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Discerning biodegradation and adsorption of microcystin-LR in a shallow semi-enclosed bay and bacterial community shifts in response to associated process

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ABSTRACT

Hepatotoxic microcystins (MCs) produced by cyanobacteria pose serious risks to aquatic ecosystems and human health, to understand elimination pathways and mechanisms for MCs, especially in a shallow and semi-enclosed eutrophic area, is of great significance. This study succeed in discerning biodegradation and adsorption of microcystin-LR (MCLR) mediated by water and/or sediment in northern part of Meiliang Bay in Lake Taihu, China, and among the first to reveal the shifts of indigenous bacterial community composition in response to MCLR-biodegradation in sediment by Illumina high-throughput sequencing (HTS). Results confirmed that biodegradation predominantly governed MCLR elimination as compared to adsorption in study area. Through faster biodegradation with a rate of 49.21 $\mu g\,L^{-1}\,d^{-1}$, lake water contributed more to overall MCLR removal than sediment. Sediment also played indispensable role in MCLR removal via primarily biodegradation by indigenous community (a rate of 17.27 $\mu g\,L^{-1}\,d^{-1}$) and secondarily adsorption ($<$ 20% of initial concentration). HTS analysis showed that indigenous community composition shifted with decreased phylogenetic diversity in response to sediment-mediated MCLR-biodegradation. *Proteobacteria* became predominant (39.34–86.78%) in overall composition after biodegradation, which was mostly contributed by sharp proliferation of *β-proteobacteria* (22.76–74.80%), and might closely link to MCLR-biodegradation in sediment. Moreover, the members of several genera belonging to *α-proteobacteria*, *β-proteobacteria* and *γ-proteobacteria* seemed to be key degraders because of their dominance or increasing population as MCLR degraded. This study expands understanding on natural elimination mechanism for MCs, and provides guidance to reduce MCs' biological risks and guarantee ecosystem safety in aquatic habitats.

1. Introduction

Eutrophication has increased the emergence and prevalence of harmful cyanobacterial blooms in freshwater ecosystems (O'Neil et al., 2012; Pereira et al., 2015). Microcystins (MCs) are the most frequently occurring cyanotoxins produced during blooms, and pose extremely intoxicating effects on public health and ecosystem functioning (Sivonen and Jones, 1999; Peng et al., 2010). As a family of cyclic heptapeptides, MCs are synthesized intracellularly by several cyanobacterial genera and can be eventually released into surrounding water via cell lysis caused by natural senescence or physical stress (Ross et al., 2006). Due to their toxicity, clarification on the elimination and detoxification pathways of MCs entering water bodies has aroused growing concern.

In virtue of the cyclic structure, MCs are physic-chemically stable, and recalcitrant to hydrolysis or oxidation even at high temperature or low pH (Dziga et al., 2013). Also, large concentration of humic acids present in aquatic system may prevent MCs from transformation by sunlight (i.e., photolysis) (Schmidt et al., 2014). Biodegradation is widely regarded as a primary pathway accounting for natural MCs elimination and detoxification in water phase (Li et al., 2012). Multiple studies have verified that MCs can be readily degraded by microbial community indigenous to natural water bodies (Jones and Orr, 1994; Cousins et al., 1996; Christoffersen et al., 2002). Aside from biodegradation, adsorption to sediment matrix is another major pathway for MCs removal in aquatic habitats. It is well documented that natural sediment often acts as an important sink for MCs via dual pathways as adsorption

Abbreviations: MCs, microcystins; MCLR, microcystin-LR; DGGE, denatured gradient gel electrophoresis; FW, fresh weight; HPLC, high performance liquid chromatography; PCR, polymerase chain reaction; HTS, high-throughput sequencing; OTU, operational taxonomic unit; GAC, granular activated carbon

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(Babica et al., 2006; Mohamed et al., 2007; Wu et al., 2011) and biodegradation (de Vries et al., 2012; Song et al., 2014), greatly affecting the environmental fate of MCs. To date most studies only focused on the role of either biodegradation or adsorption in MCs removal. However, relatively little attention has been devoted to compare the contribution of dual major pathways, biodegradation and adsorption, to MCs elimination mediated by lake water and sediment in one aquatic system. This issue is greatly significant to predict which pathway or phase fulfills the dominant role and exerts larger contribution in attenuation of MCs, especially for the case in semi-enclosed freshwater area which is susceptible to anthropogenic eutrophication and MCs-contamination.

Meiliang Bay situates in the northwest of Lake Taihu, a typical shallow (mean depth: 1.9 m) lake in China, and provides water resource for drinking, agriculture and aquaculture use to surrounding residence, closely relevant to economic growth at regional and larger scales (Liu et al., 2011). Due to rapid development and anthropogenic actions, this area has become seriously eutrophic and plagued with toxic blooms with increasing frequency over recent years. The north end of this bay shapes up as a semi-enclosed water area weakly influenced by wind wave turbulence, causing a poorer dilution of toxic pollutants by water mixing than other areas, and has higher risk of MCs-contamination (Zhou et al., 2015; Hu et al., 2016). To clarify the contribution of dual major pathways, biodegradation and adsorption, to MCs removal in this semi-enclosed area is thus of great ecological necessity to manage MCs' negative effects and guarantee regional water safety and public health.

It is noticeable that a variety of bacteria may associate with MC-biodegradation process in nature. To understand this process, a few studies have surveyed the shifts of bacterial community structure synchronized with MC-biodegradation in natural water (Christoffersen et al., 2002), biofilter facility (with plastic media as biofilm carrier) (Li et al., 2011a) or the body of flagellate *Monas guttula* (Li et al., 2011b), using denatured gradient gel electrophoresis (DGGE). Despite these research progresses, the following concerns deserve special attention:

Firstly, sediment-mediated biodegradation is a crucial pathway for MC-removal. Consequently, sediment is always used as filling media in practical biofiltration to purify MC-contaminated water, with the participation of indigenous bacterial community (de Vries et al., 2012). Yet, how the indigenous community responses to MC-biodegradation process in natural sediment remained poorly revealed. Addressing such question would be helpful in explanation on mechanisms dictating the interactions between microbial community and MCs in sediment, and instructive to water treatment practice.

Secondly, the former studies (Christoffersen et al., 2002; Li et al., 2011a, 2011b) mentioned community shifts in response to biodegradation based solely on DGGE band profiles, without any information on taxonomic composition. Moreover, due to technical drawback, DGGE yield low resolution “snapshot” of community structures and might bias comprehensive and actual characterization of community, even if sequencing procedure could be applied with DGGE. In comparison, newly-developed Illumina high-throughput sequencing (HTS) generates millions of DNA sequences with high coverage and enough depth, rendering it able to accurately characterize complex composition. Also, HTS is less costly than other next-generation sequencing technologies (e.g., 454 pyrosequencing) at the same sequencing depth (Li et al., 2013). Hence HTS qualifies as a cost-efficient approach to depict a highly-accurate genetic profiling of microbial community. However, its application in exploring the genetic diversity and community changes responsive to MC-biodegradation process has never been reported, and thus should be studied.

Present study collected water and sediment samples from northern region of Meiliang Bay and specified microcystin-LR (MCLR), the most common and toxic MCs variant (Dziga et al., 2013), as target toxin. Mimicking the environment of study area in laboratory experiment, the goals of this study are to (i) discriminate biodegradation and adsorption of MCLR mediated by water and sediment in study area, thus the contribution of dual pathways to MCLR elimination can be clarified; (ii) reveal the shifts of indigenous bacterial community composition in response to sediment-mediated MCLR-biodegradation using HTS to shed interaction between bacterial taxonomic diversity and MCLR in sediment. Based on this, the bacterial species potentially involved in MCLR biodegradation can be found out.

2. Materials and methods

2.1. Chemicals

MC-LR ($\geq$95% purity, Express Technology Co., Ltd., China) was dissolved in methanol to prepare a stock solution with the concentration of 50 μg mL^{-1} and stored at −20 °C. Chromatographic grade methanol was used to prepare the mobile phase in high performance liquid chromatography (HPLC) analyses (Li et al., 2014). Other chemicals were of analytical grade except as specified by the kits.

2.2. Field sampling

Lake water was collected in July 2014 from three sites (31°32′11″N 120°10′27″E, 31°32′36″N 120°10′52″E and 31°32′57″N 120°11′10″E) in the northern part of Meiliang Bay, when and where cyanobacterial blooms had occurred. Simultaneously, sediment samples (0–15 cm) were collected using a grab sampler from the same sites where water was sampled. All materials were stored on ice immediately after sampling until being transported to laboratory within 8 h for experimental processes. The main physicochemical properties of sediment and lake water are listed in Tables 1 and 2, respectively.

2.3. Batch tests for biodegradation and adsorption of MCLR

Batch tests were conducted in a series of autoclaved test tubes. After fully-homogenizing water or sediment samples collected from different sites, the following treatments were designed: (i) to test MCLR elimination contributed by lake water, MCLR was spiked into 10 mL of original lake water at initial concentration of 150 μg L^{-1}; (ii) to test MCLR elimination contributed by lake sediment, 1.8 g sediment (fresh weight, FW) were added into 10 mL of sterile (autoclaved at 121 °C for 20 min) lake water with MCLR spiked at 150 μg L^{-1} in water phase, with consideration into both water depth of study area and surface sediment thickness; (iii) to test MCLR-adsorption contributed by lake sediment, the series was constructed following the procedure identical to treatment (ii) except for additionally spiking 0.5% sodium azide to inhibit

Table 1
Selected physicochemical properties of sediment collected.

Moisture content (%)	Organic matter (g kg^{-1} dry weight)	pH [a]	Particle size fraction (%) [b]		
			Sand	Silt	Clay
18.06	6.66	6.80	29.92	69.49	0.59

[a] pH of lake sediment was analyzed in 0.01 M $CaCl_2$ (sediment:solution=1:5, *w*/*w*).
[b] Particle size of sand, silt and clay is 20–2000 μm, 2–20 μm and $<$2 μm, respectively.