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Analysis of the bacterial community in the two typical intertidal sediments of Bohai Bay, China by pyrosequencing

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ABSTRACT

For full understanding of the bacterial community in the intertidal zones of Bohai Bay, China, we used pyrosequencing-based approach to analyze the 16S rRNA gene of bacteria in the sediments from the two typically intertidal zones – Qikou (Qi) and Gaoshaling (Ga). Results showed that, at a 0.03 distance, the sequences from the Qi sediment were assigned to 3252 operational taxonomic units (OTUs) which belong to 34 phyla, 69 classes and 119 genera, while the 3740 OTUs from the Ga sediment were affiliated with 33 phyla, 66 classes and 146 genera. Comparing the bacterial communities inhabiting in the two intertidal sediments, we observed significant difference in the dominant composition and distribution at phylum, class and genus levels. Canonical correspondence analysis (CCA) showed that the median grain size and DO were the most important factors regulating the bacterial abundance and diversity, while the other environmental factors have effects with different degree.

1. Introduction

Bacteria, as the most abundant sediment organism (Torsvik and Ovreas, 2002), play a major role in the fate of pollutants and the cycling of nutrients while these micro-organisms are susceptible to toxic pollutants (Eismann and Montuelle, 1999; Paerl et al., 2003; Powell et al., 2003). Bohai Sea is a semi-enclosed interior sea, which is located in the northeastern of China, between 37°07′–41°N and 117°35′–122°15′E. The water exchange between Bohai Sea and ocean is weak and that makes the physical self-clean capacity of Bohai Sea very poor. With the economic boom, the ecosystem of the Bohai Sea, especially in its coastal environment, has become one of the most degraded in China (Yu and Mao, 2002; SOA, 2010). Therefore, the studies about the contaminants, the biota such as macrobenthos and their responses to this heavy pollution in the ecosystem are increasingly documented (e.g. Ma et al., 2001; Zhang et al., 2009). For microbial population, the studies have mainly focused on many pollutant-related bacteria and their responses to organic pollutants and heavy metals during bioremediation (e.g. Lu et al., 2011; Wang et al., in press). To very best of our knowledge, the entire bacterial community inhabiting in the intertidal sediment along the Bohai Sea has not been reported in the literature, yet such information is important to understand the ecological function of the bacterial assemblage and reveal the biochemical processes occurring (Rutters et al., 2002; Eiler and Bertilsson, 2004; Madsen, 2011). Furthermore, it will help to develop a bioremediation strategy using the native community to overcome the chronic pollution in the area.

The use of molecular methods to describe microbial populations in natural communities has attracted considerable interest as they are not dependent on the culturability of unknown micro-organisms but instead rely on the extraction of DNA from environmental samples. Pyrosequencing is a revolutionizing microbial ecology tool, which can offer sufficient sequencing depth. It reveals microorganism community, diversity, and biogeography that are inaccessible with genetic fingerprinting, clone library, and culture-reliant methods (Novais and Thorstenson, 2011). Recently, this technique has successfully been used to characterize bacterial communities from the coastal sediment (e.g. Kim et al., 2008). Since not only the grain size but also the sediment characteristics play an important role in controlling the contaminant distribution (Zhao et al., 2010; Gao and Chen, 2012) and bacterial community (Schmidt et al., 1998), in this study, we selected two representative intertidal zones in the Bohai Bay of Bohai Sea (Qikou with muddy sediment and Gaoshaling with sandy sediment), China and applied the pyrosequencing technique to investigate the entire bacterial community within the sediment. We compared the microbial communities from the two intertidal zones, and we also analyzed the possible factors regulating the bacterial community, both of which would be of great help toward a better understanding of how the microbial community impacts the intertidal ecosystem.

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2. Materials and methods

2.1. Study sites and sediment sampling

Bohai Bay is the second largest bay of the Bohai Sea, which is located in the western region of Bohai Sea, China, covering an area of about 1.6×10^4 km^2 with an average water depth of 12.5 m. The region of the Bohai Bay is characterized by a temperate, semi-humid continental monsoon climate. Qikou (Qi) and Gaoshaling (Ga) were two typical intertidal zones of Bohai Bay, with muddy sediment and sandy sediment, respectively. Field research was carried out in September 2009, and undisturbed subsurface sediments (a mean sample at 5–10 cm depth) were collected in axenic containers from the two intertidal stations (Fig. 1). At each intertidal site, sampling started at the time when the tide was at its lowest in the daytime of that day, and three replicate samples with about 300 m distance were collected at the middle intertidal zone. Then the samples were transported to the laboratory in an icebox, and stored in the dark at −20 °C until processing. A global positioning system was used to determine the sampling positions. The temperature (*T*), dissolved oxygen (DO) and pH of sediment were determined on-site by measuring the interstitial water using a thermometer, DO and pH meter, respectively. The collection locations and the *T*, DO and pH of each sample are summarized in Table 1.

The sediments from each station were 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 total organic carbon (TOC) was determined as the weight loss (% 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 determination of heavy metals including Cu, Zn, Cd, Pb and As, 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). All the measurements were replicated three times for each sample.

2.2. DNA extraction, PCR and pyrosequencing

Genomic DNA in the sediment was directly extracted from 0.25 g sample using the UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, California, USA), according to the manufacturer's instructions. All the DNA samples were purified with AxyPrep™ DNA Gel Extraction Kit. DNA-amplification was performed with the universal 16S rRNA gene primers (*Escherichia coli* positions 8–533: 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 μM each primer, 0.5 U Pfu polymerase (TransStart™ FastPfu DNA Polymerase, TransGen Biotech), and 2.5 mM 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 (95 °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). The composition of the PCR products of the partial 16S rRNA gene was

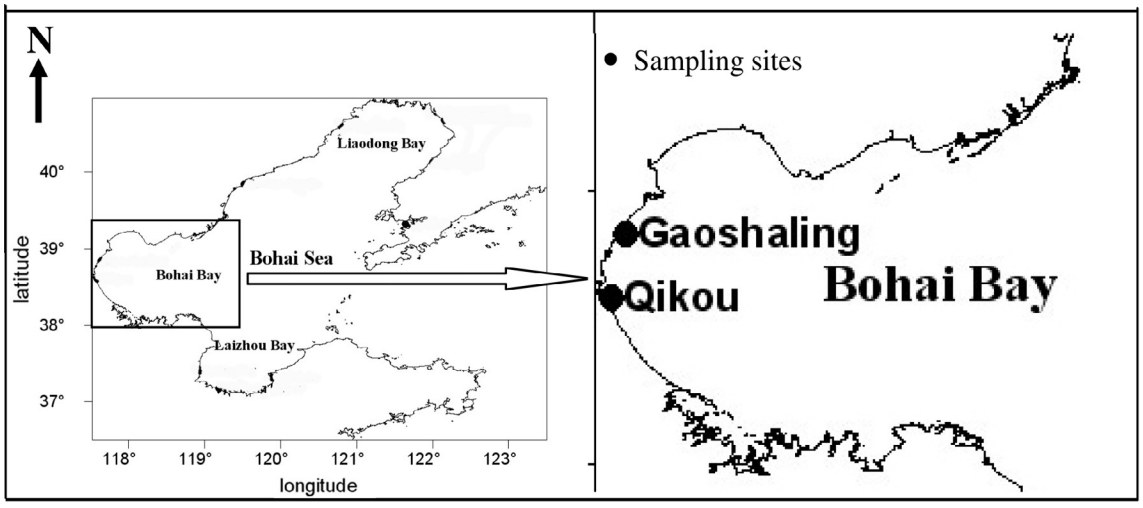

Fig. 1. Map of the study area, showing the location of the sampling stations.

Table 1
The location and environmental factors at the time of sampling.

Station ID	N	E	*T* (°C)	pH	DO (mg/L)	*W* (%)	TOC (%)	TN (μg/g)	TP (μg/g)	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	Zn (μg/g)	As (μg/g)
Qi	38°36.405′	117°35.229′	17.0	7.76	0.85	40.61	1.169	176.5	15.7	0.27	24.5	25.6	43.3	22.0
Ga	38°50.511′	117°37.538′	18.1	7.94	4.21	20.85	0.135	134.0	13.2	0.12	10.5	18.5	38.6	16.6

Qi: Qikou; Ga: Gaoshaling; *T*: temperature; DO: dissolved oxygen; *W*: water content; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus.