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Changes in the bacterial community and extracellular compounds associated with the disaggregation of *Microcystis* colonies

Wenjing Wang [a, b], Yulei Zhang [c], Hong Shen [a, *], Ping Xie [a, *], Jia Yu [c]

[a] Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, 430072, China
[b] University of Chinese Academy of Science, Beijing, 100049, China
[c] College of Fisheries, Huazhong Agricultural University, Wuhan, China

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ABSTRACT

Microcystis is a well-studied type of bloom-forming genus cyanobacteria that occurs as colonies in lakes. However, whenever *Microcystis* colonies are transferred to the laboratory, they always disaggregate into a unicellular form. The mechanism underlying this disaggregation of *Microcystis* colonies remains uncharacterized. Here, we report on the changes in morphology and the changes in the composition of the associated bacterial community of *Microcystis wesenbergii* colonies. Denaturing gradient gel electrophoresis analysis (DGGE) showed that the diversity of the associated bacterial community decreased during the disaggregation of *Microcystis* colonies. Two γ-Proteobacteria and one Bacteroidetes species from the mucilage of *Microcystis* colonies were not detected following colony disaggregation, suggesting that these species may influence *Microcystis* colony morphology. Solid phase microextraction and gas chromatography–mass spectrometry (SPME GC/MS) analysis revealed that seven of the forty-one extracellular compounds detected were exclusively present in the media of the *Microcystis* colony extracts; these compounds may be secreted by bacteria and may be a beneficial role in *Microcystis* colony maintenance. The results of this study indicate that changes in the composition of the bacterial community associated with *Microcystis* colonies are likely responsible for the disaggregation of these colonies in the laboratory.

1. Introduction

Microcystis is a bloom-forming genus of cyanobacterium. *Microcystis* species often appear as the dominant species in eutrophic lakes in summer and autumn. Under natural conditions, *Microcystis* species typically form colonies; these colonies vary in size and morphology. However, when *Microcystis* colonies are isolated from the natural field sites and cultured in artificial media, they always disaggregate into unicellular cells (Reynolds et al., 1981). *Microcystis* in the laboratory exists as a

* Corresponding authors. Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Science. No.7 Donghu south Road, Wuchang District, Wuhan City, 430072, China. Tel./fax: +86 027 68780056.
E-mail addresses: hongshen@ihb.ac.cn (H. Shen), xieping@ihb.ac.cn (P. Xie).

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unicellular form, and such cells cannot return to the colony form (Geng et al., 2013). *Microcystis* cells in nature can spontaneously aggregate into colonies; cells in these colonies are surrounded by mucilage (Klock et al., 2007); several laboratory experiments have reported that the formation of colonies or aggregates may be a *Microcystis* response to various environmental stresses such as zooplankton predation (Burkert et al., 2001), exposure to microcystins (Sedmak and Eleršek, 2006), and/or interactions with heterotrophic bacteria (Shen et al., 2011).

To date, there is a dearth of knowledge about morphological changes in *Microcystis* colonies isolated from lakes. While the physiology of colonial and unicellular *Microcystis* has been documented in a previous study (Wu and Song, 2008), yet few studies have focused on the associated bacteria and/or the detrital material during the course of *Microcystis* colony disaggregation. Shen et al. (2011) demonstrated that the interactions between *Microcystis aeruginosa* and associated heterotrophic bacterial community can induce the morphological changes in *M. aeruginosa* colonies. We hypothesized that the bacteria associated with *Microcystis* colonies may secrete extracellular compounds that influence *Microcystis* morphology in laboratory cultures. In the present study, the compositions of the associated bacteria community and extracellular compounds were evaluated during the process of *Microcystis* colony disaggregation in the laboratory. Our results contribute to deepening the understanding of *Microcystis* morphological changes in the laboratory and provide information about the mechanism of *Microcystis* colony disaggregation.

2. Materials and methods

2.1. Microcystis collection and experimental design

Microcystis samples were obtained from a sampling site (31°31′35.00″N, 120°9′45.14″ E) near Meiliang Bay in Lake Taihu, China, in July 2013. *Microcystis wesenbergii* colonies were isolated using an Olympus BX50 microscope (Japan). *Microcystis* species were classified according to the descriptions of Otsuka et al. (2000). After isolation, colonies were incubated in BG11 medium at 25 ± 1 °C with a 12 h: 12 h light:dark photoperiod at an irradiance of 50 μ Em^{-2} s^{-1}. The initial cell concentration was approximately 2×10^6 cells mL^{-1}. Samples (100 mL volume) were collected every 25 days, six times; the experiment lasted 125 days. 100 mL of fresh media was added to the cultures following sampling to sustain *Microcystis* growth. We evaluated the composition of the associated bacterial community and analyzed the extracellular compounds present in *Microcystis* colonies in an attempt to characterize mechanistic basis of the frequently observed disaggregation of *Microcystis* colonies.

2.2. DGGE analysis of the composition of bacterial community

Bacteria attached to the *Microcystis* mucilage were obtained from *Microcystis* colonies following a protocol described by Shi et al. (2010). Total DNA of the bacterial community was extracted using a bacterial DNA kit (Omega, USA). The universal primer 357F (5′-CCTACGGGAGGCAGCAG-3′) with a 40-bp GC clamp attached to its 5′ end and the 518R primer (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify 16S rRNA gene fragments by PCR. PCR experiments were performed in a 25 ml reaction volume and analyzed using DGGE gels with a Dcode system (Bio-Rad Laboratories, USA) according to the methods detailed in Shen et al. (2011). Following electrophoresis, gels were stained with a 1: 10,000 diluted solution of GelRed (Biotium, USA) for 30 min. The products were removed from the gel, purified using a Gel Recovery Purification Kit (AxyPrep™, USA), ligated into the pMD18-T plasmid vector system (Takara, Japan), and transformed into *Escherichia coli* DH5α-competent cells. Positive clones were sequenced by the Sino Genomax Genomics Company (Wuhan, China).

2.3. Analysis of extracellular compounds

Filtrates that included extracellular compounds were separated from *Microcystis* colonies using 0.22-μm membrane filters (Millipore, USA). The compounds in the filtrates were extracted using dichloromethane (CH_2Cl_2) and these extracts were then analyzed via gas chromatography mass spectrometry with an Agilent 7890A+5975C GC/MS system (Agilent, USA) following the procedures described by Zhang et al. (2013). Compounds were identified based on spectral searching against the NIST08.1 and the Wiley 275 libraries. Authentic reference standards were analyzed with the same analytical platform and compared with the mass spectra, retention times, and relative peak areas of the sample peaks (Graham et al., 2002).

2.4. Statistical analysis

Cluster analysis of DGGE bands was performed using the NTSYS-pc program (Exeter software, USA). A phylogenetic tree was constructed with a neighbor-joining method in MEGA 6. The partial sequences of the 16S rRNA genes obtained in this study were deposited in the DNA Data Bank of Japan with the following accession numbers: AB922603–AB922613.

3. Results and discussion

Three strains of *M. wesenbergii* were isolated from *Microcystis* bloom areas in Meiliang Bay of Lake Taihu, China. After cultivation in the laboratory, we confirmed that the *Microcystis* morphology changed; colonies disaggregated into a