Marine Pollution Bulletin 131 (2018) 481–495

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Sediment bacterial community structures and their predicted functions implied the impacts from natural processes and anthropogenic activities in coastal area

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ARTICLE INFO

Keywords:
Bacterial community structures
Predicted functions
Nitrogen metabolism
PICRUSt
Coastal area

ABSTRACT

Coastal ecosystem structures and functions are changing under natural and anthropogenic influences. In this study, surface sediment samples were collected from disturbed zone (DZ), near estuary zone (NEZ), and far estuary zone (FEZ) of Hangzhou Bay, one of the most seriously polluted bays in China. The bacterial community structures and predicted functions varied significantly in different zones. Firmicutes were found most abundantly in DZ, highlighting the impacts of anthropogenic activities. Sediment total phosphorus was most influential on the bacterial community structures. Predicted by PICRUSt analysis, DZ significantly exceeded FEZ and NEZ in the subcategory of Xenobiotics Biodegradation and Metabolism; and DZ enriched all the nitrate reduction related genes, except *nrfA* gene. Seawater salinity and inorganic nitrogen, respectively as the representative natural and anthropogenic factor, performed exact-oppositely in nitrogen metabolism functions. The changes of bacterial community compositions and predicted functions provide a new insight into human-induced pollution impacts on coastal ecosystem.

1. Introduction

Coastal ecosystems provide human beings with critical ecological services and economic benefits in the world (Lv et al., 2016). However, with rapid economic development, they are exposed to increasing environmental pressure (Zhang et al., 2016b). The coastal sediments are always under the influences of geological, hydrodynamical, physico-chemical, chemical, and biological processes (Koster and Meyer-Reil, 2001). In contaminated coastal areas, sediments act as both sink and source of a large number of contaminants, such as nutrients (Zhou et al., 2017), heavy metals (Dou et al., 2013), and persistent hydrophobic organics (Lofrano et al., 2017). On the other hand, a rich variety of microbes including bacteria, archaea, and eukaryotic microorganisms live in the sediments and play important roles not only in the natural biogeochemical processes, but also in the mineralization of organic contaminants and the biotransformation of other pollutants (Diaz and Rosenberg, 2008; Wada et al., 2012).

The structure, composition, and diversity of sediment microbial community are evidently affected by natural environmental factors, including salinity (Lv et al., 2016), temperature (Du et al., 2011), pH (Zhang et al., 2017), and dissolved oxygen (Wang et al., 2013). Human-induced disturbances may also alter the changes of sediment microbial community (Sun et al., 2013). By analyzing 16S rRNA gene sequences, Todorova et al. (2014) identified the biodegradative potential of the adapted bacterial community in the oil-polluted sediments of Black Sea harbor; Zhao et al. (2014) found that high copper exposure caused an obvious reduction in culturable bacterial counts in the sediments of Jiaozhou Bay, China; and Lu et al. (2016) showed that Gamma- or Delta-proteobacteria dominated in the sediments of 3 wastewater-polluted estuaries in the south of Zhejiang Province, China.

Although microbial community is functionally redundant, its functional composition would change under long-term exposure to pollution. Recently in the study of microbial ecology, whole-metagenome shotgun sequencing has been applied, as it provides much information about microbes' functional potentials. By analyzing metagenomic profiles, a few studies attempted to reveal the variation of microbial community functions caused by human-induced pollution in coastal areas. Jeffries et al. (2016) chose the highly urbanized Sydney Harbour, Australia as a model system to investigate the influence of allochthonous nutrient inputs on the shift of microbial phylogenetic and functional biogeography. Meziti et al. (2016) collected surface water samples from the Kalamas River, Greece and compared the microbial gene

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https://doi.org/10.1016/j.marpolbul.2018.04.052
Received 6 January 2018; Received in revised form 20 April 2018; Accepted 20 April 2018

diversity with other aquatic habitats, from which they found that the samples during the decreased water flow months were dominated by sewage inputs and soil-related organisms. However, metagenomic sequencing and splicing may generate errors due to the non-standardized methods. So far limited functional variations are identified to be significant between the samples from polluted coastal areas and those from non-polluted areas (Fortunato and Crump, 2015; Jeffries et al., 2016).

Since 2013 a series of bioinformatic approaches called 'predictive metagenomics' has been developed, including PICRUSt (Langille et al., 2013), Tax4Fun (Aßhauer et al., 2015), and FAPROTAX (Louca et al., 2016). Based on the presumption that phylogeny and function are sufficiently linked, these computational approaches used marker gene (usually 16S rRNA gene for microbes) data and a database of reference genomes to predict the functional composition of a metagenome. By using PICRUSt analysis, Lv et al. (2016) found that nitrogen, methane, and energy metabolisms were shifty along a successional series of tidal flats in the Yellow River Delta; Jeanbille et al. (2016) showed the predictive functional response of benthic microbial communities to chronic polyaromatic hydrocarbons (PAHs) contamination in coastal sediments. K. Wang et al. (2016) demonstrated the importance of geographic distance and environmental condition in driving benthic prokaryotic diversity in coastal areas, where anthropogenic perturbations were heavy.

In this study, we chose Hangzhou Bay as the target coastal area. High-throughput sequencing of 16S rRNA gene was used to investigate the sediment bacterial compositions; and the sequence data were further employed in PICRUSt analysis to predict their functional profiles.

Hangzhou Bay is located in the southeast of China, being the inlet of the Qiantang River and the south adjacent area of the Yangtze River Estuary. With the rapid urbanization and industrialization over the past decades, the bay has been suffering from severe pollution originated mainly from anthropogenic activities, such as wastewater discharge, ports and bridges construction, and coastal mariculture. According to the official monitoring bulletin, inorganic nitrogen and labile phosphate were the primary pollutants (Zhejiang Province Ocean and Fisheries Bureau, 2016), leading to the eutrophication of the bay (Gao et al., 2011). Some studies reported high level of heavy metals (Fang et al., 2016) and persistent organic pollutants (POPs) (Adeleye et al., 2016) in the bay. In such a polluted coastal area, bacterial communities are considered more tolerant toward environmental changes (Xu et al., 2014; Dai et al., 2016; Zhang et al., 2016a; Tao et al., 2017), as bacteria usually have great versatility in resource utilization and metabolism (Székely and Langenheder, 2014). However, how the bacterial community functions vary specifically under long-term anthropogenic stress remains unknown. The purposes of this study were to investigate the spatial variation of bacterial community functions by a new bioinformatic tool and reveal the in-depth linkages between bacterial community and environmental factors in a polluted coastal area.

2. Materials and methods

2.1. Sampling sites and samples collection

Twelve sampling sites in Hangzhou Bay were chosen, as shown in Fig. 1. Among them, HC2 and HC4 are in the effluent-receiving area of a wastewater treatment plant (WWTP) in an industrial park; the other HB-series sites, radiating from the offshore coast to the bay mouth with about 220 km across, are the national sites for routine monitoring. Notably, HB1 is vulnerable to the land-sourced pollution and the influence of the Qiantang River; and HB7 is close to the Yangshan Port, the China's largest containers' deepwater port under construction from 2005 till now. Therefore, we merged HB1 and HB7 with HC2 and HC4, as a group in the disturbed zone (DZ). We also merged the site under the influence of the Yangtze River input (HB4) with the sites still being affected by the input of the Qiantang River (HB2, HB5, and HB9), as a group in the near estuary zone (NEZ). Then the rest of the sites (HB3, HB6, HB8, and HB10) were grouped, as they are in the far estuary zone (FEZ).

The sediments at the 12 sites were sampled in April 2015. At each site, triplicate surface sediment (0–5 cm deep) samples were collected within a 10 m × 10 m area using a grab sampler (Van Veen, Hydro-Bios, Germany). The sediment samples (n = 36) were packed in airtight sterile plastic bags. All the samples were stored at −20 °C at sea and − 70 °C after transportation to our laboratory. At the same time, the overlying seawater samples of each site was collected using an organic glass deepwater sampler; and all samples were preserved in polyethylene bottles at 4 °C and transported to our laboratory.

The sediment properties and seawater qualities of all samples were analyzed according to the determination methods shown in Table S1.

2.2. DNA extraction and PCR amplification

The total DNA in the sediment was extracted from each 0.5 g sample in triplicate using an EZNA® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The extracted DNA concentration was determined with a NanoDrop ND-2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The extracted DNA was stored at −20 °C for further analysis.

The bacterial 16S rRNA gene was amplified for constructing community library. PCR (polymerase chain reaction) amplifications were performed using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) targeting the V1-V3 region of the bacterial 16S rRNA gene (Dai et al., 2016). The PCR was performed in triplicate with a 20 μL mixture, containing 4 μL of 5 × FastPfu Buffer, 2 μL of dNTPs (2.5 mM), 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. Thermal program was as follows: an initial denaturation at 95 °C for 3 min; followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s; and a final extension at 72 °C for 10 min.

2.3. Illumina MiSeq sequencing

The amplicons were extracted from 2% agarose gels, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified by a QuantiFluor™-ST (Promega, Madison, WI, USA). The purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP119947).

2.4. Processing of sequencing data

The raw sequence data were processed on the Quantitative Insights Into Microbial Ecology (QIIME v.1.9.1 http://www.qiime.org/) platform (Caporaso et al., 2010). Briefly, all the raw sequence data obtained were assigned to the samples based on their barcodes. Reads with an average quality value < 20 and without universal primer sequences were filtered off. Following quality control, removal of chimeras and nontargeted sequences, all sequences were aligned with the Greengenes 2013 database (gg_13_8; http://greengenes.lbl.gov/) (DeSantis et al., 2006).

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff by using the UPARSE with usearch7 (http://drive5.com/uparse/) (Edgar, 2013). Chimera checking was performed using the UCHIME algorithm (Edgar et al., 2011), which is the fastest and most sensitive chimera checking algorithm.

The prediction of metagenomic functions of bacteria was performed by a bioinformatic tool that predicts gene family abundances based on 16S rRNA gene sequences, Phylogenetic Investigation of Communities