Original article

Microbial diversity from the continental shelf regions of the Eastern Arabian Sea: A metagenomic approach

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ABSTRACT

The marine microbiome is a complex and least-understood habitat, which play a significant role in global biogeochemical cycles. The present study reported the culture-independent assessment of microbial diversity from the Arabian Sea (AS) sediments (from Gujarat to Malabar; at 30 m depth) by using metagenome sequence analysis. Our results elucidated that bacterial communities in the Malabar coastal region are highly diverse than the Gujarat coast. Moreover, Statistical analysis (Spearman rank correlation) showed a significant correlation co-efficient value ($r = P < 0.001$) between microbial communities and physicochemical parameters (salinity and dissolved oxygen) in the water column. A total of 39 bacterial phyla were recorded from the eastern side of AS, of which six phyla *Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, Firmicutes,* and *Planctomycetes* were found to be the most dominant group. The most dominant genus from Valapad region (Malabar Coast) was found to be *Halomonas* sp., while other regions were dominated with *Psychrobacter pulmonis*. The subsequent Principal Coordinate Analysis (PCoA) showed 99.53% variance, which suggests that, highly distinct microbial communities at Valapad (Malabar Coast) sampling location than other sites. Moreover, the microbial metabolic activity analysis revealed the important functions of microbial communities in the AS are hydrocarbon degradation, polymer degradation, nutrient oxidation and sulphate reduction (biodegradation process). Further extended studies are needed to be carried out for better understanding the functional diversity of microbial communities from the marine sediments.

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1. Introduction

Marine continental shelf regions microbial communities are complex, highly diversie and play a significant role of organic matter decomposition in sediments. The deep-sea biosphere (Pelagic and benthic) is one of the largest and most understudied ecosystems (Jorgensen et al., 2011). The global prokaryotic biomass in the deep-subsurface sediments are low, the abundance and diversity of microbial communities varies regionally in the continental shelf region (Kallmeyer et al., 2012). The shelves microbial communities played a vital role in ecological processes such as biogeochemical cycling and nutrient dynamics in the marine environment (Jiao et al., 2010). Moreover, the microbial diversity in the ocean is much higher in the sediments and undertake nutrient regeneration by degrading the settled dead organisms (Zinger et al., 2011). Besides, the microbial communities are sensitive to water quality changes and respond quickly as an indicator to detect the changes in marine ecosystems (Lai et al., 2006).

Over many decades, the marine scientific explorations in the Northern Indian Ocean such as living and non-living resources, coastal pollution index and ecological niches are commissioned by Ministry of Earth Sciences (MoES), India, the Council of Scientific and Industrial Research (CSIR) - National Institute of Oceanography (India) and other institutions (Qasim et al., 1988). The Indian scientific explorations have brought a shred of vast evidence to

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limelights the complex and crucial role of marine microbes in the goods and services such as food web, biogeochemistry, bioremediation etc. In recent years, the coastal urbanization and anthropogenic activities (Shipping and industrial discharges) are causing an impact on marine ecosystems (microbes to mammals) in a regional level (Nogales et al., 2011).

Halpern et al., (2008) stated that one-third of the oceans (about 41%) are predicted to be under the category of medium to high impact polluted coastal ecosystems. The major anthropogenic activities such as coastal urbanization, industries, maritime transport (shipping), oil extraction and refinery, tourism, and aquaculture are affecting both seawater column and the marine sediments by benthic-water flux (upwelling process; Halpern et al., 2008). The impact of pollution on coastal microbial communities is a complex process from multiple stressors such as natural and anthropogenic pollutions (Nogales et al., 2011). These stressors have an effect on coastal ecosystem, which may cause changes in the marine ecosystem from microbes to higher animals. Moreover, the effect of pollution on microbial communities might cause negative effects on ecosystem functioning and act as a bioindicator for environmental conditions (Nogales et al., 2011). The abundance of microbial communities is higher due to the availability of nutrients and industrial pollutions, which leads to the high temporal variability of microbial community composition and abundance in the marine environment (Nogales et al., 2011). Moreover, pollution indicate is the main determinant of microbial community composition in the marine environment (Thiyagarajan et al., 2010). Nonetheless, early studies in Indian coastal areas revealed that half of the coastal areas are found to be moderate and highly polluted areas by human-activities, coastal urbanization, shipping and aquaculture activities. The influence of pollutions on bacterial diversity were well documented by Ramaiah et al., (1996), Divya et al., (2010, 2011), Anas et al., (2016) and functional gene diversity in the pelagic and seawater of Indian coastal regions and elsewhere were studied by using classical microbiology approaches (Woebken et al., 2008; Ward et al., 2009; Luke et al., 2016).

Last two decades, marine microbial ecologists have studied the sediment microbial communities by using cultivable methods. However, the methods of cultivability are time-consuming laborious process, and the diversity coverage ranged from 5% (freshwater) to 25% (marine sediment) with the availability of lab infrastructure and expertise manpower. Therefore, the microbiologists adopted the non-cultivable 16S ribosomal ribonucleic acid (rRNA) sequence-based tools to understand the microbial communities structure and diversity from a particular environment. To understand microbial diversity and environmental health index, the ribosomal genes or intergenic regions of ribosomal operons (16S rRNA gene and internal transcribed spacer) using V3- V4 primers region with specificity at the domain level (i.e. Bacteria or Archaea) were studied successfully in worldwide marine sediments. Moreover, These methods limited to provide hug data on the microbial communities structure, diversity index, novelty enzymes for industrial application (Dahllof, 2002). Presently, the sequencing of 16S rRNA genes or high-throughput sequencing database helps to obtain phylogenetic data on the composition of microbial communities at the species level (Forney et al., 2004; Cardenas and Tiedje, 2008).

Metagenomics is a revolutionary concept in the aspect of studying the evolution of microbial biodiversity, adaptation and their ecological niches (Risenfield et al., 2004; He et al., 2007; Handelsman et al., 2007). Last decade, several studies have been documented by using a metagenomics approach to explore the microbial diversity, community structure or composition, and seasonal variation in marine environments (Gilbert et al., 2009; Gilbert and Dupont, 2011; Gilbert et al., 2012; Won et al., 2017). Moreover, metagenomic approaches of the microbial communities

in manganese mining revealed their role in the natural manganese geochemical cycle (Gosh and Das, 2018). The microbial diversity play an important role in oceanic productivity, major biomass and nutrient recycling in the coastal and marine environment (Nair et al., 2017). The metagenomic studies on microbial communities in the coast of AS are lacking. Therefore, an attempt has been made in the present study to investigate metagenomic insights of the microbial diversity from the continental shelf region of AS (from Gujarat to Kerala) by using Next-Generation Sequencing (NGS) technology.

2. Materials and methods

2.1. Study Area

In the present study, surface sediment samples were collected from a uniform depth of 30 m from four selected stations covering from Gujarat to Malabar coastal regions in the AS (Fig. 1). Generally, the northern Indian Ocean is more productive than the southern Indian Ocean due to the land lock areas in the northern side of the Indian Ocean. Nevertheless, anthropogenic activities are also responsible for the high biomass productivity in the northern Indian Ocean. These processes include several factors such as coastal upwelling current pattern and carbon flux from adjacent land areas, which together influence the coastal biogeochemistry of northern Indian Ocean (Chinni et al., 2019).

2.2. The Arabian Sea (AS)

The AS in the northwestern part of the Indian Ocean is situated between 5 and 21°″ N and 80-90°″ E, and monsoon is known to affect the western coastal region every year and they cause numerous changes in the coastal physiochemical properties and local hydrography patterns. The AS is one of the most productive regions in the world ocean. The AS receives $1.7 \times 10^{11} \mathrm{m}^2$ of freshwater discharging every year and -5×10^8 ton of sediments deposit annually from rivers sources (Chinni et al., 2019; Fig. 1).

2.3. Sample collection and physiochemical analysis

Surface sediment samples were collected during a 'FORV Sagar Sampada' (Cruise No. 374001) cruise in the AS (May 2018) and sampling points are shown in Fig. 1. A conductivity–temperature–depth (CTD) system was deployed to record various physicchemical properties of the water column including depth, oxygen, turbidity, salinity, density, pressure and chlorophyll.

2.4. Metagenomic DNA isolation:

Metagenomic DNA was isolated from the marine sediment samples collected from different locations by using a commercially available kit (Nucleospinsediment). The extracted DNA was stored at $-40 °C$ until further downstream processing. The triplicate DNA samples in each sediment sample were pooled together (for more coverage of microbial taxon). The pooled DNA samples were quantified using Qubit 2.0 (fluorometer, Thermo Fischer Scientific, USA) and Nanodrop spectrophotometer (Nanodrop 2000, Thermo Fischer Scientific, USA), and 20 ng/μl of DNA was used for 16S rRNA sequences analysis. Primers for the amplification of the 16S rDNA gene-specific (bacterial V3-V4 region), forward-5′-GCCTACGGGN GGCWGCAG and reverse primer R'-5′-ACTACHVGGGTATCTAATCC were designed at Eurofins Genomic Lab facility, Bangalore, India. PCR conditions included 95 °C initial denaturation for 4 min, followed by 30 cycles of 94 °C for 45 sec, 61.3 °C, for 30 sec, and 72 °C for 30 sec, and a final extension at 72 °C for 7 min. The ampli-
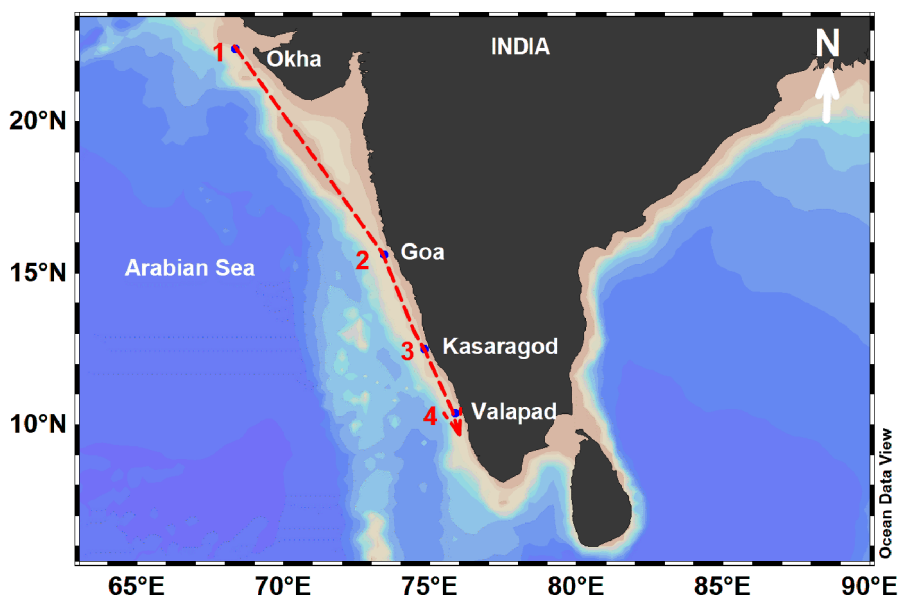
Fig. 1. Map showing the sampling locations of 4 sampling sites (St-1 Okha; St-2 Goa; St-3 Kasaragod; St-4 Valapad) along the Arabian Sea, India.

fication was examined by 2% agarose gel electrophoresis and purified using QiagenMinElute PCR purification kit. The purified PCR product was diluted to 5 mM final concentration using resuspension buffer (Illuminainc. CA, USA). Further, the PCR amplicon was denatured for 5 min, and buffered with 0.2 N NaOH and HT1 buffer (Illuminainc. CA, USA). The purified pooled PCR amplicon libraries were seen into an IlluminaHiseq 2500 platform (Illuminainc. CA, USA) and run in 2X250bp mode using HiSeq Rapid SBS Kit v2 in an IlluminaHiseq 2500 system platform NGS ((Illuminainc. CA, USA) at Eurofins Genomics India Pvt. Ltd at Bangalore.

The obtained raw IlluminaMiSeq gene sequence data were further pre-processed with the software PRINSEQ-lite (0.19.0), which include primer walks sequences trimming, eliminating ambiguous reads and removal of poor quality sequences such as < 27 bp and < 200 bp for further analysis.

2.5. Statistical analyses

Statistical analysis of sequences similarity was performed using Mothur v.1.33.3 software (Schloss et al., 2009). The sequences were classified into operational taxonomic units (OTUs) and richness were calculated through rarefaction, good coverage, alpha diversity (Chao1 index) and Shannon's index (Tao et al., 2014). Moreover, the Unifrac metric was used to analyze the beta diversity (Lozupone and Knight, 2005). To evaluate the sequences similarities of different bacterial communities' composition, the principal coordinate analysis (PCoA) was carried out based on the weight of UniFrac distance. To compare the first 15 genera of each sample, a heat map was generated by using the Heatmap package in Qlucoreomics 3.5 (64 bit) software. The Spearman rank correlation analysis was performed to identify the significant correlation coefficient (r-value) between the study sites and environmental factors (Primer 7 version).

3. Results and Discussion:

The marine microbiome plays an important role in bioremediation (pollution control), major/minor elemental recycle and nutrient cycle in the coastal ecosystem. In this study, microbial diversity in the AS coastal sediments was investigated from 4 different sites covering from Gujarat to Kerala coast. The microbial diversity from collected sites was assessed by following 16S rRNA amplicon-based high throughput sequencing with the help of IlluminaHiseq2500 platform. In this study, a total of 1,10,857 raw reads were obtained from 30 m deep sediment samples collected at four different locations of AS (Gujarat to Kerala). The metagenomic DNA samples were preprocessed (Trimming, denoising, and removing chimeras) and 77, 346 effective 16S rRNA sequences have remained with an average length of 535 bp. The present study identified a total of 10,345 bacterial OTUs and the sequences were clustered with 3 percentage dissimilarity (Table 1). All the effective sequences were classified into 34 bacterial groups and 4 archaeal groups. The highest number of bacterial sequences (26,434 sequences) was found to be present in the Valapad (Malabar Coast) sample, and the lowest number of sequences (8, 258 sequences) was observed in the Kasargode sample (near the municipal area).

3.1. α-diversity:

The four samples sequence coverage was varied from 83.49% to 90.26% (Table 1). The obtained OTUs data of each sample were shown in the rarefaction curves (Fig. S1). According to the OTUs numbers, the highest richness was found in the Valapad samples, followed by the Okha, and Karasgod than the Goa sample. The Shannon index also showed a similar pattern to the OTUs numbers. Besides, the Shannon index and Chao1 values of Valapad sample were higher than Goa sample.

3.2. β-diversity:

The four sediment samples were cluster into two groups, namely the pristine environment and polluted environment (Fig. 2). The type of pristine or unpolluted environment of Valapad sediment sample was apparently one of the influencing factors (water physiochemical factors) in the first principal coordinate plot (PCo1) and contributed 97.64% to the total variation. The bacterial communities such as *Proteobacteria* (54.81%), *Gammaproteobacteria* (44.91%) and *Oceanospirillales* (34.08%) were associated with pristine coastal sediment sample and more abundant than the deciduous tree.

Table 1
Bacterial richness indices of the 4 samples off Indian coast of Arabian sea.

Samples ID	No. of effective sequences/ NCBI acession number	No. of OTUs[a]	Coverage (%)	Chao1[a]	Shannon index
Valapad	26,434 / SAMN12586596	4053.80	89.73	5899.34	6.836
Kasargode	8,258 / SAMN12586595	1833.63	83.49	3463.15	6.160
Goa	24,434 / SAMN12586594	1912.25	90.26	3867.20	6.114
Okha	18,216 / SAMN12586593	2526.58	86.46	4341.72	6.356

*Indices (OTUs[a], Chao1[a], and Shannon index) were calculated based on the randomly selected 10,345 sequences. Cutoff = 0.03.
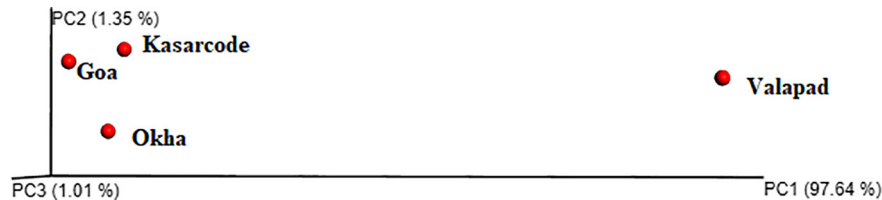

Fig. 2. Principal Coordinates plot based on the weighted UniFrac distances of all the soil samples.

4. Microbial community composition in each sediment sample:

The bacterial communities such as Proteobacteria (41.30%), *Bacteroidetes* (22.20%), *Actinobacteria* (09.07%), *Cyanobacteria* (3.70%), *Firmicutes* (19.52%) and *Planctomycetes* (2.58%) were dominant and similar across all the sampling sites at the phylum level (Figs. 3-6). However, the relative abundances of the bacteria belong to these phyla exhibited significant variations (Figs. 3 to 6). For instance, bacteria belonging to the phylum *Proteobacteria* were highly abundant in the Valapad sampling site (54.81%) than the Goa (36.32%), Kasaragod (38.01%) and Okha (36.10%) sampling sites. The similar richness in phylum *Proteobacteria* was reported across the coastal zone of AS (Nair et al., 2017) and other parts of the world (Lai et al., 2006). Whereas, the abundance of phyla *Bacteroides* (11.99%) and *Firmicutes* (9.77%) in Valapad sample was lower than other sites. Further, the major phyla like *Actinobacteria* (7.80 – 10.58%) and *Cyanobacteria* (1.28 – 2.35%) were exhibited a similar pattern of abundance across all the sampling sites. Whereas, a 3–3.5 times higher abundance of phyla *Chloroflexi* and *Planctomycetes* were found in the Valapad samples than other samples. A similar microbial distribution and abundant was observed for the major ocean microbial genera across the sampling sites (Okha, Goa and Kasaragod), namely *Psychrobacter pulmonis* (25.55–27.53%), *Prevotella copri* (7.81–8.47%), *Ruminococcaceae* unclassified (4.68–5.61%), *Prevotella stercorea* (3.82–4.02%), *Moraxellaceae* unclassified (3.54–3.60%) and *Planococcus pelagicus* (3.07–3.38). Whereas, genera *Halomonas* (34.07%) followed by *Psychrobacter pulmonics* (7.09%), *koll13* Unclassified (3.62%), *Prevotella copri* (3.61%), *Pirellulaceae* unclassified (2.90%) and *P. stercorea* (2.01%) were abundant in Valapad sample (Supplementary Table 2). The Principal Component Analysis (PCoA) showed 99.53% variance with highly distinct microbial abundance at the Valapad sampling site than the other sites (Fig. 7). Similarly, the microbial diversity from marine environmental samples like corals, estuarine water and brackish water sediments were
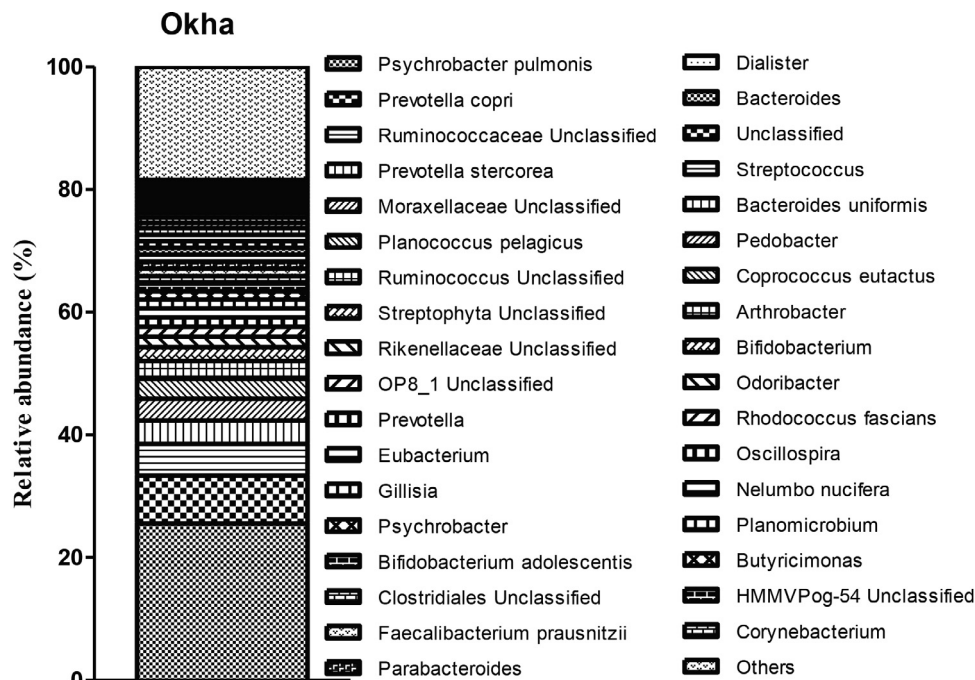

Fig. 3. Phylum-level microbial sequences diversity of sediment sample collected from Okha (St. No. 1), Gulf of Katch, Gujarat. *The taxon represented accounted for above 1% abundance in sample. Other phylum taxa had a maximum abundance of below 1% in a sample.*
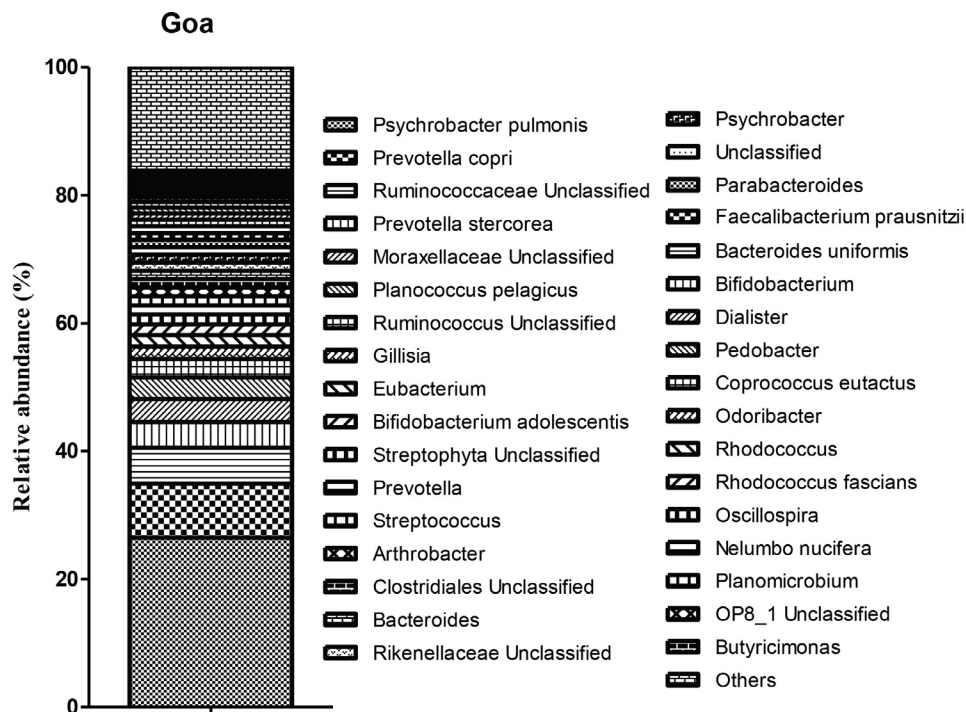
Goa

Fig. 4. Phylum-level microbial sequences diversity of sediment sample collected from Goa (St. No. 2) coast. *The taxon represented accounted for > 1% abundance in sample. Other phylum taxa had a maximum abundance of < 1% in a sample.*
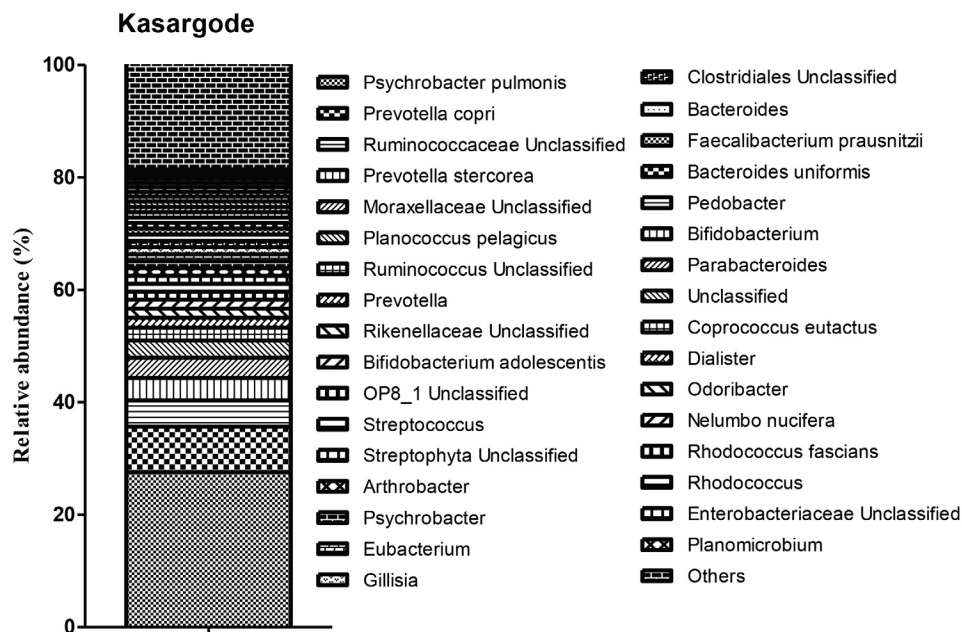
Kasargode

Fig. 5.. Phylum-level microbial 16srRNA sequence diversity of Sediment sample collected from Kasaragod (St. No. 3), Near Mangalore coast. *The taxon represented accounted for above 1% abundance in sample. Other phylum taxa had a maximum abundance of below 1% in a sample.*

reported in earlier studies (Abbondanzi et al., 2005; Hewson and Fuhrman, 2006; Kapley et al., 2007; Rowher et al., 2007).

4.1. Relationship between microbial community and environmental variables:

Marine environments are one of the most adverse environments due to their variations in the physicochemical parameters namely temperature, salinity, dissolved oxygen, chlorophyll, wind and other parameters. However, the bacterial diversity in the marine environments is capable to acclimatize in any adverse physicochemical conditions (Dash et al., 2012). Boetius et al. (2000) reported the maximum level of microbial diversity in the AS. However, the study on microbial richness across the continental slope of AS is not explored significantly. The sampling sites of Valappa, Kasargod, Goa and Okha revealed the variation with different
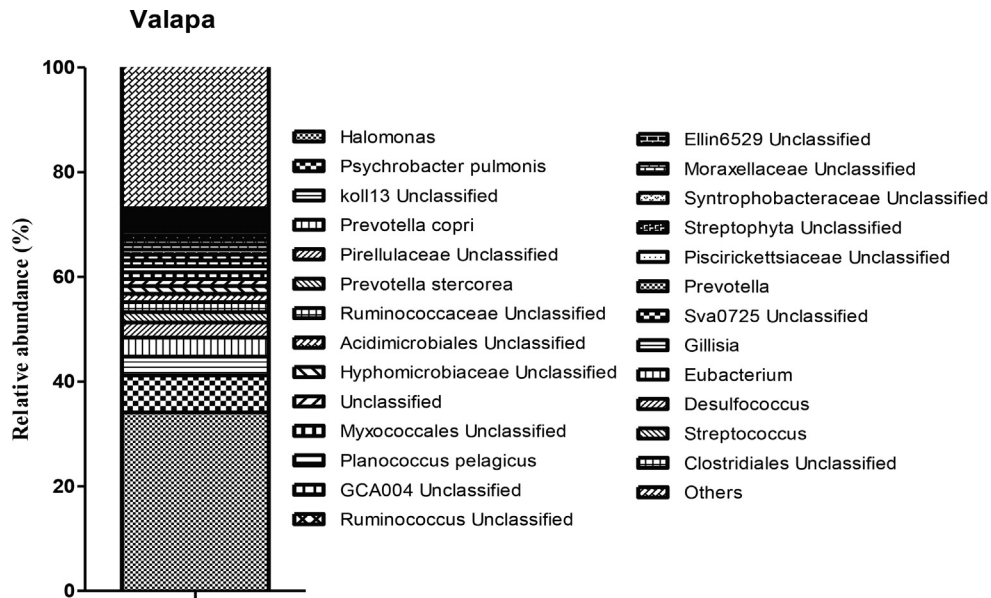
Fig. 6. Phylum-level microbial diversity of sediment sample collected from Valapad, (St. No. 4), Malabar Coast. *The taxon represented accounted for above 1% abundance in sample. Other phylum taxa had a maximum abundance of below 1% in a sample.*

physiochemical parameters (dissolved oxygen, temperature, salinity, pressure, density and turbidity) and the variations were found in different depths coving north (Gujarat) to south (Kerala) coast of India (Fig. 1). The mixed layer depth (MLD) was relatively deep in the north (21° N; MLD 0 ∼ 30 m) and shallow in the south (9° N; MLD 0 ∼ 30 m) region (Supplementary Figs. 3-7). The water temperature and salinity (1 to 30 m) were ranged between 26.7 and 24.51 °C and 36.18–3.92 PSU in the north, 29.39–28.85 °C; 34.65–34.99 PSU in central and 30.72–29.30 °C; 34.28–34.47 PSU in southern regions respectively (Fig. 7a-b). A gradual increase in temperature was observed from north to southern sampling stations. However, the DO values decreased from south to north at 30 m and increased from south to north at the surface, the values were ranged from 4.53 to 2.89 ml/L in the north, 4.38–3.32 ml/L in central and 4.11–3.58 ml/L in southern regions (Fig. 7c-e).

We found that the values of turbidity were higher in the northern and lower in the middle and southern regions. A significant correlation co-efficient (r-value) between physicochemical parameters and the microbial communities were studied by using the Spearman rank correlation co-efficient analysis (Tables S1 to S4; Wei et al., 2017; Stratford et al., 2014; Spatharis et al., 2011). This analysis elucidated that, microbial communities and physicochemical parameters in the water column were significantly correlated with each other's (p < 0.001). As a result, the correlation between salinity and microbial communities showed a positive strong significant correlation co-efficient (r = 0.981–1) in Goa and Kerala coast, whereas negative correlation at the Gujarat coast (r = -0.98). However, Dissolved oxygen (r = -0.77–0.98) was negatively significant correlation co-efficient with microbial communities (Tables S1 to S4). This result could be the reason for the chemical composition of seawater salinity.

The "Bray–Curtis" dissimilarity matrix for the bacterial community composition, the "Euclidean" dissimilarity matrices for geographic distance and environmental variables were studied by using Primer 7 software package. The cluster analysis shows that the study stations such as Valapad, Kasargode and Okha are formed one group with 85% similarity and the station Goa joined the cluster with 75% similarity (Fig. S2). The % similarity between the study stations could be due to the variation in geographical sediment nature (availability of organic matter, nutrients and minerals).

The western continental shelf of India is shallow and the sediments are classified as terrigenous, biogenic, and chemogenic sediments (Rao and Wagle 1997). In general, the western continental shelf of India is considered as a potential organic deposition region due to the enormous quantity of primary production occurs (Jacob et al., 2007). Moreover, the biogenic sediments occur between Gujarat (Okha) and Mangalore, the mixed terrigenous and biogenic sediments occur between Mangalore and Kollam (Rao and Wagle 1997). The Valapad region experiences the Mudbank phenomenon during the southwest monsoon. The Mud banks are formed by sedimentation of a huge amount of organic matter from rivers and estuaries discharges, which is usually appeared from Mangalore to the Kollam coast of India (Rao et al., 1980). The organic depositions on the continental shelf are important for carbon accumulation and play a significant role in the biogeochemical cycle (Jacob et al., 2007). In recent years, the sedimentation in the Goa coast is increased due to extensive iron ore and Manganese mining activities (Sebastain et al., 2017). From 2002 to 2011, the average annual Iron ore mining was around 28 million tonnes (Mt) and the mining waste was three-fold of the production value (Sebastain et al., 2017). Kurian et al. (2009), reported that the inner shelf of Goa has shown an enormous increase in productivity from the last five decades with the response to anthropogenic nutrients input.

5. Prediction of biodegradation bacteria communities as pollution indicator in AS

The vast biodiversity of marine bacteria is significant to the functional role of their performance in the marine environment (Rohwer et al., 2001). The functional role of marine bacteria in nutrient regeneration (nutrient cycle), carbon sequestration, food web sustainability, biodegradation, bioremediation and well adaptation to environmental changes (Climate change) (Parvathi et al., 2019; Nair et al., 2017).

In recent years, pollution in the marine environment is increased gradually due to various anthropogenic factors like urbanization, sewage and industrial wastewater discharges in the coastal areas. As a result, the diverse organisms from microbes to

mammals in the marine environment are adapting to the adverse conditions. Moreover, the marine environment found to be a good reservoir for many human pathogens (bacteria) which produces many unique enzymes and functioning in biodegradation and bioremediation process. Besides, the metabolic potentials in the marine microbes are an effective mechanism to eliminate marine pollutants. Among pollutants, Crude oil is the most important organic pollutant in the marine environment. Annually, around 1.7–8.8 X 106 tonnes of petroleum hydrocarbons are unconfined

slicking into marine environments (McKew et al. 2007). These organic pollutants can be degraded by the oil-degrading bacteria present in the marine environment. The oil and plastic degrading bacteria in the marine environment include namely *Acinetobacter, Marinococcus, Methylobacterium, Micrococcus, Nocardia, Planococcus*, and *Rhodococcusruber* (Sakalle and Rajkumar 2008). Conversely, a more potent oil-degrading bacteria were isolated from the AS sediments, which is capable to degrade 39% of oil in 8 days under laboratory conditions (Mukherji et al. 2006). Biological pro-
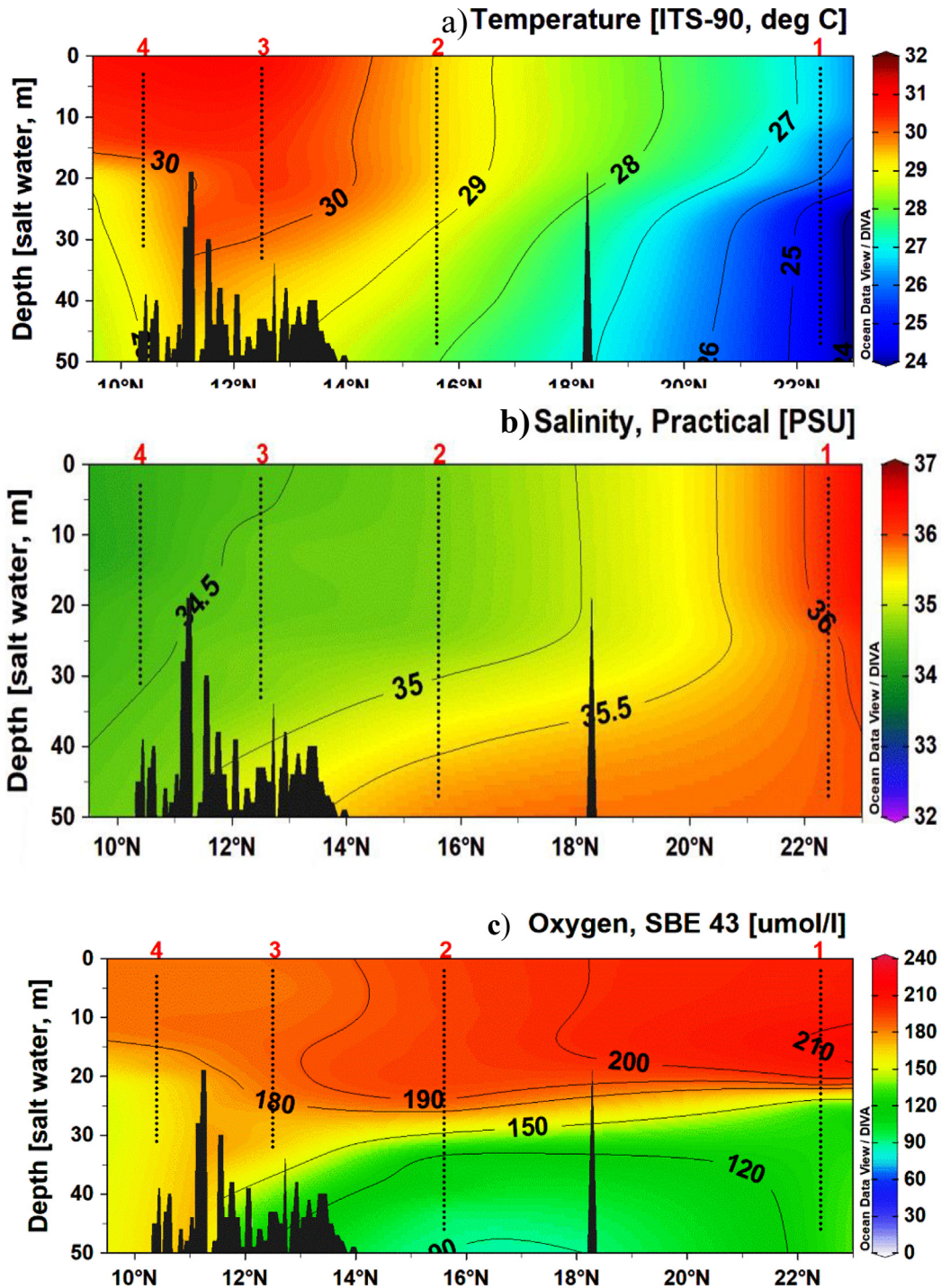

Fig. 7. Vertical profiles of hydrographic parameters from the CR-374001 cruise. (a) temperature [°C], b) salinity [PSU], c), dissolved oxygen [μmol/L], d) turbidity [NTU], and e) chlorophyll. Station numbers are depicted along the top of the figure. The cruise started from Gujarat towards Thiruvananthapuram and was completed at Kochi.
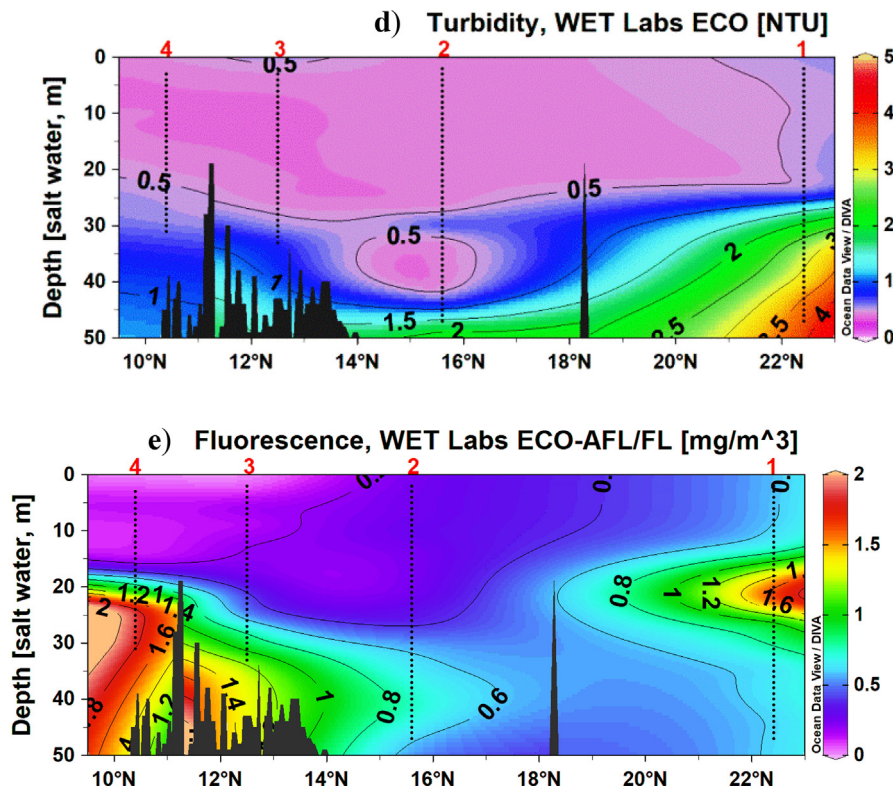
d) Turbidity, WET Labs ECO [NTU]

e) Fluorescence, WET Labs ECO-AFL/FL [mg/m^3]

Fig. 7 (*continued*)

ductivity in the Indian Ocean basin was attributed to the higher level of the benthic bacterial population which was reported in the range from 0.48 to 1.21×105 CFU/g (LokaBharathi and Nair 2005). Moreover, most of the bacterial communities in the Indian Ocean were categorized under six major taxonomic groups, namely, α, β, and γ *Proteobacteria, Actinobacteria, Bacilli,* and *Flavobacterium* (Santiago-Vazquez et al. 2007). The marine bacterial communities have a wide range of biodegradation and bioremediation potentials which are beneficial to the marine environment. They respond very quickly to pollutants and physicochemical conditions in the marine environment (Parvathi et al., 2019). As a result, the bacterial communities act as a bioindicator and play an important role in biodegradation and bioremediation. The microbial community index alters due to the external impacts like pollutant from land to marine environment.

The present study hypothesizes that the bacterial communities in the continental shelf region of AS (30 m) play an important role in the biodegradation and bioremediation process. This study was undertaken by using metagenomic data to quantify the bioindicator microbial community from the study location in the AS. The results showed the variations in the plastic and oil degradation bacterial diversity which involved in various ecological activities namely bioremediation and biodegradation. The metabolic functions of the dominant microbial communities are hydrocarbon degradation (22.38%), sulfate reduction (21.41%), polymers degradation (19.21%), metals degradation (17.36%), dehalogenation (10.26%), sulphide oxidation (6.28%), and lignin degradation (3.07%).

Among the identified bacterial communities, most dominate species of hydrocarbon degradation bacteria are *Psychrobacterpulmonis, Planococcuspelagicus, Psychrobacter*spp., *Micrococcus* spp., and *Streptococcus* spp. Moreover, all these bacterial communities are found in all the study sites. The previous studies were reported that Actinobacteria and Proteobacteria are mainly capable of con-

ducting hydrocarbon metabolism (Vikram et al., 2016; Parvathi et al., 2019). Besides, the similarity assemblage between bioremediation species was formed two major cluster groups, which are named as "a" and "b".

Group "a" formed 25–90% similarity cluster with crude oil-degrading bacteria, petroleum, diesel degradation bacteria and plastic degradation bacteria. Whereas group "b" formed 20–85% similarity cluster with crude oil-degrading bacteria, plastic degradation bacteria and pollutant bacteria. In general, the results showed that the Oil and plastic degrading bacterial communities have a strong association with each other (Fig. 8). This bacterial diversity in the coastal environment is due to the nature of sediments (Wei et al., 2017).

In addition, the four sediment samples were clustered into two groups namely pollution and non-pollution environment, which elucidated that the microbial community composition is frequently related to the type of sediments and surrounding environment (Fig S2). Likewise, similar studies (water and sediment quality index) were reported from other parts of the world (Wei et al., 2017; Griffiths et al. 2011; Chu et al. 2010; Wei et al., 2017).

The present study revealed that different coastal environments are influenced by different physiochemical variables, which induced some changes in the microbial community structure. The physiochemical parameters (seawater) such as salinity and DO showed a significant correlation with a particular coastal environment and influenced on specific bacterial communities.

The unpolluted environment was the main influencing factors for the microbial community composition in the Valapad and Kerala coast. Moreover, the bacterial communities in the three different study sites in the AS (Okha, Kasargode and Goa) were showed more similar bacterial diversity to each site than the pristine coastal environment (unpolluted study site) namely Valapad coast (Kerala). Best our knowledge, this is the first report on bacterial diversity in marine sediments (30 m depth) from continental shelf
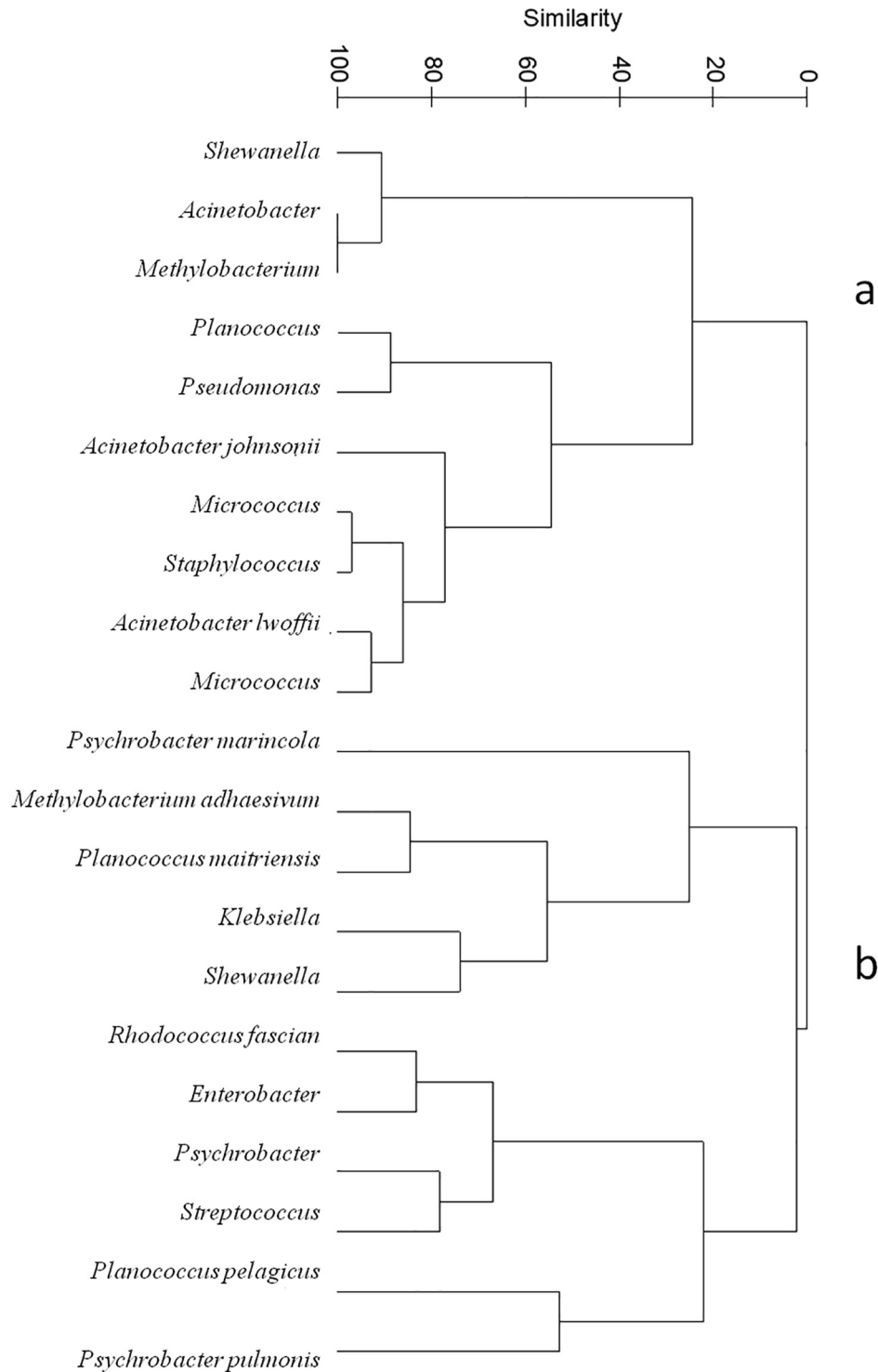
Fig. 8. Pollution Indicator bacterial communities relationship analysis using cluster statistics.

regions of the AS. Moreover, future studies need to be carried out on seasonal variation of microbial communities for better understanding their functional role in the marine sediments of AS.

Conflict of interest statement

We declare that we have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.06.011.

References

Abbondanzi, F., Campisi, T., Focanti, M., Guerra, R., Iacondini, A., 2005. Assessing degradation capability of aerobic indigenous microflora in PAH-contaminated brackish sediments. Mar. Environ. Res. 59, 419–434. https://doi.org/10.1016/j.marenvres.2004.06.006.

Anas, A., Nilayangod, C., Jasmin, C., Vinothkumar, S., Parameswaran, P.S., Nair, S., 2016. Diversity and bioactive potentials of culturable heterotrophic bacteria from the surficial sediments of the Arabian Sea. Biotech 6, 238.

Boetius, A., Ferdelman, T., Lochte, K., 2000. Bacterial activity in sediments of the deep Arabian Sea in relation to vertical flux. Deep Sea Res. Part II Top. Stud. Oceanogr. 47, 2835–2875. https://doi.org/10.1016/S0967-0645(00)00051-5.

Chinni, V., Singh, S.K., Bhushan, R., Rengarajan, R., Sarma, V.V.S.S., 2019. Spatial variability in dissolved iron concentration in the marginal and open waters of the Indian Ocean. Marine Chemistry 208, 11–28. https://doi.org/10.1016/j.marchem.2018.11.007.

Chu, H., Fierer, N., Lauber, C.L., Caporaso, J.G., Knight, R., Grogan, P., 2010. Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. Environ Microbiol. 12 (11), 2998–3006. https://doi.org/10.1111/j.1462-2920.2010.02277.x.

Cardenas, E., Tiedje, J.M., 2008. New tools for discovering and characterizing microbial diversity. Curr. Opin. Biotech 19, 544–549.

Dahllöf, I., 2002. Molecular community analysis of microbial diversity. Curr. Opin. Biotech. 13, 213–217.

Dash, H.R., Mangwani, N., Chakraborty, J., Kumari, S., Das, S. 2012. Marine bacteria: potential candidates for enhanced bioremediation. Appl. Microbiol. Biotechnol., DOI 10.1007/s00253-012-4584-0

Divya, B., Soumya, K.V., Nair, S., 2010. 16S rRNA and enzymatic diversity of culturable bacteria from the sediments of oxygen minimum zone in the Arabian Sea. Antonie Van Leeuwenhoek 98, 9–18.

Divya, B., Parvathi, A., Bharathi, P.L., Nair, S., 2011. 16S rRNA based bacterial diversity in the organic-rich sediments underlying oxygen-deficient waters of the eastern Arabian Sea. World J. Microbiol. Biotechnol. 27, 2821–2833.

Forney, L.J., Zhou, X., Brown, C.J., 2004. Molecular microbial ecology: land of the one-eyed king. Curr. Opin. Microbiol 7, 210–220.

Qasim, S.Z., Sen Gupta, R., Kureishy, T.W., 1988. Pollution of the seas around India. Proc. Indian Acad. Sci. 97 (2), 117–131.

Gilbert, JA. Field D. Swift P., Newbold, L., Oliver, A., Smyth, T., Somerfield, PJ., Huse, S., Joint, I., 2009. The seasonal structure of microbial communities in the Western English Channel. Environ. Microbiol. 11, 3132–3139

Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbrück, L., Reeder, J., Temperton, B., Huse, S., McHardy, A.C., Knight, R., Joint, I., et al., 2012. Defining seasonal marine microbial community dynamics. ISME J. 6, 298–308.

Gilbert, J.A., Dupont, C.L., 2011. Microbial metagenomics: beyond the genome. Ann Rev Mar Sci. 3, 347–371.

Ghosh, S., Das, A.P., 2018. Metagenomic insights into the microbial diversity in manganese- contaminated mine tailings and their role in biogeochemical cycling of manganese.Sci. Rep. 8, 8257.

Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environ Microbiol. 13 (6), 1642–1654. https://doi.org/10.1111/j.1462-2920.2011.02480.x. PMID: 21507180.

Halpern, B.S., Walbridge, S., Selkoe, K.A., et al., 2008. A Global Map of Human Impact on Marine Ecosystems. Science 319, 948–952. https://doi.org/10.1126/science.1149345.

Handelsman, J. et al., 2007. Committee on Metagenomics: Challenges and Functional Applications. National Academy of Sciences, Washington, DC.

Hewson, I., Fuhrman, J.A., 2006. Improved strategy for comparing microbial assemblage fingerprints. Microb. Ecol. 51, 147–153. https://doi.org/10.1007/s00248-005-0144-9.

He, Z. et al., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME. J. 1, 67–77.

Jorgensen, B.B. et al., 2011. Deep subseafloor microbial cells on physiological standby. Proc. Natl. Acad. Sci. US,108, 18193–18194.

Jacob, J., Chandramohankumar, N., Jayaraj, K.A., Raveendran, T.V., Balachandran, K. K., Josep, T., Nair, M., AuchuthanKutty, C.T., Nair, K.K.C., 2007. Biogeochemistry of the Surficial Sediments of the Western and Eastern Continental Shelves of India. J. Coast. Res. 24 (5), 1240–1248.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., et al., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593–599. https://doi.org/10.1038/nrmicro2386. PMID:20601964.

Kallmeyer, J., Pockalny, R., Adhikari, R.R., Smith, D.C., D'Hondt, S., 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. Proc. Natl. Acad. Sci. USA 109, 16213–16216.

Kurian, S., Agnihotri, R., Borole, D.V., Naqvi, S.W.A., Ferreira, A.N.A.M., Vale, C., 2009. Possible solar control on primary production along the Indian west coast on decadal to centernnial time scale. J. Quat. Sci. 24, 109–116.

Lai, L, C., Kosorukoff, A. L., Burke, P. V., Kwast, K. E., 2006. Metabolic-State-Dependent Remodeling of the Transcriptome in Response to Anoxia and Subsequent Reoxygenation in Saccharomyces cerevisiae. Eukaryot Cell. 5(9): 1468–1489. doi: 10.1128/EC.00107-06.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71 (12), 8228±35. https://doi.org/10.1128/AEM.71.12.8228-8235.2005.

Luke, C., Speth, D.R., Kox, M.A., Villanueva, L., Jetten, M.S., 2016. Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone. Peer J 4, e1924.

Parvathi, A., Jasna, V., Aswathy, V.K., Nathan, V.K., Aparna, S., Balachandran, K.K., 2019. Microbial diversity in a coastal environment with co-existing upwelling and mud-banks along the south west coast of India. Molecular Biology Reports. https://doi.org/10.1007/s11033-019-04766-y.

Ramaiah, N., Raghukumar, S., Gauns, M., 1996. Bacterial abundance and production in the central and eastern Arabian Sea. Current Sci. 71 (11), 878–882.

Rao, D.S., Mathew, K.J., Gopinathan, C.P., Ragunathan, A., Murty, A.V.S., 1980. Mud banks and coastal erosion in relation to fisheries. Technical and Extension series. 19, 1–10.

Rao, V.P., Wagle, B.G., 1997. Geomorphology and surficial geology of the western continental shelf and slope of India: A review. Current Sci. 73 (4), 330–350.

Rohwer, F., Breitbart, M., Jara, J., Azam, F., Knowlton, N., 2001. Diversity of bacteria associated with the Caribbean coral Montastraeafranksi. Coral Reefs 20, 85–91. https://doi.org/10.1007/s003380100138.

Riesenfeld, C.S., Schloss, P.D., Handelsman, J., 2004. Metagenomics: genomic analysis of microbial communities. Annu. Rev. Genet. 38, 525–552.

Kapley, A., Siddiqui, S., Misra, K., Ahmad, S.M., Purohit, H.J., 2007. Preliminary analysis of bacterial diversity associated with the Porites coral from the Arabian Sea. World J. Microbiol. Biotechnol. 23, 923–930. https://doi.org/10.1007/s11274-006-9315-1.

Mukherjee, S., Das, P., Sen, R., 2006. Towards commercial production of microbial surfactants. Trends in Biotechnology 24, 509–515.

McKew, B.A., Coulon, F., Yakimov, M.M., Denaro, R., Genovese, M., Smith, C.J., Osborn, A.M., Timmis, K.N., McGenity, T.J., 2007. Efficacy of intervention strategies for bioremediation of crude oil in marine systems and effects on indigenous hydrocarbonoclastic bacteria. Environ Microbiol 9 (6), 1562–1571.

Nair, H.P., Puthusseri, R.M., Vincent, H., Bhat, S.G., 2017. 16S rDNA-based bacterial diversity analysis of Arabian Sea sediments: A metagenomic approach. Ecol. Genet. Genomics 3–5, 47–51. https://doi.org/10.1016/j.egg.2017.09.001.

Nogales, B., Lanfranconi, M.P., Pina-Villalonga, J.M., Bosch, R., 2011. Anthropogenic perturbations in marine microbial communities. FEMS Microbiol Rev. 35, 275–298.

Sakalle, K., Rajkumar, S., 2008. Isolation of Crude Oil Degrading Marine Bacteria and Assessment for Biosurfactant Production. The Internet Journal of Microbiology 7 (2), 1–7.

Santiago-Vázquez, L.Z., Brück, T.B., Brück, W.M., Duque-Alarcón, A.P., McCarthy, P.J., Kerr, R.G., 2007. The diversity of the bacterial communities associated with the azooxanthellatehexacoralCirrhipatheslutkeni. The ISME Journal 1, 654–659.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., et al., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Sebastain, T., Nath, B.N., Naik, S., Borole, D.V., Pierre, S., Yazing, A.K., 2017. Offshore sediments record the history of onshore iron ore mining in Goa. Mar. Pollut. Bull. 114, 805–815.

Spatharis, S., Roelke, D.L., Dimitrakopoulos, P.G., Kokkoris, G.D., 2011. Analyzing the (mis)behavior of Shannon index in eutrophication studies using field and simulated phytoplankton assemblages. Ecol Indic. 11 (2), 697–703.

Stratford, J.P., Beecroft, N.J., Slade, R.C., Gru-Èning, A., Avignone-Rossa, C., 2014. Anodic microbial community diversity as a predictor of the power output of microbial fuel cells. Bioresour Technol. 156, 84–91. https://doi.org/10.1016/j.biortech.2014.01.041.

Tao, Y., Li, J.B., Rui, J.P., Xu, Z.C., Zhou, Y., Hu, X.H., 2014. Prokaryotic communities in pit mud from different-aged cellars used for the production of Chinese strong-flavored liquor. Appl Environ Microbiol. 80 (7), 2254–2260. https://doi.org/10.1128/AEM.04070-13.

Thiyagarajan, V., Tsoi M.M.Y Zhang, W., Qian, P.Y., 2010. Temporal variation of coastal surface sediment bacterial communities along an environmental pollution gradient. Mar Environ. Res 70: 56–64.

Vikram, S., Guerrero, L.D., Makhalanyane, T.P., Le, P.T., Seely, M., Cowan, D.A., 2016. Metagenomic analysis provides insights into functional capacity in a hyperarid desert sediment niche community. Environ Microbiol 18, 1875–1888. https://doi.org/10.1111/1462-2920.13088.

Ward, B.B., Devol, A.H., Rich, J.J., Chang, B.X., Bulow, S.E., Naik, H., et al., 2009. Denitrification as the dominant nitrogen loss process in the Arabian Sea. Nature. 461, 78–81.

Wei, Z., Hu, X., Li, X., Zhang, Y., Jiang, L., Li, J., Guan, Z., Cai, Y., Niao, X., 2017. The rhizospheric microbial community structure and diversity of deciduous and evergreen forests in Taihu Lake area. China. PLoSONE 12 (4), e0174411. https://doi.org/10.1371/journal.pone.0174411.

Woebken, D., Lam, P., Kuyper, S, M.M., Naqvi, S., Kartal, B., Strous, M., et al., 2008. A microdiversity study of anammox bacteria reveals a novel Candidatusscalinduaphylotype in marine oxygen minimum zones. Environ. Microbiol. 10, 3106–3119

Won, N., Kim, K.H., Kang, J.H., Park, S.R., Lee, H.J., 2017. Exploring the Impacts of Anthropogenic Disturbance on Seawater and Sediment Microbial Communities

in Korean Coastal Waters Using Metagenomics Analysis. Int. J. Environ. Res. Public Health. 14, 130. https://doi.org/10.3390/ijerph14020130.

Zinger, L., Amaral-Zettler, L.A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B., et al., 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS One 6, e24570. https://doi.org/10.1371/journal.pone.0024570. PMID:21931760.