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Morphospecies and genospecies of *Microcystis* during blooms in eutrophic Lake Taihu (China) in autumn

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ABSTRACT

Microcystis colonies collected in a hypertrophic lake (Lake Taihu, China) in October and November 2012 were divided into five subsamples according to colony size (<75, 75–150, 150–300, 300–500, and >500 μm). All the subsamples collected in November were dominated with *Microcystis ichthyoblabe* and the percentages of *M. ichthyoblabe* exceeded 83%. The percentages of *Microcystis aeruginosa* of the subsamples in >500 μm class collected in October was 93.5%. For the sample collected in October, the percentage of *M. ichthyoblabe* was more than 58% in <75, 75–150, 150–300 μm classes. The 16S rDNA as well as some polysaccharide biosynthesis-related genes were analyzed to understand the phylogeny of *Microcystis* species. There was no variant site presented in each *Microcystis* subsample but a single nucleotide polymorphism (SNP) was found in 16S rDNA alignment tested using *MSR1* in subsamples between the two months in the current study. Our results also showed that samples collected in two months can by divided into two parts by the phylogenetic analysis using two polysaccharide biosynthesis-related genes (*espL* and *TagH*). All the results suggested that 16S rDNA was valuable to identify seasonal succession of *Microcystis* genospecies and the diversity of *Microcystis* morphospecies would be explained by these polysaccharide biosynthesis-related genes.

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1. Introduction

Cyanobacterial blooms caused a series environmental and ecological problems in lakes and reservoirs worldwide (Paerl and Otten, 2013). Thereinto, *Microcystis* sp. is a common bloom-forming cyanobacteria. Multiple species have been recorded in this genera based on morphological taxonomy. Moreover, physiological activities such as growth and toxin production varied among these species (Imai et al., 2009; Watanabe et al., 1988, 1989). Thus, knowledge of the diversity and distribution of *Microcystis* species is important to understand the dynamics of *Microcystis* blooms.

Many researchers investigated the seasonal variation of *Microcystis* species in some lakes based on morphological characteristics. Interestingly, the seasonal succession of *Microcystis* species was similar in Lake Suwa (Park et al., 1993), Lake Biwa (Ozawa et al., 2005), Hirosawa-no-ike Pond in Japan (Yamamoto and Nakahara, 2009), Lake Taihu (Li et al., 2013) and Lake Chao in China (Jia et al., 2011) in which the environmental factors differed greatly. This coincidence was suggested as a

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colonial morphological change induced by mucilage solubilization (Li et al., 2014). Similar morphological changes have also been reported by Otsuka et al. (2000). Therefore, the current taxonomy of *Microcystis* depending upon morphological characteristics must be reviewed by means of molecular genetic analyses.

Phylogenetic analysis based on 16S rDNA is a commonly used method for microbiologic taxonomy because it was considered as one of the most valid criteria for determining relationships among closely related organisms (Weisburg et al., 1991). However, measured by 16S rDNA sequence, a great genotypic homogeneity was found among *Microcystis* colonies with conspicuous morphological differences (Lepère et al., 2000; Otsuka et al., 2000). Moreover, Otten and Paerl (2011) indicated that *Microcystis wesenbergii* could be identified within four different *Microcystis* morphospecies based on 16S–23S rDNA-ITS sequences. Thus, more information on the relationship between *Microcystis* colonial morphology and 16S rDNA sequence was still necessary to understand the phylogeny of *Microcystis* species.

Our previous study reported that solubilisation of mucilage induced changes in *Microcystis* colonial morphology (Li et al., 2014). Forni et al. (1997) determined the amount of polysaccharide produced and the monosaccharide composition of the mucilage produced by different *Microcystis* morphospecies and found obvious differences among these morphospecies. These findings suggested that extracellular polysaccharide (EPS) related significantly to *Microcystis* colonial morphology. Gan et al. (2012) reported that some polysaccharide biosynthesis-related genes could influence the EPS production and colony formation of *Microcystis*. Thus, it was hypothesized that some polysaccharide biosynthesis-related genes related significantly to *Microcystis* colonial morphology and this relationship would be useful to integrate morphological characteristics and molecular genetics of *Microcystis*.

The aim of this study was to make insight into current taxonomy of *Microcystis* depends upon morphological characteristics by means of molecular genetic analyses. The 16S rDNA as well as some polysaccharide biosynthesis-related genes were analyzed to understand the phylogeny of *Microcystis* species.

2. Materials and methods

2.1. Samples collection

Sampling was carried out in the area where *Microcystis* scums occurred in Meiliang Bay of Lake Taihu, China, on 20 October and 20 November 2013. Water samples were collected at a depth of 30 cm below the lake surface into 5 L plastic bottles directly. The samples were then stored in a cooler at 4 °C and transported to the laboratory for analysis. The collected samples on 20 October and 20 November 2013 were named M-O and M-N, respectively.

2.2. Analysis of colony size and morphology

The sample was poured gently through sieves (divided into five classes: <75 μm, 75–150 μm, 150–300 μm, 300–500 μm and >500 μm). Each class was re-suspended in 300 mL of BG-11 medium. The sieved samples of M-O and M-N were named M-O-Ci and M-N-Ci where i from 1 to 5 represented classes from small to large.

The photomicrographs of each subsample were taken using an Olympus C-5050 digital camera coupled to an Olympus CX31 optical microscope. The percentages of various *Microcystis* morphospecies in the total *Microcystis* biovolume of each subsample were calculated by measuring colony size of different *Microcystis* colonies in the photomicrographs obtained above. The photomicrographs were analyzed using the UTHSCSA ImageTool v3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). The length and width of *Microcystis* colonies were measured. The biovolume of *Microcystis* colony was calculated as volume $= \pi/6\,(\text{length} \times \text{width})^{3/2}$ as it is very difficult to measure the thickness of *Microcystis* colonies directly. A minimum of 300 colonies per sample were analyzed. *Microcystis* morphospecies were identified referring to Yu et al. (2007).

2.3. PCR amplification and sequencing

DNA for polymerase chain reaction (PCR) templates was extracted from the samples in different season and different colony size by using the modified procedure (Murry and Thompson, 1980). And the quantity and quality were determined by Nanodrop-2000. Six pairs of primers were used for amplification and sequencing in all samples. The PCR was carried in a 30 μL reaction mixture with 15 μL mixture buffer (From TIANGEN company), 5 μL DNA (20 ng μL^{-1}), 1 μL Forward primer (5 pmol), 1 μL Reverse primer (5 pmol) and 8 μL ddH_2O. The protocol for the PCR was one cycle of 94 °C for 5 min, then 36 cycles of 94 °C for 30 s, 50 °C for 30 s (*MSR1*, *MSR2* and *MSR3*) or 56 °C for 30 s (*16S*, epsL and *TagH*), and 72 °C for 45 s, followed by 72 °C for 10 min. Four μL reaction mixture was used to detect by agarose electrophoresis and the rest was used for sequencing in Tianyihuiyuan biotechnology company.

Table 1 shows the primers applied in the current study which were cited by Otsuka et al. (1998) and Gan et al. (2012). *MSR1*, *MSR2*, *MSR3* and *16S* were used for 16S rDNA amplification, epsL for polysaccharide deacetylase family protein and *TagH* for polysaccharide export ABC-transporter ATP-binding subunit.

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