Acta Oceanol. Sin., 2015, Vol. 34, No. 10, P. 122–131
DOI: 10.1007/s13131-015-0719-3
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Diversity and distribution of bacterial community in the coastal sediments of Bohai Bay, China

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Received 17 June 2014; accepted 25 September 2014

Abstract

In order to understand the diversity and distribution of the bacterial community in the coastal sediment of the Bohai Bay, China, high-throughput barcoded pyrosequencing of the 16S rRNA gene was used. Metagenomic DNA was extracted from the sediment samples, and was sequenced using a 454 GS FLX Titanium system. At 97% similarity, the sequences were assigned to 22 884 operational taxonomic units (OTUs) which belonged to 41 phyla, 84 classes, 268 genera and 789 species. At the different taxonomic levels, both the dominants and their distribution varied significantly among the six coastal sediments. Proteobacteria was the first dominant phylum across all the six coastal sediments, representing 57.52%, 60.66%, 45.10%, 60.92%, 56.63% and 56.59%, respectively. Bacteroidetes was the second dominant phylum at Stas S1, S2 and S4, while Chloroflexi was the second dominant phylum at Stas S3, S5 and S6. At class level, γ-Proteobacteria was the first dominant class at Stas S1, S2, S4 and S6, while δ-Proteobacteria became the first dominant class at Stas S3 and S5. In addition, a large proportion of unclassified representatives have distributed at the different taxonomic levels. Canonical correspondence analysis (CCA) results indicated that the sediment texture, water depth (D), dissolved oxygen (DO), total nitrogen (TN) and nine EPA priority control polycyclic aromatic hydrocarbons (PAHs) including naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene and indeno[1,2,3-cd]pyrene were the important factors in regulating the bacterial community composition. Those results are very important to further understand the roles of bacterial community in the coastal biogeochemical cycles.

Key words: Bohai Sea, coastal zone, aromatic hydrocarbon, bacteria, biodiversity, pyrosequencing

Citation: Wang Liping, Zheng Binghui, Lei Kun. 2015. Diversity and distribution of bacterial community in the coastal sediments of Bohai Bay, China. Acta Oceanologica Sinica, 34(10): 122–131, doi: 10.1007/s13131-015-0719-3

1 Introduction

Marine sediments are complex systems affected by the interaction of geological, hydrological, physicochemical and biological factors; they act as a reservoir of adsorbed nutrients, pesticides, toxic materials and also heavy metals (Köster and Meyer-Reil, 2001). Sediments contain a huge number and diversity of microorganisms that play critical roles in the functioning of marine ecosystems, such as in the process of environmental detoxification, recycling of organic matter to benthic food webs and biogeochemical cycles (Cabello et al., 2004; Barbier et al., 2011; Schlesinger, 1997). In recent years the analysis of amplified and sequenced 16S rRNA genes has become an important method to study environmental microbial structure and diversity (Roesch et al., 2007; Lauber et al., 2009; Lemos et al., 2011). The use of high throughput pyrosequencing allows not only the assessment of the taxonomic diversity of environmental microorganisms, but also a more detailed analysis of microbial communities (Roesch et al., 2007; Lim et al., 2010; Tripathi et al., 2012). Recently, pyrosequencing has been successfully used to analyze microbial communities in source water (Pinto et al., 2012), soil (Lauber et al., 2009), raw sewage (McLellan et al., 2010), marine water (Qian et al., 2011), coastal sediment (Kim et al., 2008; Wang et al., 2013) and activated sludge (Zhang et al., 2012; Guo and Zhang, 2012). The previous studies have indicated that the use of this method may give a better insight into the structure of environmental microbial communities.

The Bohai Bay is located in the west of the Bohai Sea, China, which is a typical semi-enclosed coastal sea. This bay is the second largest bay of the Bohai Sea covering an area of about 1.6×10^4 km^2 with an average water depth of 12.5 m. Defining the diversity and distribution of the indigenous microbial communities in the Bohai Bay sediment is a long-standing challenge. Understanding the microbial structure and diversity in the coastal sediment of the Bohai Bay is essential to understand the microbial process underlying in the coastal ecosystem. In previous studies, Wang et al. (2013) investigated the bacterial community in the two typical intertidal sediments of the Bohai Bay by pyrosequencing. The objective of the present work was to characterize the microbial community in the coastal sediments of the Bohai Bay using 454-pyrosequencing, and tried to reveal the pos-

Foundation item: The Central Basic Scientific Research Project in the Public Welfare for the Scientific Research Institutes under contract No. gyk5091301.

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sible factors regulating the microbial community.

2 Materials and methods

2.1 *Sample collection and analytical methods*

Figure 1 gives the location of the six sampling stations. S1(S4), S2(S5) and S3(S6) located on the 5 m, 10 m and 15 m depth isoline, respectively. All of them distributed near the estuary of the Haihe River that was a main river flowing to this bay. Field research was carried out on September 18–19, 2012, and the coastal sediments were collected by Box-type sampler from the six stations (Fig. 1). Then the sub-samples at about 5 cm depth were collected in axenic containers and transported to the laboratory in an icebox, and stored in the dark at –20°C until next processing. A global positioning system was used to determine the sampling positions. The temperature (*T*), dissolved oxygen (DO) and pH in the overlying water of the sediment were determined on-site using a Multi-parameter water quality meter (YSI, USA).

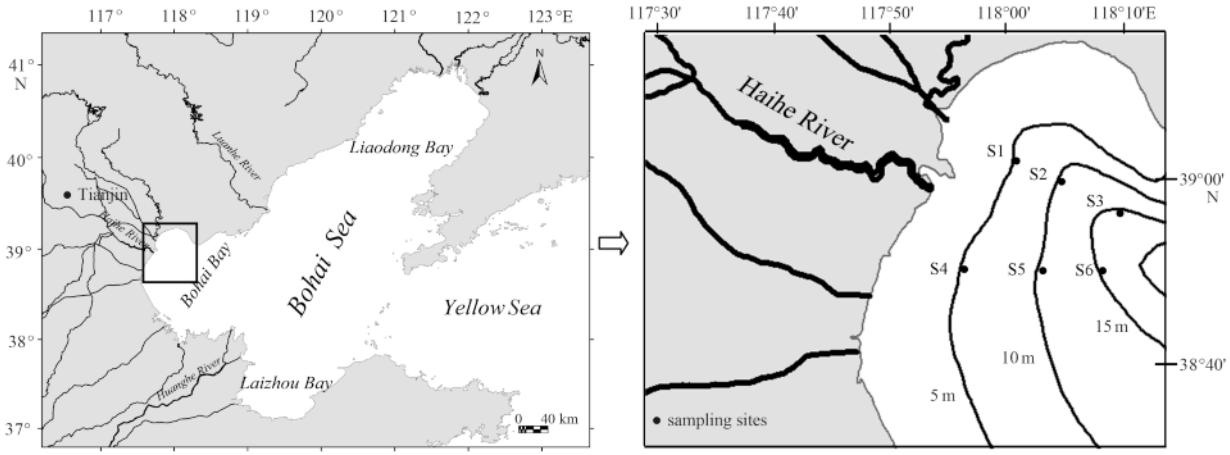

Fig. 1. Map of the study area, showing the location of the sampling stations. 5 m, 10 m and 15 m mean the depth isoline.

The sub-samples were taken from each station and homogenized in a sterilized beaker prior to the analysis. The grain size of the sediment was determined using a laser diffraction particle sizer (LS 13320, Beckman Coulter). The organic matter (OM) was determined as the weight loss (percentage of the dry weight) after ignition (2 h at 550°C), and the water content (%) was calculated as the difference between the wet and dry weights (24 h at 60°C). Sediment samples used to analyze total nitrogen (TN) and total phosphorus (TP) were digested with alkaline potassium persulphate. TN was determined by the UV spectrophotometric method, and TP was determined using the acidic molybdate-ascorbic acid spectrophotometric method.

For the metal determination including Cr, Cu, Zn, As, Cd, Sn, Sb and Pb, sediments were dried at room temperature, and sieved through a 100-mesh nylon sieve. 0.5 g sediment aliquots were digested in closed Teflon beakers by ultrapure HNO_3/HF mixtures at 120°C and evaporated to dryness. The residue was then dissolved in HNO_3/H_2O_2, evaporated to dryness again, and finally dissolved in 1% HNO_3. The metal content was analyzed by ICP-MS (Perkin-Elmer, USA).

The polycyclic aromatic hydrocarbon (PAH) content was determined by an Agilent 7890A GC equipped with 5975C mass selective detector (MSD) under the selected ion monitoring mode (SIM) as described previously (Qiao et al., 2006). The concentrations of 16 EPA priority control PAHs were presented on the basis of sediment dry weight.

2.2 *DNA extraction and PCR amplification*

DNA was extracted from sediment samples using the PowerMax Soil DNA Isolation Kits (MO BIO Laboratories InC, Carlsbad, CA) following the manufacturer's instruction. Extracted DNA was examined on Gel-red-stained 1% agarose gels using gel electrophoresis and purified using AxyPrep™ DNA Gel Extraction Kit. DNA-amplification was performed with the universal 16S rRNA gene primers (*Escherichia coli* positions 8 to 533: 8F 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 5′-TTA CCG CGG CTG CTG GCA C-3′, Baker et al., 2003). The PCR mixture (final volume, 50 μL) contained 10 μL 5-fold reaction buffer (TransStart™ FastPfu Buffer, TransGen Biotech), <100 ng of sediment DNA, 0.4 μmol/L each primer, 0.5 U Pfu polymerase (TransStart™ FastPfu DNA Polymerase, TransGen Biotech), and 2.5 mmol/L dNTPs. For each sample, three independent PCRs were performed using a MG96+ Thermal Cycler (LongGene Scientific Instruments Co., Ltd). The PCR conditions were as follows: 95°C for 3 min; 25 cycles of denaturation (95°C; 0.5 min), annealing (55°C; 0.5 min), and extension (72°C; 0.5 min); followed by the final elongation (72°C; 10 min). Then the DNA was quantified using a TBS-380 Mini-Fluorometer (Promega Corporation, CA, USA).

2.3 *Pyrosequencing and sequence analyses*

PCR products were submitted to Shanghai Majorbio Biopharm Technology Co., Ltd. for pyrosequencing using a 454/Roche GS-FLX Titanium Instrument (Roche, NJ, USA). After trimming of the barcodes and primers, sequences less than 200 bp or containing ambiguous bases were excluded. Then chimeric sequences were identified using UCHIME and removed (Edgar et al., 2011). Finally the high-quality sequences were used for further analyses. Sequences with similarities of greater than 97% were clustered into one operational taxonomic unit (OTU) using the MOTHUR program (Schloss et al., 2009). The community richness and diversity indices (Chao1 estimator, Ace, Shannon index, Simpson diversity indices and Good's coverage) and rarefaction curves were obtained using the MOTHUR program (Schloss et al., 2009). The sequences were aligned and compared with the Bacterial SILVA database (SILVA version 106; http://www.arb-silva.de/documentation/backgroud/release-106/). All of the sequences generated in this study can be downloaded from the NCBI Short Read Archive, submission number: SRA04771.

2.4 *Statistical analyses*

The similarity factor (Jaccard index by the Jest calculations, C_{jaccard}) was obtained by calculating the OTUs distribution structure within the six bacterial communities.

$$C_{\text{jaccard}} = 1 - \frac{S_{\text{AB, Chao}}}{S_{\text{A, Chao}} + S_{\text{B, Chao}} - S_{\text{AB, Chao}}}, \quad (1)$$

where $S_{\text{AB, Chao}}$ is the shared OTUs number (diversity index "Chao", the same in the following) in Samples A and B; $S_{\text{A, Chao}}$ is the OTUs number in Sample A; $S_{\text{B, Chao}}$ is the OTUs number in Sample B. A Sharedtree with shared and unique OTUs was used to depict the similarity and difference among the six bacterial communities.

The sequences of the top 20 dominant genera in the six sediment samples were selected to construct the phylogenetic tree using FastTree software (http://www.microbesonline.org/fasttree/) with approximately-maximum-likelihood. Canonical correspondence analysis (CCA) was used to evaluate the relationship between the bacterial diversity, the dominant phyla revealed by pyrosequencing of 16S rRNA genes and sediment properties. All the factors were standardized using log(N+1) transformed before the analysis. Sediment characteristics were represented by vectors. The larger vectors and the vectors forming smaller angles with an ordination axis were strongly correlated with that axis. High score of a given bacterial diversity or dominant phylum abundance on an axis indicated a strong correlation of diversity or phylum abundance to the axis and consequently to sediment.

3 Results

3.1 *Environmental conditions of sampling sites*

The various factors were measured to describe the environmental conditions at each sampling site. The water depth varied between 5.5–16.8 m. The water temperature (T), pH, salinity, DO in the six stations when the samples were collected ranged from 23.0°C to 23.6°C, 7.98 to 8.08, 24.8 to 28.4 and 5.28 mg/L to 6.29 mg/L, respectively. The OM, TN and TP in the six sediment samples varied between 1.48%–1.92%, 0.527–0.934 mg/g and 0.063–0.076 mg/g, respectively. The particle specific surface area (SSA) of the six sediment samples varied between 0.73–1.88. According to the classification of Shepard (1954), the sediment from Sta. S1 was dominated by silt-sand, and the sediment from Sta. S2 was dominated by sand-silt. The other four stations belonged to clay-silt. The eight metals ranged from 49.60 mg/kg to 68.73 mg/kg for Cr, 17.31 mg/kg to 33.62 mg/kg for Cu, 53.40 mg/kg to 111.90 mg/kg for Zn, 15.54 mg/kg to 24.00 mg/kg for As, 0.32 mg/kg to 0.70 mg/kg for Cd, 2.14 mg/kg to 2.75 mg/kg for Sn, 0.92 mg/kg to 1.63 mg/kg for Sb, 13.38 mg/kg to 23.79 mg/kg for Pb, respectively. In the six stations, the total PAH concentrations (sum of the 16 EPA priority control pollutants) in sediments ranged from 86.33 ng/g to 846.77 ng/g dry weight (dw). Anthracene (An) was detected only at Sta. S2 (6.05 ng/g dw); Chrysene (Chr) was detected only at Stas S3 (16.44 ng/g dw) and S4 (29.52 ng/g dw); Benzo[*k*]fluoranthene (BkF) was detected only at Stas S1 (11.78 ng/g dw) and S2 (13.92 ng/g dw); Benzo[*ghi*]perylene (BgP) was detected only at Stas S1 (0.36 ng/g dw), S2 (0.13 ng/g dw) and S3 (0.07 ng/g dw). Other twelve PAH compositions were detected in all the six stations, and their concentrations were showed in Table 1.

3.2 *Bacterial diversity and composition*

A total of 72 708 high-quality partial 16S rDNA sequences were obtained by 454-pyrosequencing analysis, and 22 884 OTUs (at the 97 % level, corresponding to taxonomically valid species) were obtained from the six sediment samples (Table 2). OTUs at a sequence similarity of 97% were determined to calculate the richness, diversity and rarefaction curves of the microbial communities (Table 2, Fig. 2). The rarefaction curves showed a similar pattern for all the samples (Fig. 2), and suggested that the bacterial community was well represented since they became gentle while the number of sequences analyzed increased. Good's coverage estimations revealed that 80.47%–85.34% of the species (at the 97% level) were obtained in all the samples, while rarefaction curves suggested that the sequencing effort was not large enough to capture the complete diversity of these communities. The non-

Table 1. The PAH concentrations (ng/g) in the sediments

Station ID	Nap	Acy	Ace	Fl	Phe	Flu	Pyr	BaA	BbF	BaP	DBA	InP
S1	27.00	2.99	5.13	38.29	177.92	112.44	133.73	22.04	50.64	23.38	33.81	88.10
S2	32.76	4.36	9.22	64.81	326.26	130.87	117.79	32.25	64.42	5.24	22.42	22.32
S3	15.04	2.45	5.47	32.42	137.88	64.47	35.15	14.39	39.12	2.62	9.63	3.82
S4	26.46	2.89	3.47	21.91	144.60	165.70	152.18	28.64	91.99	3.74	33.60	29.83
S5	1.09	1.34	2.61	24.01	137.08	90.41	61.17	20.99	48.88	7.81	17.08	9.13
S6	19.26	4.48	3.55	11.58	74.72	77.00	56.89	12.54	37.70	25.42	0.00	26.23

Notes: NaP represents naphthalene, Acy acenaphthylene, Ace acenaphthene, Fl fluorine, Phe phenanthrene, Flu fluoranthene, Pyr pyrene, BaA benzo[a]anthracene, BbF benzo[b]fluoranthene, BaP benzo[a]pyrene, DBA dibenzo[a,h]anthracene, and InP indeno[1,2,3-cd]pyrene.

Table 2. The summary of the bacterial community richness and diversity

Station ID	Reads	97%					
		OTU	Ace	Chao	Shannon	Coverage	Simpson
S1	10 585	2 961	6 255 (6 006, 6 530)	5 276 (4 980, 5 615)	7.06 (7.03, 7.09)	0.851 960	0.003 0 (0.002 7, 0.003 2)
S2	13 934	4 618	13 439 (12 981, 13 922)	9 443 (8 954, 9 987)	7.55 (7.53, 7.58)	0.804 722	0.001 8 (0.001 7, 0.002 0)
S3	11 940	4 024	11 119 (10 726, 11 536)	7 793 (7 383, 8 253)	7.57 (7.54, 7.59)	0.808 543	0.001 2 (0.001 2, 0.001 3)
S4	12 835	3 916	10 252 (9 885, 10 641)	7 438 (7 048, 7 877)	7.37 (7.35, 7.40)	0.830 074	0.002 0 (0.001 9, 0.002 1)
S5	12 526	3 669	7 080 (6 797, 7 384)	6 165 (5 872, 6 496)	7.34 (7.32, 7.37)	0.853 425	0.002 2 (0.002 0, 0.002 3)
S6	10 888	3 696	9 794 (9 428, 10 182)	6 952 (6 586, 7 363)	7.39 (7.37, 7.42)	0.807 403	0.001 9 (0.001 8, 0.002 0)

Notes: Reads are the high-quality sequences after filtering; values in bracket are 95% confidence intervals as calculated by MOTHUR.

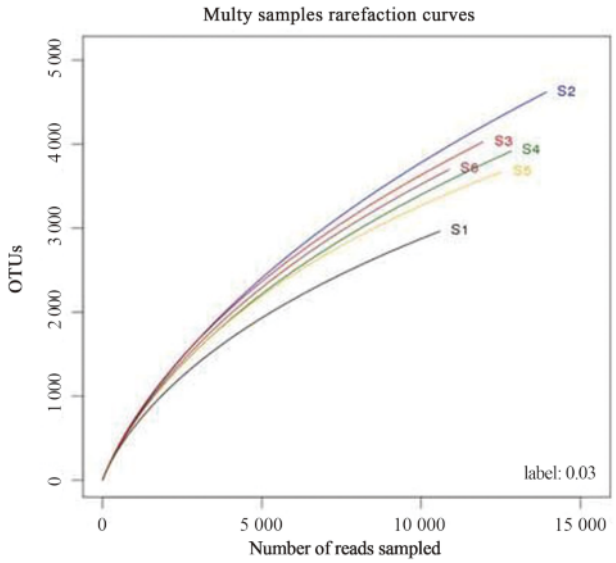

Fig. 2. Rarefaction curves based on pyrosequencing of the six bacterial communities. The OTUs were defined by 97% similarity.

parametric richness indexes of Chao and Ace, evaluated at 97% similarity, showed a similar comparative trend in predicting number of OTUs for each sample. Sample from Sta. S2 had the highest richness (Ace=13 439, Chao=9 443), while sample from Sta. S1 had the lowest one (Ace=6 255, Chao=5 276). The highest sediment bacterial diversity (Shannon=7.57) was found at Sta. S3, while the lowest one was at Sta. S1 (Shannon=7.06). The Simpson index varied between 0.012–0.030.

At 97% similarity, the sequences from the six sediment samples were classified from phylum to species according to the Mothur program using the default setting. These sequences/OTUs were assigned to 41 phyla, 84 classes, 268 genera and 789 species. The phylogenetic classification of sequences at phylum and class levels from the six coastal sediments was summarized in Fig. 3. Proteobacteria (45.10%–60.92%) was the largest phylum across all the six investigated samples, and other phyla making up more than 1% in all of the six libraries included Bacteroidetes, Chloroflexi, Planctomycetes, Acidobacteria, Nitrospirae and Gemmatimonadetes (Fig. 3a). γ-Proteobacteria was the most abundant class at Stas S1, S2 and S4 with the percentage of 27.86%, 30.78% and 30.58%, respectively. In contrast, δ-Proteobacteria was the most abundant class in the other three stations with 24.81%, 31.88% and 26.07%, respectively. Other classes with percentage >1% in all the six libraries included α-Proteobacteria, Phycisphaerae, Nitrospira, Anaerolineae, Holophagae and Gemmatimonadetes (Fig. 3b). Figure 4 showed a phylogenetic tree that was constructed based on the 16S rRNA gene sequences of the top 20 dominant genera at the six libraries. Total ten predominant genera were observed across all the six bacterial communities. Among them, four genera including *JTB255_marine_benthic_group*, *Desulfobulbus*, *Marinicella*, *Sva0081_sediment_group* and *Anaerolineaceae_uncultured* made up larger than 1%, and *JTB255_marine_benthic_group* was the most abundant genus accounting for 16.44%, 17.45%, 7.00%, 15.26%, 8.67% and 14.64%, respectively (Fig. 4).

Table 3 showed the top 20 predominant species in the six libraries. For Stas S1 and S2, the percentages of the top 20 predominant species varied between 0.31%–3.34% and 0.32%–2.04%, respectively. And Sva1033_uncultured_bacterium was the most dominant species both in the S1 and S2 bacterial libraries. At Sta. S3, the top 20 predominant species ranged from 0.44% to 4.89%, and MSBL9_uncultured_bacterium was the most abundant species. At Sta. S4, they varied between 0.41%–1.91%, and *Nitrospiraceae_uncultured_bacterium* was the most dominant species. At Stas S5 and S6, the percentages were 0.34%–3.65% and 0.38%–2.31%, respectively. And *Syntrophobacteraceae*_uncultured δ-Proteobacterium was the most abundant species both in the S5 and S6 bacterial communities.

3.3 *The results of CCA*

Figure 5a was the CCA results between the Shannon, dominant phylum abundance and the basic physical-chemical factors. Figure 5a showed that Axis 1 was affected mainly by D, DO, TN, SSA, clay%, silt% and sand% with correlation coefficient (F) = –0.655 5, 0.615 4, 0.646 8, –0.784 8, –0.781 7, –0.633 3 and 0.688 7, respectively. While Axis 2 was affected by salinity and TP with F=–0.860 0 and 0.883 5, respectively. All the eight metals we concerned had no significant effects both on Axis 1 and Axis 2 (F=–0.022

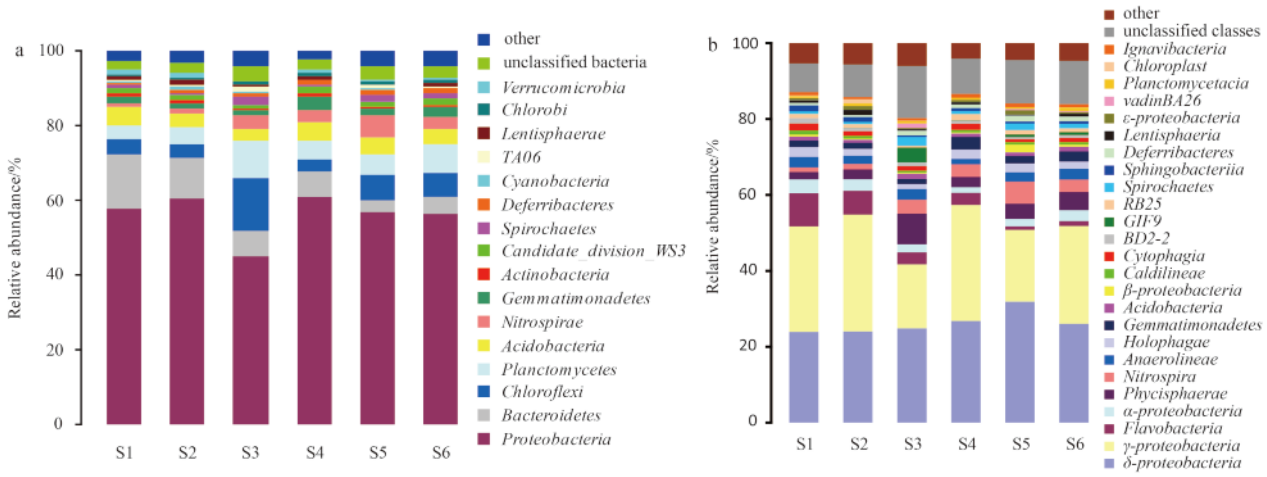

Fig. 3. Bacterial community compositions at phylum (a) and class (b) levels revealed by pyrosequencing, the relative abundance was defined as the percentage of the species sequences in total high-quality sequences in sample, classified using SILVA databank. Phyla/classes making up less than 1% of total composition in all of the six libraries were classified as "other", respectively.

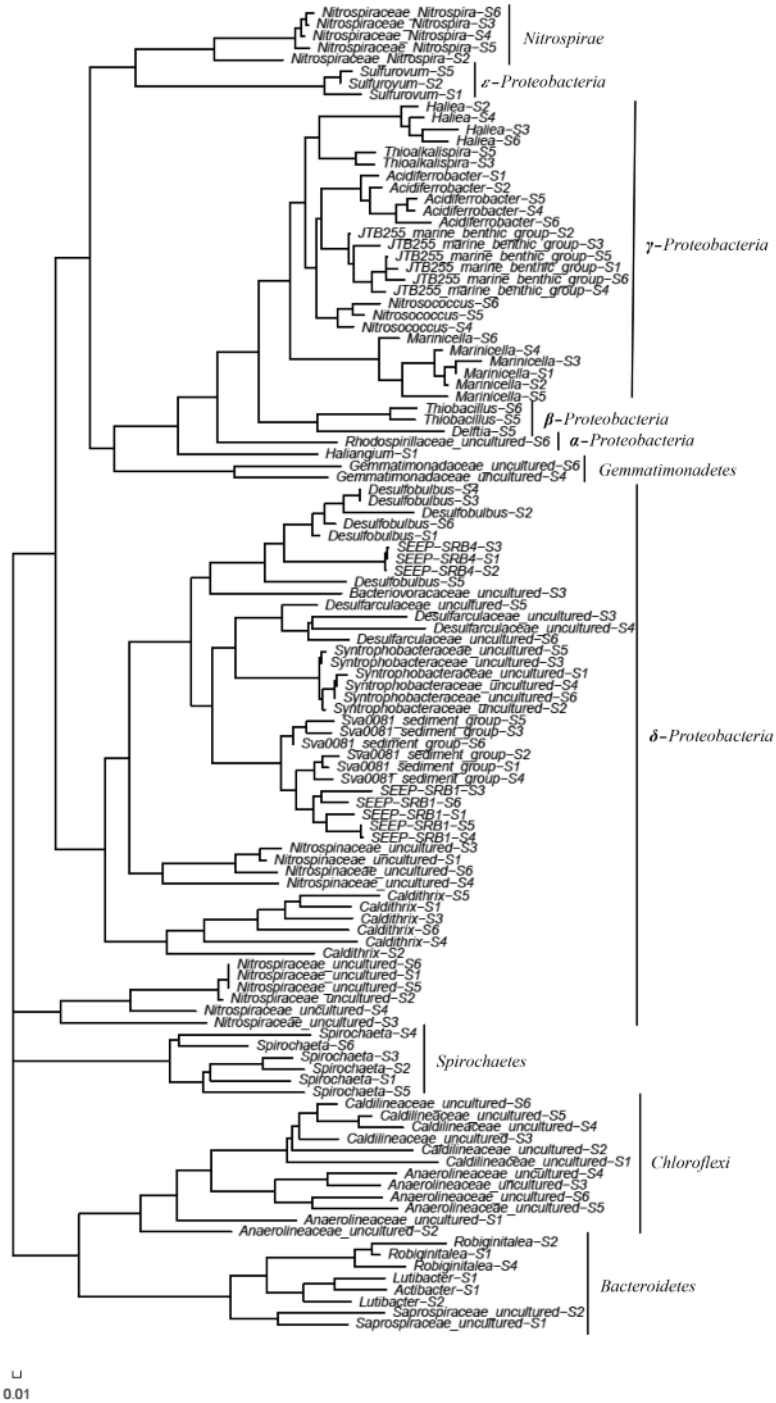

Fig. 4. Phylogenetic tree of the top 20 dominant genera at the six sediment samples based on 16S rRNA gene sequences. Branch lengths correspond to sequence differences as indicated by the scale bar.

4–0.455 1). Thus, the seven environmental variables had significantly effects on the microbial community. According to the correlation coefficient, the SSA was the most important factor, followed by clay%, sand%, D, TN, silt% and DO. Although salinity and TP had larger effects on Axis 2, there was no significant effect on the phylum abundance. Figure 5b was the CCA results between the Shannon, dominant phylum abundance and PAH pollution. It showed that all the PAH compositions were located in a positive direction of Axis 1. Total nine compositions including Nap, Acy, Ace, Fl, Phe, Flu, Pyr, BaA and InP had significantly effects on Axis 1 with correlation coefficient (F) = 0.698 2, 0.613 8, 0.725 0, 0.647 8, 0.729 4, 0.658 8, 0.773 2, 0.676 8 and 0.696 7, respectively. In contrast, only Ace had significantly effect on Axis 2 with F=–0.629 8, but Ace had no significant effect on the phylum abundance. Most of the bacterial phyla were grouped close to the biplot center indicating relatively weak effect of the studied sediment properties on them. In addition, the Shannon was near the biplot center indicating that the studied sediment properties had no significant effect on bacterial diversity.

Table 3. The top 20 predominant species in the six coastal sediments of the Bohai Bay

	S1	Percentage/%	S2	Percentage/%	S3	Percentage/%
1	*Sva1033_uncultured_bacterium*	3.34	*Sva1033_uncultured_bacterium*	2.04	*MSBL9_uncultured_bacterium*	4.89
2	*Desulfobulbus _uncultured_bacterium*	1.57	*Desulfobulbus_uncultured _bacterium*	1.83	*GIF9_uncultured _bacterium*	3.80
3	*Marinicella_uncultured _sediment_bacterium*	1.18	*Marinicella_uncultured _sediment_bacterium*	1.07	*Sva1033_uncultured _bacterium*	2.61
4	*Robiginitalea_uncultured _Bacteroidetes_bacterium*	1.04	*JTB255_marine_benthic_group _uncultured_sediment_bacterium*	1.02	*Syntrophobacteraceae _uncultured_δ-proteobacterium*	2.40
5	*SEEP-SRB4_uncultured _bacterium*	0.87	*Heterosigma_akashiwo*	0.88	*MSB-3A7_sediment_group _uncultured_bacterium*	1.04
6	*Syntrophobacteraceae_uncultured _δ-proteobacterium*	0.82	*Syntrophobacteraceae _uncultured_δ-proteobacterium*	0.81	*OPB95_uncultured _bacterium*	0.97
7	*Candidate_division_OD1 _uncultured_bacterium*	0.79	*JTB255_marine_benthic_group _uncultured__γ-proteobacterium*	0.71	*vadinBA26_uncultured _bacterium*	0.97
8	*JTB255_marine_benthic_group _uncultured_sediment_bacterium*	0.75	*MSB-3A7_sediment_group _uncultured_bacterium*	0.63	*BD7-8_marine_group _uncultured_γ-proteobacterium*	0.85
9	*Nitrospiraceae_uncultured _bacterium*	0.50	*BD7-8_marine_group _uncultured__γ-proteobacterium*	0.52	*SEEP-SRB1_uncultured _bacterium*	0.81
10	*JTB255_marine_benthic_group _uncultured γ-proteobacterium*	0.48	*Sva0081_sediment_group _uncultured_bacterium*	0.47	*Sva0081_sediment_group _uncultured_bacterium*	0.80
11	*Actibacter_uncultured _Bacteroidetes_bacterium*	0.44	*Candidate_division_OD1 _uncultured_bacterium*	0.45	*Candidate_division_OD1 _uncultured_bacterium*	0.75
12	*BD7-8_marine_group _uncultured_bacterium*	0.43	*JTB255_marine_benthic_group _uncultured_Pseudomonas_sp.*	0.44	*Desulfobulbus_uncultured _bacterium*	0.72
13	*MSB-3A7_sediment_group _uncultured_bacterium*	0.41	*SEEP-SRB4_uncultured _bacterium*	0.42	*Marinicella_uncultured _sediment_bacterium*	0.70
14	*BD7-8_marine_group _uncultured_γ-proteobacterium*	0.40	*Maritimimonas_uncultured _Bacteroidetes_bacterium*	0.39	*Spirochaeta_uncultured _bacterium*	0.64
15	*Sva0081_sediment_group _uncultured_bacterium*	0.39	*BD2-11_terrestrial_group _uncultured_bacterium*	0.38	*Desulfarculaceae_uncultured _bacterium*	0.63
16	*BD2-11_terrestrial_group _uncultured_bacterium*	0.36	*Muricola_uncultured _bacterium*	0.38	*Thioalkalispira_uncultured _γ-proteobacterium*	0.58
17	*Formosa_uncultured _bacterium*	0.35	*Candidate_division_WS3 _uncultured_bacterium*	0.37	*Nitrospiraceae_uncultured _bacterium*	0.54
18	*Maritimimonas_uncultured _Bacteroidetes_bacterium*	0.34	*Nitrospiraceae_uncultured _bacterium*	0.37	*SEEP-SRB4_uncultured _bacterium*	0.49
19	*Sandaracinaceae_uncultured _δ-proteobacterium*	0.32	*BD7-8_marine_group _uncultured_bacterium*	0.36	*BD7-8_marine_group _uncultured_bacterium*	0.47
20	*BS1-0-74_uncultured _actinobacterium*	0.31	*Haliea_uncultured _bacterium*	0.32	*Sva0081_sediment_group _uncultured δ-proteobacterium*	0.44
	S4	Percentage/%	S5	Percentage/%	S6	Percentage/%
1	*Nitrospiraceae _uncultured_bacterium*	1.91	*Syntrophobacteraceae_uncultured δ-proteobacterium*	3.65	*Syntrophobacteraceae _uncultured δ-proteobacterium*	2.31
2	*Syntrophobacteraceae_uncultured _δ-proteobacterium*	1.78	*Nitrospiraceae_uncultured _bacterium*	2.74	*Desulfobulbus_uncultured _bacterium*	1.65
3	*Desulfobulbus_uncultured _bacterium*	1.68	*MSBL9_uncultured _bacterium*	1.91	*Nitrospiraceae_uncultured _bacterium*	1.62
4	*Sva1033_uncultured _bacterium*	1.48	*Desulfobulbus_uncultured _bacterium*	1.68	*Sva1033_uncultured _bacterium*	1.51
5	*Sva0081_sediment_group _uncultured_bacterium*	1.43	*Sva0081_sediment_group _uncultured_bacterium*	1.66	*MSBL9_uncultured _bacterium*	1.44
6	*MSBL9_uncultured_bacterium*	0.77	*Sva0081_sediment_group _uncultured_δ-proteobacterium*	1.63	*Sva0081_sediment_group _uncultured_bacterium*	1.10
7	*D.43F-1404R_uncultured _δ-proteobacterium*	0.76	*GIF9_uncultured _bacterium*	0.69	*MSB-3A7_sediment_group _uncultured_bacterium*	0.97
8	*BD2-11_terrestrial_group _uncultured_bacterium*	0.72	*Candidate_division_OD 1_uncultured_bacterium*	0.65	*GIF9_uncultured _bacterium*	0.71
9	*Marinicella_uncultured _sediment_bacterium*	0.69	*Thiobacillus_uncultured _deep-sea_bacterium*	0.59	*Sva0081_sediment_group _uncultured_δ-proteobacterium*	0.71
10	*BD7-8_marine_group _uncultured_γ-proteobacterium*	0.65	*D.43F-1404R_uncultured δ-proteobacterium*	0.58	*BD7-8_marine_group _uncultured__γ-proteobacterium*	0.64

to be continued

Continued from Table 3

	S4	Percentage/%	S5	Percentage/%	S6	Percentage/%
11	*Sandaracinaceae_uncultured _δ-proteobacterium*	0.65	*Sh765B-TzT-29_uncultured _δ-proteobacterium*	0.56	*Candidate_division_OD1 _uncultured_bacterium*	0.64
12	*BD7-8_marine_group _uncultured_bacterium*	0.61	*BD7-8_marine_group _uncultured_bacterium*	0.52	*D.43F-1404R_uncultured _δ-proteobacterium*	0.63
13	*Sva0081_sediment_group _uncultured δ-proteobacterium*	0.55	*SEEP-SRB1_uncultured _bacterium*	0.48	*JTB255_marine_benthic_group _uncultured_Pseudomonas_sp.*	0.58
14	*MSB-3A7_sediment_group _uncultured_bacterium*	0.51	*Spirochaeta_uncultured _bacterium*	0.42	*Sh765B-TzT-29_uncultured _δ-proteobacterium*	0.57
15	*Sh765B-TzT-29_uncultured δ-proteobacterium*	0.51	*PHOS-HE36_uncultured _deep-sea_bacterium*	0.40	*BD2-11_terrestrial_group _uncultured_bacterium*	0.51
16	*Robiginitalea_uncultured _Bacteroidetes_bacterium*	0.48	*TM6_uncultured_bacterium*	0.38	*Marinicella_uncultured _sediment_bacterium*	0.45
17	*PHOS-HE36_uncultured _deep-sea_bacterium*	0.47	*Thioalkalispira_uncultured _γ-proteobacterium*	0.38	*Thiobacillus_uncultured _deep-sea_bacterium*	0.42
18	*Nitrosococcus_uncultured _sediment_bacterium*	0.44	*SEEP-SRB1_uncultured δ-proteobacterium*	0.37	*JTB255_marine_benthic_group _uncultured_γ-proteobacterium*	0.39
19	*Acidiferrobacter_uncultured _γ-proteobacterium*	0.41	*BD2-11_terrestrial_group _uncultured_bacterium*	0.34	*PAUC43f_marine_benthic _group_uncultured_bacterium*	0.38
20	*JTB255_marine_benthic_group _uncultured__γ-proteobacterium*	0.41	*BD7-8_marine_grou p_uncultured_γ-proteobacterium*	0.34	*Sva0725_uncultured _bacterium*	0.38

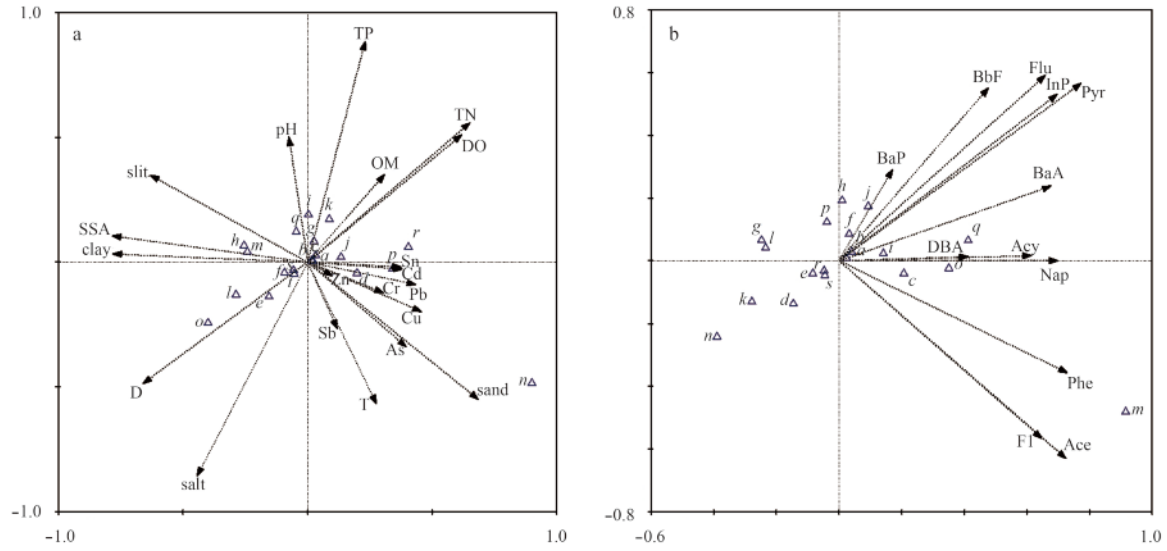

Fig. 5. CCA analysis between the bacterial community characteristics and the basic physical-chemical factors (a), and PAH composition (b). *a* represents Shannon, *b* Proteobacteria, *c* Bacteroidetes, *d* Chloroflexi, *e* Planctomycetes, *f* Acidobacteria, *g* Nitrospirae, *h* Gemmatimonadetes, *i* Actinobacteria, *j* Candidate_division_WS3, *k* Spirochaetes, *l* Deferribacteres, *m* Cyanobacteria, *n* TA06, *o* Lentisphaerae, *p* Chlorobi, *q* Verrucomicrobia, *r* unclassfied bacteria, and *s* other. Among the environmental factors, D represents water depth, salt salinity, T water temperature, sand sand%, silt silt%, clay clay%, DO dissolved oxygen, OM organic matter, TN total nitrogen, TP total phosphorus, and SSA specific surface area. The PAHs composition: Nap represents naphthalene, Acy acenaphthylene, Ace acenaphthene, Fl fluorine, Phe phenanthrene, Flu fluoranthene, Pyr pyrene, BaA benzo[a]anthracene, BbF benzo[b]fluoranthene, BaP benzo[a]pyrene, DBA dibenzo[a,h]anthracene, and InP indeno[1,2,3-cd]pyrene.

4 Discussion

Second generation sequencing technology (454-pyrosequencing) has provided more comprehensive information about microbial communities due to its capacity to identify a greater number of sequences than traditional DNA approaches (Kwon et al., 2011; Zhang et al., 2011). Recently it has been applied to reveal the characterization of complex microbial communities in a range of environments (Dowd et al., 2008; Gomes et al., 2011; Roh et al., 2010; Wang et al., 2013). Here we used pyrosequencing to detect the microbial community in the coastal sediments of the Bohai Bay, China. The results showed that both the richness and diversity were different among the six stations (Table 2), and the community compositions at the different taxonomic levels were diversified (Fig. 3, Fig. 4 and Table 3). Furthermore, a large of unclassified representatives was obtained and their percentages increased with the depth of classification (Fig. 3). However, the rarefaction curves of the six samples have not been saturated and different samples required varied minimal sequencing amounts to reach the saturated stage (Fig. 2). In the previous studies, higher diversity at the phylum level was detected using pyrosequencing comparing with the results based on Sanger sequencing based analysis of 16S rRNA gene clone libraries (Zeng et al., 2013). Therefore pyrosequencing offered the ability to detect more unknown and low abundance sequences than tra-

ditional clone library approaches, which should assist in the discovery of new species. The sheer number of sequences generated by pyrosequencing was able to ensure that minor microorganisms that composed only a small portion of the population were not missed.

At the different taxonomic levels, there were obvious differences in the species composition and their distribution among the six microbial communities (Fig. 3, Fig. 4 and Table 3). Hierarchical cluster analysis was used to identify the differences of six bacterial community structures. There were three clusters (Fig. 6). It showed that the bacterial community at Stas S1 and S2 were clustered together, suggesting a similar community structure between the two sediments. Whereas the bacterial community at Stas S4, S5 and S6 were clustered together, suggesting a similar community structure among the three sediments. In contrast, the bacterial community at Sta. S3 formed a cluster by itself. The three clusters were well separated from each other, indicating that there were clear distinctions in the community structure among the three clusters.

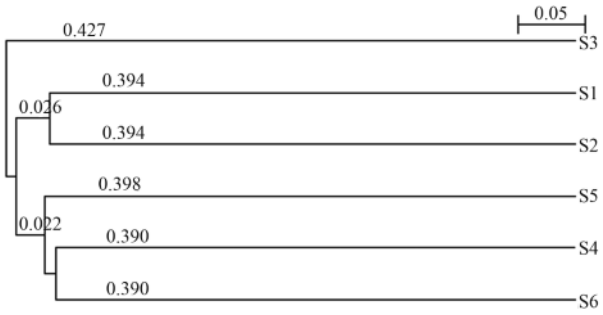

Fig. 6. The similar comparison of bacterial community structures among the six sediment samples. The sharedtree was made using the software package MOTHUR 1.15.0 based on the C_{jaccard}. The scale bar represents the unit of branch length, and the length of each branch represents the distance of the dissimilarity between the bacterial communities.

In order to reveal the role of environmental variables on the bacterial communities, here CCA was used (Fig. 5). A clear effect of sediment texture (SSA, clay%, silt%, sand%), D, DO and TN on some dominant phylum abundance were observed, whereas eight metals have slight effects on these dominant phylum abundance as indicated by their small loading score on Axis 1 (Fig. 5a). For PAH pollutions, total nine compositions including Nap, Acy, Ace, Fl, Phe, Flu, Pyr, BaA and InP had significantly effects on the dominant phylum abundance. In contrast, all the environmental variables have no significant effects on the diversity (Shannon) (Fig. 5). Those results suggested that the abundance change of dominant phyla caused by environmental pressure did not induce a distinct shift of bacterial community diversity. In most systems, organisms are exposed to a wide range of "contaminants" concurrently. In the presence of multiple stressors, a shift of some species which is sensitive to some environmental variables would occur. However, community diversity has the potential to return to that of an undisturbed state once the stressor is removed, although some community members may have changed (Ager et al., 2010). In a chronically polluted system, diversity has been shown to be a poor indicator of ecosystem stress as it can recover due to divergence and proliferation of tolerant species (Gillan et al., 2005). Our results indicated that the bacterial composition might be more sensitive than the diversity to our concerned environmental variables in the studied coastal regions.

The roles of sediment texture, DO and pH in microbial community has been widely reported (Freitag et al., 2003; Girvan et al., 2003; Böer et al., 2009; Tripathi et al., 2012; Wang et al., 2013). Some studies indicated that salinity and rainwater addition were major factors in controlling microbial diversity, function, and community composition in aquatic environments (Crump et al., 2004; Teira et al., 2013). The strong influence of heavy metals on bacterial community structure has been also widely reported in the literatures both in the fields (Ellis et al., 2001; Gillan et al., 2005) and under the laboratory conditions (Magalhães et al., 2011; Roane and Kellogg, 1996). However, from our investigative analyses, no significant correlations between pH, salinity, metals and sediment bacterial community have been detected. It indicated that sediment texture (SSA, clay%, silt% and sand%), D, DO as well as TN were responsible for driving the observed changes in bacterial community composition. This may be partly because the pH and salinity range in the sampled environment was not very broad (pH 7.98–8.08, salinity 24.8–28.4). At the same time, the metal contents did not varied sharply enough and their roles might be obscured. In addition, our study has found that TN has significant effect on the bacterial community (Fig. 5a), which was in accordance with the previous studies (Wobus et al., 2003; Kolukirik et al., 2011). Here D was also a key factor in controlling the bacterial community composition, which was never reported in the previous studies. CCA results showed that there were significant relationships between D and DO (p<0.05, not showed here). Therefore, it was likely that the effect of D was induced by affecting the DO content that was a key factor in regulating the bacterial community.

PAHs are a group of organic pollutants composed of two or more fused aromatic rings, produced by natural and anthropogenic sources. PAHs present in sediment may exhibit a toxic activity towards different organisms. Microorganisms, being in intimate contact with the sediment environment, are very sensitive to low concentrations of contaminants and respond rapidly to environment perturbations (Andreoni et al., 2004). Therefore, the microbial communities that chronically exposed to PAH tend to be dominated by those organisms capable of use PAH as carbon and energy source (Gallego et al., 2007). Even in the previously unpolluted areas there is a proportion of microbial community composed by PAH degrading bacteria that are able to degrade PAH (Surridge et al., 2009). The specific response of a microbial community varies from site to site, as well as from laboratory to field studies (Röling et al., 2002). Most of the commonly studied PAH-degrading bacteria from marine environments have belonged to Proteobacteria (Yakimov et al., 1998, 2003) and Cyanobacteria (Sorkhoh et al., 1992; Raghukumar et al., 2001). Here Nitrospirae, Deferribacteres, Spirochaetes, TA06, Verrucomicrobia, Lentisphaerae and Cyanobacteria had been significant related with PAH pollution, while Proteobacteria had weak relationship with PAH pollution (Fig. 5b). In addition, most PAH-degrading species from marine environment identified in the previous studies (Haritash and Kaushik, 2009) had not been observed in the studied region, and those results were consistent with the fact that a majority of coastal sediment bacteria are unknown. Therefore, the PAH-degraders in the coastal sediment of the Bohai Bay had differed from the isolates studied so far, suggesting that the studied region might harbor different groups of PAH-degrading bacteria with high potential for PAH degradation.

5 Conclusions

Pyrosequencing was a powerful tool to elucidate the microbial community in the coastal sediment. Among the studied six coastal stations, high bacterial diversity was detected and there were significant differences in the dominants and their abundances at the different taxonomic levels. A large proportion of unclassified representatives distributed in the different taxonomic levels, and more efforts would be necessary to investigate their functions in the coastal ecosystem. The environmental conditions including PAH pollutions played key roles in controlling the bacterial communities. In the coastal region of the Bohai Bay, the bacterial community structure might be more sensitive than the diversity to the existing environmental pressure.

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