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Recruitment-promoting of dormant *Microcystis aeruginosa* by three benthic bacterial species

Wansheng Zou[a,b], Zhi Wang[a,e,*], Qisheng Song[c], Shaoxian Tang[a], Yuande Peng[d]

[a] College of Bioscience and Biotechnology, Hunan Agriculture University, 1# NongdaRoad, Changsha, 410128, Hunan, China
[b] Collaborative Innovation Center for Efficient and Health Production of Fisheries in Hunan Province, Hunan University of Arts and Science, Changde, 415000, Hunan, China
[c] Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA
[d] Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha 410205, Hunan, China
[e] School of Life Science, Hunan Normal University, Changsha, 410000, Hunan, China

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ABSTRACT

The frequent occurrence of *Microcystis aeruginosa* blooms benefit from the dormant *Microcystis* cells, which will be recruited from sediment into overlying water to form a dominant population and algal blooms when external environmental conditions are suitable. Previous studies have unveiled factors involved in *M. aeruginosa* recruitment and bloom initiation, including nutrition, illumination, temperature, and hydrodynamic force. In this study, three dominant benthic bacterial species isolated from Lake Chongtian with frequent blooms-forming were identified through next generation sequencing (NGS) techniques, and laboratory experiments were conducted on the recruitment of dormant *M. aeruginosa* cells *via* co-culture with these bacteria at 10 °C, 15 °C, 20 °C and 25 °C. The results showed that the bacterial strains in sediment proliferated quickly before recruitment of dormant *M. aeruginosa* cells, subsequently significantly promoted the recruitment of dormant *M. aeruginosa via* allelochemical (metabolite) production, lower N:P values and lower dissolved oxygen concentrations in the sediment-water interface, and enhanced photosynthesis of *M. aeruginosa* cells. Furthermore, dormant *M. aeruginosa* was recruited from sediment at 10 °C when bacterial activity was present, but not recruited when bacterial activity was absent. At 15 °C,20 °Cand 25 °C, there were no remarkable differences in the recruitment rate of dormant *M. aeruginosa* cells among all bacterial groups, although their recruitment rate were significantly higher than that at 10 °C.These findings suggested that, under laboratory conditions, three benthic bacteria not only had a great influence on promoting the recruitment of dormant *M. aeruginosa* cells under desirable temperatures, but also can spur recruitment of dormant *M. aeruginosa*cells from sediment at lower temperature (10 °C).

1. Introduction

Blooming cyanobacteria, such as *Microcystis aeruginosa*, is the most common and harmful freshwater algae (Preston et al., 1980; Paerl and Otten, 2013). Toxic blooms of *M. aeruginosa* occur or recur frequently in many freshwater ecosystems, such as lakes, reservoirs, ponds, swamps, and even slow-flowing rivers due to eutrophication (Stone, 2011). They are often dominant compared with other phytoplankton species during algal blooms and produce toxins that pose a serious threat to aquatic organisms and humans (Ghadouani et al., 2004; Neilan et al., 2013).The life history of *M. aeruginosa* is identical to that of most other *Microcystis* spp. under natural conditions (Cires et al., 2013). It is widely agreed that *M. aeruginosa* cells in the water column will sink to the surface of the sediment and become dormant, living through extremely disadvantageous environmental conditions such as low temperature, faint irradiance, poor nutrition, and other adverse factors (Delphine and Warwick, 2014). When environmental conditions are favorable, benthic *M. aeruginosa* cells are recruited to the water column from the sediment and form *Microcystis* blooms (Mission and Latour, 2012; Tan, 2012). A lack of benthic *Microcystis* recruitment would reduce freshwater blooms by at least 50% (Neilan et al., 2013) as cells in the sediment are the main source for the formation of *M. aeruginosa* blooms (Reynolds et al., 1981). Consequently, recruitment of *M. aeruginosa* from the sediment is a crucial factor contributing to the formation of bloomsin eutrophic waters(Brunberg and Blomqvist, 2003; Karlson-Elfgren et al., 2003).

The interrelations of cyanobacteria and bacteria in sediment are very complex and extremely important for aquatic ecosystem (Daft et al., 1975; Takamura et al., 1984; Berg et al., 2009). To unveil the

* Corresponding author at: College of Bioscience and Biotechnology, Hunan Agriculture University, 1# NongdaRoad Changsha, Hunan, 410128, China.
E-mail addresses: zwsksy@163.com (W. Zou), wangzhispider@hunau.edu.cn (Z. Wang), Songq@missouri.edu (Q. Song), 1239011@hunau.edu.cn (S. Tang), maleisuo@caas.cn (Y. Peng).

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mechanism of *M. aeruginosa* recruitment and bloom initiation, to understand the impact of *Microcystis* blooms on ecosystems, and to develop possible solutions, many studies have been performed. Both field investigations and laboratory studies have revealed that temperature and light play important roles in the recruitment process of *M. aeruginosa* from sediment (Ye et al., 2011; Yang et al., 2016). Recruitment of *Microcystis* cells from sediment is significantly increased under strong illumination and at relatively high temperatures under eutrophic conditions. Over-enrichment of nutrient sources, such as nitrogen and phosphorus, is fundamental for *Microcystis* recruitment from the sediment. Interestingly, that a low N:P ratio is favorable for the recruitment of *Microcystis* has been reported in some studies (Sabour et al., 2009). Also, the number of *Microcystis* resuspended in the water column from sediment by wind or water flow may play a key role in recruitment (Blottiere et al., 2014). Similarly, bioturbation has also been proven to enhance the recruitment of benthic *Microcystis* to the water column (Yamamoto, 2010; Karlson et al., 2012). These observations partially illuminate the mechanism and development of *Microcystis* recruitment and bloom formation. Recently, more and more attention had been paid to bacterial communities in some eutrophic waters because they are seemingly associated with *Microcystis* blooms (Berrendero et al., 2016). The sediment is a complex benthic ecosystem with plentiful aquatic organisms, especially bacteria. These bacteria are very active and play important roles in the process of nutrient-recycling and energy-flow in aquatic ecosystems (Falkowski et al., 2008).The population structure of the microbial community and dynamic changes, especially of the dominant bacteria, can optimize the community structure and regulate community function (Zhao, 2014).

In this study, sediment was sampled from the frequent bloom-forming Lake Chongtian. Next generation sequencing (NGS) technique (Illumina, USA)was used to determine the microbial community composition and 16S rDNA to identify dominant OTUs. Three strains of bacteria (named *E.sp013, Ba.spD06, and Ba.spD24*) were isolated before and after bloom formation from Lake Chongtian sediment using agar-plate culture method. Similarity analysis of the DNA sequence (compared with GenBank) showed that strains *Ba.spD06* and *Ba.spD24* belong to *Bacillus* (97% and 99% sequence homology, respectively), while strain *E.sp013* belongs to *Exiguobacterium* spp. (99% sequence homology). The dominant bloom-forming cyanobacterium found in Lake Chongtian is *M. aeruginosa,* according to analytical results of several blooms. To explore the effects of the three dominant benthic freshwater bacteria on the recruitment of *M. aeruginosa* from the sediment, and to find potential factors that can relieve blooms, simulation experiments on the recruitment of *M. aeruginosa* cells from sediment were performed in the presence or absence of the three bacterial strains.

2. Materials and methods

2.1. Sediment

Sediment was sampled from Lake Chongtian, which has frequently suffered from blooms caused by *M. aeruginosa* in the past few years, utilizing a KC Kajak sediment core sampler (6.5-cm diameter, KC-Denmark), and the upper 3 cm of the sediment core were sliced into enamel dishes. Sediment obtained was pre-filtered through a steel mesh with a 125-μm diameter pore size to remove protozoans and large particles, then dispensed into sterilized plastic sacks and preserved in a cold polyethene box with ice (temperature 4 °C). Sediment collected was sterilized at 121 °C for 25 min to eliminate the impact of other plankton and flora on the experiment.

2.2. Culture solution

To support enough nutrition for the growth of *M. aeruginosa* and other bacteria, the culture solution for the recruitment experiments was modified lake water by adding 0.16 g $L^{-1}$$NaNO_3$ and 0.07 g $L^{-1}$$K_2HPO_4$. Lake water was sampled using a Micros water sampler (Monotube, HYDRO-BIOS, Germany) from the same site of Lake Chongtian, pre-filtered using 10-μm diameter pore size mesh (Yapei, China), and preserved using methods similar to those used for the sediment (4 °C storage). Twenty liters of surface sediment and 400 liters of lake water were collected by repeated sampling, according to the procedure mentioned above, and transported to the laboratory. Modified lake water was sterilized (121 °C, 20 min).

2.3. Bacteria

Three dominant bacterial strains(*E.sp013, Ba.spD06,* and *Ba.spD24*) obtained from the sediment of Lake Chongtian (**Supporting information Fig. S1**) were activated and cultured to the exponential growth phase using beef peptone liquid medium at 35 °C (Chang and Webb, 2017). Mineral salt solution ($(NH4)_2SO_4$: 0.5 g L^{-1}; KCl: 0.3 g L^{-1}; $FeSO_4$: 0.03 g L^{-1}) was added to the liquid medium to improve growth (Berg et al., 2009). The bacteria were filtered from the culture medium using a cellulose acetate membrane (0.22 μm, Φ47 mm, Japan), and acclimated in the culture solution mentioned above for 12 h.

2.4. Dormant M. aeruginosa cells

The dominant *Microcystis* forming bloomsis *M. aeruginosa* in Lake Chongtian (**Supporting information Fig. S2)**. These cells of *M. aeruginosa* isolated from overlying water were incubated in BG11 liquid media (provided by the Chinese Academy of Sciences) and cultured to the exponential growth phase under laboratory conditions (25 °C, 50 μmol photons $m^{-2}s^{-1}$). Then they were gently placed in a refrigerator at 4 °C in the dark for three and half months. When the upper solution was removed, dormant *M. aeruginosa* cells remained.

2.5. Design of recruitment experiments

The bacterial strains used in recruitment experiments with dormant *M. aeruginosa* cells comprised four groups: *E.sp013* (single strain), *Ba.spD06* (single strain), *Ba.spD24* (single strain), and a mixture group (mixed strains). Under aseptic conditions, the concentrations of the three strains were diluted to the same concentration (3×10^9 cfu ml^{-1}) by adding culture solution before mixing with sediment. In the mixture group, the volume ratio of three bacterial strains was 1:1:1 ($V_{E.sp013}$:$V_{Ba.spD06}$:$V_{BaspD24}$ = 1:1:1). Ten milliliters(ml)of diluted bacterial solution and 10 ml of dormant *M. aeruginosa* cells were mixed with 380 ml sediment, and mixed sediment was transferred into sterilized cylindrical glassware (SCG: Φ15 cm, H50 cm) with a sterile sealing membrane (cellulose acetate: Φ25 cm). Immediately, 6320 ml of modified lake water was poured gently into each SCG along the walls so as not to disturb the surface sediment. For a control (CK), 10 ml of modified lake water was added instead of diluted bacteria. All SCGs with mixed sediment were placed in an artificial climate chamber (MMM Climacell, Germany) with irradiance of 15 μmol photons $m^{-2}s^{-1}$ (equivalent to the light intensity on surface sediment in Lake Chongtian in spring), light: dark 12 h:12 h, at 10 °C, 15 °C, 20 °C, and 25 °C. Each temperature condition had 3 replicates and was cultured for 21 days. At the beginning of the experiments, sediments were sampled from SCG to determine the initial value of chlorophyll *a* (Chl *a*: 0.7 μg g^{-1}fresh sediment weight (FW)), photosynthetic efficiency (F_v/F_m = 0.1), and bacterial density (6×10^7 cfu g^{-1} FW). Water was sampled from the sediment-water interface (SWI) to determine the concentrations of dissolved inorganic nitrogen (DIN: 9.44 mg L^{-1}), soluble reactive phosphorus (SRP: 0.51 mg L^{-1}), dissolved oxygen (DO: 5.64 mg L^{-1}), and metabolites.

2.6. Determination of chlorophylla and bacteria concentration

For all experiments, samples were collected from the sediment and