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Lysis of *Aphanizomenon flos-aquae* (Cyanobacterium) by a bacterium *Bacillus cereus*

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Abstract

A phytoplankton-lytic (PL) bacterium, *Bacillus cereus*, capable of lysing the bloom-forming cyanobacterium *Aphanizomenon flos-aquae* was isolated from Lake Dianchi of Yunnan province, China. This bacterium showed lytic activities against a wide range of cyanobacteria/algae, including *A. flos-aquae*, *Microcystis viridis*, *Microcystis wesenbergi*, *Microcystis aeruginosa*, *Chlorella ellipsoidea*, *Oscillatoria tenuis*, *Nostoc punctiforme*, *Anabaena flos-aquae*, *Spirulina maxima*, and *Selenastrum capricornutum*. Chlorophyll *a* contents, phycocyanin contents, and photosynthetic activities of the *A. flos-aquae* decreased evidently in an infected culture for a period. Bacterium *B. cereus* attacked rapidly *A. flos-aquae* cells by cell-to-cell contact mechanism. It was shown that the lysis of *A. flos-aquae* began with the breach of the cyanobacterial cell wall, and the cyanobacterial cell appeared abnormal in the presence of the PL bacterium. Moreover, transmission electron microscope examinations revealed that a close contact between the bacterium and the cyanobacterium was necessary for lysis. Some slime extrusions produced from *B. cereus* assisted the bacterial cells to be in close association with and lyse the cyanobacterial cells. These findings suggested that this bacterium could play an important role in controlling the *Aphanizomenon* blooms in freshwaters.
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Keywords: Phytoplankton-lytic; *Bacillus cereus*; *Aphanizomenon flos-aquae*; Biological control; Blooms

1. Introduction

Recently, the eutrophication of freshwater environments and the occurrence of cyanobacterial and algal blooms have received a great deal of attention. They cause a wide range of social, environmental, and economic problems, such as deterioration of water quality, decrease of the aesthetic value of the affected water, and damage of aquaculture industries (Angeline et al., 1994; Shi et al., 2004). In recent years, blooms of *Aphanizomenon flos-aquae*, a common species of cyanobacteria, have increased in the world. *A. flos-aquae* can produce toxins that cause paralytic shellfish poisoning, which often cause the death of fish, shellfish, and other organisms in the natural environments (Ferreira et al., 2001; Jin et al., 2000; Underdal et al., 1999). Therefore, there is an urgent need for the development of techniques for controlling and reducing the impacts of *A. flos-aquae* blooms.

It is now widely accepted that bacteria may play an important role in regulating the phytoplanktonic biomass in freshwater environments (Choi et al., 2005). Some bacteria have algicidal effects and are involved in the termination and decomposition of cyanobacterial and algal blooms, while others promote bloom formation (Fukami et al., 1992, 1997). In general, the lytic bacteria can be categorized into direct and indirect attack types. The indirect attack type bacteria lyse algal cells through the extracellular production of algicidal substances (Lee et al., 2000), while another type of bacteria kill algal cells by cell-to-cell contact mechanism (Imai et al., 1995; Mitsutani et al., 1992). Although some phytoplankton-lytic (PL) bacteria have been isolated from natural environments and their lytic

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abilities have been investigated, detailed information about the mechanisms of algal cell lysis is still limited.

In this study, we isolated a PL bacterium *Bacillus cereus* from Lake Dianchi of Yunnan province, China, and investigated its lytic activities and mechanism. To our knowledge, this is the first report about the lytic effects of *B. cereus* on *Aphanizomenon*.

2. Materials and methods

2.1. Isolation, identification, and toxicity testing of PL bacteria

A bacterial strain DC22 showing high PL activities was isolated from Lake Dianchi of Yunnan Province, China, in 2003, by a double-layer agar technique (Shilo, 1970). The strain was cultivated in BG-11 medium (Steiner et al., 1971) plus 6 g/l tryptic soy broth (Walker and Higginbotham, 2000), which consist of 3.4 g pancreatic digest of casein, 0.6 g enzymatic digest of soybean meal, 0.5 g dextrose, 1 g sodium chloride, and 0.5 g dipotassium phosphate. The isolated bacterium was cultured at 30 °C for 24 h and stocked at 4 °C until use. It was submitted to the China Center Type Culture Collection (CCTCC) for identification based on its physiological and biochemical characters. Toxicity testing was performed by mouse bioassays according to microbiological methods (Shen et al., 2002) using male KM mice 18–20 g (Laboratory Animal Research Center, Wuhan University).

2.2. Cyanbacteria/algae

Cyanbacterial species of *A. flos-aquae* (FACHB 943), *Microcystis viridis* (FACHB 102), *Microcystis wesenbergii* (FACHB 107), *Microcystis aeruginosa* (FACHB 905), *Oscillatoria tenuis* (FACHB 247), *Nostoc punctiforme* (FACHB 252), *Anabaena flos-aquae* (FACHB 245), and *Spirulina maxima* (FACHB 438), and two green algae: *Selenastrum capricornutum* (FACHB 271), *Chlorella ellipsoidea* (FACHB 41), were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), located in China. All the strains were grown in BG-11 medium at 25 °C with illumination at 150 μmol photons/m^2/s under a 16L/8D cycle. Axenic cultures of cyanobacteria/algae were prepared by repeated disaggregation, centrifugation and single-cell isolation (Rippka, 1988; Shirai et al., 1990).

2.3. Host range of the PL bacterium

All the cyanobacteria/algae listed above were selected for determining the host range of bacterium DC22. The lytic ability of bacterium DC22 was confirmed by determining the changes of the chlorophyll *a* (Chla) contents of the cyanobacteria/algae in an infected culture for 7 days. Chla was measured at $\lambda = 665$ and 649 nm by spectrophotometer (UV-3000, England) after extraction with ethanol (Wintermans and Mots, 1965). The axenic cultures (45/100 ml flask) of each cyanobacterium/alga, which had been cultivated for one week, were inoculated with 5 ml of bacterial cultures (approximately 1.0×10^8 cells/ml). Controls consisted of the cyanobacterial/algal cultures that received 5 ml of BG11 medium only. Incubation conditions were similar to the former (Section 2.2). The bacterial cells were determined by direct counting at magnification of 1000× by using a haemacytometer and a Nikon microscope (E600, Japan).

2.4. PL effects of bacterium DC22 on A. flos-aquae

After *A. flos-aquae* was grown for 1 week, 5 ml of bacterial cultures were inoculated into the cyanobacterial cultures (45/100 ml flask). The PL activity of bacterium *B. cereus* was investigated by measuring the changes of Chla and phycocyanin (PC) contents of the cyanobacterium in an infected culture for a period. PC was determined with the procedures described by Bennett and Bogorad (1973).

2.5. Photosynthetic activity

Photosynthetic activity was measured with a phytoplankton analyzer (PHYTO-PAM, Walz GmbH, Germany). Cyanobacterial cells were dark-adapted for at least 15 min before measuring the fluorescence parameter F_v/F_m (photosystem II activity). The minimal fluorescence (F_0) was obtained at low measuring light frequency, whereas maximal fluorescence yield (F_m) was determined after exposure to saturating flash of light. The photochemical efficiency of PSII (F_v/F_m) was estimated as the ratio of the variable ($F_v = F_m - F_0$) to the maximal fluorescence yield.

2.6. Microscopy

The PL process of bacterium DC22 was investigated with the aid of an Axiophot 2 photomicroscope (Carl Zeiss, Jena, Germany). For transmission electron microscopy (TEM) analysis, the samples were fixed with 3% glutaraldehyde in cacodylic acid buffer for 1 h at 4 °C, followed by rinsing six times in cacodylic acid buffer. Then, cells were postfixed with 2% osmium tetroxide in cacodylic acid buffer for 3 h at 4 °C. Fixed cells were dehydrated through and acetone series and were finally embedded in Spurr's resin. Sections were cut using a diamond knife on an ultramicrotome (MT-1; Sorvall, Norwalk, USA), and stained with uranyl acetate and lead citrate. Observations were made using a transmission electron microscope (H-700H; Hitachi, Tokyo, Japan).

2.7. Statistical analysis

Data for each group were evaluated by one-way ANOVA (Spss 6.0.1 for Windows) and the least significance difference by Tukey's Honest Significant Difference test (Zar, 1984).

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