

Sediment bacterial communities are more complex in coastal shallow straits than in oceanic deep straits*

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Abstract Straits are ideal models to investigate the bacterial community assembly in complex hydrological environments. However, few studies have focused on bacterial communities in them. Here, comparable bacterial communities in coastal shallow Bohai Strait (BS) and oceanic deep Fram Strait (FS) were studied. The Shannon and Chao1 indices were both higher in BS than in FS. The relative abundances of the classes Deltaproteobacteria and Bacilli and the family Halieaceae were higher in BS than in FS, in contrast to the families OM1_clade and JTB255_marine_benthic_group, revealing typical characteristics of bacterial communities in coastal and oceanic regions. Cluster analysis based on the Bray-Curtis index showed that samples were clustered by depth layer in FS and BS, indicating that structures of bacterial communities would differ with increasing water depth in straits. Additionally, the cluster relationships among samples in abundant and rare communities were both similar to those in entire communities. However, the dissimilarities among samples showed a descending order as rare communities, entire communities and abundant communities. Network analysis indicated that the BS network was obviously more complex than the FS network. Filamentous bacteria Desulfobulbaceae exhibited high degree values in BS but not in FS, indicating key roles of Desulfobulbaceae in the BS. Our study provides different and common evidences for understanding microbial ecology in coastal shallow and oceanic deep straits.

Keyword: bacterial communities; Bohai Strait; Fram Strait; currents; microbial ecological network

1 INTRODUCTION

The bacterial communities are major decomposers, secondary producers (Li and Gao, 2012) and major drivers of biogeochemical processes in the marine (Fuhrman et al., 2015). It is therefore important to investigate the marine bacterial communities. One fundamental question is the bacterial community assembly laying emphasis on the structures, diversities, abundant and rare bacteria and coexist relationships (Pedrós-Alió, 2012; Nemergut et al., 2013; Lynch and Neufeld, 2015). According to the niche theory (Pontarp et al., 2012), the structures and diversities of bacterial communities are determined by environmental variables. The hydrological environments in the marine exert great effects on the environmental variables and the microbial dispersion (Gilbert et al., 2012), suggesting that the assembly of

marine bacterial community should be related to the hydrological environments such as currents. In the water body, for instance, regions with strong currents and large thermal fluctuations selected microbial communities with the greatest capacities for flexibility and evolution (Doblin and van Sebille, 2016), and the taxonomic and functional richness increased with depth, possibly resulting from vertical stratification with changes in physicochemical parameters (Sunagawa et al., 2015). Thus, these findings implied that water movements will exert effects on the microbial communities. Since sediment transportation

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is carried by currents (Shao et al., 2007), and the structures of bacterial communities in the sediments of Fram Strait with West Spitsbergen Warm Current (WSWC) and East Greenland Cold Current (EGCC) were similar along the direction of currents (Jacob et al., 2013), we inferred that the currents might influence the bacterial communities in the area they flowed along. However, investigations on the bacterial communities in sediments in the regions with strong currents remain sparse.

Additionally, the abundances of bacterial communities are affected not only by environmental variables but also by coexisting bacteria in the habitats. Various relationships among bacteria (e.g., competition and symbiosis) exert distinct influences on the parties (Hibbing et al., 2010). The widespread biofilm also provides excellent evidence (Singh et al., 2006). Bacterial associations thus should be included to contribute better understandings of the bacterial communities assembly (Faust and Raes, 2012; Nemergut et al., 2013; Coutinho et al., 2015). Recently, network analysis, which was used to investigate the associations among different phylotypes, has been introduced into bacterial communities in soil and marine environments (Chaffron et al., 2010; Zhou et al., 2011a; Barberan et al., 2014).

The straits, which separate and connect two large bodies of water, are influenced by strong tidal currents. According to the morphology, oceanic and atmospheric forcing, and flow characteristics of straits, straits have been classified into different types including shallow strait and deep strait (Li et al., 2015). The Bohai Strait (BS) is the foremost channel for the materials exchange between the Bohai Sea and the Yellow Sea. It is a typical shallow strait with depth ranged from 14 m in the south to 80 m in the north. The regional current pattern described as “north-in-south-out” of the BS is the important factor controlling the sediment transportation in the BS (Li et al., 2016). Meanwhile, the BS adjacent to well-developed economic zones including Dalian and Yantai in the north and south, respectively, is an important fishery and navigable seaway. The anthropogenic influences exacerbate the complexity of environments in the BS. Thus, the BS is a representative to investigate the bacterial communities in the sediments with currents and anthropogenic influences. Despite some studies have investigated the microbial communities in the deep strait such as Fram strait (Jacob et al., 2013) and Bransfield Strait (Signori et al., 2014), few studies

were reported in the coastal shallow straits. The Fram Strait is typical deep strait with depth around 2 500 m. It is the only deep-water connection between the Arctic Ocean and the World Oceans. Considering the little studies on the microbial ecology in straits, BS and FS are pretty much the extremes, at least for which samples probably exist, although there are lots of straits with the depth ranged from 20 m to 2 500 m including Strait of Hormuz, Strait of Gibraltar and Strait of Messina. Comparative studies on bacterial communities between in the BS and FS may provide more understanding on the ecological roles of bacterial communities in the straits.

This study aimed to investigate the structures, biogeographic patterns and bacterial associations of bacterial communities in surface sediments of BS in winter compared to those in FS. Our study will provide more evidences for the bacterial ecology in straits representing hydrologically complex environments.

2 MATERIAL AND METHOD

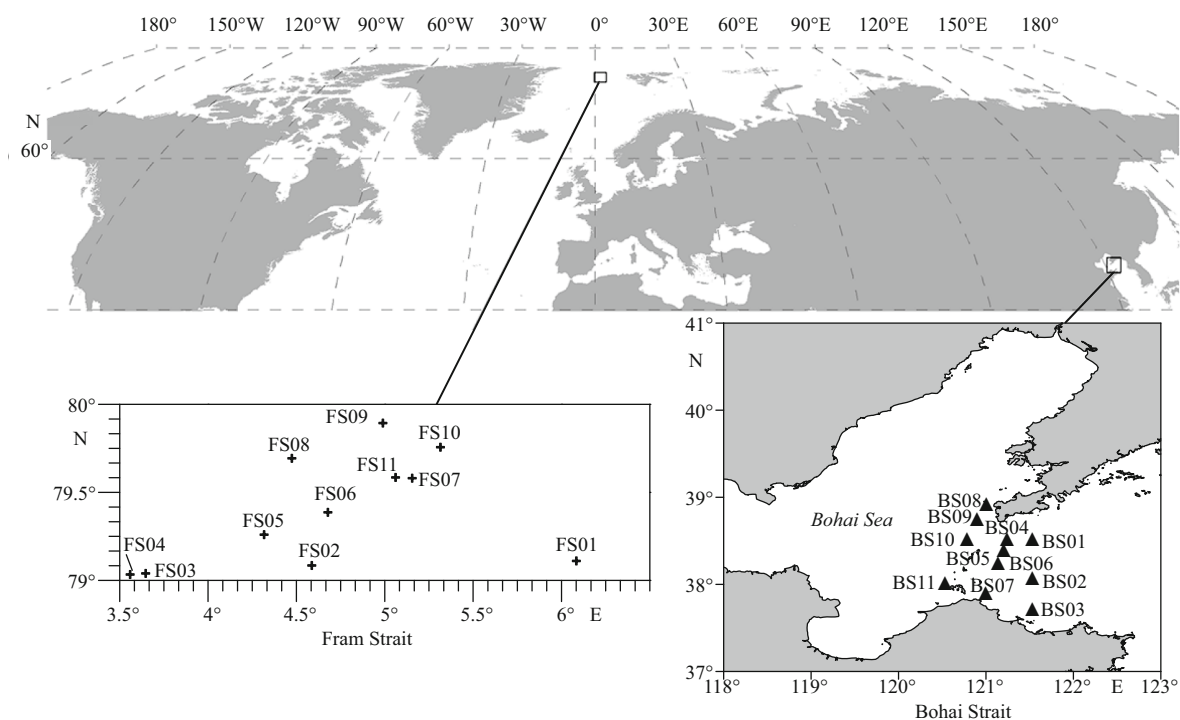
2.1 Sediment sampling, DNA extraction, PCR amplification, and Illumina-Miseq sequencing

Eleven stations were sampled to cover the BS (Fig.1). At the time of sampling, composite samples of surface sediments were collected between December 17, 2013, and December 21, 2013. At each station, sediments were sampled in triplicates for the 0–5 cm surface layer using box grab. The sediment samples were transferred to sterile polythene bags, mixed, and stored at -80°C in the dark until processing. Information regarding the depth of the stations was obtained from a navigational report. Eleven stations in the Fram Strait (FS) were selected for comparative analysis (Table 1). The samples in FS were collected in July 2009. For the environmental variables, only depth was both detected in the BS and FS.

Bacterial genomic DNA was extracted using MoBio PowerSoil™ kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. Three replications were extracted and mixed together for each sample. Eleven DNA extracts were obtained. The universal primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were used to amplify the V4–V5 region of the bacterial 16S rRNA gene using PCR. After extracting and purifying amplicons from 2% agarose gels using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union

Table 1 Summary of environments, sequencing, and alpha diversities of survey stations

Strait	Station	Environment			Based on original dataset		Based on subsampled dataset	
		Lat. (°N)	Lon. (°E)	Depth (m)	Filtered sequences	Good's coverage (%)	Observed OTUs	Shannon (mean±std)
Bohai Strait	BS01	38.5	121.5	52	23 755	95.041	3 052	6.214±0.017
	BS02	38.05	121.5	32	18 096	93.872	2 546	6.062±0.015
	BS03	37.7	121.5	19	36 113	96.957	3 114	5.892±0.018
	BS04	38.517 1	121.223 6	57	27 822	95.928	2 938	5.809±0.019
	BS05	38.369 58	121.163 9	50	27 890	96.805	2 196	4.841±0.022
	BS06	38.232 9	121.107 8	24	44 172	97.827	2 963	5.986±0.018
	BS07	37.900 12	120.968 8	19	23 858	95.146	2 767	5.816±0.018
	BS08	38.898 57	120.979 3	51	20 042	93.499	2 971	6.091±0.017
	BS09	38.736 6	120.867 8	70	28 810	96.154	2 639	5.006±0.021
	BS10	38.499 9	120.760 4	35	21 023	93.969	3 047	6.251±0.016
	BS11	37.991 8	120.494 6	17	17 462	93.586	2 498	5.927±0.015
Fram Strait	FS01	79.133 889	6.096 111 1	1 284	9 966	92.194	1 506	5.592±0.002
	FS02	79.108 056	4.598 888 9	1 895	10 890	91.883	1 690	5.705±0.007
	FS03	79.063 056	3.658 888 9	3 105	11 604	95.329	1 177	4.625±0.01
	FS04	79.056 944	3.571 111 1	3 535	20 653	96.030	1 997	5.451±0.017
	FS05	79.283 056	4.328 888 9	2 401	23 096	95.973	2 182	5.572±0.017
	FS06	79.41	4.69	2 545	17 850	95.042	1 859	5.342±0.015
	FS07	79.603 889	5.166 944 4	2 802	16 159	94.034	1 878	5.269±0.015
	FS08	79.716 944	4.486 111 1	2 339	17 066	94.269	1 995	5.537±0.015
	FS09	79.916 944	5.001 111 1	2 786	12 186	93.328	1 482	4.935±0.011
	FS10	79.78	5.326 944 4	2 637	14 776	93.665	1 717	4.934±0.015
	FS11	79.608 056	5.073 055 6	2 473	15 022	93.656	1 794	4.998±0.015

**Fig.1 Locations of the eleven survey stations in the Bohai Strait and Fram Strait (plotted with Surfer 11.0™)**

Map drawing No. GS(2016)2945 (accessed from the National Administration of Surveying, Mapping and Geoinformation of China).

City, CA, US), the purified amplicons were quantified using QuantiFluor™-ST (Promega, Fitchburg, WI, USA). An Illumina-Miseq platform (Illumina Inc., San Diego, CA, USA) was applied for paired-end sequencing (2×250) according to the standard protocols in Majorbio (Shanghai, China).

2.2 Sequence data processing

The FS sequence data were downloaded from the GenBank Sequence Read Archives (www.ncbi.nlm.nih.gov) under BioProject ID: PRJNA208712. The sequences were obtained using 454 pyrosequencing of the 16S rRNA gene's V4–V6 region described previously (Jacob et al., 2013). The BS and FS sequence data were then transformed into operational taxonomic unit (OTU)-by-sample tables using Mothur software (version 1.36.1) (Schloss et al., 2009) according to the standard operating procedure (Kozich et al., 2013) and previous description (Jacob et al., 2013). Because of the different amplification region, the sequence data from BS and FS were processed separately. There were three alterations: (i) the latest release of the Silva reference (downloaded from http://www.mothur.org/wiki/Silva_reference_files, Release 123) with more reference sequences was applied for assigning taxonomic information; (ii) singletons containing only one sequence across all samples are more likely to be artefacts, and we preferred to remove them to reduce the impact of spurious errors for both FS and BS. Each OTU was filtered to contain more than two sequences from at least two samples (Zhou et al., 2011b; Flynn et al., 2015); (iii) as the primers we used are supposed to amplify members of the bacteria, thus those OTUs affiliated to archaea, chloroplasts and mitochondria, which might be undesirable and random mistakes or have no functional role in microbial communities (Kozich et al., 2013) were removed.

2.3 Comparative analysis between FS and BS

First of all, the following data processing was executed separately for the BS and FS. Those OTUs with average relative abundance (RA) of less than 0.1% were considered to be rare (Coveley et al., 2015; Lynch and Neufeld, 2015). Shannon and chao1 indices were applied to estimate the α -diversity. Because sequencing depth has great influence on estimated diversity (Magurran, 2003) and the sequencing depths of FS and BS were different, original datasets were subsampled to 9 900 sequences

per sample (the lowest number of sequences in samples was 9 966). The Bray-Curtis index was applied to evaluate dissimilarities in community composition within samples. Cluster analysis was applied based on the average distance matrices, which were respectively calculated with entire OTUs, rare OTUs, and abundant OTUs of subsamples. Subsampling and calculating the three indices were executed 10^3 times to obtain the mean values. For the taxonomic composition, high Good's coverage across all samples was necessary (Magurran, 2003). The original datasets with higher Good's coverage were thus normalized into RA. The RAs of OTUs were summarized at the class, family, and genus levels. *T*-test was used to analyze the significant differences in the RA at different levels between in FS and BS. OTUs that occurred in fewer than five samples in FS or BS were removed, considering the following reasons: (i) the 11 samples were considered to be parallel; and (ii) the network with reduced complexity facilitated the determination of core communities. Datasets with 9900 rarefied sequences per sample were used to calculate the Spearman correlation coefficients ($P < 0.05$, adjusted with “Benjamini-Hochberg”) to explore the abundance associations among OTUs in FS and BS. Those correlations were visualized into the networks, in which the nodes and connections represented the bacterial populations and the correlations. Based on network topology, the keystone species in the bacterial communities can be discerned (Zhou et al., 2011a). The degree defined as the number of connections connected to one node was used to evaluate the influence of the nodes in the network (Barberan et al., 2014). Significant correlations between two species were often related to their direct or indirect interactions (Faust and Raes, 2012; Barberan et al., 2014; Lupatini et al., 2014; Coutinho et al., 2015). An OTU with a higher degree value, which means more OTUs were correlated with the OTU, was thus considered to perform stronger influence (directly or indirectly) on the abundances of the correlated members in the network. R software (version 3.3.2) was employed to perform calculations and to plot the results.

3 RESULT AND DISCUSSION

3.1 The data were comparable

The survey stations are shown in Fig.1. Some measures have been taken to make sure that the data of FS and BS were comparable: (i) the sequence data

was processed in the same methods; (ii) considering the different amplification region, the sequence data of FS and BS were processed separately; (iii) considering that the sequencing depth affects estimating alpha- and beta-diversity, subsampled datasets with same sequences were used; (iv) comparative analysis was performed on the taxonomic level with relative abundances, when Good's coverage (BS: 95.34%±1.5%, FS: 94.13%±1.39%) indicated that sequencing captured majority of the bacterial diversity in the samples (Table 1).

3.2 The bacterial community in BS was more complex than that in FS

First, after quality and OTU filtering, 289 043 and 169 268 16S rRNA gene sequences with 5 309 and 4 313 OTUs, identified at a 3% dissimilarity cutoff were obtained across samples in FS and BS, respectively (Table 1). The Shannon and Chao1 values were both higher in BS (Shannon: 5.81±0.46, Chao1: 3 160.49±393.84) than those in FS (Shannon: 5.27±0.35, Chao1: 2 599.45±339.86, $P<0.01$, Table 1), indicating that the bacterial communities in BS were more diverse and richer than those in FS.

Second, the taxonomic compositions in BS and FS showed typical characteristics of bacterial communities in coastal and oceanic regions, respectively. At class level, the relative abundance of Deltaproteobacteria was higher in BS (20.04%±5.63%) than in FS (7.62%±1.07%, $P<0.05$, Table 2). The major contributors at family level were Desulfobacteraceae and Desulfobulbaceae. At a global scale, the relative abundance of Deltaproteobacteria in sediments tended to decrease from the coastal sea to the open sea (Liu et al., 2015). At family level, the Haliaceae (*Halieta* genus and unclassified in BS, *Halioglobus* genus and unclassified in FS) affiliated to Gammaproteobacteria is aerobic anoxygenic phototrophic bacteria (AAPB) and mainly inhabits in coastal sea. Members of this family require dissolved organic carbon (DOM) as carbon source, and are capable of using bacteriochlorophyll *a* and carotenoids to harvest light and utilize light as additional energy source (Spring et al., 2015). Its relative abundance in BS was 3.78%±2.05% while the number in FS was 0.92%±0.24%. Taking into account the availability of DOM and light limitation for AAPB, the shallow depth and vertically well mixed water are benefit for the colonization of AAPB in BS. The family OM1 clade affiliated to the class Acidimicrobiia and the family JTB255_marine_benthic_group affiliated to

Gammaproteobacteria showed higher relative abundance in FS (4.38%±0.72% and 20.18%±4.63%) than in BS (0.57%±0.18% and 6.89%±4.70%, Table 2). Both of them were more sequence-abundant in polar, cold regions (Bienhold et al., 2016).

Moraxella and *Psychrobacter* were psychrophiles with widespread distributions in deep seas such as in the Japan Trench and cold Antarctic environments (Maruyama et al., 2000). However, the genera *Acinetobacter*, *Moraxella*, and *Psychrobacter* affiliated to Moraxellaceae were not observed in FS but in BS. Meanwhile, our previous study had isolated one crude oil-degrading *Acinetobacter* sp. strain HC8-3S from sediments of the Bohai Sea (Lin et al., 2014; Liu et al., 2016). Likewise, the genus *Pseudomonas* in the family *Pseudomonadaceae*, which is able to colonize a wide range of niches (Madigan et al., 2014), was abundant in BS but not detected in FS, despite that *Pseudomonas* has been previously found in deep seas (Pan and Hu, 2015; Yoshida et al., 2015). Therefore, further explorations on these unusual phenomena might be helpful to investigate the microbial ecology in straits.

Third, compared to the FS, the BS was more susceptible to the anthropogenic influences. At class level, the relative abundance of Bacilli in BS was higher (8.77%±8.09%) than that in FS (7.62%±1.07%, $P<0.05$, Table 2). The genus *Lactococcus* (7.18%±6.53% in BS; not observed in FS), which contributed greatly to the high abundance of Bacilli in BS, was found to be abundant in fish and shellfish (Itoi et al., 2014). Since the Bohai Sea is an important fishing farm in China, the fishery breeding was supposed to influence the bacterial communities in the surface sediments of BS.

3.3 The structures of bacterial community differed with increasing water depth

The tidal current scour in straits may lead to depth gradients. In the BS, the depth gradients are due to the scour of coastal and tidal currents (Li et al., 2016). Depth may shape the composition and structure of microbial communities in the global ocean (Sunagawa et al., 2015). In BS, cluster analysis based on Bray-Curtis shows that the samples were separated by their depth layer (Fig.2a), 15–20 m (BS11, BS07 and BS03), 21–50 m (BS10, BS08, BS02 and BS06) and 50–70 m (BS09, BS05, BS04 and BS01). The results shown in Fig.2d were same to what described by Jacob et al. (2013). They indicated significant differences of bacterial community compositions and

Table 2 Summary of the classes with significantly different RA between in the FS and BS groups, and the major contributors at family and genus levels

Class (mean±std, %)			Major family (mean±std, %)			Major genus (mean, %)	
Class	BS	FS	Family	BS	FS	BS	FS
Acidimicrobiia	0.85± 0.26	5.12± 0.82	Acidimicrobiaceae	0.18±0.09	0.10±0.04	<i>Illumatobacter</i> (0.18)	<i>Illumatobacter</i> (0.10)
			OM1_clade	0.57±0.18	4.38±0.72	Unclassified (0.57)	Unclassified (4.38)
			Sva0996_marine_group	0.07±0.04	0.61±0.15	Unclassified (0.07)	Unclassified (0.61)
			Hyphomicrobiaceae	0.09±0.86	0.41±0.14	<i>Filomicrobium</i> (0.05), <i>Cohaesibacter</i> (0.02), <i>Pseudahrensia</i> (0.02)	<i>Filomicrobium</i> (0.05), <i>Ahrensia</i> (0.07), <i>Roseobacter_clade_NACII-7_lineage</i> (0.16)
			Phyllobacteriaceae	0.08±0.03	0.19±0.06	Unclassified (0.27)	Unclassified (0.19)
Alphaproteobacteria	3.07± 1.27	6.45± 0.55	Rhodobacteraceae	0.32±0.14	0.45±0.13	<i>Andersenella</i> (0.04), <i>Methyloceanibacter</i> (0.03)	Unclassified (0.19)
			Rhodobiaceae	0.10±0.05	0.19±0.03	<i>Deftuviococcus</i> (0.16), <i>Pelagibius</i> (0.1)	<i>Pelagibius</i> (0.44)
			Rhodospirillaceae	0.42±0.22	0.54±0.11	Unclassified (1.38)	Unclassified (0.03)
Anaerolineae	1.44± 0.55	0.03± 0.01	Anaerolineaceae	1.44±0.55	0.03±0.01	<i>Leuconostoc</i> (0.62)	NA
			Leuconostocaceae	0.62±0.57	0	<i>Brochothrix</i> (0.4)	NA
Bacilli	8.77± 8.09	0.002± 0.004	Listeriaceae	0.4±0.4	0	<i>Macrococcus</i> (0.01), <i>Staphylococcus</i> (0.001)	<i>Staphylococcus</i> (0.002)
			Staphylococcaceae	0.02±0.02	0	<i>Lactococcus</i> (7.18)	NA
			Streptococcaceae	7.55±6.57	0	<i>Nitrosomonas</i> (0.02)	<i>Nitrosomonas</i> (1.28)
Betaproteobacteria	0.57± 0.29	1.80± 0.19	Nitrosomonadaceae	0.07±0.05	1.29±0.26	NA	<i>Azospira</i> (0.47)
			Rhodocyclaceae	0	0.47±0.13	<i>Janthinobacterium</i> (0.21)	NA
			Oxalobacteraceae	0.21±0.23	0	<i>Reichenbachiella</i> (0.26)	<i>Fulvivirga</i> (0.091), <i>Reichenbachiella</i> (0.28), Unclassified (0.54)
Cytophagia	1.24± 0.60	1.75± 0.28	Flammeovirgaceae	1.07±0.53	0.64±0.14	<i>Desulfatiglans</i> (0.45)	NA
			Rhodothermaceae	0.09±0.04	0.54±0.07	<i>SEEP-SRB1</i> (0.53), <i>Sva0081_sediment_group</i> (2.34)	<i>Sva0081_sediment_group</i> (0.004), <i>SEEP-SRB1</i> (0.007)
			Desulfarculaceae	0.45±0.24	0	<i>Desulfobulbus</i> (4.38)	<i>Desulfobulbus</i> (0.05)
Deltaproteobacteria	20.04± 5.63	7.62± 1.08	Desulfobacteraceae	4.46±1.23	0.13±0.13	Unclassified (1.70)	NA
			Desulfobulbaceae	5.63±2.24	0.06±0.04	<i>Flavobacterium</i> (0.40), <i>Lutimonas</i> (0.29), <i>Myroides</i> (0.7)	<i>Actibacter</i> (0.97), <i>Gilvibacter</i> (0.35)
			Sva1033	1.70±2.11	0	<i>Haliea</i> (1.00)	<i>Halioglobus</i> (0.42)
Flavobacteriia	2.80± 1.13	4.04± 1.39	Flavobacteriaceae	2.33±1.29	3.61±1.27	Unclassified (6.89)	Unclassified (20.18)
			Haliaceae	3.78±2.05	0.92±0.24	<i>Acinetobacter</i> (0.50), <i>Moraxella</i> (0.71), <i>Psychrobacter</i> (0.90)	NA
			JTB255_marine_benthic_group	6.89±4.70	20.18±4.63	<i>Pseudomonas</i> (5.45)	NA
Gammaproteobacteria	29.11± 8.11	38.98± 545	Moraxellaceae	2.10±1.93	0	Unclassified (0.01)	Unclassified (0.20)
			Pseudomonadaceae	5.45±5.0	0	Unclassified (0.85)	Unclassified (0.57)
			NS72	0.01±0.01	0.20±0.06	<i>Nitrospira</i> (0.63)	<i>Nitrospira</i> (0.28)
Holophagae	1.69± 0.77	1.06± 0.18	Sva0725	0.85±0.45	0.57±0.11	<i>Urania-1B-19_marine_sediment_group</i> (0.44) <i>Blastopirellula</i> (0.22), <i>Pir4_lineage</i> (0.99), <i>Pirellula</i> (0.14), <i>Planctomyces</i> (0.27), <i>Rhodopirellula</i> (0.71)	<i>Urania-1B-19_marine_sediment_group</i> (0.32) <i>Blastopirellula</i> (0.14), <i>Pir4_lineage</i> (0.21), <i>Planctomyces</i> (0.26)
			Nitrospiraceae	0.96±0.26	0.28±0.07	Unclassified (0.004)	Unclassified (1.24)
Phycisphaerae	4.02± 1.12	1.34± 0.19	Phycisphaeraceae	0.68±0.18	0.57±0.14	NA	<i>Rubritalea</i> (0.54) <i>Luteolibacter</i> (0.46), <i>Persicirhabdus</i> (0.13), <i>Roseibacillus</i> (0.57)
			Planctomycetaceae	3.39±1.50	1.92±0.93		
Planctomycetacia	3.40± 1.50	1.93± 0.94	DEV007	0	1.24±0.29		
			Rubritaleaceae	0	0.54±0.41		
Verrucomicrobiae	0.01± 0.01	3.21± 0.89	Verrucomicrobiaceae	0.01±0.01	1.16±0.41		

Only the classes that met the following criteria are shown here: (i) RA significantly differed between in the FS and BS groups, (ii) average RA at class level was more than 1% in at least one group, (iii) they could be further classified at the family level. For clarity, the families and genera who's RAs accounted for more than 10% in total class or family were considered major. The values in the table represented the relative abundances of the classes, families and genera in total sequences of BS or FS.

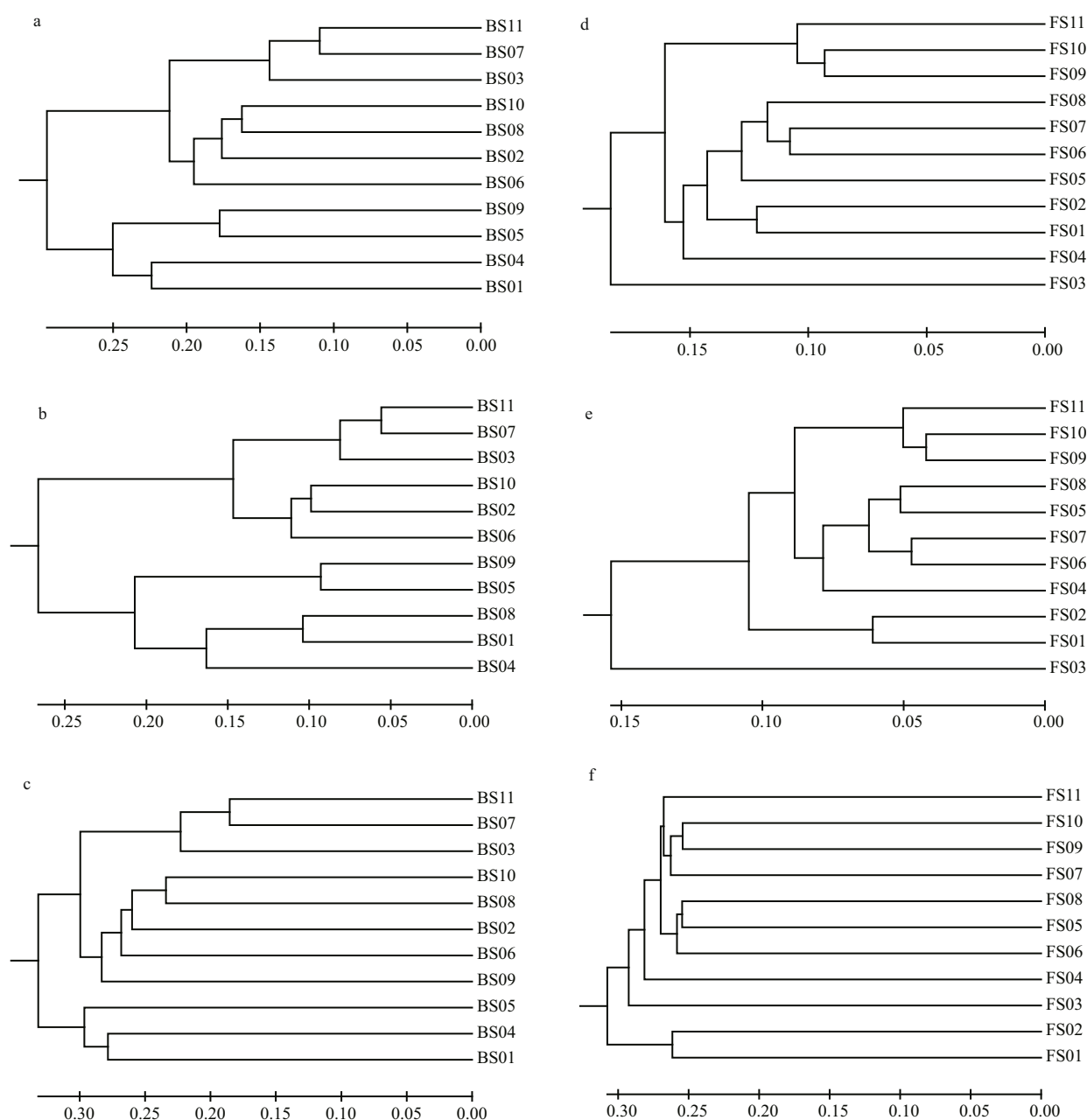


Fig.2 Dendrograms of cluster analysis based on the Bray-Curtis index among survey stations in BS and FS

a. entire bacterial communities in BS; b. abundant compositions in BS; c. rare compositions in BS; d. entire bacterial communities in FS; e. abundant compositions in FS; f. rare compositions in FS.

structures along the bathymetric transect. Remarkably, Gong et al. have revealed that depth shapes α and β diversities of the single-celled eukaryote in surficial sediments in the Bohai Sea and Yellow Sea (Gong et al., 2015).

The rare OTUs termed as rare bacterial biosphere were acknowledged to make up a great fraction of the bacterial communities (Jacob et al., 2013; Coveley et al., 2015; Lynch and Neufeld, 2015). Approximately 97% of the OTUs were considered to be rare in this study. In total of 5 185 and 4 178 OTUs were

considered to be rare and their relative abundances were $40.92\% \pm 5.6\%$ and $27.74\% \pm 3.94\%$ in BS and FS, respectively (data not shown). The cluster relationships among samples in abundant and rare communities (Fig.2b, c) were both similar to those in entire communities (Fig.2a). However, the dissimilarities among samples showed a descending order as Fig.2c, Fig.2a and Fig.2b. For instance, the distances between BS07 and BS11 were approximately 0.1 in Fig.2b, more than 0.2 in Fig.2a and about 0.35 in Fig.2c. Similar tendencies were found in FS

(Fig.2d, e, f). These findings were likely due to that microbes with high abundance have great effects on the structure and functions (Campbell and Kirchman, 2013; Zhang et al., 2013) while the more diverse rare communities were the major contributors to the diversity of communities (Lynch and Neufeld, 2015). Additionally, comparison between BS and FS indicated that dissimilarities among samples were lower in FS than in BS, in contrast to depth gradients in them (Table 1). It could be due to the more complex environmental shapers in BS than in FS.

3.4 Filamentous bacteria *Desulfobulbaceae* play key roles in BS

Bacteria do not exist in isolation but form complex ecological interaction webs (Faust and Raes, 2012), and the associations within the bacterial community are important elements of the structure of bacterial communities. The impacts of interaction may be positive, negative, and neutral. For instance, syntrophy between two species can benefit both species involved (positive impact), while competition results in negative impacts on both (Hibbing et al., 2010; Molloy, 2014). These interactions can be reflected by their abundance correlations (Faust and Raes, 2012). Numerous studies on the microbial biogeography (Campbell and Kirchman, 2013; Jacob et al., 2013; Buttigieg and Ramette, 2014; Coveley et al., 2015; Piontek et al., 2015) and the niche theory (Pontarp et al., 2012) known as the habitat filtering have indicated that members with positive correlations among them respond similarly to the environmental conditions in the habitats. That is, if two species showed positive correlations in their abundance across multiple environments or samples, it can be interpreted as evidence that they occupy similar ecological niches (Coutinho et al., 2015). Based on these observations, significant correlations between two species were often related to the direct or indirect interactions between the two species (Faust and Raes, 2012; Barberan et al., 2014; Lupatini et al., 2014; Coutinho et al., 2015).

In this work, 1 976 and 975 OTUs in BS and FS were used to calculate Spearman correlation coefficients. The number of significant correlations for BS and FS were 164 422 and 31 163. It is difficult to draw a clear conclusion from the huge and complex network (1 976 nodes and 164 422 edges in BS network, 975 nodes and 31 163 edges in FS network). In order to better interpret the network and increase the confidence for detecting only strong interactions,

correlations with $|r| \geq 0.9$ ($P \leq 0.05$) were extracted to construct a new network (Fig.3a, b). There are 1 303 nodes (65.94%) and 2 642 edges in the BS network. The numbers in FS network were 390 nodes (40%) and 315 edges. That is, 60% of the OTUs in FS did not perform strong abundance correlations with others, while the percentage in BS was 34.06%. Obviously, the BS network was more connected than the FS network. Therefore, we inferred that the bacterial community in the BS might be more interactive than that in the FS.

To evaluate the taxonomic importance in the extracted network, degree value of nodes were calculated. Notably, about 75.5% of the nodes in the extracted network of BS were unclassified (the percentage in the extracted network of FS was 87.7%). The degree values were hence summarized at the family level. For clarity, OTUs unclassified at the family level were omitted in the following discussions. As shown in the Fig.3c & d, there were 4 characteristics determining the importance of the involved families, including the relative abundance, the number of OTUs, the max degree values, and the sum degree values. As mentioned, microbes with high abundance have great effects on the structure and functions (Campbell and Kirchman, 2013; Zhang et al., 2013). In most case, the high number of OTUs was related to high relative abundance. The max degree values indicated the importance of the most important OTU affiliated to the families. The sum degree values revealed the importance of the families. In the extracted network of BS (Fig.3c), 98 OTUs were affiliated to the family Planctomycetaceae with degree sum of 291. However, the highest degree value of these OTUs was only 16. In contrast, the three members of the family Streptococcaceae exhibited high max degree values (the three degree values were 45, 42, and 35). The Planctomycetaceae, Flavobacteriaceae, and the JTB255_marine_benthic_group families were found in the FS network at the same time, suggesting that these three widely distributed families might play universal roles in constructing the bacterial communities. For the family Desulfobulbaceae, the number of OTUs, the relative abundance and the max degree value was 27, 5.14% and 30, respectively.

Additionally, taxon assignment on lower taxonomic levels is not always accurate, which might confuse the ecological signals and artefacts (Faust and Raes, 2012). To consider links and patterns above a certain taxonomic rank (for example, above family) were

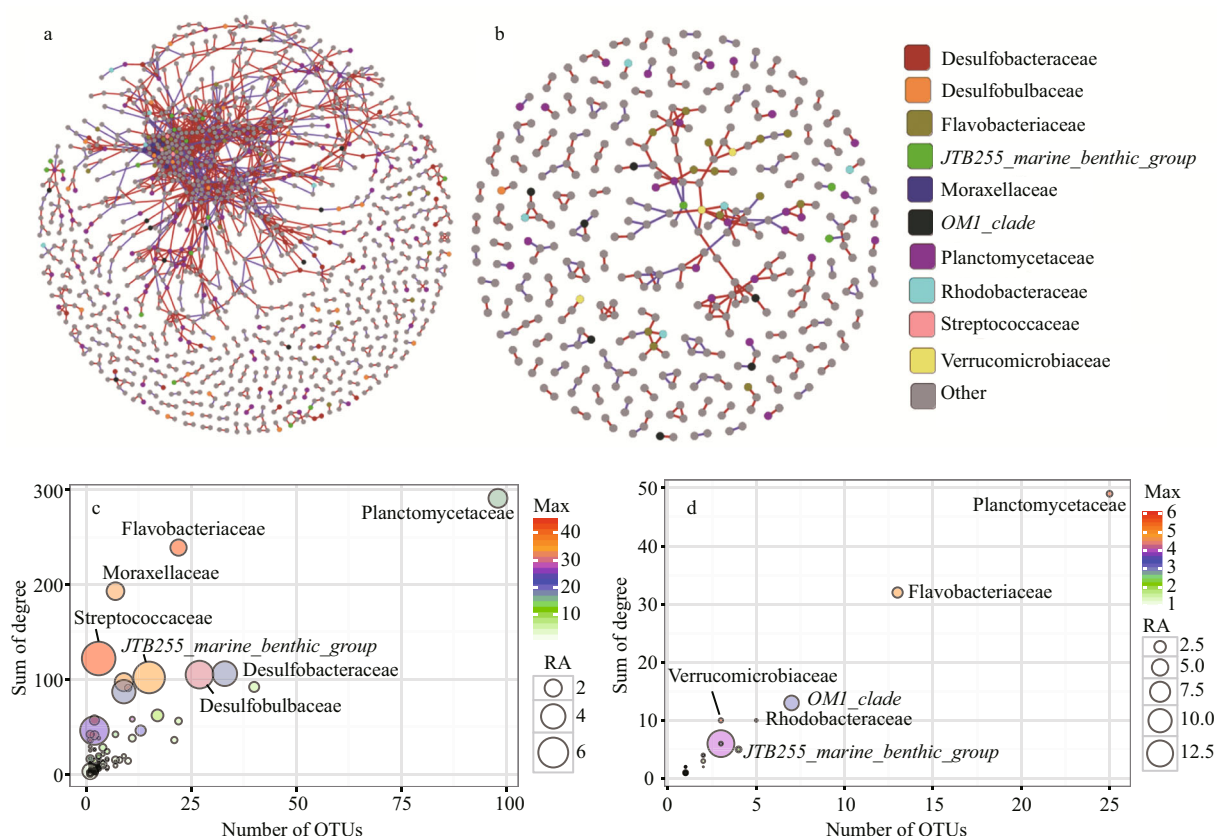


Fig.3 a. BS; b. FS. Networks reveal Spearman correlations among the abundance of filtered OTUs. The nodes represent OTUs; c. BS; d. FS. The bubble diagram describes the number of OTUs affiliated to the family, the sum of degree values, its RA, and the max degree value performed by the OTUs affiliated to the family

Different colors of nodes represent different families. The families other than the first ten families with high RAs were grouped into “Others.” Nodes connected by red edges are positively correlated, and those connected by blue edges are negatively correlated ($P < 0.05$).

Table 3 Summary of correlations at the family level

Straits	Number of pairs	Most connective families (NCF)	Pairs with highest NC (NC)	
			Positive	Negative
BS	385 pairs within 98 families	Planctomycetaceae (45), Desulfobulbaceae (29), Flavobacteriaceae (29), Family_Incertae_Sedis (25), JTB255_marine_benthic_group (23), Streptococcaceae (22)	Moraxellaceae-Flavobacteriaceae (24), Streptococcaceae-Flavobacteriaceae (17), Streptococcaceae-Moraxellaceae (15), Moraxellaceae-Pseudomonadaceae (13), Desulfobacteraceae-Planctomycetaceae (9)	Desulfobulbaceae-Moraxellaceae (11), Desulfobulbaceae-Flavobacteriaceae (7), Family_Incertae_Sedis-Flavobacteriaceae (6), JTB255_marine_benthic_group- Flavobacteriaceae (6), Flavobacteriaceae-MidBa8 (6)
		Planctomycetaceae (9), Flavobacteriaceae (9), Verrucomicrobiaceae (6), Rhodobacteraceae (3), Flammeovirgaceae (3), Cryomorphaceae (3)	Planctomycetaceae-Flavobacteriaceae (5), Rhodobacteraceae-Flavobacteriaceae (3), Flavobacteriaceae-Flammeovirgaceae (2), Nitrospinaceae-Planctomycetaceae (2), Flavobacteriaceae-Cryomorphaceae (1)	JTB255_marine_benthic_group- Cryomorphaceae (1), Flavobacteriaceae-Oligoflexaceae (1), JTB255_marine_benthic_group- Planctomycetaceae (1)
FS	24 pairs within 19 families	Planctomycetaceae (9), Flavobacteriaceae (9), Verrucomicrobiaceae (6), Rhodobacteraceae (3), Flammeovirgaceae (3), Cryomorphaceae (3)	Planctomycetaceae-Flavobacteriaceae (5), Rhodobacteraceae-Flavobacteriaceae (3), Flavobacteriaceae-Flammeovirgaceae (2), Nitrospinaceae-Planctomycetaceae (2), Flavobacteriaceae-Cryomorphaceae (1)	JTB255_marine_benthic_group- Cryomorphaceae (1), Flavobacteriaceae-Oligoflexaceae (1), JTB255_marine_benthic_group- Planctomycetaceae (1)

therefore suggested (Freilich et al., 2010). In this work (Table 3), 285 and 24 pairs of correlated families in BS and FS were observed, respectively. Planctomycetaceae, Flavobacteriaceae, and Desulfobulbaceae were the three most connected families in BS (degree values were 45, 29 and 29 in that order). In FS, they were Planctomycetaceae, Flavobacteriaceae, and Verrucomicrobiaceae (degree

values were 9, 9, and 6 in that order). Additionally, the numbers of connections (NC) between two families at the OTU level were summarized to estimate the strength of connections between the two families. For instance, the positive NC value between Flavobacteriaceae and Streptococcaceae was 17, while the NC value between Flavobacteriaceae and Moraxellaceae was 24 (Table 3). In FS and BS, the

highest NC values were both connected to Flavobacteriaceae. Flavobacteriaceae was a major component of microbial biomass in the marine pelagic zone, strongly coupled to phytoplanktonic primary production (photosynthesis), and has been reported to be responsible for a major fraction of organic matter remineralization in the oceans (Bowman, 2006). The shared pairs of positively correlated families consisted of Flammeovirgaceae-Planctomycetaceae, Flavobacteriaceae-Cryomorphaceae, and Phycisphaeraceae-Planctomycetaceae.

3.5 Limitations and future works

We firstly note that only one representative of each type of strait was picked in this study due to the lack of data, which might undermine the convincing of this study. Additionally, the environmental variables, for instance, temperature and nutrients which were generally accepted as the drivers of bacterial community structures were not detected in FS. The geographic distance especially latitude gradient was an important factor driving the variability of bacterial communities (Liu et al., 2015) when the locations of FS and BS were obviously different. Thus, it was difficult to accurately test what drove the differences of bacterial communities between FS and BS. More work such as the investigation on the bacterial communities in other straits and more detailed environmental variables are in request.

Network inference approaches cannot distinguish between true ecological interactions and other nonrandom processes (Faust and Raes, 2012). For instance, both syntrophy and niche overlap might lead to positive correlations in abundances between two species, despite the fact that syntrophy benefits both species while niche overlap leads to competition (Molloy, 2014; Coutinho et al., 2015). Thus, the summary of links and patterns at the family level in this study were just preliminary data for further exploration (e.g., metabolic activities or potential) to differentiate the true ecological interactions from other nonrandom processes.

4 CONCLUSION

In this study, the bacterial communities in the surface sediments of the Bohai Strait were investigated and compared with that in the Fram Strait. Both of the coastal shallow Bohai Strait and the oceanic deep Fram Strait are influenced by strong currents. Our results suggested that the bacterial communities in

Bohai Strait were more complex than what in Fram Strait. The structures of bacterial communities in the two straits differed significantly with increasing water depth as the results of tidal current scour in the straits. Rare compositions contributed to the bacterial seed bank while abundant compositions presented greater effects on the entire bacterial community structures. The bacterial community in BS might be more interactive than that in the FS. Filamentous bacteria from the family Desulfobulbaceae played a key role in BS but not in FS. Planctomycetaceae and Flavobacteriaceae were the most connected families in both FS and BS.

5 DATA AVAILABILITY STATEMENT

The 16S rRNA sequences have been deposited in the NCBI database under accession number PRJNA304670 (biosample accession numbers are from SAMN05206979 to SAMN05206989).

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7 CONFLICT OF INTEREST

There are no conflicts of interest.

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