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Bacterial communities of four adjacent fresh lakes at different trophic status

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ABSTRACT

Knowing the microbial compositions in fresh lakes is significant to explore the mechanisms of eutrophication and algal blooms. This study reported on the bacterial communities of the four adjacent fresh lakes at different trophic status by Illumina MiSeq Platform, which were Tangxun Lake (J1), Qingling Lake (J2), Huangjia Lake (J3) and Niushan Lake (J4) in Wuhan, China. J1 had the highest salinity and phosphorus. J2 was abundant in TC (Total Carbon)/TOC (Total Organic Carbon.), calcium and magnesium. J3 had the highest content of nitrogen, iron and pollution of heavy metals. High-throughput sequencing analysis of the 16S rRNA gene revealed that the eutrophic lakes (J1, J2 and J3) were dominated by Cyanobacteria (46.1% for J1, 40.8% for J2, 33.4% for J3) and the oligotrophic lake (J4) was dominated by *Actinobacteria* (34.2%). An increase of *Cyanobacteria* could inhibit the growth of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*. Functional inferences from 16S rRNA sequences suggested that J4 had more abundant bacteria with regard to substrate metabolism than J1, J2, and J3. *Burkholderia* and *Fluviicola* might be a suggestion of good water quality. The results demonstrated that the bacterial community could well reflect the water quality of the four lakes.

1. Introduction

Freshwater lakes are one of the most significant ecosystems on earth but human-related activities have altered the ecosystem gradually by increasing the flow of both inorganic nutrients and organic substances into the ecosystem, which have been always associated with the civilization and urbanization. Although a wastewater treatment process can diminish the nutrients discharge, the accumulated oversupply of nutrients can result in eutrophication (Ji et al., 2015), which leads to overgrowth of plants and algae. Plants or algae are always directly bound up with bacteria (Ramanan et al., 2016), which play an indispensable role in the decomposition and cycling of varying compounds in nutrient-rich eutrophic lakes (Bai et al., 2012). Bacteria community can reflect the water quality of a particular lake and its composition is varied with season (Newton and Mcmahon, 2011).

For lakes, sediments samples were usually adopted to disclose the microbial components (Sheng et al., 2016; Weise et al., 2016). Few literatures reported the microbial community in lake water. While microbes in lake water would be more reasonable to reflect the water quality since the microorganisms live in the water directly. Moreover, experiments were generally designed and conducted at different sites of one lake (Sheng et al., 2016). While microbial community would vary with geographical locations of lakes. So, it makes sense and is necessary to study the microbes from different lake waters in the same district.

Pyrosequencing analysis of 16S rRNA genes is one of an effective ways to explore the microbial community of an environmental sample (Molina et al., 2016), which allows for in-depth characterization of complex microbial communities (Pascault et al., 2014). By using quantitative PCR assay and pyrosequencing analysis of 16S rRNA, Kou et al. (Kou et al., 2016) reported that *Burkholderiales, Gallionellales, Myxococcales, Desulfuromonadales, Sphingobacteriales, Nitrospirales, Xanthomonadales* were identified as the major taxa in Poyang Lake, the largest freshwater lake in China. Pyrosequencing of 16S rRNA genes revealed that *Proteobacteria, Actinobacteria, and Bacteroidetes* were the 3 dominant phyla in Taihu Lake, China (Tang et al., 2015). The main bacterial communities in Lake Baikal, Russia were the phyla *Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes, Acidobacteria* and *Cyanobacteria* (Kurilkina et al., 2016).

In this study, four adjacent lakes at different trophic status were studied, which were named Tangxun Lake, Qingling Lake, Huangjia Lake and Niushan Lake. The four lakes located in the west of Wuhan City, China. The water areas were 47.6, 6.0, 8.5 and 99.0 km^2, for Tangxun Lake, Qingling Lake, Huangjia Lake and Niushan Lake, respectively. Illumina high-throughput sequencing was applied to reveal the bacteria communities. The carbon, nitrogen and phosphorus and metals in the lake water were also tested. We aimed to find the differences of bacteria compositions in the four lake waters and to associate the bacteria with water quality of the four lakes.

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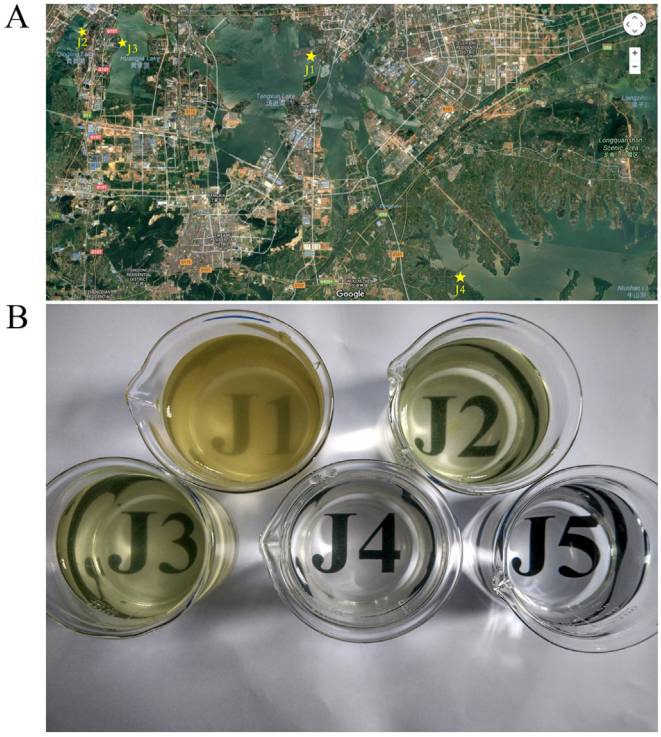

Fig. 1. A. Map of the west of Wuhan City of China showing the four lakes and sampling sites: J1, Tangxun Lake; J2, Qingling Lake; J3, Huangjia Lake; J4, Niushan Lake. B. An illustration shows the four samples and tap water: J1, Tangxun Lake; J2, Qingling Lake; J3, Huangjia Lake; J4, Niushan Lake; J5, tap water.

2. Materials and methods

2.1. Study sites and sampling

The four sampling sites were indicated in Fig. 1A, which were J1 (30°26′8.6″N, 114°22′29.0″E), J2 (30°27′1.8″N 114°15′7.4″E), J3 (30°26′42.4″N, 114°16′15.9″E) and J4 (30°20′12.7″N, 114°27′11.0″E) for Tangxun Lake, Qingling Lake, Huangjia Lake and Niushan Lake, respectively. Surface water was collected at 9–11 a.m. on June 20, 2017, when the lakes were relatively steady and at normal water levels, since it was sunny every day in a week before sampling day. The temperatures of each water of J1, J2, J3 and J4 when sampling were 26.5 °C, 26.9 °C, 27.1 °C and 26.8 °C, respectively. The collected water samples were analyzed and tested for its general properties in 4 h.

2.2. DNA extraction and measurement of water properties

We passed 100 mL water through a 25 mm diameter, 0.2 μm filter (Osmonics, Livermore, California, USA) on the vacuum filtration equipment and the filters were immediately kept at −20 °C and used for DNA extraction with the E.Z.N.A. soil DNA isolation kit (OMEGA Biotek Inc., Norcross, GA, USA) as described in the manufacturer's instructions. Ammonium nitrogen (NH_4^+-N), total nitrogen (TN) and phosphate (PO_4^{3-}P) of the samples were determined according to the Standard Methods (APHA, 2005). Concentrations of F^-, Cl^-, NO_3^-, NO_2^- and SO_4^{2-} were determined by ion chromatograph (DIONEX-ICS-600, USA). Typical metals and semimetals were tested by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using an IRIS Advantage ER/S spectrophotometer with 3 replications. All of the biochemical parameters of water samples were repeated 3 times.

2.3. Illumina Miseq sequencing

The V4 region of bacterial 16S rRNA genes with 283 bp was amplified using a broadly conserved primer set (520F:5′-GCACCTAA YTGGGYDTAAAGNG-3′, 802R:5′-TACNVGGGTATCTAATCC-3′). Q5 High Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) was used for DNA amplification. The PCR mixture of 25 μL contained the following: 0.25 μL polymerase, 5 μL 5 × Reaction buffer, 5 μL 5 × High GC buffer, 0.5 μL dNTPs, 1 μL forward primer, 1 μL reverse primer, 1 μL template DNA, and 11.25 μL ultrapure water. Thermal cycling consisted of initial denaturation at 98 °C for 30 s followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 50 °C for 30 s, and an extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The amplicons were gel purified using a DNA purification kit (Axygen Biosciences, Inc., CA, USA) and quantified with Nanodrop 2000 spectrophotometer. After purification and quantification of the PCR product, a genomic DNA library was constructed according to protocols provided by Illumina with the TruSeq Nano kit (Illumina, San Diego, CA). DNA library quality was verified on an Agilent Bioanalyzer with a High Sensitivity DNA chip. Subsequently, 300 bp paired-end sequencing was conducted for the DNA libraries with Miseq Reagent Kit v3 (600-cycles-PE) on the Illumina MiSeq platform at Personal Biotechnology Co., Ltd. of China. 16S rRNA gene sequences are available at NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra/, accession numbers SRR5895944 for J1, SRR5895943 for J2, SRR 5895946 for J3, and SRR 5895945 for J4).

The raw sequences obtained from Illumina sequencing were examined by using a sliding window approach to remove short and low-quality reads. Overlapping paired reads were combined to produce a modified FLASH source code (Magoc and Salzberg, 2011). QIIME software package was utilized to further analyze the high-throughput sequencing data (Caporaso et al., 2010). The sequences were aligned using the software QIIME ver. 1.17.0 and assigned to operational taxonomic units (OTUs) using with a 97% threshold of pairwise identity. The representative sequences from each OTU were subjected to the RDP-II Classifier of the Greengenes databases. Alpha diversity and OTU networks were generated using QIIME ver. 1.17.0. Differences in OTUs abundance at phylum and genus levels were determined using Metastats (White et al., 2009) via a web interface (http://metastats.cbcb.umd.edu/detection.html). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013) was used to predict the functional content of the bacterial operational taxonomic units. Bacterial functional profiles were compared to Kyoto Encyclopedia of Genes and Genomes (KEGG) modules level 2.

3. Results

3.1. Water properties

The four samples and tap water were presented in Fig. 1B. Values of pH of the four samples differed slightly (6.83–6.87). It indicated that J1 (Tangxun Lake) had the highest chromaticity and turbidity. J2 (Qingling Lake) and J3 (Huangjia Lake) had similar chromaticity and turbidity. While J4 (Niushan Lake) showed the best water quality among the four lake water samples. Contents of typical metals/semimetals and main pollutants in water were depicted in Table 1. In contrast, J4 (Niushan Lake) was low in nutrients such as carbon, nitrogen and phosphorus, as the TOC, TN and TP were 1.1, 0.55 and 0.04 mg/L. Moreover, Niushan Lake had fewer concentrations of metals and typical anions (F^-, Cl^- and SO_4^{2-}) than other lakes generally. So we considered Niushan Lake as oligotrophic.

Although J1, J2 and J3 samples all showed eutrophication characteristics, they had different nutrient elements. The water sample of Tangxun Lake (J1) had an extremely higher P load (1.07 mg/L PO_4^{3-}P) than other samples. The salinity of Tangxun Lake (J1) could not be neglected as shown by concentrations of sodium and chloride ions. Fe