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Characterization of an algicidal bacterium *Brevundimonas* J4 and chemical defense of *Synechococcus* sp. BN60 against bacterium J4

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ABSTRACT

As part of efforts to enhance the strategies explored to eliminate the adverse impacts of cyanobacterial blooms, we isolated an algicidal bacterium, J4, from Lake Taihu. Analysis of 16S rDNA sequence revealed that strain J4 belonged to the genus *Brevundimonas*. Bacterium J4 exhibited algicidal activity mainly through excretion of extracellular algicidal compounds that were further extracted with methanol and purified by silica gel chromatography and high performance liquid chromatography (HPLC). The compounds showed thermal stability, strong polarity and water solubility in J4 cultures. Study on the algicidal activity of J4 against two dominant cyanobacterial bloom-forming species in Lake Taihu showed that J4 exhibited lower algicidal rate against *Synechococcus* sp. BN60 (48.6%, t = 6 days) than against *Microcystis aeruginosa* 9110 (91.8%, t = 6 days). Additionally, rapid reduction in cell density of J4 was observed in co-cultures of *Synechococcus* sp. BN60 and bacterium J4 but not observed in co-cultures of *M. aeruginosa* 9110 and bacterium J4 during algicidal process, which was the main reason why the algicidal rate of J4 against BN60 was lower than against 9110. The reduction in cell density of J4 resulted from inducible production of antimicrobial-like compound secreted by *Synechococcus* sp. BN60 in co-cultures of *Synechococcus* sp. BN60 and bacterium J4, which reflected a kind of chemical defense from cyanobacteria (BN60) against algicidal bacteria (J4). However, *M. aeruginosa* 9110 had no chemical defense against J4, suggesting that whether cyanobacterial chemical defense occurs or not between cyanobacteria and algicidal bacteria depends on specific cyanobacteria–algicidal bacteria pairs. These results show that not only one-sided algicidal effect but also two-sided reciprocal inhibition interactions exist between algicidal bacteria and cyanobacteria, indicating the complexity of cyanobacteria–algicidal bacteria interactions in Lake Taihu and the need to take the cyanobacterial defensive responses into consideration when assessing potential use of algicidal bacteria.

1. Introduction[1]

Increases in human population and economic development have brought heavy nitrogen/phosphorus loads into waterways, consequently causing many freshwater bodies become eutrophic. Cyanobacterial blooms can be one consequence of eutrophication and frequently occur around the world, often causing large economic losses, e.g. by killing massive fish and reduction of water resources, and seriously threatening humans and livestock health by the accumulation of toxic compounds in food or drinking water (Guo, 2007; Huisman et al., 2005; Wang et al., 2012; Xie, 2008). Accordingly, many researchers have proposed and investigated various strategies for bloom control, including best options for management, such as controlling nutrient input to prevent blooms (Conley et al., 2009; Sengco, 2009), and supplementary methods, such as physical, chemical and biological efforts at stemming blooms (Sengco, 2009; Sigee et al., 1999). However, physical and chemical means are challenging because of their costs and unknown potential environmental damages (Churro et al., 2009; Sengco, 2009). Recently, algicidal bacteria have been associated with termination of cyanobacterial blooms (Manage et al., 2001; Rashidar and Bird, 2001; Zhang et al., 2012) and consequently considered as potential bio-agents for bloom control. Because of their potential effectiveness and species specificity, several algicidal bacteria have been isolated and further investigated their potential use in controlling cyanobacterial blooms (Feng et al., 2013; Hee-jin et al., 2005; Manage et al., 2000; Ren et al., 2010).

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[1] HPLC: high performance liquid chromatography.

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Lake Taihu, the third largest lake in China and a typical shallow freshwater body (surface area: 2338 km^2; mean depth: 1.9 m (Wu et al., 2007)), has gradually become hypereutrophic during the last two decades and consequently undergone annual cyanobacterial blooms since 1987 (Ma et al., 2008), in which *Microcystis* and *Synechococcus* are the dominant genera (Chen et al., 2003; Ye et al., 2011). These cyanobacterial blooms cause huge economic losses. For example, a cyanobacterial bloom in Lake Taihu from May 29 to June 4, 2007 caused an estimated US$ 400 million loss in the reduction of drinking water sources and tourism income (Liu et al., 2011). In this study, as part of efforts to enhance the strategies explored to eliminate the adverse impacts of cyanobacterial blooms, a bacterial strain J4 with strong algicidal activity against *Microcystis aeruginosa* was isolated from Lake Taihu. Surprisingly, during comparison of the algicidal effect of J4 against *M. aeruginosa* 9110 and *Synechococcus* species BN60, we had also observed a cyanobacterial defensive response from *Synechococcus* sp. BN60 against the algicidal bacterium J4, resulting in a cell density reduction of J4 in co-cultures of J4 and BN60.

Van Donk et al. (2011) found that some cyanobacteria and algae have defense responses such as migration, morphological change, cyst formation, and production of bioactive compounds against adverse conditions such as nutrient limitation, competition, or herbivorous zooplanktons. Certain algae could potentially produce cysts as defense responses to algicidal bacteria (Mayali et al., 2007; Nagasaki et al., 2000). These defensive responses enhance their survival and partially shape the structure of their populations in nature (Anderson et al., 2012; Van Donk et al., 2011). Unquestionably, algicidal bacteria also represent a source of mortality for cyanobacteria. In the present study, we focused on the cyanobacterial defensive responses against algicidal bacteria.

2. Materials and methods

2.1. Cyanobacterial cultures

Microcystis aeruginosa 9110 and *Synechococcus* sp. BN60 were isolated from Lake Taihu. Both cyanobacterial strains were axenic. All cyanobacterial cultures used in this study were incubated in 250 mL Erlenmeyer flasks with 100 mL BG11 medium (Stanier et al., 1971) at 25 °C, under 40 μmol photons m^{-2} s^{-1}, a 12-h light:12-h-dark cycle and amended with fresh medium every month.

2.2. Isolation and identification of algicidal bacteria

Algicidal bacteria were isolated during a cyanobacterial bloom in Meiliang Bay of Lake Taihu. Water samples were collected at the Taihu Ecosystem Research Station (31°24′N, 120°13′E) of Meiliang Bay from 0.5 m below the water surface during October 2009. They were collected with a sterile sampler and transported to the laboratory on ice within 4 h.

An aliquot (10 mL) of water samples was inoculated into 100 mL log-phase *Microcystis aeruginosa* 9110 cultures. When the cyanobacterial cell density was below 20% of the control (10 mL sterile water instead of water sample added) during the cultivation, 10 mL co-cultures (*M. aeruginosa* 9110 cultures inoculated with water samples from Lake Taihu) were inoculated into another fresh log-phase *M. aeruginosa* 9110 culture. To promote the possibility of isolating algicidal bacteria, this process was repeated until the consistent reduction of *M. aeruginosa* 9110 density to below 20% of the control within 6 days after inoculation.

An aliquot of co-cultures on day 6 in the last incubation cycle (fifth cycle) was tenfold serially diluted with sterile water and 0.1 mL aliquots of each dilution were spread onto beef extract-peptone agar plates (10 g L^{-1} peptone, 3 g L^{-1} beef extract, 5 g L^{-1} sodium chloride, 1.5% (w/v) agar). The plates were incubated at 30 °C until colonies appeared. Individual colonies of distinct morphology were selected, purified using the method described by Yamamoto and Suzuki (1990) and then cryopreserved at −70 °C in 30% (v/v) glycerol. For screening of algicidal bacteria, bacterial isolates were grown in beef extract-peptone medium (30 °C, 200 rpm) for 24 h and then an aliquot (10 mL) of each bacterial culture was inoculated into 100 mL log-phase *Microcystis aeruginosa* 9110 cultures respectively. Log-phase *M. aeruginosa* 9110 culture (100 mL) inoculated with 10 mL bacterial medium served as a control. The growth of *M. aeruginosa* 9110 was monitored daily by measuring the biomass. Algicidal activity was calculated using the equation described in Section 2.6. The bacterial strains with strong algicidal activity (algicidal rate $A > 80\%$, $t = 6$ days, see Section 2.6) were further analyzed.

Identification of algicidal bacterial strains was accomplished by analysis of their 16S rDNA sequences as previously described (Tian et al., 2012).

2.3. Determination of algicidal mode

Bacterium J4 was incubated in beef extract-peptone medium at 30 °C, 200 rpm for 24 h. Bacterial cultures were centrifuged at 12, 000 × *g* for 20 min and the supernatants were passed through 0.22-μm polycarbonate filters to obtain cell-free filtrates. Heat-treated cell-free filtrates were obtained by autoclaving at 121 °C for 20 min. Bacterial cells were collected by centrifugation (5000 × *g*, 20 min), washed twice with sterile water and re-suspended in an equal amount of water. An aliquot (10 mL) of bacterial cultures, cell-free filtrates, heat-treated cell-free filtrates and re-suspended bacterial cells in water was inoculated into 100 mL log-phase *Microcystis aeruginosa* 9110 cultures respectively and cultivated at 25 °C, under 40 μmol photons m^{-2} s^{-1} and a 12-h light:12-h dark cycle. *M. aeruginosa* 9110 culture (100 mL) inoculated with 10 mL bacterial medium acted as a control. The algicidal rates ($t = 6$ days) of differently treated J4 cultures were calculated according to the change of cell density of *M. aeruginosa* 9110 on day 6 after inoculation of J4. Significant differences in algicidal rate were determined using one-way ANOVA with SPSS version 19.0 (IBM, USA).

2.4. Interactions between bacterium J4 and each of two cyanobacterial species (M. aeruginosa *9110 and* Synechococcus *sp. BN60)*

To compare the interactions between bacterium J4 and each of the two cyanobacterial species, an aliquot (10 mL) of stationary-phase J4 cultures (cell density 1.3–1.5 × 10^{10} CFU mL^{-1}) was inoculated into 100 mL log-phase cultures of *Microcystis aeruginosa* 9110 and 100 mL log-phase cultures of *Synechococcus* sp. BN60 respectively. The controls were 100 mL log-phase cyanobacterial cultures inoculated with an equal volume of beef extract-peptone medium instead of J4 cultures. The additional control was 100 mL BG11 medium inoculated with 10 mL J4 cultures. All tests and controls were incubated at 25 °C, under 40 μmol photons m^{-2} s^{-1} and a 12-h light:12-h dark cycle. Cell density of *M. aeruginosa* 9110 and chlorophyll-*a* concentration of *Synechococcus* sp. BN60 were evaluated daily and then algicidal rate was calculated according to Section 2.6. In the meantime, cell density of algicidal bacterium J4 was also monitored daily by the CFU method performed on beef extract-peptone agar plates (Su et al., 2007).