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Relationship between extracellular polysaccharide (EPS) content and colony size of *Microcystis* is colonial morphology dependent

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1. Introduction

Large colony formation is important to the occurrence of blooms of *Microcystis* (Yamamoto et al., 2011). Large colonies migrate more quickly than small ones (Nakamura et al., 1993); and are better able to float to the surface and form blooms (Wu and Kong, 2009). Colony formation is also a strategy to resist predation by zooplankton (Cyr and Curtis, 1999; Yang et al., 2006, 2009). Furthermore, colony morphology was also helpful in reducing the growth inhibition of *Microcystis* from *Microcystis* inhibiting substances (Wu et al., 2007; Park et al., 2009) and high light intensity (Wu et al., 2011; Zhang et al., 2011). The mechanisms behind *Microcystis* colony formation are important in understanding the formation of *Microcystis* blooms.

Colonial *Microcystis* lose their colonial morphology and exist as free-living cells in axenic laboratory cultures following long-term cultivation (Zhang et al., 2007). To reveal the mechanisms of *Microcystis* colony formation, the effects of different environmental factors on colony formation of *Microcystis* have been extensively investigated. Previous research has demonstrated that both biotic and abiotic factors and their combined effects could induce colony formation in *Microcystis* (Wang et al., 2010). These factors included predation by zooplankton (Yang et al., 2008), stimulation by heterotrophic bacteria (Shen et al., 2011) and *Cylindrospermopsis raciborskii* (Mello et al., 2012), temperature, light intensity (Li et al., 2013a), nutrient concentration (Yang and Kong, 2013), calcium level (Wang et al., 2011) and heavy metals (Bi et al., 2013). However, the morphology of the colonies formed in the above studies still differed from that of the colonies existed under natural conditions.

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Extracellular polysaccharides (EPS) cohere single algal cells into colonies (Plude et al., 1991) (Kessel and Eloff, 1975). The production of EPS was related to the colony formation in algae, such as *Chlorella pyrenoidosa* (Yang et al., 2010) and *Scenedesmus obliquus* (Liu et al., 2010). An increase in EPS content was reported to be important to *Microcystis* colony formation (Yang et al., 2008). A significant relationship between EPS content and *Microcystis* colony size was also observed (Liu et al., 2011; Li et al., 2013a). Many scientists believed that the cellular EPS content of large colonies would be much higher than that of smaller ones (Xu et al., 2013). Since colonies obtained in the laboratories differ from those under natural conditions, it is important to determine whether a significant positive relationship between EPS content and *Microcystis* colony size exists in the environment. To answer this question, we analyzed the relationship between EPS content and *Microcystis* colony size in Lake Taihu, a well-studied eutrophic shallow lake in China.

2. Materials and methods

2.1. Sample collection

Sampling was carried out in *Microcystis* bloom areas in Meiliang Bay of Lake Taihu, China, from July to November 2012. This area (coordinates: 31° 24′–31° 28′N; 120° 10′–120° 12′E) was previously described by Li et al. (2014a). The samples were collected at a depth of 30 cm below the lake surface into 1 L plastic bottles, and immediately fixed with formalin [2% (v/v)] for the laboratory analysis.

2.2. Microscopic examination

Samples were shaken thoroughly, and photomicrographs of the samples were taken using a digital camera (Olympus C-5050) coupled to an optical microscope (Olympus CX31). The photomicrographs were analyzed via the UTHSCSA ImageTool v3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). A minimum of 200 colonies per sample were analyzed to determine the percentages of *Microcystis* colonies in each size class and the percentages of various *Microcystis* species in the total *Microcystis* biovolume (*M. aeruginosa*, *Microcystis wesenbergii*, *Microcystis flos-aquae* and other/unidentified *Microcystis*). A sphere volume calculation formula was used to calculate the biovolume of *Microcystis* colonies.

2.3. Sample sieving

Samples were divided into six size classes (<75 μm, 75–100 μm, 100–150 μm, 150–300 μm, 300–500 μm and >500 μm) using a series of sieves. Each class was re-suspended in 300 mL of BG-11 medium. Afterwards, six 10 or 20 mL re-suspended samples of each class were filtered through GF/C (Whatman, UK) filters.

2.4. EPS analysis

Three filters were used for EPS content analysis using the method described previously (Li et al., 2013a). Briefly, the filters were re-suspended in 10 mL of distilled water added to the centrifuge tubes, and the pH was adjusted to 10. The centrifuge tubes were incubated in water at 45 °C for 4 h and subsequently re-centrifuged at 11,550× *g* for 15 min. EPS content of the supernatants was assayed by the anthrone sulfuric acid method in triplicate.

2.5. Assessment of biomass

Total organic carbon (TOC) was used as a proxy for *Microcystis* biomass so cellular EPS content was expressed as EPS/TOC. The other three filters of each size class were freeze-dried. The TOC content of these dried filters was analyzed by a TOC analyzer (Shimazu TOC-CPN SSM-5500).

3. Results

A total of ten samples were collected and divided into three groups depending on *Microcystis* colonial morphology. Table 1 shows the proportion of colonies in each size class and in different colonial morphologies for each sample. Sample A was dominated by *M. wesenbergii* with most colonies larger than 150 μm. Colony size in sample B was similar to sample A, but *M. flos-aquae* made up 98.3% of the sample. Sample C was evenly split between *M. flos-aquae* and *M. aeruginosa*.

Fig. 1 shows the EPS content of *Microcystis* in each size class. The maximum EPS content of sample A appeared in the moderate size class (75–300 μm). The maximum and minimum values for EPS content (EPS/TOC) were 0.15 and 0.07, respectively. EPS content (EPS/TOC) of sample B increased from 0.03 to 0.05 along with increasing colony size. Relationships between EPS content and colony size in sample C were similar to those in sample A.

The average EPS content of sample A in each class represents EPS values for *M. wesenbergii* while sample B represents values for *M. flos-aquae*. The EPS content of *M. aeruginosa* can be calculated from sample C-4 with the EPS values of *M. wesenbergii* and *M. flos-aquae*. Fig. 2 shows the EPS content of different colonial morphologies of *Microcystis* in each size class.