



Bacterial community structure in response to environmental impacts in the intertidal sediments along the Yangtze Estuary, China



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ABSTRACT

This study was designed to investigate the characteristics of bacterial communities in intertidal sediments along the Yangtze Estuary and their responses to environmental factors. The results showed that bacterial abundance was significantly correlated with salinity, SO_4^{2-} and total organic carbon, while bacterial diversity was significantly correlated with SO_4^{2-} and total nitrogen. At different taxonomic levels, both the dominant taxa and their abundances varied among the eight samples, with *Proteobacteria* being the most dominant phylum in general. Cluster analysis revealed that the bacterial community structure was influenced by river runoff and sewerage discharge. Moreover, SO_4^{2-} , salinity and total phosphorus were the vital environmental factors that influenced the bacterial community structure. Quantitative PCR and sequencing of sulphate-reducing bacteria indicated that the sulphate reduction process occurs frequently in intertidal sediments. These findings are important to understand the microbial ecology and biogeochemical cycles in estuarine environments.

1. Introduction

Coastal marine environments, especially estuaries, are complex systems affected by the interactions of geological, hydrological, physicochemical and biological factors (Zhang et al., 1999; Jiao et al., 2007). In this ecological system, microorganisms play important roles in various biological and ecological processes, including organic matter decomposition (Feng et al., 2009), carbon cycling (Jiao et al., 2014), nitrogen fixation (Barlett and Leff, 2010), and sulphate reduction (Santegoeds et al., 1998). Both the water and sediments act as reservoirs for pollutants from land-based sources via river runoffs and sewage outfalls, such as nutrients (Liu et al., 2003), antibiotics (Shi et al., 2014), polycyclic aromatic hydrocarbons (Liu et al., 2001), pesticides (Liu et al., 2008), heavy metals (Lin et al., 2002), and antibiotic resistance genes (Mao et al., 2014). Such pollutants in water can be diluted or removed by tides. On the contrary, pollutants in sediments can exist for a relatively long time, inevitably affecting the sediment-borne microbial communities and their functions (Wang et al., 2016). Therefore, investigation of the diversity and abundance of microorganisms, especially of bacteria, is becoming increasingly important to predict the environmental changes.

Nowadays, the 454 pyrosequencing and Illumina MiSeq sequencing

methods are widely used to study the 16S rRNA diversity of bacteria in various environmental samples, such as biofilms (X.F. Li et al., 2014; Y.F. Li et al., 2014; Guo et al., 2017), soil (Lauber et al., 2009), sediment (Feng et al., 2009), and water (Sun et al., 2014). Many researchers have found that the environmental parameters can affect the diversity, abundance and structure of bacterial community. For example, Liu et al. (2015) found that the sediment median size and dissolved oxygen content could regulate the microbial community in the four marginal seas of northern China, and Yu et al. (2012) found that bacterial communities were correlated with the soil organic matter and vegetation. In addition, for some bacterial communities, the dominant environmental factor affecting the community composition and structure may be relatively simple, such as salinity for halophilic microbes (Bouvier and Giorgio, 2002), nitrogen content for denitrifiers (Hou et al., 2014; Y.L. Zheng et al., 2014) and sulphate for sulphate-reducing prokaryotes (Muyzer and Stams, 2008; He et al., 2015). However, the effects of urban rivers, estuaries and marine tides on the intertidal bacterial community structure have seldom been studied (B.H. Zheng et al., 2014).

The Yangtze Estuary is located offshore from the mouth of the Yangtze River into the East China Sea and is mixed with the freshwater from the Yangtze River. The Taiwan warm current from the south and

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the Yellow Sea coastal water from the north (Chen et al., 1999) form a complex hydrological environment in this region. Moreover, the intertidal ecosystems in the Yangtze Estuary are seriously affected by pollutants from urban river runoff and sewage outfalls (Liu et al., 2001; Kuotung et al., 2009; Shi et al., 2014). Recently, studies of the microbial ecology in the Yangtze Estuary and its adjacent areas have been increasing in number (Zhang and Jiao, 2007; Feng et al., 2009; Sun et al., 2014; Liu et al., 2015; Ye et al., 2016; Guo et al., 2017). However, the total microbial communities in intertidal sediments along the Yangtze Estuary have not yet been explored. Therefore, the objectives of this study were to investigate the bacterial community structures and compositions in the intertidal sediments along the Yangtze Estuary, compare the populations among the different sampling sites, and reveal the possible environmental factors influencing the abundance, diversity and composition of the bacterial communities in the intertidal sediments in the study area.

2. Materials and methods

2.1. Sample collection

To comprehensively investigate the bacterial communities and their responses to environmental factors in the intertidal sediments along the Yangtze Estuary, eight sampling sites were selected (Fig. 1). The Xupu (XP) and Donghainongchang (DH) sites are located at a tidal flat that is mainly affected by the tides of Yangtze River water and sea water. The Qiyakou (QYK), Liuhekou (LHK), Sanchakou (SCK), and Chaoyang-nongchang (CY) sites are located at the junction of the Yangtze River and the Qipu River, Liu River, Chuanyang River, and a nameless urban river, respectively. These sites are all affected by the tides and urban river runoffs. The Shidongkou (SDK) site is located near a wastewater treatment plant (WWTP) that receives domestic sewage. The Wusongkou (WSK) site is located at the junction of the Yangtze River and the Huangpu River, the largest river across Shanghai city. In January 2016, the surficial sediment samples (0–5 cm deep) were collected in triplicate using sterile shovels. All the samples were immediately transferred into sterile plastic bags and stored in an icebox in the field.

After being transported to the laboratory, the sediments were homogenized completely. A portion of each sample was sub-packaged into a 1.5 mL sterile tube and stored at -20°C for DNA extraction. The remaining portion of each sample was stored at 4°C for the analysis of physicochemical properties.

2.2. Physicochemical properties analyses

Environmental parameters are listed in Table 1, including salinity, pH, grain size, total phosphorus (TP), total nitrogen (TN), SO_4^{2-} and total organic carbon (TOC). Salinity and pH were measured with a portable water quality analyzer (HQ 40d, HACH, USA), after fresh sediments were mixed with CO_2 -free deionised water at a volume ratio of 1:2.5 (Y.L. Zheng et al., 2014). Sediment grain size was measured using a LS 13 320 Laser grain sizer (Beckman Coulter, USA). TP was assayed using the ascorbic acid-molybdate blue method and measured using a spectrophotometer (Hou et al., 2014) after sediments were digested by the mixed solution of $\text{H}_2\text{SO}_4\text{-HClO}_4$. TN was determined by using a CN thermal combustion furnace analyzer (Elementar analyzer vario Max CN, Germany) after sediments were leached with a 1 M HCl solution (X.F. Li et al., 2014; Y.F. Li et al., 2014). The concentration of sedimentary SO_4^{2-} was measured with an auto discrete analyzer (Easy-Chem Plus, Systema, Italy) with the method of Method EASY-SO4-01 rev 0 after sediments were leached with deionised water (Yang et al., 2013). TOC contents were determined by a total organic carbon automatic analyzer (SSM-5000A, Shimadzu, Japan). The moisture content of sediments was measured based on the weight loss of a known amount of wet sediment dried at 80°C to a constant weight (Y.L. Zheng et al., 2014). All physicochemical parameters were analyzed in triplicate, and the concentrations of TP, TN, SO_4^{2-} and TOC were expressed in dry weight.

2.3. Illumina MiSeq sequencing analysis of bacterial communities

Microbial genomic DNA of the sediment samples was extracted in triplicate using the OMEGA Mag-Bind Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). The V4–V5 region of the 16S rRNA gene was

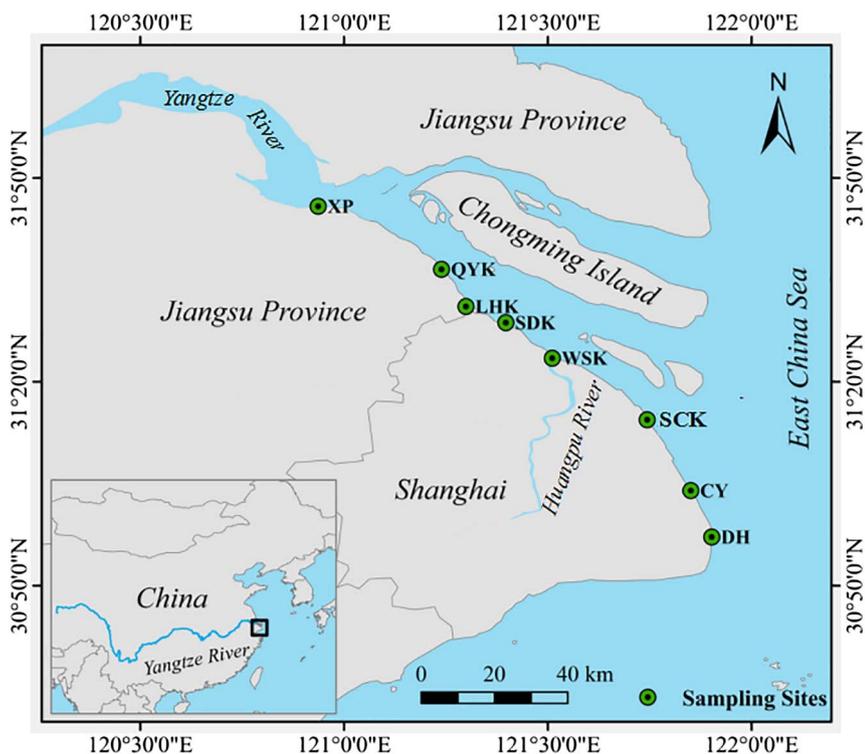


Fig. 1. Sampling sites along the Yangtze Estuary.

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