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Seasonal dynamics of photosynthetic activity of *Microcystis*, genotype abundances and microcystin concentrations in Meiliang Bay, Lake Taihu

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ABSTRACT

Lake Taihu is the third largest freshwater lake (surface area: 2338 km^2, mean depth: 1.9 m) in eastern China, and it is also the primary drinking water source for 30 million residents in the lake basin. In recent decades, Lake Taihu has annually experienced lake-wide cyanobacterial blooms. However, the seasonal changes of photosynthetic activity of *Micricystis*, genotype abundances and microcystin and their relationship with environmental factors are still poorly understood. In the present study, surface water samples in Meiliang Bay were collected monthly from January to December in the year of 2014, at the same time, we investigated the photosynthetic activity of *Micricystis,* the abundances of total and toxic *Microcystis* genotypes, and microcystins (MCs) concentrations using pulse amplitude modulation (PAM), quantitative real-time PCR (qPCR), and high performance liquid chromatography (HPLC) techniques, respectively. It was found that the maximum and effective quantum yields both had an obvious seasonal patterns. In winter season, photosynthetic activity were not detectable, Then, it increased fast from spring to summer, at the last, it gradually decreased from fall to winter. The level of non-photochemical quenching (NPQ) increased from March to August, when it showed a maximum, decreasing thereafter toward December. The abundances of total and toxic *Microcystis* genotypes ranged from $1.91 \pm 0.15 \times 10^5$ to $8.64 \pm 0.56 \times 10^7$, from $2.38 \pm 0.25 \times 10^4$ to $5.67 \pm 0.43 \times 10^6$ copies/mL, respectively, meanwhile, the toxic proportion ranged from 12.5 ± 1.3 to 65.6 ± 3.8%, and they all showed similar change patterns with increasing first and then decreasing later. The extacellular and intracellular MCs concentrations varied from 0.17 ± 0.05 to 1.82 ± 0.35 μg/L, from 0.59 ± 0.18 to 14.36 ± 1.62 μg/L, respectively. The correlation analysis suggested that photosynthetic capacity and NPQ of *Microcystis* had positive correlation with water temperature. Meanwhile, there were strong positive correlation among the total and toxic *Microcystis* genotypes, toxic proportion and intracellular MCs, and they were significant positively correlated with water temperature. In addition, intracellular MCs also have strong positive correlation with total phosphorus. In conclusion, the photosynthetic activity of *Microcystis* was corresponded to the dynamics of *Microcystis* bloom, and water temperature may be a key environmental factor which determined *Microcystis* photosynthetic activity, total and toxic *Microcystis* genotypes abundances as well as intracellular MCs in Lake Taihu.

1. Introductions

Nowadays, toxic cyanobacterial blooms mostly dominated by *Microcystis* spp. have increasingly occurred in a large number of freshwater lakes on a global level as a result of eutrophication. Such blooms are of particular concern due to their negative impacts on water quality and human health. Therefore, the mechanism of *Microcystis* bloom formation has became a research hotspot in aquatic environmental field [23]. Kong and Gao proposed four-phase development hypothesis on the process of the *Microcystis* bloom-forming in large shallow eutrophic lakes [15]. In this literature, the authors pointed that the physiological state of *Microcystis* is of importance to understand the mechanism of *Microcystis* water-blooming formation [15]. As an autotroph species, *Microcystis* rely heavily upon on the photosynthetic energy conversion using oxygenic photosynthetic systems, similar to plants. Therefore, photosynthetic activity is regarded as the most common indicator of eco-physiological state of *Microcystis.* Chlorophyll a fluorescence, which can provide tremendous information on photosynthesis, is a fast, non-invasive probe of photosynthetic activity, and its changes can be used to reflect the photosynthetic characteristics [45,46]. Until now, there has been many papers reporting on changes of cyanobacterial competence measured by Phyto-PAM fluorescence instrument in lab and field experiment, which play an important role in understanding the mechanism of *Microcystis* bloom formation and its physiological adaption [40,44].

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Microcystins (MCs) are a chemically diverse group of cyanotoxins which present a threat to humans as well as various forms of aquatic life, and some evidence of tumor-promoting activity of microcystins also exists [32]. In order to protect human health, the safe concentration threshold of MCs set by WHO is < 1.0 μg/L based on MC-LR equivalent. In natural freshwater ecosystems, Blooms of MC-producing *Microcystis* are usually comprised of toxic and non-toxic strains, and the tremendous variability in toxin level in cyanobacterial blooms is suggested to be due to the successive replacement of toxigenic and nontoxigenic *Microcystis* strains [16,30]. However, it is almost impossible to distinguish toxic species from non-toxic species using traditional light microscopy because many strains of *Microcystis* have identical appearances. It is known that MCs are synthesized nonribosomally by an integrated peptide-polyketide synthetase system encoded by the microcystin synthetase (*mcy*) gene cluster [14]. As a result, PCR analysis based on *mcy* gene has become a fast useful tool for identifying toxic and non-toxic *Microcystis* in environmental samples [24]. Real-time quantitative PCR (qPCR) is a high sensitive technique, which has been widely used to monitor and quantify potential toxic and non-toxic genotypes worldwide. It can facilitate to reveal the dynamic mechanism of MCs concentrations based on qPCR data [8,12,27].

Lake Taihu, the third largest freshwater shallow lake in east China (surface area: 2338 km^2, mean depth: 1.9 m), which located in dealt of Yeangze river area with high developed economy, and it is the primary drinking water source for 30 million residents in the lake basin and Shanghai (the largest city in China). Lake Taihu has been in a eutrophic state since the 1980s, and it is currently considered to be hypertrophic. In the past three decades, *Microcystis* bloom has been annually occurring in Lake Taihu [47]. Zhang et al. (2008) has investigated the diurnal changes of photochemical responses of phytoplankton in the large shallow Taihu Lake in relation to light and mixing [44]. Furtherly, the spatial distribution of photochemical activity of *Microcystis* were also measured by Li et al. (2013) [18]. However, the seasonal changes of *Microcystis* photosynthesis in Lake Taihu is still unclear. Now, the variation of MCs concentration of *Microcystis* bloom and its relationship with environmental factors have been investigated by many researchers [6,28,36–37,42], but the regulated mechanism of MCs contents is poorly understood. In this study, the seasonal changes of photosynthetic activity, *Microcysti* community and MCs concentration were measured by Phyto-PAM fluorescence, qPCR and HPLC methods, respectively, and these results will helpful to provide scientific basis for the prevention and control of *Microcystis* bloom and ecological risk assessment in Lake Taihu.

2. Material and method

2.1. Site and water sampling

The sampling site (31°44′ N, 120°18′ E) is located in Meiliang Bay which is the most hypertrophic parts of Lake Taihu. Water samples were collected monthly from surface water by a sterile sampler from January to December of 2014. Each sample was preserved in a sterile bottle and transported to the laboratory for further analysis.

2.2. Environmental factors

The physical factors were measured in situ, where the water temperature, pH, dissolved oxygen (DO), and turbidity were all measured using a water quality monitoring system (YSI6600, USA). Dissolved inorganic nitrogen and phosphorus (NH_4^+, NO_3^- and PO_4^{3-}) were determined using continuous flow analyzing system (SKALAR, Netherlands). Total phosphorus (TP) was determined by Ammonium molybdate spectrophotometric method (GB11893-89, China), and total nitrogen (TN) by alkaline potassium persulfate digestion-UV spectrophotometric method (GB11894-89, China). Chlorophyll-a was determined spectrophotometrically by acetone method [39].

2.3. Measurements of photosynthetic activity

The photosynthetic activity of *Microcystis* cells was measured by pulse-amplitude-modulated fluorometry (Phyto-PAM, Germany) with the software PhytoWin v2.10. Before measurement, 3 ml water samples were dark-adapted for 15 min. The fluorescence parameters, including F_0 (the minimum fluorescence), F_m (the maximum fluorescence), F_m' (the maximum fluorescence in the light adapted state) and F_s (the current instantaneous fluorescence in the light-adapted steady-state), obtained according to analysis methods described in the literature [18]. Then, photosynthetic activity parameters were calculated according to the following equations: $F_v/F_m = (F_m - F_0) / F_m$, $\Delta F/F_m' = (F_m' - F_s) / F_m'$ and $NPQ = (F_m - F_m') / F_m'$. F_v/F_m is the maximum optical quantum yield, $\Delta F/F_m'$ is the effective quantum yield and NPQ is the non-photochemical quenching.

2.4. Real-time quantitative PCR

Water samples were filtered onto GF/C filters and immediately stored at −20 °C until extraction. Total DNA was extracted using the method as described previously [20].

The real-time PCR assay was used to quantify two genetic elements, the phycocyanin intergenic spacer (*PC-IGS*) and microcystin synthetase gene (*mcy*D) regions. The external standards were prepared using genomic DNA of *Microcystis aeruginosa* PCC7806 obtained from the FACHB-Collection (Freshwater Algal Culture Collection of institute of Hydrobiology, China). Cells from a known volume of the *M. aeruginosa* PCC 7806 culture were filtered through GF/C filters, and the DNA extraction was as described above. The DNA copy numbers of two genes above were calculated by Vaitomaa et al. [33]. A 10-fold dilution series of the DNAs (8.27×10^6–8.27×10^1 copies/μL) was prepared and amplified with the *PC-IGS* and *mcy*D gene real-time PCR assays.

The real-time PCR was performed with the *Mastercycler realplex* 4 system (Eppendorf, Germany) using 25 μL (total volume) of a reaction mixture, containing 12.5 μL of SYBR Premix EX Taq™ (TaKaRa, Japan), 10 μmol of each primer, 10.5 μL of distilled water, and 1 μL of the template DNA. Amplification was performed according to Li et al.(2014) [21]. The melting temperature for the real-time PCR products was determined using the manufacturer's software. All of the samples were amplified in triplicate.

2.5. Measurement of microcystin concentrations

Water samples of 200–500 ml were filtered using a Whatman GF/C filter. Filter papers and filtrate were stored at −20 °C for subsequent intracellular and extracellular microcystin analysis. Intracellular microcystin were extracted from filter papers with 5% acetic acid solution followed by 80% aqueous methanol [26]. MCs in organic solvent and filtrate were concentrated and purified through C_{18} SPE column.

MCs were analyzed using high performance liquid chromatography with photodiode array detection (Agilent 1200, Agilent, PaloAlto, CA, USA) equipped with an ODS column (Agilent Eclipse XDB-C18, 5 μm, 4.6 mm × 150 mm), using a gradient of 40 to 60% Phosphate buffer (pH = 3.0) methanol at a flow rate of 1 mL/min. Column temperature was maintained at 40 °C and injection volume was 10 μl. MCs were identified using their characteristic UV spectra under the wavelength of 238 nm. Total MC concentrations were quantified as the sum of all MC peaks using MC-LR, -RR, and -YR standards (Sigma, Germany).

2.6. Statistical analysis

The graphs in the paper were constructed by Origin8.0 software. The relationships between the photosynthetic activity, *Microcystis* abundance and MCs concentrations and the environmental factors were analyzed with SPSS softwater using a Pearson correlation. Before the correlation analysis, a logarithmic transformation was conducted for a