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Bar-Coded Pyrosequencing Reveals the Bacterial Community during *Microcystis* water Bloom in Guanting Reservoir, Beijing

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Abstract

An understanding of bacterial community composition is a fundamental goal of identifying ecological efficiency caused by *Microcystis* bloom in aquatic system. In this study, we employed bar-coded 454 pyrosequencing approach to investigate the bacterial community variation during a *Microcystis* water bloom in Guanting Reservoir, Beijing. More than 140,000 sequences were generated and assigned to 7133 operational taxonomic units (OTUs) which belong to 18 phyla through Ribosomal Database Pyrosequencing (RDP) database. *Microcystis* was detected at low abundance in July then broke out and became the exclusive dominant genus in September. Meanwhile, bacteria composition changed dramatically after the *Microcystis* water bloom reflected by the decrease of bacteria abundance, diversity and evenness indices. The classify results indicated that dominant species in July were *Pelagibacter*, *Haliscomenobacter*, *Rhodobacter* and *Fluviicola*. In September, *Methylotenera*, *Flavobacterium* and *Methylophilus* were the dominant genera. It is worth noticing that all the dominant genera in July was more or less related to nitrogen and phosphorus cycling while in September, dominant genera *Flavobacterium* was reported as *Microcystis* lysing bacterium and may be a symptom of the coming postbloom phase of *Microcystis* water bloom in Guanting Reservoir.

Keywords: Guanting Reservoir; *Microcystis* bloom; Bacterial community; 16S rRNA; 454 pyrosequencing.

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1. Introduction

Microcystis water blooms in eutrophic aquatic environment such as rivers, lakes and reservoirs have been widely reported in the last decades. It constitutes a serious risk to aquatic ecosystems by consuming dissolved oxygen in the water and producing harmful secondary metabolites named Microcystin [1, 2]. Bacteria play an important role in this process, they can embed in the mucilage formed by *Microcystis*, symbiosis with *Microcystis* cells by supplying nutrients to young healthy colonies [3, 4] and benefit from using nutrients when they decayed [5]. On the other hand, aquatic bacteria such as *Sphingomonads* [6, 7] can also degrade Microcystins. However, the detailed interrelationship between *Microcystis* bloom and bacterial community is still uncertain.

Molecular tools to monitor the bacterial diversity of complex microbial assemblages have developed in the last decade using 16S rDNA based approaches recommended by Amann et al. [8]. Methods usually used are polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) [9,10], fluorescence in situ hybridization (FISH) [11], Terminal restriction fragment length polymorphism (T-RFLP) [12]. Recently, high-throughput 454 FLX pyrosequencing was introduced as a new approach capable of better revealing the taxonomic diversity within extant microbial communities [13-15]. The use of 454 pyrosequencing is revolutionary because it can provide a sufficient number of sequences to look in depth at microbial diversity in virtually any type of samples [16-18].

In this study, we employed 454 bar-code pyrosequencing of the V3 region of the 16S rRNA gene to investigate the bacterial community variation during *Microcystis* water bloom in a eutrphic reservoir in Beijing. Aim to provide detailed biological information to identify the interrelationship between bacteria community and *Microcystis* bloom.

2. 2. Methods and materials

2.1. Study sites and sample collection

Guanting Reservoir was built in 1954, located in the northwest of Beijing. It used to be a main drinking water resource of Beijing with a storage capacity of 410 million m^3 [19]. It has suffered from extensive eutrophication since 1980s, severe *Cynaobacteria* water bloom formed during summer annually [20]. *Microcystis* is the dominated species in early autumn [21]. Triplicate of surface water (0-10cm) was collected using a polymethyl methacrylate sampler from the dam area of Guanting Reservoir in middle July and September in 2009. The sampling site was reported extremely eutrophic and severe *Microcystis* bloom occurred during late summer and early autumn [20].

2.2. Bar-code pyrosequencing and data processing

For DNA extraction, water samples were filtered and the filters were immediately frozen and stored at −20°C. Triplicate of genomic DNA of each sample was extracted using Omega Water DNA Kit (Omega) according to the manufacturer's protocol. DNA was then visualized on 1.2% (wt/vol) agarose gels and photometrically measured at 260nm to assess its purity and molecular size.

For bar-code pyrosequencing, DNA of each sample was amplified using primers constructed by adding a unique barcode [21] to the 27F primer sequence (AGAGTTTGATCCTGGCTCAG) and reverse 543R primer sequence (ATTACCGCGGCTGCTGGC) for each sample. PCR products were then gel purified using EZ Spin Column PCR Product Purification Kit according to the manufacturer's protocol. All the prepared PCR products were submitted to Beijing Institute of Genomics, Chinese Academy of Sciences for sequencing on a Roche FLX 454 pyrosequencing instrument.

Sequence information was processed through the proprietary Roche software to generate sequences then processed through the pipeline initial process of Ribosomal Database Pyrosequencing (RDP, http://rdp.cme.msu.edu) database. Richness and diversity of each sample was estimated by Chao1, Shannon's diversity and evenness (E) indices.

3. Results

3.1. Distribution of taxa and phylotypes

More than 140,000 acceptable-quality sequences were obtained with an average length of 485bp. After trimming 23416 sequences were obtained, 17107 from July and 16298 from September. When clustered at 97% similarity level, sequences were assigned to 2327, 1215 operational taxonomic units (OTUs) respectively. As shown in table 2, nearly equal numbers of sequences were detected in July and September. After classified through RDP database, the OTUs number achieved in July was almost two times higher than in September. Bacterial richness and diversity were estimated using Chao1 [22], Shannon's diversity (H') [23] and evenness (E).

Table 1. Estimated OTU richness, diversity indices for 16S rRNA libraries of Guanting Reservoir water samples

Season	Reads	OTUs	Chao1	H'	E
Jul.	17107	2327	4,916.32	5.424	0.699
Sep.	16298	1215	2,525.91	3.329	0.469

Of all the classifiable sequences, 16 phyla were identified (Fig. 1). The dominant phyla in July were *Cyanobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, representing 32.3%, 30.2%, 18.9% 14.2% of the total sequences. In September, *Cyanobacteria* and *Proteobacteria* bacteria became the dominant phyla accounted for 75.8%, 14.3% of the sequences there. For detailed analyses, sequences belong to *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes* phyla were selected because of their high abundance in water samples.

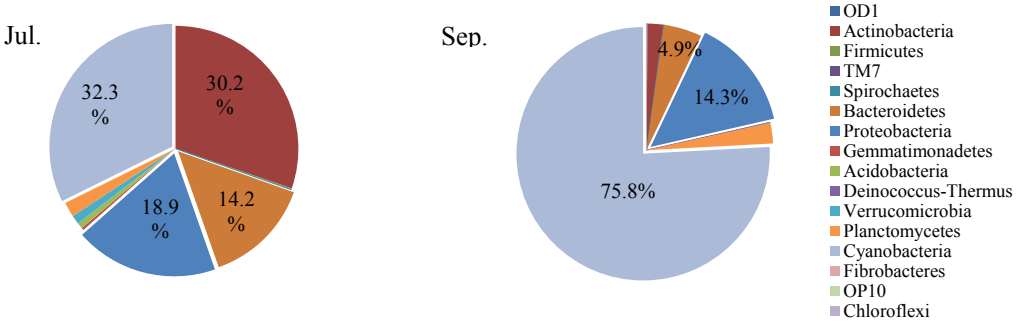

Fig. 1. Phylum distribution of bacterial communities from water samples selected from Guanting Reservoir in July and September. Proportions were calculated based on the sequences classified using RDP Classifier with a confidence threshold level of 80%.

3.2. Variability in bacterial community diversity

Cyanobacteria was the most dominant and bloom forming phylum during the sampling period, hence we distinguished it and discuss its community individually. Due to the lack of specific taxonomic

information from the RDP taxonomical system, we selected representative sequences of each *Cyanobacteria* genus and classified through the National Center for Biotechnology Information (NCBI) database using NCBI's Blastn tool.

Cyanobacteria community differed at genus level between July and September. In July 6 genera were identified, GpIIa (*Synechococcu*), GpI (*Anabaena*), and GpXIII (*Planktothrix*) dominant and accounted for 96.1% of the classified *Cyanobacteria* sequences. Other sequences were assigned to GpXI (*Microcystis*), GpX (*Limnococcus limneticus*) and GpVI (*Pseudanabae*). Most of the species declined remarkable, the most dominant genus GpIIa (*Synechococcu*) was even extinct in September. GpXI (*Microcystis*) sequences increased from only 44 to 11523 and became the exclusive dominant genus (comprised 99.4% of classified *Cyanobacteria* sequences) in September.

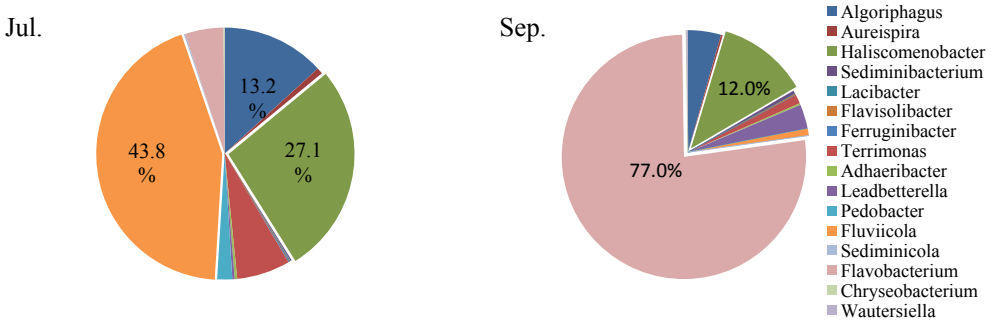

Fig. 2. Genera level composition of *Bacteroidetes* bacterial community based on classification of 16S rRNA sequences of bacteria from the Guanting Reservoir in July and September.

In Guanting Reservoir, 36 different *Proteobacteria* species were detected in July. *Pelagibacter* dominant the system followed by *Methylotenera*. In September 32 genera were detected, *Methylotenera* bacteria increased to 61.5% of sequences, *Methylophius* was also found abundant there. *Bacteroidetes* bacteria were abundant in July and decrease sharply in September. As shown in Figure 2, among 14 species detected in July *Fluviicola* was the most dominant specie followed by *Haliscomenobacter* and *Algoriphagus*. In September, *Fluviicola* bacteria decreased and *Flavobacterium* become the dominant specie followed by *Haliscomenobacter*.

4. Discussion

The bacterial community in prebloom phase and bloom phase were very different in phylum level and deeper taxonomic level. *Cyanobacteria* were the dominant phylum in Guanting Reservoir during the sampling time. In July, most of *Cyanobacteria* sequences were assigned to phyla such as GpIIa, GpI, and GpXIII. According to the abundance of *Microcystis* sequences, the *Microcystis* water bloom process was divided into two phases: prebloom phase in July and bloomphase in September.

Betaproteobacteria was equally the same abundance with *Alphaproteobacteria* in prebloom phase increased and became dominant after *Microcystis* water bloom occurred [11]. Richness and diversity indices indicated that there were more bacterial genera before *Microcystis* water bloom and the bacterial community was steadier at that phase.

When classified at genous level, there were 73 certain genera detected in July and only 55 in September. The dominant genera existed before *Microcystis* bloom were *Pelagibacter*, *Fluviicola* and *Methylotenera*. *Pelagibacter* within SAR11 lineages was the most abundant genus in July. It involved in phosphate metabolism in ocean ecosystem [24]. They now can be detected in many fresh water systems

[25,26]. To our knowledge, this is the first time SAR11 lineages were detected in Guanting Reservoir. *Fluviicola* was reported containing denitrifying species [27]. *Haliscomenobacter* can be found in aerobic phosphorus-removal ecosystem, wastewater treatment plant [28]. All these bacteria are reported to have more or less relationship with nitrogen or phosphorus cycling.

Dominant bacterial genera detected after *Microcystis* water bloom were *Methylotenera*, *Flavobacterium* and *Methylophilus*. *Methylotenera* have been detected in anaerobic sediment in Guanting Reservoir [24]. The recently study implicated *Methylotenera*-like species in playing a role in methanol-linked denitrification in lake sediment [29]. *Flavobacterium* has been reported exist in the sediment of Guanting Reservoir [30] and it also be well known as a kind of *Microcystis* lysing bacterium [31]. The existence of *Flavobacterium* bacteria may be a symptom of the coming postbloom phase of *Microcystis* water bloom in Guanting Reservoir.

5. Conclusion

The bar-coded pyrosequencing technique generated great number of sequences thus provided detailed understanding of bacterial community variation during *Microcystis* water bloom in Guanting Reservoir. Community differences were observed by phylotype distribution at each taxonomic level between the two seasons. The dominant genera existed before *Microcystis* bloom were *Pelagibacter*, *Fluviicola* and *Methylotenera*, all of which are related to nitrogen or phosphorus cycling. After the water bloom, bacterial composition changed the dominant genera were *Methylotenera*, *Flavobacterium* and *Methylophilus*. *Methylotenera* play an important role in methanol denitrification pathway. *Flavobacterium* may be a symptom of the coming postbloom phase. Further researches could focus on monitoring bacterial community during an entire *Microcystis* bloom process and determining the interrelationship between environmental factors and bacterial composition.

6. Acknowledgement

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