Ecotoxicology and Environmental Safety 166 (2018) 192–199

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of nutrient temporal variations on toxic genotype and microcystin concentration in two eutrophic lakes

Min Wang[a,b,c], Wenqing Shi[b], Qiuwen Chen[b,*], Jianyun Zhang[b], Qitao Yi[b], Liuming Hu[b]

[a] School of Life Sciences, University of Science and Technology of China, Hefei 230026, China
[b] Center for Eco-Environmental Research, Nanjing Hydraulic Research Institute, Nanjing 210029, China
[c] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Keywords:
Toxic *Microcystis*
Microcystin
Environmental factors
Nitrogen
Phosphorus

ABSTRACT

Harmful cyanobacterial blooms are a growing threat to freshwater ecosystems worldwide due to the production of microcystin (MC), which can have detrimental effects on water quality and human health. The relations between MC-producing *Microcystis*, MC production, and environmental variables especially nutrient conditions in eutrophic lakes, Lake Taihu and Lake Yanghe, were investigated during the bloom season of 2015. Results showed that toxigenic cells contributed to 8.94–75.68% and 7.87–58.69% of the total *Microcystis* in Lake Taihu and Lake Yanghe, respectively. The dynamics of toxigenic cells and MC production were positively associated with NH_3-N concentration in Lake Taihu, while positively associated with the concentrations of TP, TDP and PO_4-P in Lake Yanghe, indicating that the dominant nutrient factor affecting the toxic blooms was nitrogen in Lake Taihu, whereas it was phosphorus in Lake Yanghe. The significant relationship between TLR eq (total MC after transformation of MC-RR and MC-YR into MC-LR) and Chlorophyll-*a* (Chl-*a*) concentration implied that Chl-*a* could be an alternative measure to predict MC risk in the two lakes, and the safe threshold value of Chl-*a* was proposed as 25.38 and 31.06 μg/L in Lake Taihu and Lake Yanghe, respectively.

1. Introduction

Cyanobacterial blooms that frequently occur in freshwater ecosystems have gained increasing attention worldwide (Gobler et al., 2016; Reichwaldt and Ghadouani, 2012; Zhu et al., 2016). The main concern regarding the presence of cyanobacterial blooms is the production of cyanotoxins by some species. Among the diverse cyanotoxins, highly prevalent hepatotoxic microcystin (MC) exhibits inhibitory activity against serine-threonine protein phosphatases 1 (PP1) and 2 A (PP2A), causing illness and mortality in animal species (Carmichael, 1992; Kaebernick and Neilan, 2001), and posing a threat to human health (Azevedo et al., 2002). The MC cyanotoxin is mainly produced by the *Microcystis*, *Anabaena*, *Nostoc*, *Oscillatoria*, and *Planktothrix* genera (Nishizawa et al., 2000) via an integrated multifunction enzyme complex encoded by the microcystin synthetase (*mcy*) gene cluster (Christiansen et al., 2003; Rouhiainen et al., 2004; Tillett et al., 2000). To date, at least 246 MC variants with different toxicity have been reported (Meriluoto et al., 2017).

Microcystis is one of the most pervasive bloom-forming cyanobacteria in freshwater bodies, and is known to be major MC producers (Fleming et al., 2002; Ouellette et al., 2006). Attribute to its ability to form buoyant colonies, *Microcystis* can vertically migrate to access ample nutrient and optimize utilization of radiant energy, forming extremely large blooms covering the water surface (Harke et al., 2016; Xiao et al., 2017). *Microcystis* blooms have been reported in 108 countries on every continent except Antarctica, 79 of which have also been confirmed the presence of MC (Harke et al., 2016). Both MC-producing and non-MC-producing *Microcystis* coexist within the blooms (Kurmayer et al., 2003; Wang et al., 2012). As they are morphologically and taxonomically indistinguishable (Meissner et al., 1996), it can be difficult to discriminate between strains by traditional microscopic methods (Dittmann et al., 1997). In recent years, the development and application of quantitative real-time polymerase chain reaction (qPCR) methods have solved this problem. So far, qPCR with primers targeting various *mcy* genes has been successfully applied in freshwater bodies worldwide (Baxa et al., 2010; Pimentel and Giani, 2013; Rintakanto et al., 2005; Vaitomaa et al., 2003), enabling researchers to precisely and rapidly identify and quantify the abundance of toxic strains among the cyanobacterial population, thereby elucidating the spatial and temporal dynamics of the toxic genotypes in natural assemblages.

Plenty of studies have been conducted to explore the relationship between MC production and environmental variables, including both

* Corresponding author.
E-mail address: qwchen@nhri.cn (Q. Chen).

https://doi.org/10.1016/j.ecoenv.2018.09.095
Received 13 July 2018; Received in revised form 19 September 2018; Accepted 21 September 2018

abiotic factors (water temperature, pH, rainfall, nutrients and trace metals), and biotic factors (algal biomass, zooplankton grazing and cyanophages) (Davis et al., 2009; Gobler et al., 2007; Liu et al., 2011; Sevilla et al., 2010; Singh et al., 2015; Srivastava et al., 2012; Wang et al., 2016; Yoshida et al., 2008). Among various environmental factors, anthropogenic eutrophication is considered as a primary contribution to the occurrence of toxic cyanobacterial blooms. Phosphorus (P) input was found to be the major factor responsible for increased *Microcystis* density and enhanced *mcyD* transcripts (Hu et al., 2016; Rintakanto et al., 2009). The increase in inorganic nitrogen (N) and/or P promoted the proliferation of toxic *Microcystis* strains and potentially yielded higher MC concentration (Davis et al., 2010). Under N-limited conditions, significant decreases were observed in the transcripts of *mcy* genes and MC quota (Harke and Gobler, 2013). However, N limitation has also been reported to enhance the individual *mcy* genes transcriptions as well as MC production (Ginn et al., 2010; Pimentel and Giani, 2014). In view of these different and even contradictory results, the exact mechanisms of nutrients regulating the MC synthesis are still disputable. As nutrient conditions vary seasonally, cyanobacteria undergo periods from nutrient replete to nutrient limitation. We hypothesized that the temporal variations of nutrients triggered physiological changes in cyanobacteria and affected the mechanisms of MC production.

To test the hypothesis, field investigations were conducted in two eutrophic, *Microcystis*-dominated lakes, Lake Taihu and Lake Yanghe. The occurrence and dynamics of toxic *Microcystis* and MC concentration, coupled with the environmental variables, were analyzed in both lakes across spatial and temporal scales to explore the relationships between MC production, toxic *Microcystis* population and nutrient conditions.

2. Materials and methods

2.1. Study area and sample collection

Lake Taihu (119°08′-122°55′ E, 30°05′-32°08′ N) is a shallow lake located in the Yangtze River delta, experiencing a subtropical monsoon climate. It has a surface area of 2338 km^2, with a corresponding volume of 4.4×10^9 m^3 and an average water depth of 1.9 m. It is a primary freshwater source to local people in the lake basin for irrigation, aquaculture, industries, recreation and tourism. The Taihu basin has a complex and well-developed river network, with over 200 main rivers crisscrossing the basin (Yi et al., 2017). Due to the rapid economic development of Taihu basin, it has been in a eutrophic state since the 1980s, especially in the northern lake areas (Wang et al., 2012). Five sampling sites were selected in the study, that is, Zhushan Bay (T1), Meiliang Bay (T2), Gonghu Bay (T3), lake center (T4) and west coast (T5), respectively (Fig. 1).

Lake Yanghe (119°03′-119°18′ E, 39°56′-40°18′ N) is an important water source for Qinhuangdao city in northern China, experiencing a temperate monsoon climate. It has a surface area of 13 km^2, with a corresponding volume of 0.75×10^8 m^3 and an average depth of 5.7 m. The major inflow discharges of Lake Yanghe are the West River and East River. Since the 1980s, the water body has experienced intense eutrophication and annual large-scale harmful cyanobacterial blooms in summer (Li et al., 2015). In this study, the water intake (Y1) and lake center (Y2) were selected as sampling sites (Fig. 1).

Surface water samples (0–0.5 m depth) were collected monthly from the sampling sites of each lake from May to September 2015 in Lake Yanghe, and May to October 2015 in Lake Taihu (except June because of heavy rain). All samples were collected in triplicate and mixed well, then kept in 2 L acid washed glass bottles and immediately transported to the laboratory in an ice-box.

2.2. Environmental parameter analyses

Water temperature (WT), pH, dissolved oxygen (DO), and conductivity were measured in situ using a multi-parameter water quality sonde (YSI EXO2, USA). Water transparency was obtained with a Secchi disk. Nutrient concentrations were determined according to the methods of Huang (2000). Water samples used for the quantification of total phosphorus (TP) and total nitrogen (TN) were digested by alkaline potassium sulfate. The samples for the determination of total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), orthophosphate (PO_4-P), ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N), and nitrite nitrogen (NO_2-N) were filtered through GF/C glass fiber filters (pore size, 1.2 μm; Whatman, UK). Dissolved inorganic nitrogen (DIN) was the sum of NH_3-N, NO_3-N and NO_2-N, and dissolved organic nitrogen (DON) was calculated by subtracting DIN from TDN. The suspended solids (SS) in the water column were measured using the gravimetric method (Huang, 2000). Chlorophyll-*a* (Chl-*a*) concentration was estimated according to Li et al. (2012).

2.3. Conventional PCR

For DNA extraction, water samples (50–100 mL) were filtered using GF/F glass fiber filters (pore size, 0.7 μm; Whatman, UK), with the filters then maintained at − 80 °C in polyethylene centrifuge tubes until analysis. Genomic DNA of the algae community was isolated by the traditional phenol-chloroform method (Rintakanto et al., 2005). The extracted DNA was re-suspended in 50 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), followed by concentration and purity analyses (NanoDrop-2000, Thermo Fisher Scientific, USA).

The extracted DNA was initially examined to assess the presence of *Microcystis* by targeting *cpcBA* gene (Kim et al., 2010), and MC-producing *Microcystis* by targeting *mcyB* gene (Nonneman and Zimba, 2002) (Table 1). The PCR analyses were performed in a MJ Mini thermal cycler (Bio-Rad Laboratories, USA) with a final volume of 50 μL, containing 2 μL of DNA template, 1 μL of each primer (10 pmol), 4 μL of dNTP mixture (2.5 mM), 5 μL of 10 × buffer, and 2.5 U of *Ex Taq* DNA polymerase (Takara, Japan). The amplification program was set as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 90 s, and a final extension at 72 °C for 5 min. All PCR products were visualized on 1.5% agarose gels stained with ethidium bromide under UV light.

2.4. Quantitative real-time PCR

To determine the *cpcBA* and *mcyB* gene copy numbers in the samples, external standards were prepared using *M. aeruginosa* PCC 7806 offered by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. Genomic DNA of the reference strain was extracted from the cells of a known volume culture, and its concentration and purity were determined spectrophotometrically as described above. The copy numbers of the genomic DNA were then calculated according to (Vaitomaa et al., 2003), assuming that each genome had one copy of the *cpcBA* (Kim et al., 2010) and *mcyB* gene (Tillett et al., 2000). Subsequently, serial dilutions (10^2–10^8 copies/μL) of the genomic DNA were prepared to establish the linear regression equations. Detailed parameters for the different standard curves were summarized in Table 1. The amplification efficiency (*E*) was calculated by $E = 10^{-1/S}$ -1, where *S* is the slope of the linear regression.

The quantitative real-time PCR (qPCR) assays were carried out in triplicate on the Thermal Cycler Dice Real Time System TP 800 (Takara Bio Inc., Japan). All reactions contained 12.5 μL of 2 × SYBR *Primer EX Taq* II (Takara, Japan), 1 μL of each forward and reverse primer (Table 1), 1 μL of template DNA from the standards or environmental samples, and adjusted to a final volume of 25 μL with sterile water. The qPCR programs consisted of an initial pre-heating step for 30 s at 95 °C,