view of bacilli shaped bacteria (*Wobalchia* sp.)

Figure 7 PICRUSt showing prominent metabolic predictions of the bacterial communities in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples.

Supplementary Figure 1 Rarefaction analysis of the 16S rRNA gene sequences from the bacterial communities in the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples. The analysis was performed using the free online analytic rarefaction 1.3 software (Holland 2003, Analytic Rarefaction 1.3. www.uga.edu/~strata/software/).

Supplementary Figure 2 Generalized heat map with dendrogram representing the 16S rRNA gene sequences from the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples. The color key represents centred and scaled counts of each taxon.

Supplementary Figure 3 Gel picture showing the presence of *Wobalchia* sp. in deep-sea nematode Lanes M: 100 bp molecular marker (Bangalore Genei, Bangalore, India), 1: DNA extracted from deep-sea sediment sample. 2: Negative control (sterile nuclease-free water), 3, 4 and 5: DNA samples from nematodes from deep-sea sediments.

Table 1: Geographic location and physicochemical characteristics of coastal and deep-sea sediment samples. Samples collected from two coastal stations are represented as SD1, and SD2 and those from three deep-sea stations are represented as SD4, SD5, and SD6. Total Phosphate (TP), total Nitrogen (TN), and organic carbon (OC) are represented in percentages.

Stations	Latitude (°N)	Longitude (° E)	Depth (m)	Sand %	Clay %	Silt %	TP mg/g	TN mg/g	TC mg/g
SD1	16°35'05.62''	82°25'18.5''	50	20.61	17.85	61.54	0.021	0.10	0.87
SD2	15°43'50.40''	81°07'52.0''	80	18.44	13.15	68.41	0.034	0.01	0.49
SD4	14°43'48.46''	84°04'39.0''	3171	7.17	28.17	64.66	0.062	0.11	1.34
SD5	13°37'41.14''	83°52'46.5''	3278	5.07	23.94	70.99	0.050	0.12	1.88
SD6	14°15'56.12''	82°07'26.0''	3325	6.73	20.31	72.96	0.090	0.12	2.07

Table 2: Summary of the operational taxonomic units (OTUs) of coastal (SD1, and SD2) and deep-sea sediment samples (SD4, SD5, and SD6). The number of raw and processed reads using Illumina Miseq sequencing is indicated for all the samples. The OTU diversity and richness estimates of sediment samples are represented using various indices, such as ACE, Chao1, Goods coverage, Simpson, and Shannon.

OTU reads	COASTAL SEDIMENTS		DEEP SEA SEDIMENTS		
No. of reads	SD1	SD2	SD4	SD5	SD6
Total raw reads	0.12 million	0.12 million	0.27 million	1.12 million	0.67 million
processed reads	91,183	94,968	257,154	1,053,569	629,859
OTU Richness and diversity					
No. of species identified	514	776	949	1325	1142
Shannon diversity index	1.841	2.894	1.312	1.303	1.243
ACE	1126.78	1552.34	1319.51	1343.36	1200.55
Chao 1	1090.89	1506.73	1335.87	1355.56	1238.58
Simpson	0.928	0.95	0.65	0.65	0.63
Goods coverage	0.9969	0.9961	0.9967	0.9966	0.9968

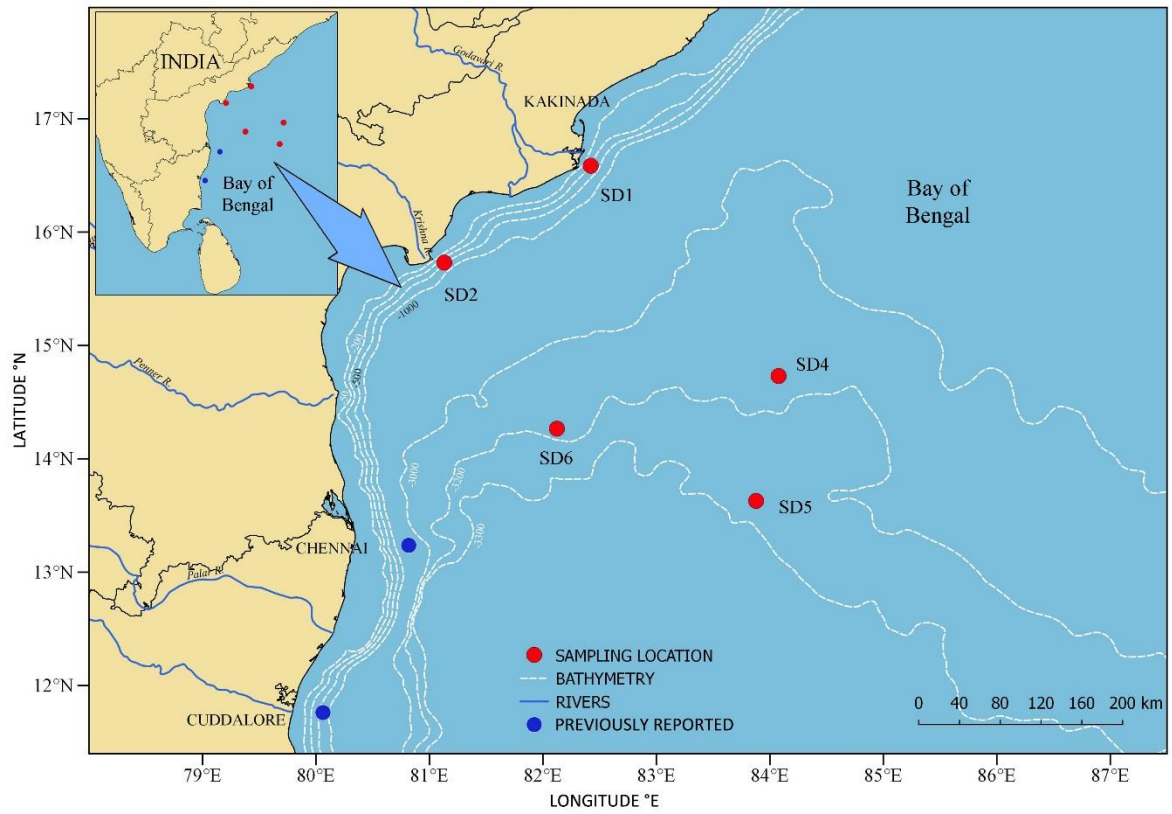


Figure 1

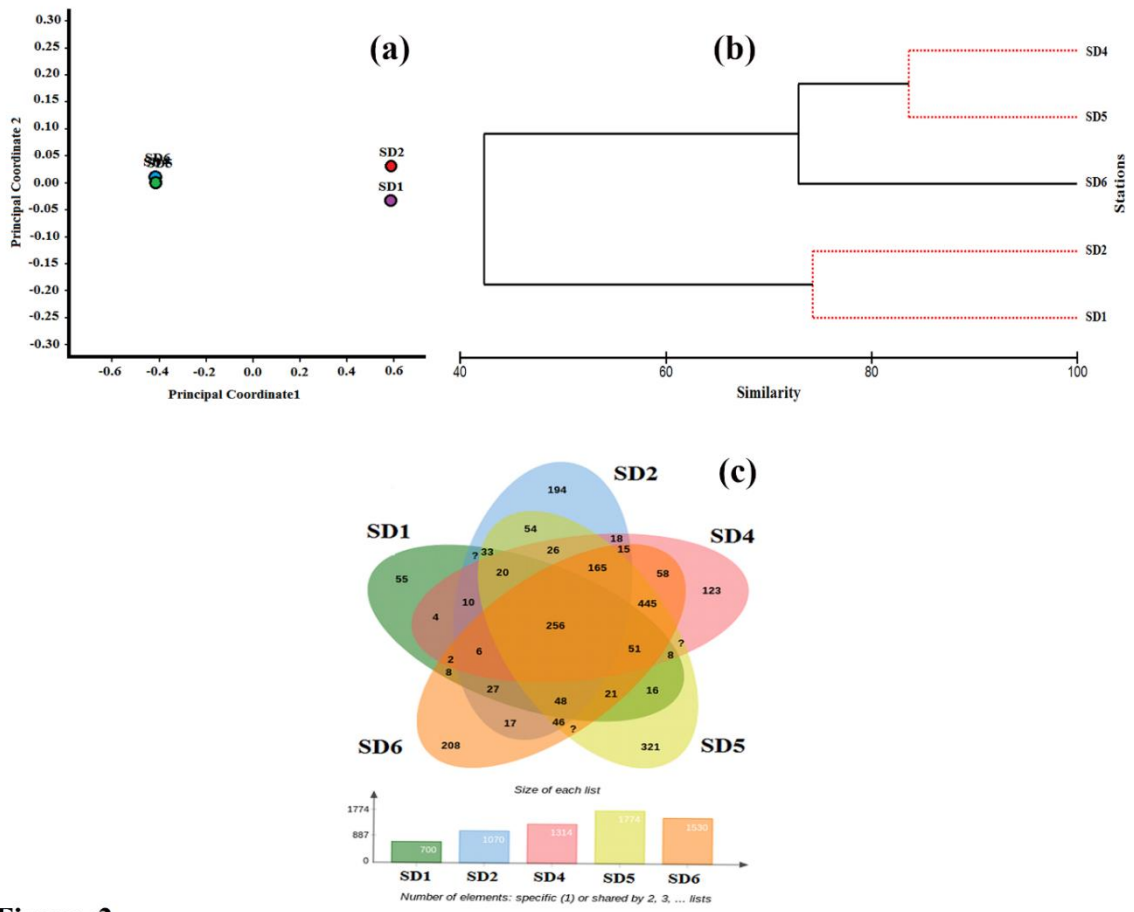


Figure :2

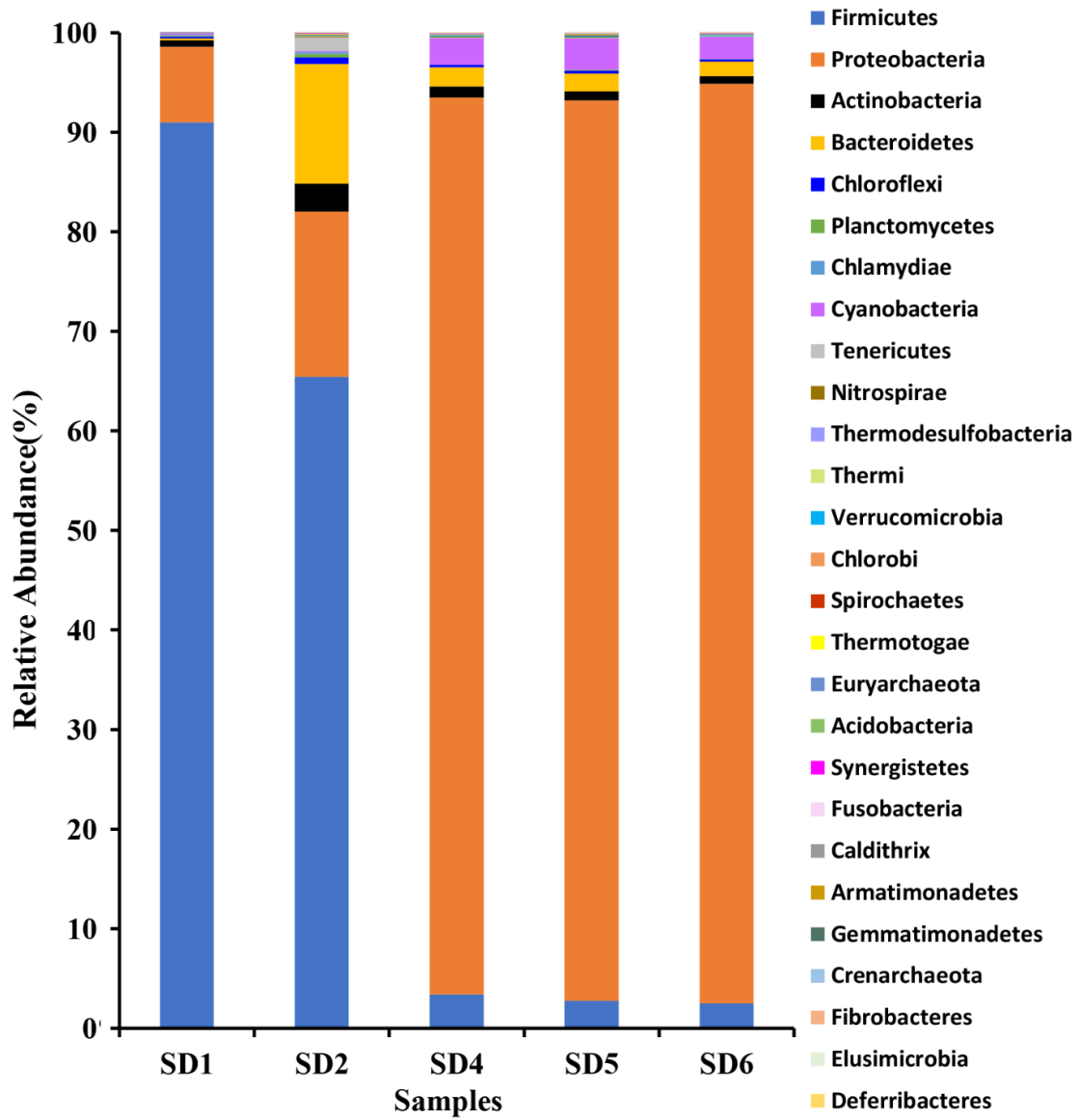


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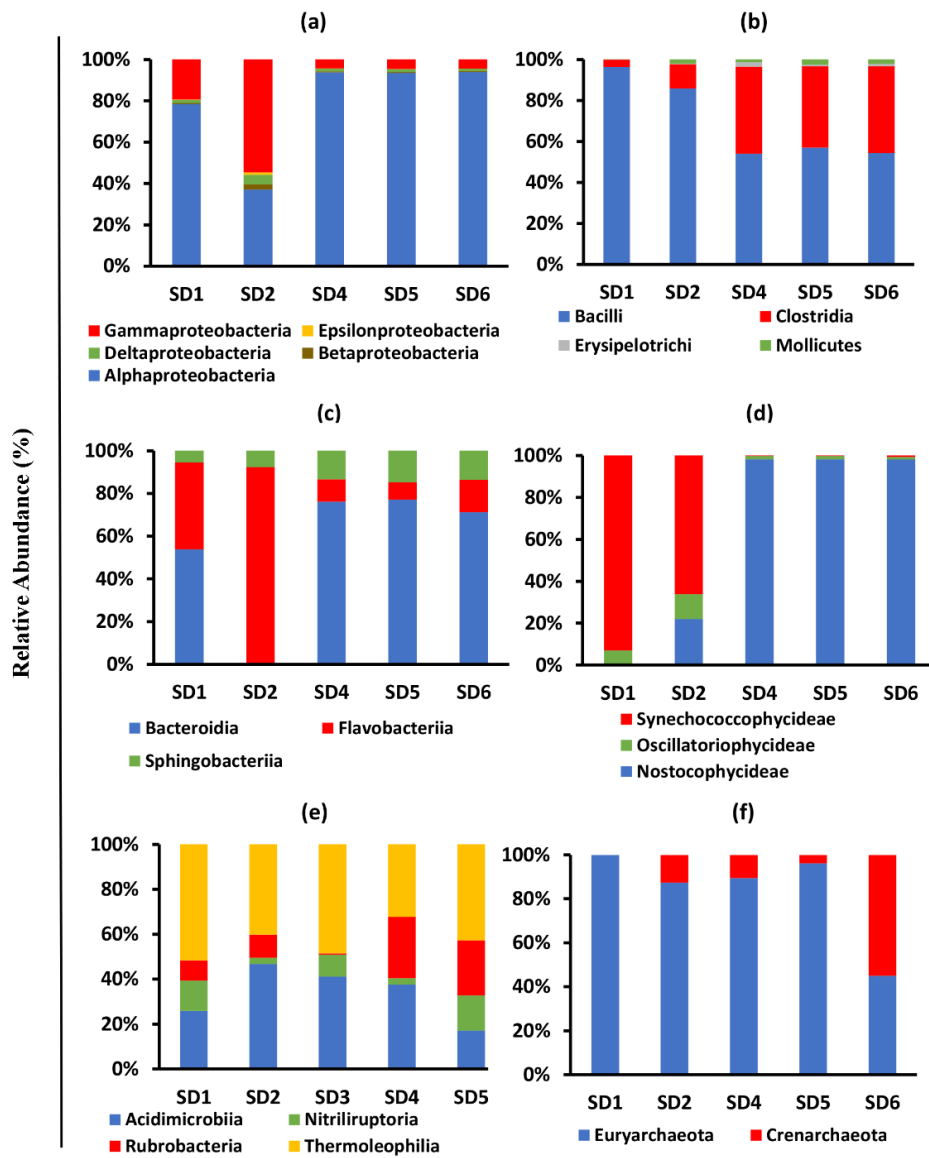


Figure 4

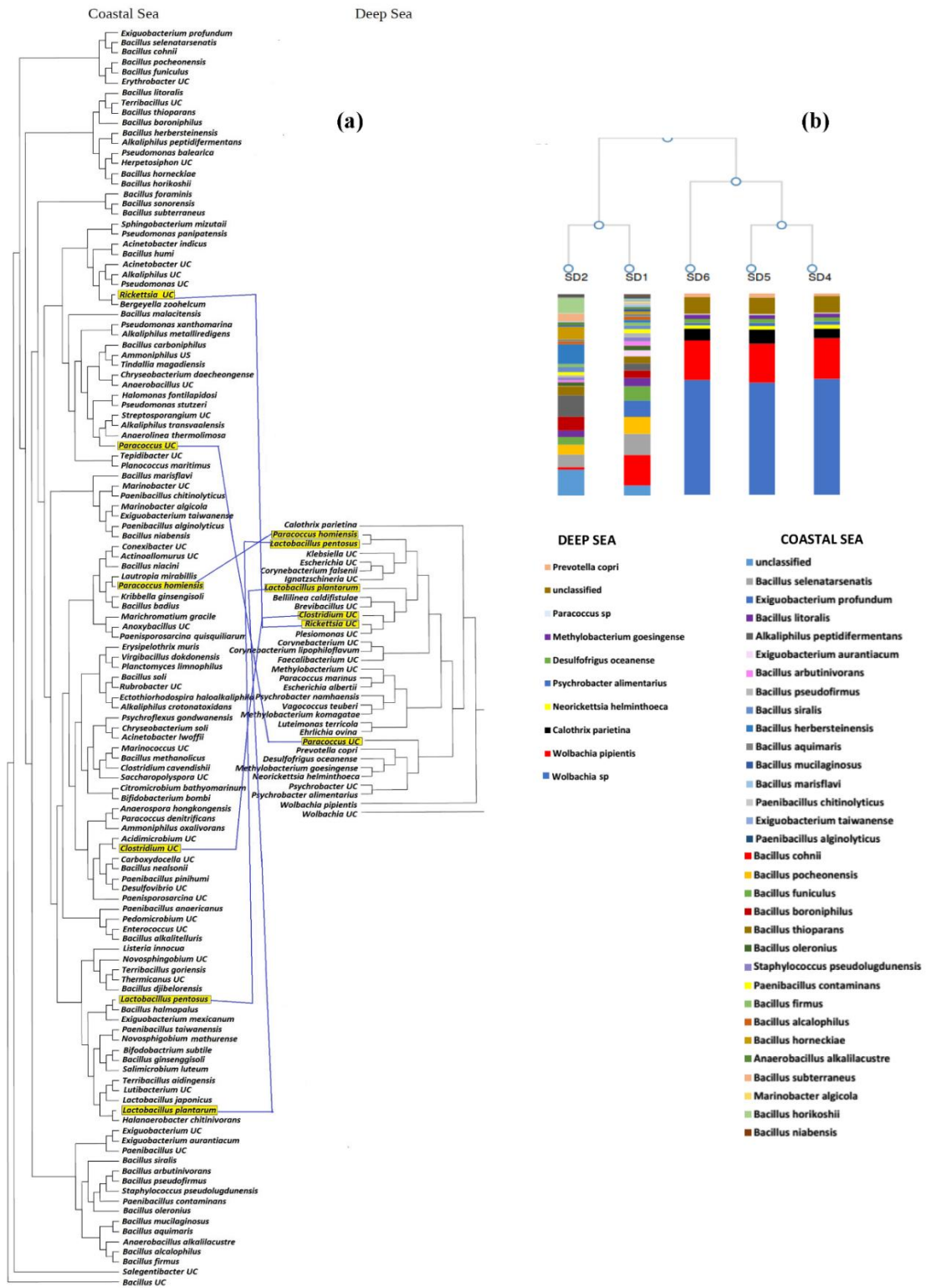


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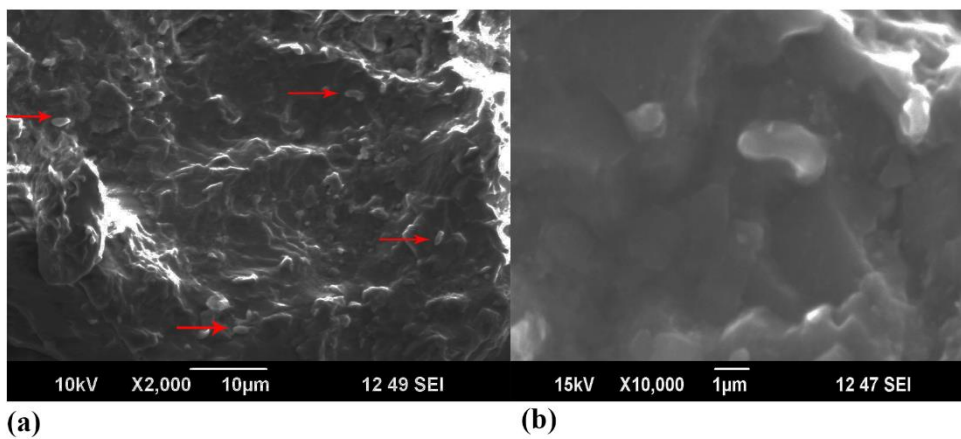


Figure 6

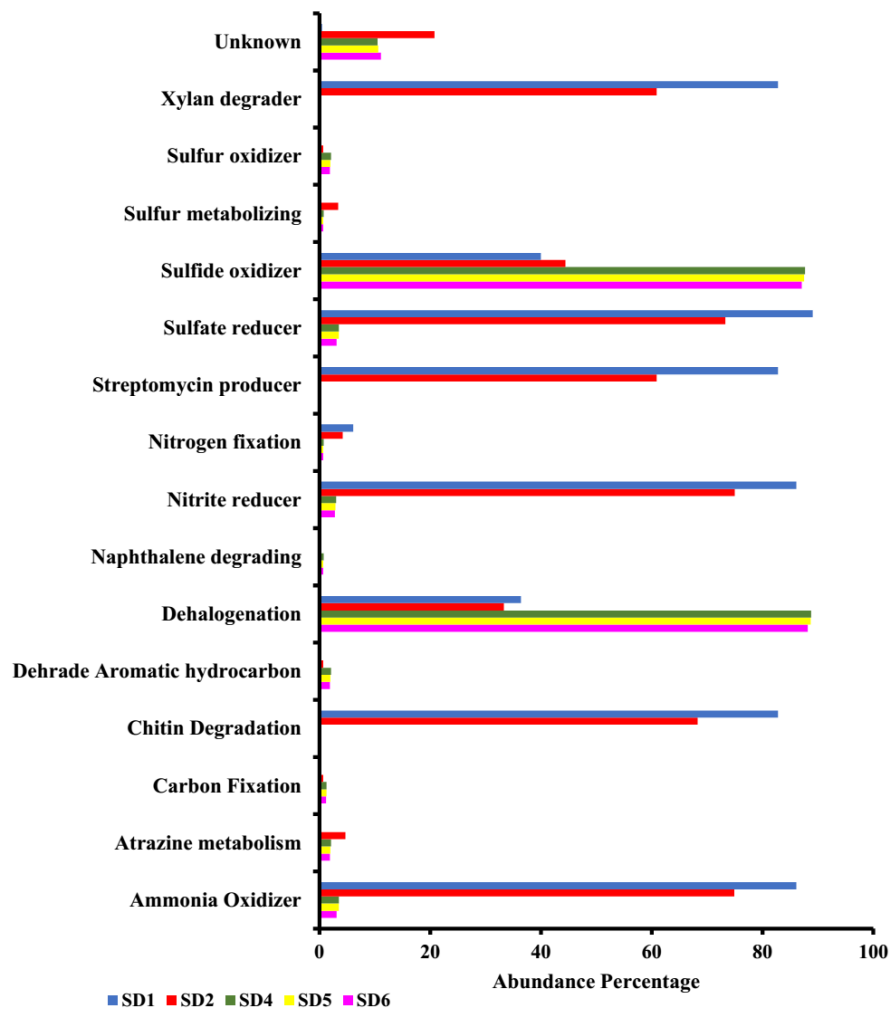
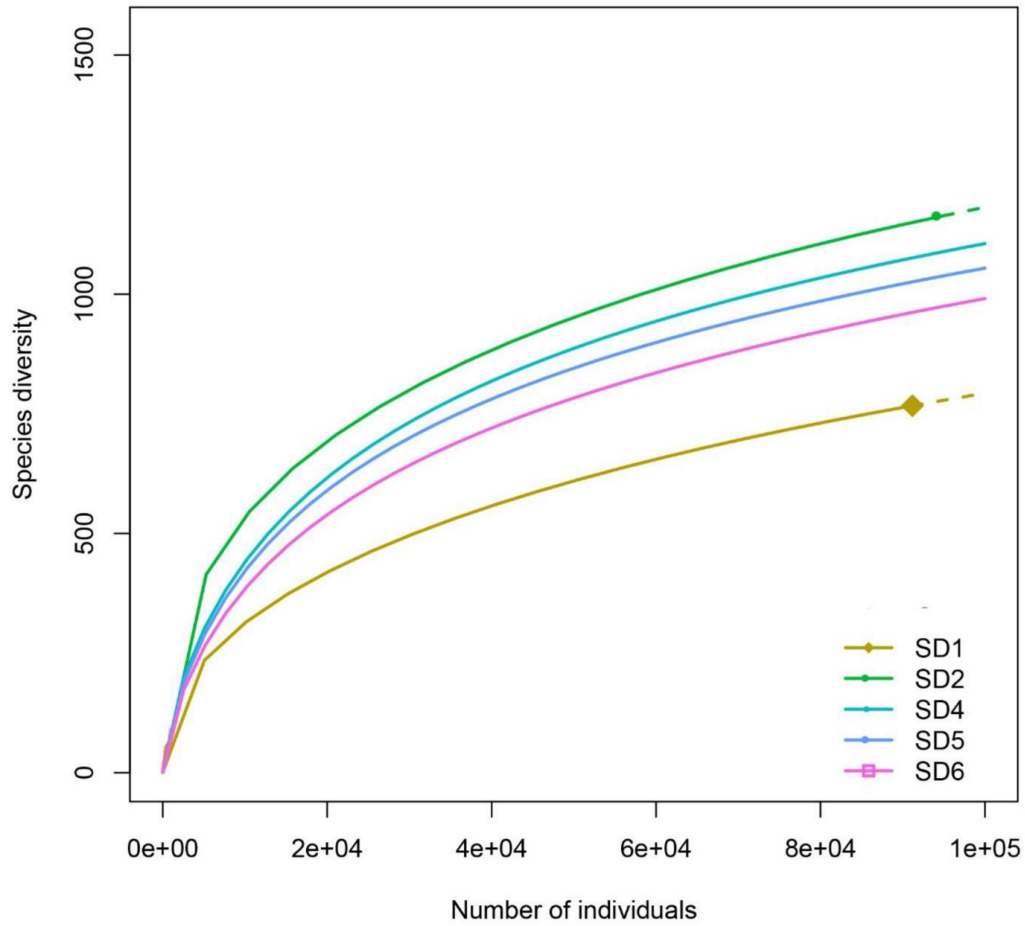
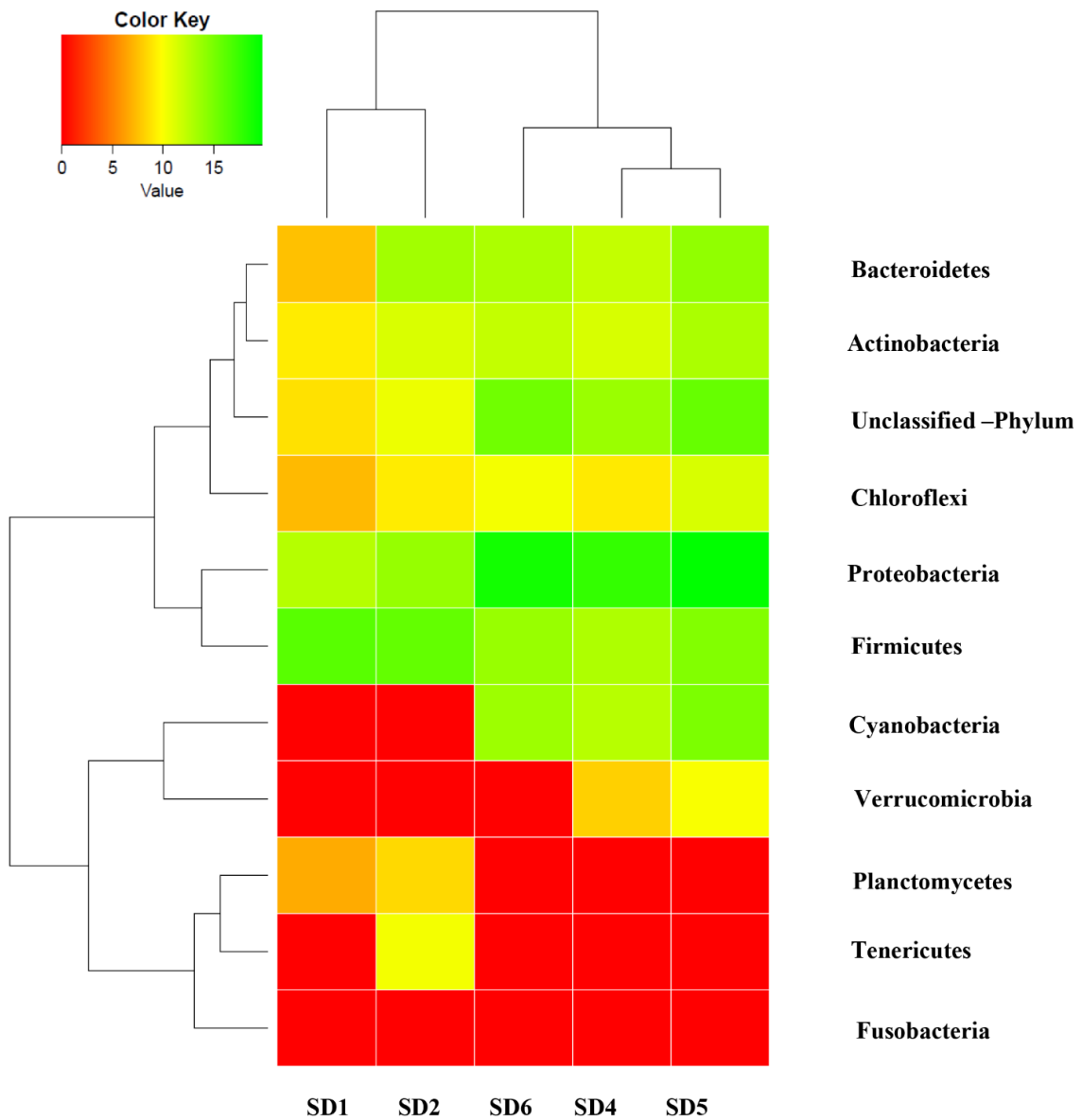


Figure: 7



Supplymentary Figure:1

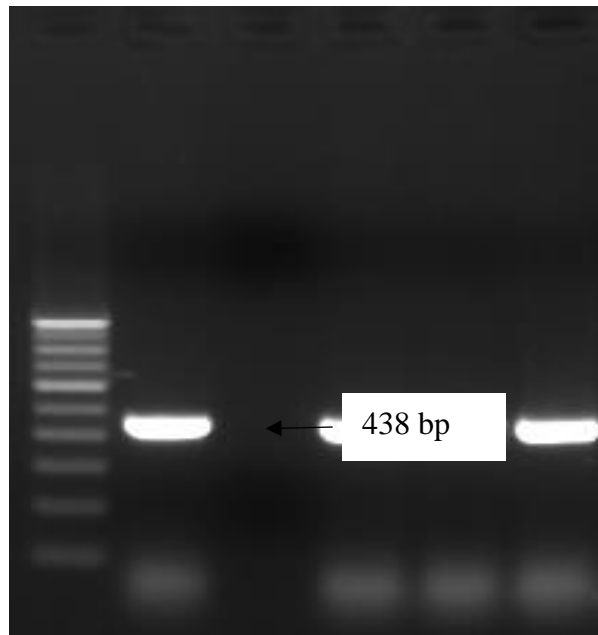
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Supplementary Figure:2

Supplementary Figure: 2. Generalized heat map with dendrogram representing the 16S rRNA gene sequences from the coastal (SD1 and SD2) and deep-sea sediment (SD4, SD5 and SD6) samples. The color key represents centered and scaled counts of each taxon.

M 1 2 3 4 5



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