Isolation, identification and characterization of phytoplankton-lytic bacterium CH-22 against *Microcystis aeruginosa*

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**A B S T R A C T**

A bacterial strain named CH-22 showing phytoplankton-lytic activity against bloom-forming cyanobacterium *Microcystis aeruginosa* was isolated from Lake Chaohu of Anhui Province, China. The isolated strain was identified as *Pseudomonas putida* by morphology and homology research based on 16S rDNA. The phytoplankton-lytic activity was confirmed by reduction in cell number and chlorophyll *a* concentration of *M. aeruginosa* in an infected culture for a defined period. The initial bacterial and *M. aeruginosa* densities affected the phytoplankton-lytic activity significantly. When the 15% (150 μL/mL) concentration of bacterial cultures was infected, the highest phytoplankton-lytic activity reached to 98.8% after 7 days. When the initial *M. aeruginosa* density was less than 3 × 10^6 cells/mL, about 90.0% of chlorophyll *a* was removed. Obvious reduction in phycocyanin concentration in the treated *M. aeruginosa* suggests that isolated strain may possibly inhibit the synthesis of photosynthetic apparatus. Supernatants of bacterial cultures showed higher phytoplankton-lytic activity, suggesting that phytoplankton-lytic bacterium *P. putida* indirectly attacked *M. aeruginosa* cells by secretion of extracellular antialgal substances, which is characterized as anti-heat shock. The isolated *P. putida* also showed effective phytoplankton-lytic activity against a wide range of phytoplankton. These results suggest that indigenous bacteria isolated from eutrophic lake may be employed to regulate the ecological balance between the phytoplankton and bacteria, and consequently, to reduce the occurrence of cyanobacterial blooms in freshwaters.

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**Introduction**

Recently, eutrophication of freshwater environment and occurrence of cyanobacterial blooms have received a great deal of attention in China. Till now, more than 60% of the lakes in China have been eutrophicated and suffered from cyanobacterial blooms (Pan et al., 2006a; Yang et al., 2008). They have caused a lot of social, environmental and economic problems, such as deterioration of water quality and damage of aquaculture industries (Shi et al., 2006; Hong et al., 2009). Several species of cyanobacteria, e.g., *Microcystis, Anabaena* and *Aphanizomenon*, are very harmful as they can produce and release toxins that cause illness or death for wildlife and do harm to human health as well (Svrcek and Smith, 2004; Song et al., 2007; Kagalou et al., 2008; Zhang et al., 2009; Pan et al., 2008). Therefore, there is an urgent need to develop efficient techniques so as to control and reduce the adverse impact of these cyanobacterial blooms.

Actually, in order to reduce bloom biomass, several control techniques have been put forward such as employing yellow loess (Sun et al., 2004) and clay (Sengco and Anderson, 2004; Pan et al., 2006b; Zou et al., 2006). Although these methods may be effective, yellow loess and clay can cause secondary effects on bottom-dwelling organisms (Pierce et al., 2004; Zou et al., 2006). The application of chemical algicidal agents such as copper sulfate (Steidinger, 1983), hydrogen peroxide, and triosyn are also effective in controlling cyanobacterial blooms within a short period (Shen et al., 2004; Kim et al., 2009), but these methods either require high-energy inputs or damage the aquatic environment (Lam et al., 1995). Commercially available flocculants such as polyaluminum chloride and polyferric chloride are normally not applicable in treating natural waters because they pose potential risks to environmental ecological safety (Pan et al., 2006a).

Since phytoplankton-lytic bacteria are found to be involved in termination and decomposition of cyanobacterial blooms, they become of a particular interest (Hare et al., 2005; Shi et al., 2009; Kim et al., 2009) recently. It is generally accepted that bacteria can affect phytoplankton dynamics in water environment, either negatively or positively (Choi et al., 2005). In various situations...
bacteria exhibit antagonistic effects against microalgae, and some species of bacteria can promote bloom formation (Fukami et al., 1997; Shi et al., 2006). It is found that phytoplankton-lytic bacteria inhibit algal growth effectively through either direct attack that requires cell-to-cell contact (Imai et al., 1995; Shi et al., 2006) or indirect attack mediated by extracellular compounds (Imai et al., 1995; Wang et al., 2005; Kang et al., 2005). Many reports have concerned that phytoplankton can be inhibited by phytoplankton-lytic bacteria isolated from marine and freshwaters, and so focused on bloom-forming species Aphanothece flos-aquae and diatom Stephanodiscus hantzschii in the late autumn (Mu et al., 2007; Shi et al., 2006; Kim et al., 2009). Cyanobacteria, M. aeruginosa, as a dominant species in highly eutrophic shallow lakes such as Lake Taihu, Chaohuo and Dianchi (Yang et al., 2008) in China, have received attention for being recognized as such phytoplankton-lytic bacteria. Recently, Ahn et al. (2003) reported that a culture broth of B. subtilis completely inhibited the growth of M. aeruginosa, and Mu et al. (2007) reported that secreted metabolites of B. fusiformis showed phytoplankton-lytic activity against M. aeruginosa, Chlorella and Scenedesmus. However, in general, there is still lack of detailed research on the interaction between M. aeruginosa and phytoplankton-lytic bacterium, Pseudomonas putida.

In the present study, we reported the identification and characterization of a phytoplankton-lytic bacterium, P. putida CH-22, and its phytoplankton-lytic effect against the dominant species (M. aeruginosa) of the cyanobacterial blooms in some shallow lakes in China.

Material and methods

Phytoplankton culture

Cyanobacterial species of M. aeruginosa (FACHB 905), Aphanothece flos-aquae (FACHB 943), M. viridis (FACHB 102), M. wesenbergii (FACHB 107), Anabaena flos-aquae (FACHB 245), green alga Chlorella ellipsoidea (FACHB 41) Scenedesmus quadricauda, were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), Wuhan, China. The growth medium of above species was BG11 (Stanier et al., 1971). Inocula of each group were grown in batch culture in 250 mL Erlenmeyer flasks containing 150 mL liquid medium (sterilized by autoclaving at 15 psi for 20 min) with three replicates. The experiments were conducted at 28 °C and illuminated with cool white fluorescent light at about 60 μE/m²/s with a 16/8 h light/dark cycle. In order to reduce any effect caused by minor difference in photon irradiance, the flasks were shaken manually three times each day. M. aeruginosa at exponential phase (about 1.0 x 10⁸ cells/mL) was used unless specially mentioned. Cultures were maintained under axenic condition and at similar temperature and light conditions to that as described previously.

Isolation and screening of phytoplankton-lytic bacteria

The water samples were collected from the eutrophic freshwater Chaohu Lake (Anhui, China) where cyanobacterial blooms previously occurred in July 2008. Samples were serially diluted with sterile water and 0.1 mL aliquots of each dilution were spread onto modified medium (Lu et al., 2007) agar plates, which consist of 1.0 g ammonium dihydrogen phosphate, 0.2 g magnesium sulfate, 0.5 g sodium citrate, 1.0 g potassium hydrogen phosphate and 15 g agar (liquid medium absent agar, sterilized by autoclaving at 15 psi for 20 min) per liter, followed by incubation at 28 °C till identifiable colonies appeared after two days. Individual colonies of distinct morphology were streaked onto agar plates for purification and frozen at – 70 °C in 15% glycerol. Isolated bacteria were pre-cultured into 100 mL flask of 50 mL modified liquid medium at 28 °C, at 200 rpm for 24 h and the growth curve was detected by measuring the absorbance at 600 nm (A600nm). To test the phytoplankton-lytic effect on M. aeruginosa, 10% volume bacterial culture was incubated into 250 mL flask with 135 mL M. aeruginosa and the total volume was 150 mL, with the control containing only the modified medium. The flasks were incubated for 5 days with similar temperature and light regime to that described previously (see Section ‘Phytoplankton culture’). Phytoplankton-lytic activity was determined by the changes in chlorophyll a concentration compared with the control. Chlorophyll a was measured at 665 and 649 nm in the absorption spectra by spectrophotometer (UV-2550, Japan) after extraction with 95% ethanol (Sartory and Grobberal, 1984). The phytoplankton-lytic activity or the inhibitory rate was calculated by the following equation (Kim et al., 2009):

\[
\text{Phytoplankton-lytic activity} \% = \frac{1 - \text{Tt} / \text{Ct}}{} \times 100
\]

where Tt (treatment) and Ct (control) refer to the concentrations of chlorophyll a of treated sample and control sample, respectively.

Identification of the bacterium by phylogenetic analysis

Morphological observations were carried out using photomicroscope and scanning electron microscope (SEM). Under photomicroscope, the bacterium was observed by Gram staining (Bergey et al., 1994). For SEM observation, the bacteria cells were harvested and pre-fixed with buffered 2.5% glutaraldehyde (pH 7.2) after being air-dried and later post-fixed with buffered 1% Osmium tetroxide at room temperature for 1 h. The sample was then cleansed with the Milloning buffer, dehydrated through a series of 50–100% ethanol solutions. The filter was then mounted on the stub, coated with gold and examined under a scanning electron microscope (JEOL JSM-35CF). Morphological changes of M. aeruginosa with treatment or without treatment with CH-22 were observed by SEM.

The isolated strains were identified by polymerase chain reaction (PCR) amplification of the 16S rDNA gene, BLAST analysis, followed by comparison with sequences in the GenBank nucleotide database. The bacterial cells were lysed by heating at 96 °C for 10 min, and then they were immediately cooled on ice, centrifuged and suspended in TE buffer with lysozyme (10 mg/mL). The total DNA was extracted using a DNA extraction kit according to the manufacturer’s instructions (Genetech, China). PCR was performed in a total volume of 25 μL solution containing 100 ng DNA, 10 mmol dNTP, 10 pmol PCR universal primers 27F (5’-GAGAGTTTGATCCCTGCGCA-3’) and 1541R (5’-AAGTTTGATCCACGAGGTTA-3’), 2.5 μL PCR buffer and 4 U Taq polymerase with 30 thermal cycles of amplification (1 min for 94 °C, 1 min for 55 °C, 1 min for 72 °C) followed by hot water bath in thermostat at 72 °C for 10 min. The PCR product was purified and cloned into pMD 18-T vector followed by sequencing, which was performed by Shanghai Genetech Biotechnological Company. A comparison of nucleotide sequences was performed using the BLAST database (http://www.ncbi.nlm.nih.gov/BLAST) at the National Center for Biotechnology Information (NCBI). Sequences were aligned using the program CLUSTAL W, and a phylogenetic tree was made using the MEGA 4.0 program.

Analysis of phytoplankton-lytic effect of CH-22 on M. aeruginosa

Effect of bacterial density

The bacterium CH-22 was incubated in liquid modified medium at 28 °C when it reached logarithmic growth phase,
**M. aeruginosa** were fed with 5%, 7.5%, 10%, 12.5% and 15% (v/v) of bacterial cultures (approximately 1.0 x 10^5 CFU/mL, determined by colony forming unit (CFU) assays). Controls consisting **M. aeruginosa** received the same volume of the modified medium. Cell number of **M. aeruginosa** was determined by counting at 400 x magnification using a haemocytometer under a Nikon microscope (E6000, Japan). The chlorophyll a concentrations were measured daily.

**Effect of initiate **M. aeruginosa** density**

The bacterial culture of CH-22 (15 mL, 1 x 10^8 CFU/mL) was added to the samples containing **M. aeruginosa** cells at different densities (3 x 10^6, 6 x 10^6, 9 x 10^6, 1.2 x 10^7 and 1.5 x 10^7 cells/mL). Controls consisting **M. aeruginosa** received the same volume of the modified medium. Incubation conditions were similar to that described above. Cell number of **M. aeruginosa** was counted and chlorophyll a concentration was measured daily as described above.

**Effect of phycocyanin concentration**

15% of bacterial culture was inoculated into **M. aeruginosa** culture. The concentration of PC were measured according to Bennett and Bogorad's method (1973) in an infected culture for 7 days and then evaluated according to the following equation:

\[ PC = A_{615} - 0.474 \times A_{652}/5.34 \text{ (mg/mL)} \]

**Isolation of the active agent produced by the bacterium CH-22**

To test which fraction of the cultures of the bacterium CH-22 inhibited **M. aeruginosa**, together with the heat resistance property of the active agent, we treated cultures of CH-22 in different ways as follows. Bacterial cells were harvested after cultivation overnight, then 15 mL supernatants of the bacterial cultures was obtained by centrifugation at 4°C with 5000g for 15 min followed by filtration through 0.22 µm Millipore membranes. The cell pellet was then resuspended in 15 mL modified medium. The 15 mL bacterial culture supernatant was heat-treated at 121°C for 30 min, whereas the bacterial pellet, which was resuspended in 15 mL modified medium was also treated at 121°C for 30 min. Then the supernatants of sonicated bacterial pellet after re-suspension together with the cell debris of the sonicated pellet were added into the 135 mL **M. aeruginosa** culture and incubated under the same conditions as described previously. Controls were added with the equal volume of the modified culture. The phytoplankton-lytic effects were examined by measuring the concentration of chlorophyll a after 7 days.

**Identification of phytoplankton taxa sensitive to the active agent**

All the phytoplankton taxa listed in Section 'Phytoplankton culture' were selected to determine the range of phytoplankton taxa sensitive to the active agent produced by CH-22. The cultures of each phytoplankton were inoculated with 15 mL of bacterial culture supernatants. Controls consisted of the cultures that received 15 mL modified medium only. Incubation conditions were similar to the former conditions. The phytoplankton-lytic activity was confirmed by determining the changes in the chlorophyll a concentration in an infected culture after 4 or 7 days.

**Results**

**Screening of phytoplankton-lytic bacteria**

A total of 54 bacterial strains were isolated. Among them, five isolates showed phytoplankton-lytic activity against **M. aeruginosa**.

The inhibitory rates against **M. aeruginosa** of strains named as CH-21, CH-22, CH-23, CH-41 and CH-5d were 79.1%, 87.6%, 84.5%, 67.3% and 78.0%, respectively. Among these five isolates, CH-22 exhibited the strongest phytoplankton-lytic activity against **M. aeruginosa**. Therefore, in the following experiments only this bacterium was further examined.

**Identification of the bacteria by phylogenetic analysis**

To identify the CH-22 strain, morphological and genetic analyses were performed. The phytoplankton-lytic bacterium CH-22 was Gram-negative, and non-pigmented in a modified agar plate. **Fig. 1** shows the SEM pictures illustrating that it is rod-shaped with the diameter from 0.7 to 2.4 µm. The growth curve is shown in **Fig. 2**.

To genetically characterize the CH-22 strain, DNA was isolated and PCR was carried out to amplify 16S rDNA. The 16S rDNA sequences of the CH-22 strain were aligned through comparison with available sequences from the GenBank database. The sequences of the CH-22 strain shared the greatest identity with those of **P. putida** IAM 1236 (D 84020) (99.3% identity). A phylogenetic tree based on bacterial 16S rDNA sequences showed...
a close relationship between CH-22 and the genus Pseudomonas (Fig. 4). The genome DNA of the strain CH-22 and amplification of CH-22 16S rDNA are shown in Fig. 3, and the sequence is available in GenBank under accession number GU060497. Together with the morphological identification, CH-22 was identified as P. putida.

Phytoplankton-lytic effect of the bacterium CH-22 on M. aeruginosa

Dependence of the phytoplankton-lytic effect on the initiate M. aeruginosa density affects

The phytoplankton-lytic activity of strain CH-22 was severely affected by the initial M. aeruginosa density. Both indicators of M. aeruginosa biomass, i.e., the chlorophyll a concentration and the cell numbers, showed the inhibitory effect of CH-22 in a similar manner (Fig. 5a and b). The higher the initial M. aeruginosa cell density, the faster the reduction of two indicators after treatment with the same concentration (10%) of bacterial culture. When initial cell density was $1.5 \times 10^7$ cells/mL, it was still $5.4 \times 10^5$ cells/mL of cell density even 7 days later after the treatment.

Dependence of the phytoplankton-lytic effect on the bacterial density

To investigate the relationship between phytoplankton-lytic activity and bacterial cell density of CH-22, different initial bacterial cells densities were added into M. aeruginosa cultures. As shown in Fig. 6a and b, with increase in density of CH-22, the cell number and the chlorophyll a concentration of M. aeruginosa decreased. Fig. 6c shows the relationship between the inhibitory rate and changes in M. aeruginosa cell number. When the 15% bacterial culture was infected for five days, the cell density decreased from $5.3 \times 10^8$ to no more than $1 \times 10^5$ cells/mL and the inhibitory rate maintained over 93.0%.

Morphological change in M. aeruginosa observed by TEM

Fig. 7 shows the SEM images of M. aeruginosa in the presence of normal cells and cells treated with bacterial culture (10%) for 7 days. Fig. 7a shows that normal M. aeruginosa cells are round and plump and have a spherical shape with a smooth exterior. Compared with the normal M. aeruginosa cells, the cells in the presence of bacterial culture are different. Fig. 7b shows that these were distorted from their normal spherical shape and appeared flattened. Moreover, some of cells were cracked and their inclusion leaked out.

Change in phycocyanin concentration in M. aeruginosa after treatment with the phytoplankton-lytic bacterium CH-22

Phycocyanin (PC) belonging to the photosynthetic apparatus has antioxidant and radical scavenging properties (Benedetti et al., 2006). As presented in Fig. 8, obvious reduction in phycocyanin concentration in the treated M. aeruginosa was detected after inoculation with CH-22. Phycocyanin concentration of the treated M. aeruginosa was reduced by 63.9%, 79.5%, 87.1%, and 94.9% after 4, 5, 6, and 7 days, respectively, while that of the control group was kept in an increasing tendency.

Identification of the active agent produced by the bacterium CH-22

Fig. 9 presents the result for the test of the active agent of the cultures of the bacterium CH-22. Bacterial culture (A), supernatants of bacterial culture (B) and supernatants of bacterial culture treated with heat (D, 121 °C, 30 min) have higher phytoplankton-lytic activity compared with the bacterial pellet with the same treatment. Moreover, the group of the bacterial cell pellet treated with heat (E) shows slight promotion on the growth of M. aeruginosa. A group with treatment of sonication, the supernatant of sonicated pellet resuspended in the modified medium has phytoplankton-lytic activity reaching to 37.3% (F) while the phytoplankton-lytic activity of the cell pellet group without sonication has the phytoplankton-lytic activity of only 29.1% (C). It means that some phytoplankton-lytic substance was released during the sonicated treatment. The cell debris of sonicated bacterial pellet group may also accelerate the growth of M. aeruginosa (∼18.3%, G).

Determination of the range of phytoplankton taxa sensitive to the active agent produced by CH-22

Fig. 10 shows the bacterium CH-22’s strong phytoplankton-lytic activity against a wide range of phytoplankton taxa. The
phytoplankton-lytic activity rates were measured against various phytoplanktons as follows: *M. aeruginosa* (89.3%), *M. wesenbergii* (76.5%), *M. viridis* (70.3%), *A. fos-aquae* (61.6%), *C. ellipsoidea* (48.5%) and *Anabaena fos-aquae* (71.3%). All species tested were susceptible to this bacterium, but *P. putida* CH-22 showed particularly strong phytoplankton-lytic activity against...
In this experiment, after 4 days of incubation, the chlorophyll a concentration reduction in *Anabaena fos-aquae* was the strongest (60.8%), *Anabaena fos-aquae* (59.1%) was subsequent and *M. aeruginosa* the least (only 51.2%). However, after 7 days of incubation, the strongest inhibitory effect was found in *M. aeruginosa* (89.3%). Ordinary phytoplankton-lytic activity was found in green algae *C. ellipsoidea*. After treatment of 7 days, the inhibitory effect was still no more than 50% (48.5%).

**Discussion**

Recently, many phytoplankton-lytic bacteria have been isolated from various freshwater regions (Kim et al., 2003; Choi et al., 2005). However, only few bacteria with phytoplankton-lytic activity against the cyanobacteria have been reported (Shi et al., 2006; Lu et al., 2009). In particular, for *M. aeruginosa*, few reports have been found to identify and characterize the phytoplankton-lytic bacteria and the active agent from cultures with the potential to lyse the species. Kang et al. (2005) reported that one kind of phytoplankton-lytic bacterium HYK0203-SK02 against *M. aeruginosa* possesses 71.4% inhibitory rate, but that study focused on diatom *S. hantzschii*, which is a bloom-forming species in South Korea in late autumn. Here, we reported for a phytoplankton-lytic bacterium CH-22 that appears to act against *M. aeruginosa*.

Many documents have reported that phytoplankton-lytic activity is significantly influenced by the growth phase of isolated bacterium and harmful algae (Su et al., 2007; Kang et al., 2005). However, in the shallow lake or marine waters, it is difficult to identify the growth phases of the cyanobacterial and indigenous phytoplankton-lytic bacteria. Therefore, in this work, the influence by initial bacterial culture and phytoplankton cell density was emphasized. The phytoplankton-lytic activity of CH-22 is obviously influenced by the volume added to the bacterial culture. For dependence on the initial *M. aeruginosa* cell density, the higher the initial cell density, the lower the phytoplankton-lytic activity. Thus the phytoplankton-lytic activity does not only depend on the bacterium CH-22 itself, but also is affected by secreted metabolites. Besides, it is dependent on both bacterium CH-22 density and initial *M. aeruginosa* cell density.

**Fig. 7.** Scanning electron microphotographs of *M. aeruginosa* treated with 10% bacterial culture of bacterium CH-22: (a) normal cells; (b) treated cells.

**Fig. 8.** Effect of bacterium CH-22 cultures on phycocyanin of *M. aeruginosa*. 10% bacterial culture was added into the *M. aeruginosa* cultures and the initial *M. aeruginosa* cell density was about $9 \times 10^6$ cells/L. Values are given as the mean ± standard deviation from triplicate assays. *Low significant differences at $P < 0.05$. **Significant differences at $P < 0.01$ (highly significant).

**Fig. 9.** Phytoplankton-lytic activity of different fractions treated by bacterium CH-22 against *M. aeruginosa* after 7 days of incubation. Each value is mean ± standard deviation from triplicate assays. (A) bacterial culture; (B) bacterial culture supernatant; (C) bacterial pellet; (D) bacterial culture supernatant treated with heat (121 °C, 30 min); (E) bacterial pellet treated with heat (121 °C, 30 min) and resuspended with modified media; (F) supernatant of sonicated bacterial pellet; (G) cell debris of sonicated bacterial pellet.

It is well known that phycocyanin participates in photosynthesis as accessory pigment in cyanobacteria. French and Violet
treated phytoplankton compared with the control. Data are expressed as the mean ± standard deviation from triplicate assays.

Also, it is known that chlorophyll plays a specific chemical role in photosynthesis in addition to acting as a light absorber to transfer energy to chlorophyll. Indeed, we found that in the treated *M. aeruginosa* there is a drastic reduction in phycocyanin concentration, which is also consistent with the previous report (Shi et al., 2006). Reduction in phycocyanin is either due to enzymatic breakdown of the cyanobacterial cell membrane, or due to inhibitory effect by the active agent secreted from bacterium CH-22 on the photosynthetic activities of the cyanobacteria.

Generally, phytoplankton-lytic bacteria can kill their prey via one of two main mechanisms: direct or indirect (Shi et al., 2006; Mu et al., 2007). In our experiment, the supernatants of bacterial cultures (B) and heat-treated supernatants (D) all show stronger phytoplankton-lytic activity, whereas the bacterial cells (E) and debris of bacterial cells (G) show low phytoplankton-lytic activity or even slightly accelerated growth of *M. aeruginosa*. These results reveal that the dominant mode of *P. putida* against *M. aeruginosa* is due to indirect interaction. Similarly, *Alteromonas* sp. K and D (Imai et al., 1995), *Pseudoalteromonas* sp. T827/2B (Baker and Herson, 1978), *Pseudoalteromonas* sp. A28 (Lee et al., 2000) were also reported as phytoplankton-lytic to the test algal species through indirect attack.

Kang et al. (2005) reported isolated strain HYK0203-SK02 can inhibit *M. aeruginosa* and diatom *S. hantzschii*, but simulated another diatom *Cyclotella* sp. HYK0210-A1. Mu et al. (2007) reported phytoplankton-lytic bacterial *B. fusiformis* against *M. aeruginosa*, *Chlorella* and *Scenedesmus*. In this work the isolated phytoplankton-lytic bacterium CH-22 can inhibit five strains of cyanobacteria and two strains of green alga, i.e., *C. ellipsoidea* and *S. quadricauda*.

Phytoplankton-lytic bacteria may play an important role in population dynamics during harmful algal blooms; at the same time studies on the bacterial–phytoplankton interactions are also helpful for a better understanding of carbon cycle models in the water ecosystem (Su et al., 2007). Therefore, in our next work we plan to investigate the mechanisms by which bacteria influence the phytoplankton dynamics.

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**References**


