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Exploring the differences of antibiotic resistance genes profiles between river surface water and sediments using metagenomic approach

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ABSTRACT

To better understand the potential genic communication and dissemination of antibiotic resistance genes (ARGs) in different environmental matrices, the differences of ARG profiles between river surface water and sediments were explored. Metagenomic analysis was applied to investigate the comprehensive ARG profiles in water and sediment samples collected from the highly human-impacted catchment of the Beijiang River and its river source. A total of 135 ARG subtypes belonging to 18 ARG types were identified. Generally, ARGs in surface water were more diverse and abundant than those in sediments. ARG profiles in the surface water and sediment samples were distinct from each other, but some ARGs were shared by the surface water and sediments. Results revealed that multidrug and bacitracin resistance genes were the predominant ARGs types in both surface water (0.30, 0.17 copies/cell) and sediments (0.19, 0.15 copies/cell). 73 ARG subtypes were shared by the water and sediment samples and had taken over 90% of the total detected ARG abundance. Most of the shared ARGs are resistant to the clinically relevant antibiotics. Furthermore, significant correlations between the ARGs and 21 shared genera or mobile genetic elements (MGEs) (plasmids and integrons) were found in surface water and sediments, suggesting the important role of genera or MGEs in shaping ARGs profiles, propagation and distribution. These findings provide deeper insight into mitigating the propagation of ARGs and the associated risks to public health.

1. Introduction

The spread of antibiotics resistance genes (ARGs) in aquatic ecosystems has attracted global concerns because of its association to the ineffectiveness of antibiotic in treating life-threatening infections (Luis Martinez, 2009; Witte, 2000). The riverine input of antibiotics not only resulted in the chemical pollution, but may also accelerate the development and spread of antibiotic resistant bacteria (ARB) and ARGs (Pruden et al., 2012, 2006). Furthermore, ARGs can be transferred between different microbial populations through horizontal gene transfer (HGT) carried out by mobile genetic elements (MGEs) like plasmids, integrons and transposons (Chen et al., 2013b; Lupo et al., 2012a, 2012b; Na et al., 2014; Pruden et al., 2006), increasing the risk of ARGs transfer from environmental and human commensal microorganisms to pathogens (Thomas and Nielsen, 2005). Therefore, ARGs have been regarded as the emerging pollutants (Levy, 1998; Pruden et al., 2006).

ARGs have been detected in various water bodies, including reservoirs (Huerta et al., 2013; Su et al., 2014), source of drinking water (Jiang et al., 2013), river (Ling et al., 2013; Luo et al., 2010), lake (Devarajan et al., 2015; Huerta et al., 2013) and ocean (Chen et al., 2013a; Zheng et al., 2011). However, few studies were conducted to investigate the differences of ARGs in the surface water and sediments in river environment. In the previous studies, Luo et al. (2010) investigated the occurrence and abundances of 7 tetracycline and 2 sulfonamides resistance genes in sediments and water in Haihe River of China. Different distribution of tetracycline and sulfonamide resistance genes were found between water and sediment samples and results indicated that sediments was an important ARG reservoir. Likewise, Na et al. (2014) investigated the occurrence of 3 sulfonamides resistance genes, *E.coli* and class 1 integrons in seawater and sediments of the Northern Yellow Sea of China. Results also showed that sediments harbored higher abundances of sulfonamide resistance genes when compared to those in water. It is suspected that the variations of ARGs

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in different environment matrices might be related to the distinct environmental conditions or the existence of special microorganisms. The disparate hydrogeochemical and biological factors would influence the transport, maintenance, amplification, and attenuation of ARG in aqueous ecosystems (Luo et al., 2010). For example, free DNA generally persists longer in sediment than in water because of the adsorption of DNases by soil and clay components, which would otherwise hydrolyze the free DNA (Luo et al., 2010; Manson et al., 2004). However, only limited number of ARGs were investigated in previous studies, the differences of the comprehensive profiles of ARGs in water and sediment remained unknown.

Some ARGs are ubiquitous in different environmental matrices, which may also be resulted from the transferable ability of genes in aquatic environments (Trevors et al., 1987). The existence of the shared ARGs between different environmental matrices indicated their higher mobility to spread in the natural environment compared to other ARGs. As for the unique ARGs in different environment, their exists would likely be more related to the existence of special microorganisms in water or sediment environment, or associated environmental conditions. Therefore, clarification of the patterns and profiles of ARGs in different environmental phases is important to better understand the propagation of environmental ARGs.

The occurrence, abundance and distribution of ARGs in a river catchment were found significantly influenced by the anthropogenic activities (Jiang et al., in press). Likewise, the ARGs profiles of surface water and sediments samples from the same locations would probably be influenced by the potential contamination sources nearby, bringing about the similar results in these two phases. Therefore, to objectively evaluate the common patterns and profiles of ARGs in different environmental matrices, different sampling locations along the river were chosen in the present study. Metagenomic approach was utilized to investigate the profiles of ARGs, especially the unique and shared ARGs subtypes in surface water and sediments of the Beijiang River and the river source. Furthermore, the profiles of integrons, plasmids and microbial community structure in surface water and sediments were identified as well. The relationships between ARGs and microbial community or MGEs from different environmental matrices were also examined.

2. Materials and methods

2.1. Sampling sites

The Pearl River Delta Region is one of the most densely urbanized regions in China. A total of 4 surface water samples and 4 sediment samples were collected in March and May 2013 in the Beijiang River and the Xijiang River of the Pearl River Delta. Surface water samples were designated as WA1, WA2, WA3 and WA4, and sediment samples were designated as SE1, SE2, SE3 and SE4 (Fig. 1). WA1 was collected at the intersection of a branch and main stream of the Beijiang River, and an intensive hydraulic engineering just 20 km far away. WA2 was collected at the downstream of the Xijiang River. WA3 was sampled at the intersection of three tributaries of Pearl River with complex dynamic environment. SE1 was sampled from a reservoir, one of the sources of the Beijiang River. SE2 was sampled in the midstream of the Beijiang River. SE3 was collected in the area where had the most densely population density and flourishing economy in the Beijiang River catchment. WA4 and SE4 were sampled from the same location of the Pearl River estuary where was intensively influenced by integrated aquaculture farms. The locations of potential pollution sources of ARGs, e.g. livestock farms, aquaculture farms, wastewater treatment plants (WWTPs), hospitals, metal smelting plants and pharmaceutical factories were marked in Fig. 1.

At least 1.5 L surface water samples (0.5 m below the surface) were collected using a glass sampler and stored in sterile PET bottles. About 400 g sediment samples were collected using a grab sampler and sealed in polyethylene zip-bags. All the collected samples were kept in the dark at 4 °C and transported to the lab within 2 days.

2.2. DNA extraction

Microorganisms in water samples were collected with a 0.22 μm membrane filter using vacuum filter device immediately after their arrivals at the lab. Sediment samples were freeze-dried and kept at − 20 °C before DNA extraction. DNA of microorganisms on the membrane filters and sediments was extracted by the E.Z.N.A. Water DNA Kit and E.Z.N.A. Soil DNA Kit (OMEGA bio-tek, USA) according to the manufacturer's protocol. The purity and yield of the extracted DNA was determined using the Nano Vue Plus Spectrophotometer (Healthcare Bio-Sciences AB, Sweden).

2.3. DNA sequencing

Approximately 3 μg of extracted DNA from each sample was used for high throughput sequencing. High-throughput sequencing was performed by the Beijing Genomics Institution (Shenzhen, China) using the Illumina Hiseq. 2000 platform with the sequencing strategy of PE100. Approximately 5 Gb (giga base pairs) was generated for each DNA sample, resulted in about 40 Gb metagenomic data. These datasets were deposited in NCBI SRA with the accession number of SRP128944.

2.4. Bioinformatic analysis

Quality of the sequencing data was first checked with FastQC (Schmieder and Edwards, 2011). The ARG-like sequences were identified using ARGs-OAP (Yang et al., 2016). A read was annotated as an ARG-like fragment if it had ≥ 90% amino acid identity and alignment length ≥ 25 amino acids. In addition, sequences of integrons and plasmids were also identified using BLASTN against the INTEGRALL database and the plasmid sequences available in the NCBI RefSeq database (Moura et al., 2009; Yang et al., 2013) to characterize the profiles of these MGEs in the Beijiang River. A read was annotated as an integron or plasmid sequence if it had a nucleotide sequence identity higher than 90% over an alignment length of at least 50 bp (Moura et al., 2009) or above 95% over a length of at least 90 bp (Zhang et al., 2011), respectively.

The microbial community in the water and sediment samples was characterized by BLASTn (Altschul et al., 1997) using Silva SSU database (version 128) with E-value cutoff of 1e-100 (Mackelprang et al., 2011). The sequences from the BLAST results were assigned to NCBI taxonomies via MEGAN (version 6) (Huson et al., 2007) by using the Lowest Common Ancestor (LCA) algorithm and the default cutoff of BLAST bitscore 50%, and 10% of the top 50 hits.

2.5. Statistical analysis

The abundances of ARGs and MGEs were presented using the unit of copies/cell. The calculation for ARG abundance was described by Yang et al. (2016). The calculation of MGE abundance was adapted from ARGs abundance calculation and shown as follows:

$$\boldsymbol{Abundance}_{MGEs} = \sum_{1}^{n} \frac{\boldsymbol{N}_{(MGE-like\ sequences)i} \times \boldsymbol{L}_{reads} / \boldsymbol{L}_{MGE\ reference\ sequence}}{N_{cell\ number}} \quad (1)$$

where, $N_{(MGE\text{-}like\ \ sequences)i}$ is the number of the MGE-like sequences annotated to one specific MGE reference sequence; L_{reads} is the length of the reads, which was 100 bp in the present study; L_{MGE} reference sequence refers to the nucleotide sequence length of the corresponding specific MGE reference sequence; $N_{cell\ number}$ represents the bacterial cell number in the sequencing data calculated from ARGs-OAP (Yang et al., 2016).