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A snapshot on spatial and vertical distribution of bacterial communities in the eastern Indian Ocean

WANG Jing[1, 2], KAN Jinjun[3], BORECKI Laura[3], ZHANG Xiaodong[1, 2], WANG Dongxiao[4], SUN Jun[1, 2*]

[1] College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin 300457, China

[2] Tianjin Key Laboratory of Marine Resources and Chemistry, Tianjin University of Science and Technology, Tianjin 300457, China

[3] Stroud Water Research Center, Avondale, PA 19311, USA

[4] State Key Laboratory of Tropical Oceanography (LTO), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

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Abstract

Besides being critical components of marine food web, microorganisms play vital roles in biogeochemical cycling of nutrients and elements in the ocean. Currently little is known about microbial population structure and their distributions in the eastern Indian Ocean. In this study, we applied molecular approaches including polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE) and High-Throughput next generation sequencing to investigate bacterial 16S rRNA genes from the equatorial regions and the adjacent Bay of Bengal in the eastern Indian Ocean. In general, *Bacteroidetes*, *Proteobacteria* (mainly *Alpha*, and *Gamma*), *Actinobacteria*, *Cyanobacteria* and *Planctomycetes* dominated the microbial communities. Horizontally distinct spatial distribution of major microbial groups was observed from PCR-DGGE gel image analyses. However, further detailed characterization of community structures by pyrosequencing suggested a more pronounced stratified distribution pattern: *Cyanobacteria* and *Actinobacteria* were more predominant at surface water (25 m); *Bacteroidetes* dominated at 25 m and 150 m while *Proteobacteria* (mainly *Alphaproteobacteria*) occurred more frequently at 75 m water depth. With increasing water depth, the bacterial communities from different locations tended to share high similarity, indicating a niche partitioning for minor groups of bacteria recovered with high throughput sequencing approaches. This study provided the first "snapshot" on biodiversity and spatial distribution of *Bacteria* in water columns in the eastern Indian Ocean, and the findings further emphasized the potential functional roles of these microbes in energy and resource cycling in the eastern Indian Ocean.

Key words: eastern Indian Ocean, water column, bacterial community, pyrosequencing

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1 Introduction

Microbes are the most abundant and diverse group of living organisms in the world (Whitman et al., 1998), and they play vital functional roles in ecosystems, including marine environments. For instance, together with zooplankton, bacteria remineralize ~90% of the settling particles in the water column, providing nutrients for marine phytoplankton as well as food for benthic organisms (Rixen et al., 2008; Suess, 1980). Marine bacteria contain metabolically diverse and versatile groups who participate in critical biogeochemical metabolisms, and recycle significant nutrients including carbon, nitrogen, sulphur, phosphorus, and iron, etc. (Goñi-Urriza et al., 1999; Jiao et al., 2007; Emerson et al., 2010). By mediating chemical transformations, marine bacteria impact the nutrient composition and energy flow in both water column and sediments (Arrigo, 2005; Fennel et al., 2005). Advanced sequence analyses revealed distinct biological patterns in marine environments: *Proteobacteria* dominated in the Atlantic Ocean and sediment (Fuhrman et al., 1993; Schauer et al., 2010), the Arctic Ocean, as well as the Pacific Ocean especially during summer; while *Bacteroidetes* became more abundant than *Proteobacteria* in the Pacific Ocean during winter (Suh et al., 2014; Han et al., 2014).

Compared to the other oceans, the Indian Ocean is relatively poorly studied. Johnson et al. (1968) explored the composition of bacteria in the Indian Ocean by isolation of the cultivable species, and additional isolation-based studies followed (Nair et al., 1994; Bharathi and Nair, 2005). More recently, sediment bacterial distribution in the central margin and two sediment cores in the equator region of the Indian Ocean were investigated (Hoek et al., 2003; Khandeparker et al., 2014). In order to explore the vital roles that microbes may play, further characterization of the composition and distribution of microorganisms in the Indian

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*Corresponding author, E-mail: phytoplankton@163.com

Ocean is needed.

The Indian Ocean is characterized by its equatorial region and its two semi-enclosed basins in the north, the Arabian Sea and the Bay of Bengal (BOB), which are both strongly influenced by monsoon (Rixen et al., 2008; Fine et al., 2008). Strong stratification induced by seasonal monsoon suppresses upwelling and therefore fewer nutrients from the deep waters are supplied, making the eastern equatorial Indian Ocean a typical oligotrophic area (Kumar et al., 2009). In the Arabian Sea, dust input, enhanced advection, and vertical eddy mixing carries small detritus to the central/eastern Arabian Sea, where intensified remineralization consumes oxygen and provides nutrients for the marine life cycle (McCreary Jr et al., 2013). Meanwhile, the oxygen depletion in the BOB is weaker than the Arabian Sea partly due to the short length of time for oxygen consuming remineralization, and also lack of detritus transport for organic matter accumulation (Kumar et al., 2004). In addition, river runoff, evaporation, seasonal advection and mixing in BOB results in low surface salinity and high surface stratification (Rao et al., 1994; Kumar et al., 2002). The oceanic circulation and heat storage variation caused by low surface salinity strengthens the vertical stratification (Nyadjro et al., 2013). Finally, BOB is also characterized by its low productivity (Madhupratap et al., 2003; Kumar et al., 2004) and high nitrification rate (Srinivas et al., 2011). Since microbes are key players in participating critical biogeochemical processes such as transformation of nitrogen, carbon, iron and sulfur, their composition and distribution characteristics in BOB are of great interest to us.

In this study, we collected water samples in the eastern Indian Ocean in both the Bay of Bengal and the equatorial area during the inter-monsoon of 2011. In order to investigate the microbial compositions and their water column distributions, molecular approaches including polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE) and high throughput sequencing analysis (454 pyrosequencing) were applied to water samples collected from different locations and layers. The high throughput sequencing data provided information on detailed community structures including the dominant and rare populations, and more importantly retrieved those uncultivable components as well. The distribution pattern and potential function of dominant groups were discussed and their interactions with ambient environments were explored. This study provided the first "snapshot" on bacterial diversity and their spatial distributions in the eastern Indian Ocean, and these results underlined the need for further investigation on the population dynamics of microbial communities and relevant functional groups (e.g., N transformation) in the eastern Indian Ocean.

2 Materials and methods

2.1 *Sampling stations*

Water samples were collected during a multidisciplinary cruise carried out in the eastern Indian Ocean by R/V *Shiyan 1* from March 30 to May 4, 2011. Four transects were investigated, including equatorial (I4), longitudinal (I3), parallel to equator (I6) and parallel to the coastline of the Sumatra (I5). Six stations were chosen for bacterial profile analysis with multiple depths at each station: I304, I405, I413, I503, I612 and I613 (Fig. 1). Stations I413 and I612, as representatives for the equatorial region and BOB respectively, were selected for pyrosequencing analysis at the depths of 25 m, 75 m and 150 m.

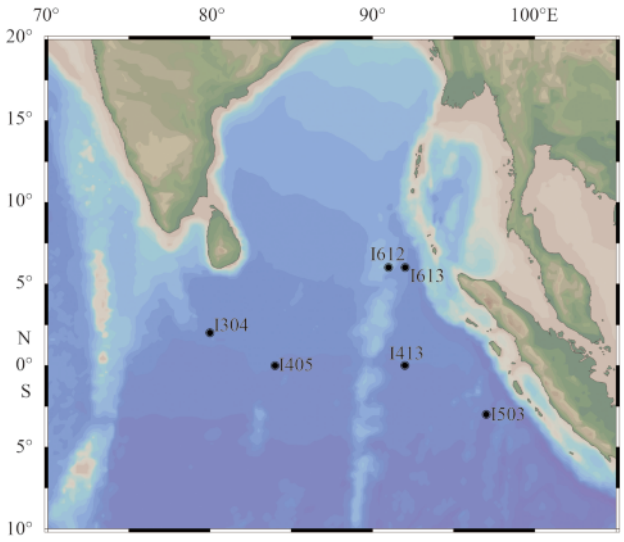

Fig. 1. Map showing the location of sampling stations in the eastern Indian Ocean.

2.2 *Sample collection and environmental parameter measurements*

Water samples were collected by Niskin bottles (General Oceanic Inc.) mounted to the Sea-Bird SBE-911 Plus V2 Conductivity-Temperature-Depth (CTD) system. For each sample, 2 L seawater were filtered through 0.22 μm GTTP filters (47 mm in diameter, Merck Millipore, Germany) under low pressure vacuum (<13 kPa). The filters were then placed into a 2 mL microtube with sterilized forceps and then frozen in liquid nitrogen immediately. The filters were later transported on dry ice to the laboratory and stored at –80°C until deoxyribonucleic acid (DNA) extraction. Hydrographic parameters including the temperature, salinity, pH and chlorophyll fluorescence were measured on site by CTD. Chlorophyll *a* (Chl *a*) concentrations were determined using fluorescence method followed by Parsons et al. (1984). Water samples were filtered through Whatman GF/F filters under a filtration vacuum of less than 100 mmHg. The filters were placed into a Petri dish box which wrap with the aluminium-foil paper, stored in the dark at –20°C until it been analyzed in the laboratory. The filter was then extracted by 90% acetone, and stored in the dark at 4°C for 24 h. The Chl *a* content was then measured using a Turner designs Thilogy™ laboratory fluorometer.

2.3 *DNA extraction and PCR-DGGE*

Total community genomic DNA was extracted from filters following previously described protocol (Kan et al., 2006b). DNA was quantified by spectrophotometric absorption at 260 nm, and the purity was assessed from absorbance ratios at 260/280 and 260/230 nm using an ND-2000 Nanodrop spectrometer (Thermal Scientific, Wilmington, DE).

16S rRNA genes of bacterial communities were amplified with primers 1070f (ATGGCTGTCGTCAGCT) and GC-clamped 1392r (ACGGGCGGTGTGTAC) (Kan et al., 2006b). The PCR reactions were performed using an automated Eppendorf Mastercycler Thermal Cycler (Perkin-Elmer, Norwalk, CT). Denaturing gradient gel electrophoresis (DGGE) was performed using the Dcode system (Universal Mutation Detection System, Biorad) as previously described (Muyzer et al., 1993; Kan et al., 2006b). Briefly, equal amounts of PCR products were loaded onto an 8% vertical polyacrylamide gel containing a 45%–65% denaturing gradient

made of urea and formamide. Gels were electrophoresed at 60°C and 70 V for 16 h and visualized with SYBR Gold staining (Life Technologies, NY). Gel images were analyzed using GelComparII v.5.10 (Applied Maths, Austin, TX, USA). A binary data matrix (presence or absence of bands) was used for all subsequent analysis to prevent patterns from being biased by variable band intensities.

2.4 *Pyrosequencing and data processing*

Pyrosequencing of V1–V3 region of 16S ribosomal RNA genes followed the previous protocol (Mosher et al., 2013): 27F oligonucleotide primer was fused to the 454 Titanium sequencing primer A adapters and reverse primer 534R was fused to variable key tags for multiplexing and to the 454 Titanium sequencing primer B. Samples were denatured (95°C, 5 min), followed by 28 cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s), and extension (68°C, 45 s) with a final extension (68°C, 7 min). Then the amplicons were pooled in equimolar concentrations and the purity, concentration and size were estimated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Waldbronn, Germany). Emulsion reactions of paired samples and sequencing were performed on a 454 Life Sciences Genome Sequencer FLX using Titanium chemistry (Roche Diagnostics, Indianapolis, IN) with the unidirectional amplicon library sequencing protocol and emPCR kit II (Roche) at the Perelman School of Medicine of the University of Pennsylvania.

Raw 454 Titanium sequences were sorted by tag sequences, and filtered for length, quality and chimera removal using the software package Mothur (Edgar et al., 2011; Schloss et al., 2009). After alignment, pre-clustering and clustering at the 97% level representative sequences for each cluster were obtained through Mothur. Sequences were uploaded into *my*RDP through the Ribosomal Database Project (RDP) and percent identity scores were obtained against type-strains through SeqMatch protocol using the National Center for Biotechnology Information (NCBI) taxonomy (Cole et al., 2009).

2.5 *NMDS (nonmetric multidimensional scaling) analyses on both PCR-DGGE and pyrosequencing data*

To better compare the population structures and explore the distribution patterns, nonmetric multidimensional scaling (NMDS) was applied to analyze data from both PCR-DGGE and pyrosequencing by following previous protocol (Kan et al., 2006a). Briefly, a binary data matrix or relative abundance data for high throughput sequencing were compared and pairwise-comparisons were calculated to obtain similarity/distance among samples. Based on the distance matrix, NMDS was performed with the multidimensional scaling (MDS) procedure of the SAS System (SAS/STAT, 2008). The differences between bacterial communities were illustrated in two-dimension MDS plots. The communities with the higher similarity were plotted closer, and communities with the lower similarity were located further apart. To judge the degree to which this ordination matches the distance matrix, the stress value of MDS was examined. Stress value less than 0.1 indicated a good ordination, with little risk of misinterpretation of banding patterns (Clarke, 1993).

3 Results

As the third largest ocean around the world, the Indian Ocean is strongly stratified and oligotrophic (Hood et al., 2009). Vertical profiles of the temperature, salinity and Chl *a* measurement at each station were shown in Fig. 2. The temperature and salinity profiles followed typical distributions except for a disturbance in salinity concentration observed at surface and subsurface layers of Stas I612 and I613, which was possibly caused by rainfall. The deep chlorophyll *a* maximum (DCM) layer varied across stations, ranging from 50 m to 75 m in depth.

3.1 *Spatial distribution of bacterial communities in the eastern Indian Ocean by PCR-DGGE*

The DGGE image showed pronounced differences in vertical and horizontal distribution patterns of bacterial communities in the eastern Indian Ocean (Figs 3 and 4). Changes in bacterial diversity (number of bands) as well as community composition (position of bands) were observed among stations and among depths within stations. Some stations (e.g., I503, I304) showed decreasing diversity with increasing depth. Other stations showed similar or increased diversity with increasing depth and most showed changes in the dominant band pattern. Some common bands were also observed at most or all stations and depths. In addition to the presence/absence of DGGE bands, the relative abundance of bacterial groups as indicated by band intensity varied with depth and location. Certain dominant/distinct bands from the DGGE gel (Fig. 3) were sequenced and identified (Table 1). All the identified bands belonged to *Alpha* and *Gammaproteobacteria*. Bands 2 and 4 (*Stenotrophomonas* sp.) were universally distributed among all the stations and Band 10 (*Sulfitobacter dubius*) occurred at most of the stations/depths. In contrast, other bands (e.g., Bands 1, 6, 7 and 8) were only presented at certain stations (Table 1 and Fig. 3).

NMDS ordination, a statistical representation of the similarity of samples based on the DGGE pattern, showed both hori-

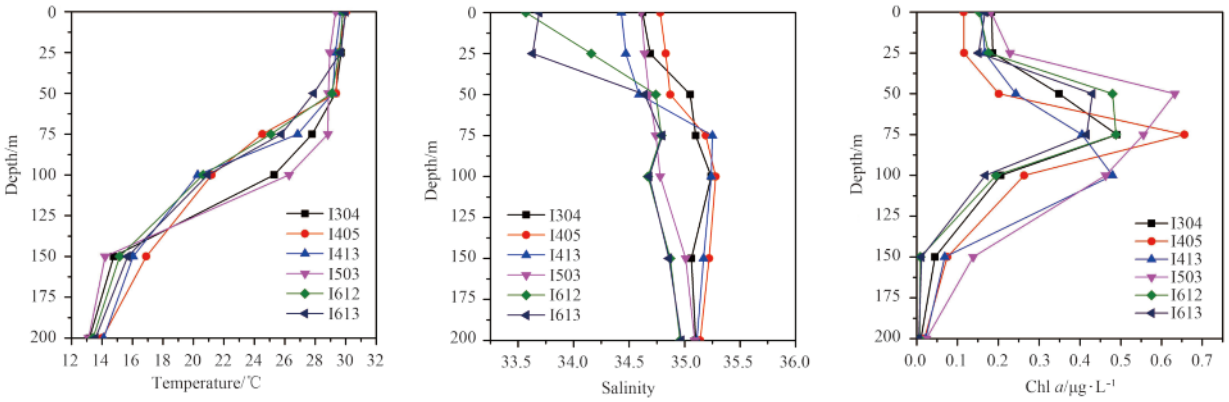

Fig. 2. Temperature, salinity and Chl *a* content in the upper 200 m water column of the six sampling stations.

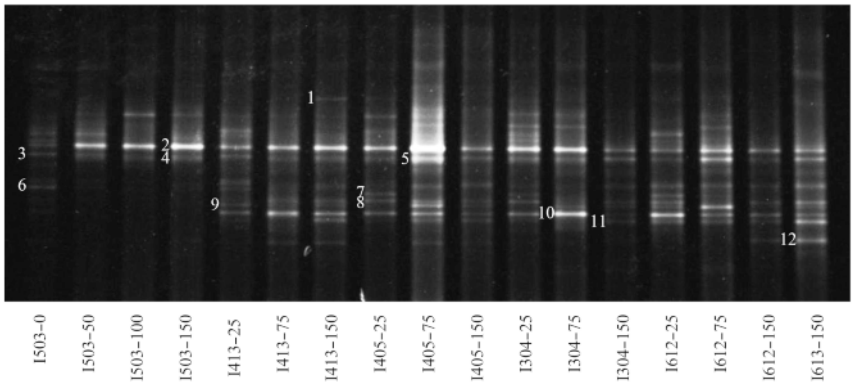

Fig. 3. DGGE fingerprints of bacterial community structure and distribution. Samples from different depths and stations were compared and analyzed.

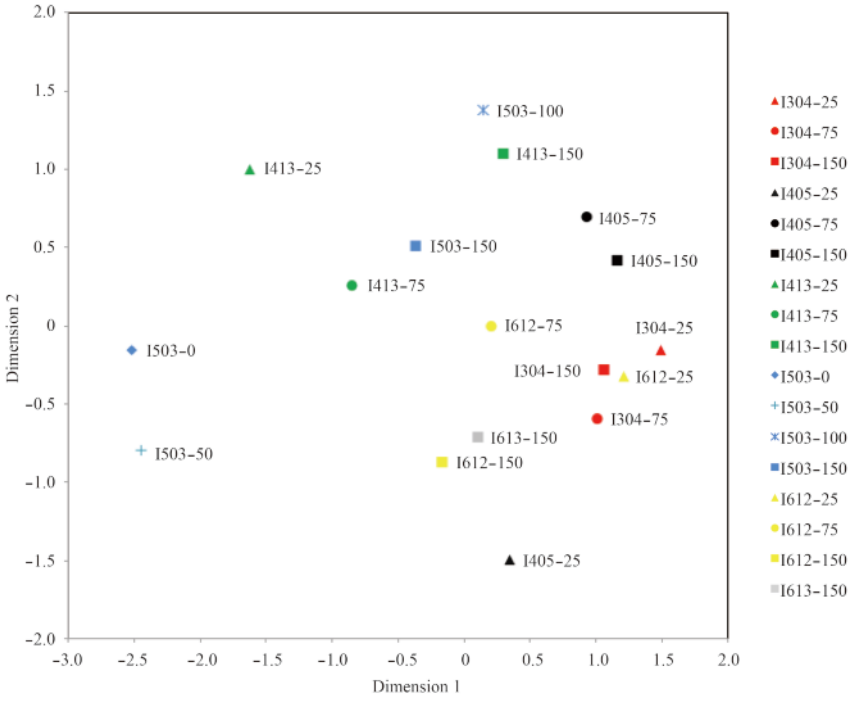

Fig. 4. NMDS ordinations of DGGE banding patterns from Fig. 3. Stations were indicated by color and depths are indicated by data point shape. Stress=0.167.

zontal and vertical distribution patterns: all the samples from different stations and depths were clustered based on location and depths as well (Fig. 4). Stations I304, I612, and I613 were the most similar, and the depths among these stations were also similar, with I612 and I613 closely grouped at 150 m, and I304 and I612 closely grouped at 25 m. At Sta. I503, samples from four depths did not share high similarity but samples at 0 m and 50 m were more closely clustered to each other than to samples from 100 m and 150 m. Deeper samples at Sta. I405 were closely related to each other and different than the 25 m sample. The samples at Sta. I413 were neither closely clustered based on location nor were they clustered to other stations based on depth.

3.2 *Community composition by pyrosequencing*

In order to further explore the bacterial composition and its distribution in the eastern Indian Ocean, Stas I413 and I612 were selected for more detailed community analyses with pyrosequencing. These longitudinal paralleled stations were chosen as representatives for equatorial region and the BOB, respectively. Both were sampled at a water depth of 25 m, 75 m and 150 m. In total, 59 775 sequencing reads were obtained from these six samples. A summary of the dominant bacterial phyla was listed in Table 2.

The community composition of each sample at phylum level was shown in Fig. 5. In general, *Bacteroidetes*, *Proteobacteria* (mainly *Alpha* and *Gamma*), *Actinobacteria*, *Cyanobacteria*, and *Firmicutes* were dominant groups among all the samples investigated. The bacterial communities showed similar vertical distribution patterns within these two stations. At 25 m, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* and *Cyanobacteria* were dominant groups at both stations. *Proteobacteria* became the most dominant at 75 m, with *Bacteroidetes* and *Actinobacteria* making up the majority of the remainder. Station I413 was more diverse at 150 m, with *Bacteroidetes* as the most dominant followed by *Proteobacteria*, *Firmicutes*, and *Actinobacteria*; while *Bacteroid-*

Table 1. Sequence identifications for DGGE bands from Fig. 3

Band	Accession	Description	Source	Phylum	Similarity/%
1	AB682295	*Oleibacter marinus* NBRC 105760	seawater off Jakarta, Indonesia	*Gammaproteobacteria*	97
2	JQ975872	*Stenotrophomonas* sp. Y007	Indian Ocean	*Gammaproteobacteria*	100
	JQ860235	*Alteromonas* sp. QHL20	Qinghai Lake		
3	HE680095	*Erythrobacter* sp. A3	Humma Salt Pan, India	*Alphaproteobacteria*	96
4	JQ975872	*Stenotrophomonas* sp. Y007	Indian Ocean	*Gammaproteobacteria*	99
	HQ342692	*Alteromonas* sp. Liv16S-L158	Indonesian Coral Reef		
5	JX407256	*Alteromonas* sp. RKVR23	associated with coral, Bahamas	*Gammaproteobacteria*	99
6	JQ739459	*Roseovarius* sp. M-M10	sediment, South Korea	*Alphaproteobacteria*	97
	GU584141	*Sulfitobacter* sp. 114Z-13	sea water, Antarctica		
7	JX075063	*Ruegeria* sp. K2	Osprey Reef, Coral Sea, Australia	*Alphaproteobacteria*	99
8	HE572674	*Phaeobacter* sp. MC A8	Strait of Messina, Italy	*Alphaproteobacteria*	99
9	JQ739459	*Roseovarius* sp. M-M10	sediment, South Korea	*Alphaproteobacteria*	99
	FJ848891	*Sulfitobacter* sp. DHVB8	Lau Basin hydrothermal vent		
10	JQ806410	*Sulfitobacter dubius* HME8274	seawater	*Alphaproteobacteria*	99
11	AB681671	*Alcanivorax* sp. NBRC 102024	seawater, Japan	*Gammaproteobacteria*	98
12	JX310256	*Sulfitobacter* sp. 199Z-19	Southern Ocean	*Alphaproteobacteria*	98

Table 2. Relative abundance (%) of bacterial groups at Stas I413 and I612

Taxon	Sample					
	I413-25	I413-75	I413-150	I612-25	I612-75	I612-150
Bacteroidetes	43.49%	18.49%	62.09%	34.44%	24.47%	49.99%
Proteobacteria	34.18%	69.55%	18.75%	28.37%	68.11%	45.01%
Cyanobacteria	3.39%	1.36%	0.39%	10.99%	0.92%	0.09%
Actinobacteria	15.50%	4.47%	4.69%	20.34%	4.26%	1.69%
Firmicutes	0.01%	0.38%	7.01%	1.17%	0.92%	0.36%
unclassified Bacteria (miscellaneous)	3.19%	5.46%	4.34%	4.09%	1.29%	0.96%
Planctomycetes	0.31%	0.33%	1.62%	0.47%	0.23%	1.12%
Acidobacteria	–	–	0.96%	–	–	0.63%
Spirochaetes	0.05%	–	–	–	–	–
SAR46 cluster	0.01%	–	0.03%	–	–	0.01%
Bacillariophyta	0.01%	–	–	–	–	0.01%
Chlorobi	–	0.02%	0.04%	–	–	0.01%
Candidate division TM7	–	0.01%	–	–	–	–
Chloroflexi	–	–	0.06%	–	–	0.08%
Thermodesulfobacteria	–	–	0.04%	–	–	0.04%

etes and *Proteobacteria* alone dominated at Sta. I612. This suggested good adaptation of the dominant groups to depth in the eastern Indian Ocean.

Diversity and community composition varied with depth in these two stations. Within the phylum *Proteobacteria*, *Alphaproteobacteria* was the major subdivision detected at all depths, but *Gammaproteobacteria* was also present at 150 m layers, despite being almost undetected at other layers. *Actinobacteria* showed a highest abundance at 25 m, and then decreased sharply at 75 m and 150 m (Fig. 5). *Cyanobacteria* showed a similar distribution pattern as *Actinobacteria*. In contrast, *Planctomycetes* was more abundant at 150 m than in shallow waters at both stations. A distinct distribution pattern was also detected for *Firmicutes*, which was quite abundant at 150 m (7%) at Sta. I413 but less than 1% for other water samples (Fig. 5).

NMDS ordination clearly echoed this pattern and demonstrated that the distribution was impacted by both depth and location. Along Dimension 1, depth determined the bacterial distribution and samples from the same layers were similar (Fig. 6). Meanwhile, location also influenced the distribution and all samples from the same station were clustered together and stations were separated along Dimension 2 (Fig. 6). Based on the variations that each ordination (74% at Dimension 1 and 19% at Dimension 2) explained, the distribution was likely driven primarily by depth and secondarily by location (Fig. 6).

4 Discussion

Highly diversified groups of bacteria were detected in the eastern Indian Ocean. As implied by 16S rRNA gene analysis, seven dominant phyla were identified in addition to unclassified bacteria. In the similar studies from other oceans, such as the South Pacific Ocean, the North Pacific Ocean, the Atlantic Ocean, the South China Sea and the Southern Ocean, similar groups of bacteria were detected. *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* were the major dominant groups, and *Planctomycetes*, *Cyanobacteria*, *Acidobacteria* and *Firmicutes* were also commonly found (Pace, 1997; Keller and Zengler, 2004). Predominance of *Proteobacteria* over other phylum at all depths has been observed in the North Pacific Ocean, the South China Sea, and the Southern Ocean (Brown et al., 2009; Du et al., 2013; Wilkins et al., 2013). During the project ICoMM for the full survey of marine microbes globally, *Alphaproteobacteria* showed an overwhelm-

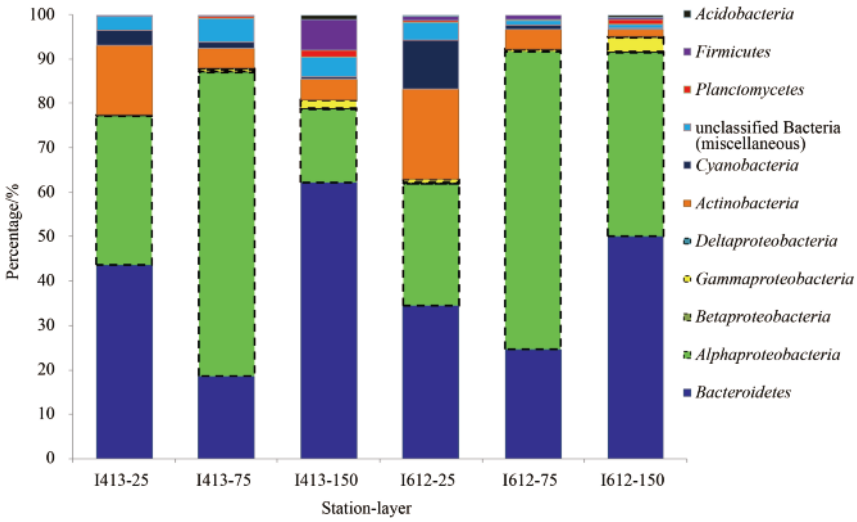

Fig. 5. Phylum level composition (relative abundance) of bacterial communities in the eastern Indian Ocean. Dashed areas of the columns represented taxa within the phylum of *Proteobacteria*.

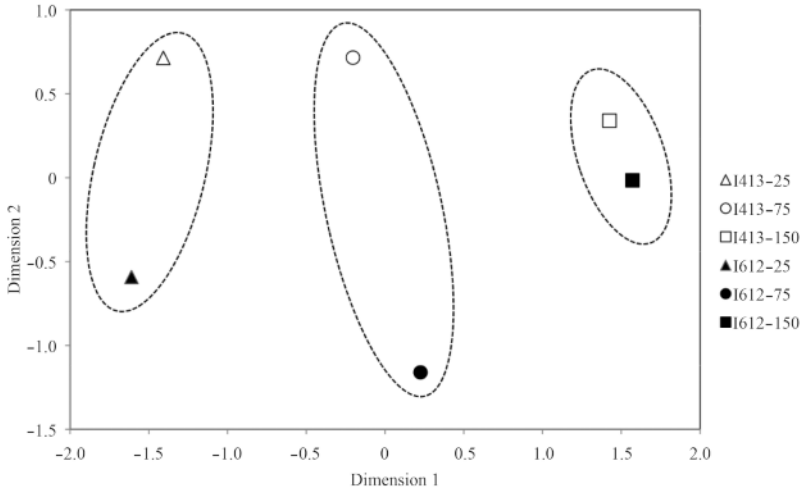

Fig. 6. Two dimensional NMDS plots for bacterial population structures (relative abundance) at genus level (97%) (Stress<0.01). Samples from Sta. I413 were marked by transparent points and samples from Sta. I612 were marked by solid points.

ing dominant presence over other groups in all the studied oceans, including water column and sediment from the Pacific Ocean, the Atlantic Ocean, the Southern Ocean, the Arctic Ocean and the Indian Ocean (Amaral-Zettler et al., 2010). In our study, *Proteobacteria* (mainly *Alphaproteobacteria*) was the most abundant group retrieved at 75 m. However, *Bacteroidetes*, which was only observed to be dominant in the Pacific during winter time (Suh et al., 2014; Han et al., 2014), was the most prevalent at 25 m and 150 m (Fig. 5). Dominance of *Bacteroidetes* over *Proteobacteria* has also been documented in coastal areas, and attributed 10%–30% of the total bacterial counts (Glöckner et al., 1999; Kirchman, 2002; Amaral-Zettler et al., 2010); but in our study, *Bacteroidetes* comprised up to 50% of the total bacterial sequences at the depth of 150 m.

The most abundant groups of bacteria in *Alphaproteobacteria* revealed in this study by both DGGE and pyrosequencing were *Sulfitobacter* sp. (7 744 counts in total), *Roseobacter* sp. (7 727 counts in total) and *Novosphingobium naphthalenivorans* (6 548 counts in total). These bacterial groups likely participated in critical processes of sulfur and carbon cycling (Moran and Armbrust, 2007), as groups of *Sulfitobacter* sp. are sulfite oxidizers and have been isolated from marine environments, such as in Mediterranean seawater, sediments, and organisms such as sea star and seagrass (Pukall et al., 1999; Ivanova et al., 2004). Marine *Roseobacter* contains genes involved in carbon monoxide oxidation, dimethylsulfoniopropionate demethylation and aromatic compound degradation (Moran et al., 2007), and therefore they are involved in sulfate transport and metabolisms in the open ocean (Buchan et al., 2004; Brinkhoff et al., 2008). Further, as a novel polycyclic aromatic hydrocarbon (PAH)-degrading bacterium, *Novosphingobium* sp. has been isolated from the Indian Ocean in deeper waters (Yuan et al., 2009), and its dominance in upper layer waters as well as its function deserves further investigation, not only in the Indian Ocean but also globally. Although not much information is available on the concentration of PAH in the eastern Indian Ocean, we suspect PAH originating from ocean carbon cycling might account for the high occurrence of *Novosphingobium naphthalenivorans* at the studied sites.

Although BOB was characterized by its low productivity

(Madhupratap et al., 2003), there was a high deposition rate of atmospheric N, P, Fe at BOB (Srinivas and Sarin, 2013) and the nitrification rate was also comparable to Arabian Sea (Srinivas et al., 2011). Several studies have been focused on investigating the distribution of *Trichodesmium* and how the high abundance of nitrogen was utilized in the Indian Ocean (Jason et al., 1995). Since bacteria are involved in almost every pathway of nitrogen transformations, we expected to recover relevant nitrifying microbes at surface water or denitrifying bacteria in the lower part of the eutrophic zone, as the oxygen was depleted along a vertical gradient. As a good example, *Thiohalomonas denitrificans* was found solely at depth of 150 m. Other denitrifying bacteria were also frequently retrieved from deep layers, such as *Thiobacillus prosperus*, *Micrococcus* sp., and *Pseudomonas* sp., etc. Another important nitrogen transformation process is anaerobic ammonium-oxidation (Anammox). Anammox bacteria were frequently found in deeper ocean layers, and *Planctomycetes* represented the most dominant Anammox bacterial group in the oxygen minimum zone in the ocean (Woebken et al., 2008). In our study, increasing relative abundance of *Planctomycetes* was detected at 150 m layer compared to 25 m and 75 m layers at both sites. However, with limited phylogenetic and metabolic information, we could not conclude that the *Planctomycetes* sequences were Anammox relevant groups. Nevertheless, with the importance of nitrogen cycling in the eastern Indian Ocean and its relevance to global nutrient balance, nitrogen transformation and the characterization of relevant functional genes in the eastern Indian Ocean deserve future studies and are under investigation in our research group.

In agreement with previous studies showing parallel effects on shaping and sustaining the microbial community structure by environmental contexts and genetic mechanisms together (Konstantinidis and DeLong, 2008), the hydrologic characters between the studied sites demonstrated a good niche separation for dominant bacterial groups. PCR-DGGE provided only low-resolution information on population dynamics of the dominant groups, however, in general the identified bands agreed well with our high throughput sequencing data, and the banding pattern showed a clear spatial and vertical distribution. Fewer bands were detected in three layers of Sta. I503, while the surface sample in Sta. I503 had similar bands pattern with other layers from different stations. During the inter-monsoon stage in eastern Indian Ocean, the area around Sta. I503 was the initial stage of the subsurface water entrainment into the mixed layer, therefore the surface water was distinct from other layers, but mixing may minimize the difference of the entrained surface water with other deeper waters in other stations. Although a decline in total bacterial count was observed with depth in the equatorial area of the western Indian Ocean (Burkill, 2002), our data did not show a significant population structure shift along a vertical profile.

The more detailed characterization of bacterial population structures by pyrosequencing for Stas I413 and I612 demonstrated a distinct distribution pattern where bacterial community were separated by depth first, and then location. The depth profiles of microbial distribution were in line with the vertical distribution of physical, chemical and biological environmental parameters (Fig. 2). Biomass of primary producers (i.e., phytoplankton) was correlated with hydrologic structure through the fluctuation of DCM layer in the equatorial eastern Indian Ocean, and the thermocline and halocline inosculated well with DCM layer ranging from 50 m to 78 m deep during the spring inter-monsoon. The phytoplankton produced high concentrations of dissolved organic matter (DOM) with low-molecular-weight, and this DOM was easily consumed by *Alphaproteobacteria* due to their high efficiency in catabolizing low molecular weight DOM. Larger polymers and organic particles were accumulated at the bottom of DCM layer or deeper, which provided feasible food sources for *Bacteroidetes* at 150 m. *Bacteroidetes* are well-known particle-associated microorganisms (Kabisch et al., 2014) and their roles as degraders in oligotrophic marine environment have been well confirmed by recent genome sequencing information (Fernández-Gómez et al., 2013; Kabisch et al., 2014). Obviously, phytoplankton along with other biogeochemical parameters, such as nutrients (Fuhrman and Steele, 2008) played vital roles affecting microbial community structure, as discussed in studies on bacterial diversity in different locations of the Indian Ocean sediment (Wu et al., 2011; Khandeparker et al., 2014).

Both *Cyanobacteria* and *Actinobacteria* were documented with the highest abundance in the euphotic zone at both sites, which was in accordance with their distributions in other oceans (Bouteiller et al., 1992; Ward and Bora, 2006; Treusch et al., 2009; Oren, 2014). Interestingly *Cyanobacteria* and *Actinobacteria* were observed in the polar freshwater and sea ice of the euphotic zone of Arctic, but were absent in the Pacific sector of the Arctic Ocean, where Han et al. (2014) argued that the coastal line and melting ice may affect the distribution of these two groups of bacteria. In our study, higher relative portion of *Cyanobacteria* and *Actinobacteria* were documented at Sta. I612 compared to Sta. I413. The low surface salinity at Sta. I612, which was possibly due to the rainfall in the sampling duration, played a role as a disturbance of freshwater input that might had impacted the presence of *Cyanobacteria* and *Actinobacteria*. As one of the most frequently isolated groups, *Firmicutes* has been detected in the Indian continental margin (Parkes et al., 2009). Across all the samples analyzed in our study, *Firmicutes* was only abundant at 150 m of Sta. I413, but rarely detected in all the other samples. This might be because of the low oxygen content of 150 m layer samples and the oligotrophic conditions in the equatorial area of the eastern Indian Ocean, since most of the *Firmicutes* species in marine environments were endospore-forming bacteria (da Silva et al., 2013) who were capable of resisting the deteriorate environment (Priest, 1993) and possibly survived in the anaerobic oligotrophic zone more easily.

Primary production provides energy and nutrient for microbes, and different groups of microbes participate in different biochemical cycles, making their metabolism pathway a complex process (Azam et al., 1994; Pace, 1997). Because of a lack of upwelling in the equatorial area during the inter-monsoon in the Indian Ocean (Schott and McCreary Jr, 2001), the stable status of organic matter at each highly stratified layer may help to maintain the similar community structure at equal depths. Thus, the vertical distributions of bacterial populations were determined by geochemical properties of the eastern Indian Ocean and metabolic characteristics for different groups of bacteria.

5 Conclusions

The bacterial composition in the eastern Indian Ocean was mainly dominated by *Bacteroidetes* and *Proteobacteria*, along with *Cyanobacteria*, *Actinobacteria* and *Planctomycetes* as important components as well. The microbial community was highly diverse in the eastern Indian Ocean and certain dominant and functionally important groups showed distinct vertical distribution patterns. To our knowledge, this work provided the first "snapshot" on bacterial population structure and its vertical dis-

tribution at the eastern Indian Ocean. Driving factors that shaped the bacteria composition and the distribution pattern in eastern Indian Ocean were related to physical, chemical and biological characteristics. Our future studies are focusing on investigating population dynamics of microbial populations and their functional roles at the eastern Indian Ocean. One approach is characterizing and quantifying functional genes relevant to nutrient cycling, such as nitrogen transformation, and relating this information to the potential roles that these microbes may play in eastern Indian Ocean.

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