WATER RESEARCH 47 (2013) 5659–5669

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Differences in microcystin production and genotype composition among *Microcystis* colonies of different sizes in Lake Taihu

Xingyu Wang [a], *Mengjia Sun* [a], *Meijuan Xie* [a], *Min Liu* [a], *Lan Luo* [a], *Pengfu Li* [a,*], *Fanxiang Kong* [b]

[a] *State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, PR China*

[b] *State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, PR China*

ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form
20 May 2013
Accepted 22 June 2013
Available online 2 July 2013

Keywords:
Lake Taihu
Microcystis
Bloom
Microcystin production
Colony size
Genotype composition

ABSTRACT

The cyanobacterium *Microcystis*, which occurs as colonies of different sizes under natural conditions, can produce toxic microcystins (MCs). To monitor the toxicity and assess the risk of *Microcystis* blooms in Lake Taihu, it is important to investigate the relationship between MC production and *Microcystis* colony size. In this study, we classified *Microcystis* collected from Zhushan Bay of Lake Taihu during blooms into four classes with size of <50 μm, 50–100 μm, 100–270 μm and >270 μm and studied their differences in MC production and genetic structure. The results showed that colonies with size of <50, 50–100, 100–270 and >270 μm produced 12.2 ± 11.2%, 19.5 ± 7.9%, 61.3 ± 12.6%, and 7.0 ± 9.6% of total MC, respectively. The proportion of cell density of colonies with size of 50–100, 100–270 and >270 μm was positively correlated with MC concentration during blooms, while that of colonies with size of <50 μm was negatively correlated. The MC cell quota tended to be higher during blooms in colonies with larger size except that of colonies with size of 100–270 μm was higher than that of colonies with size of >270 μm from June 11 to September 16. Colonies with size of <50 μm showed the highest proportion of the less toxic MC congener MC-RR, and colonies with size of >100 μm showed higher proportion of the most toxic MC congener MC-LR than colonies with size of <100 μm. Real-time PCR indicated that larger colonies had higher proportion of potential toxic genotype. Principal component analysis of PCR-denaturing gradient gel electrophoresis profile showed that *cpcBA* and *mcyJ* genotype compositions were different between colonies with size of <50 μm and colonies with size of >50 μm, and *cpcBA* genotype composition was also different among colonies with size of 50–100 μm, 100–270 μm and >270 μm. These results indicated that MC cell quota and congener composition were different in *Microcystis* colonies with different sizes in Lake Taihu during blooms, and the differences in MC production in colonies with different size resulted chiefly from the difference in their genotype composition. Therefore, the authorities of water quality monitoring and drinking water supply service in Lake Taihu should be alert that the toxicity of *Microcystis* colony with different size was different during blooms, and the high abundance of colonies larger than 50 μm could be an indicator of relatively high bloom toxicity.

* *Corresponding author*. Tel.: +86 25 83686755; fax: +86 25 83592705.
E-mail address: pengfuli@nju.edu.cn (P. Li).

0043-1354/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2013.06.040

1. Introduction

Cyanobacterial blooms in freshwater ecosystems are a global environmental and public health concern. The toxins and taste-and-odor released into the water by cyanobacteria are great health risks for animals as well as human beings and limit the utilization of recreational and drinking water (Jones and Korth, 1995; Briand et al., 2003; Graham et al., 2010). *Microcystis* spp. are the most commonly reported species responsible for toxic cyanobacterial bloom worldwide. Microcystins (MCs) released by *Microcystis* are a family of more than 80 structurally similar hepatotoxins, which can act as inhibitors of serine/threonine protein phosphatase 1 (PP1) and 2A (PP2A) (Hoeger et al., 2005). MCs are reported to be responsible for liver failure in wild animals, livestock and aquatic life (Codd et al., 1997; Chorus and Bartram, 1999; Carmichael, 2001). The presence of MCs in drinking water in the eastern region of China has been suggested to be related to primary liver cancer of local people (Ueno et al., 1996). In 1996, the liver failure and death of 50 patients exposed to MC-contaminated water during a dialysis treatment led to increasing concerns and investigation into the potential toxic and lethal effects of cyanobacterial blooms and the toxins produced by them (Jochimsen et al., 1998). Recent studies revealed that the gonads of many aquatic invertebrates and vertebrates are the second important target organ of MCs (Chen and Xie, 2005a, b; Chen et al., 2005, 2009a) and MCs were even found in the egg yolk and egg white of duck and water bird (Chen et al., 2009a).

MCs are synthesized in a mixed polyketide synthase/non-ribosomal peptide synthetase system called MC synthetase (Tillett et al., 2000). In general, MCs are stored in cyanobacterial cells during the growth and steady phase of cyanobacterial blooms (Sivonen, 1990; Rapala et al., 1997), and can be released into the surrounding water body by senescence of the blooms (Park et al., 1993). Two previous studies from Germany indicate that MC production from *Microcystis* population is related to its colony size. An investigation in the Bautzen reservoir reveals that MC concentration of *Microcystis* populations fractioned based on colony size is correlated with their colony size (Jungmann et al., 1996). A study on a natural population of *Microcystis* sp. in Lake Wannsee shows that the proportion of MC-producing genotypes and MC cell quotas are positively correlated with colony size, and the net MC production in the lake is thought to be mainly influenced by the abundance of the larger (>100 μm) MC-producing colonies (Kurmayer et al., 2003). To get a better understanding about the relationship between MC production of *Microcystis* and colony size, more investigations on MC production and genetic structure of colonies in different size are required.

Lake Taihu, the third largest freshwater lake in China, is situated in the Yangtze delta, one of the most developed areas in China. It has an area of 2250 square kilometers and mean depth of 1.9 m (Qin et al., 2007) and is an important freshwater resource for several cities, such as Shanghai, Suzhou and Wuxi. Unfortunately, as the development of local economy, Lake Taihu has been polluted since 1980's (Sun and Huang, 1993). Because of pollution and eutrophication, toxic cyanobacterial blooms consisting mainly of *Microcystis* have emerged in Lake Taihu. *Microcystis* blooms last from spring to autumn and mainly occur in Meiliang Bay, Gonghu Bay and Zhushan Bay, which are the most eutrophicated bays in Lake Taihu (Yin et al., 2011; Zhang et al., 2011). The cyanobacterial blooms in Lake Taihu have caused massive problems for industry, recreation, tourism and local drinking water supplies. In 2007, a massive bloom of the cyanobacteria *Microcystis* spp. in Lake Taihu even caused a drinking water crisis in Wuxi (Qin et al., 2010). Previous studies indicated Lake Taihu is heavily polluted during summer months by MCs, and poses serious health threats when serving as a source of drinking water and for recreational use (Song et al., 2007). What is more, due to the high-concentrations of accumulated MCs in bivalve, snail, shrimp, fish, turtle, duck, water bird and hydrophytes, it is likely unsafe to consume aquatic species from Lake Taihu (Chen and Xie, 2005a; Song et al., 2007; Chen et al., 2009a). The study of the relationship between MC production and *Microcystis* of different colony sizes will benefit water quality monitoring and risk assessments in Lake Taihu. In this study, we compared MC production and genetic structure of different *Microcystis* colony size collected from Lake Taihu during blooms. Apart from MC cell quota and proportion of potential toxic genotype in *Microcystis* colonies with different sizes, which were investigated in Lake Wannsee by Kurmayer et al. (2003), we investigated MC congener composition and *cpcBA* and *mcyJ* genotype compositions in *Microcystis* colonies with different sizes in this study.

2. Material and methods

2.1. Sampling

Samples were collected for 12 times from June 11, 2010 to October 14, 2010 in Zhushan Bay, which located in the northwest part of Lake Taihu, China (Fig. 1). Water samples were collected from the whole water column using a 2-m-long sampler. A total of 30 L of water was sampled every time. 2 L of subsample was used for chemical and physical parameter analysis. *Microcystis* colonies of different sizes in 20 L water samples were separated by sequential filtration through 270 μm, 100 μm and 50 μm mesh nylon sieves and classified into four classes with size of <50 μm, 50–100 μm, 100–270 μm and >270 μm. The sieving process was completed within minutes. All colonies were resuspended in 500 ml of GF/C (Whatman, UK) -filtered lake water, and aliquots of 25 ml were analyzed for *Microcystis* cell numbers and aliquots of 50 ml were filtered with GF/C filters. Colonies with size <50 μm remained in water samples were also filtered with GF/C filters. After filtration, filters were freeze-dried and stored at −20 °C for DNA extraction and MC determination.

2.2. Environmental variables analyses

Water temperature (WT) and pH were simultaneously measured using a YSI 6600 multi-parameter water quality sonde. Chemical oxygen demand (COD), suspended solids (SS), ammonia nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), nitrite nitrogen (NO_2^-–N), total dissolved nitrogen (TDN), dissolved inorganic phosphorus (DIP, PO_4^{3-}–P) and total dissolved