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Isolation, identification and characterization of an algicidal bacterium from Lake Taihu and preliminary studies on its algicidal compounds

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Received 11 November 2011; revised 01 February 2012; accepted 10 February 2012

Abstract
In an effort to identify a bio-agent capable of controlling cyanobacterial blooms, we isolated a bacterial strain, A27, which exhibited strong algicidal activity against the dominant bloom-forming species of *Microcystis aeruginosa* in Lake Taihu. Based on 16S rRNA gene sequence analysis, this strain belongs to the genus *Exiguobacterium*. This is the first report of an algicidal bacterial strain belonging to the genus *Exiguobacterium*. Strain A27 exhibited algicidal activity against a broad range of cyanobacteria, but elicited little or no algicidal activity against the two green algal strains tested. The algicidal activity of strain A27 was shown to be dependent on the density of the bacteria and to have a threshold density of $1.5 \times 10^6$ CFU/mL. Our data also showed that the algicidal activity of strain A27 depended on different growth stages of *Microcystis aeruginosa* (exponential > lag phase > early stationary) rather than that of the bacterium itself. Our results also suggested the algicidal activity of strain A27 occurred via the production of extracellular algicidal compounds. Investigation of the algicidal compounds revealed that there were at least two different algicidal compounds produced by strain A27. These results indicated that strain A27 has great potential for use in the control of outbreaks of cyanobacterial blooms in Lake Taihu.

Key words: algicidal bacteria; freshwater cyanobacterial bloom; *Microcystis aeruginosa*; *Exiguobacterium* sp. A27

DOI: 10.1016/S1001-0742(11)60983-2

Introduction
Lake Taihu, which is the third-largest lake in China, is a typical shallow freshwater lake located in Eastern China (surface area: 2338 km², mean depth: 1.9 m; Wu et al., 2007). Due to industrial and agricultural pollution over the last two decades, Lake Taihu has experienced severe ecological and environmental problems (Lin, 2002; Wu et al., 2007; Tian et al., 2009). A cyanobacterial bloom has occurred every summer in Lake Taihu during the last few years. The occurrence of such blooms has become a serious problem, as indicated by a heavy cyanobacterial reservoirs worldwide, problems caused by off-flavors such as geosmin or 2-methylisoborneol (MIB) are known to be particularly associated with the occurrence of planktonic and benthic cyanobacteria (Jähnichen et al., 2011).

It has been reported that *Microcystis* is the dominant genus during cyanobacterial blooms in many eutrophic lakes (Hutchinson, 1967; Chen et al., 2003; Rinta-Kanto et al., 2005; Yoshida et al., 2007). Some earlier studies have indicated that *Microcystis* and *Synechococcus* are the dominant genera comprising cyanobacterial blooms in Lake Taihu (Chen et al., 2003; Ye et al., 2011). Also, several genera of cyanobacteria, including *Microcystis*, *Planktothrix* and *Anabaena*, are known to produce microcystins (Carmichael and Falconer, 1993; Carmichael, 1994; Chorus and Bartram, 1999; Fleming and Stephan, 2001). Microcystins are the most widespread cyanobacterial toxins present in freshwater systems and can cause liver failure and death (Carmichael et al., 2001). As a result, it is essential to find an effective way to inhibit the growth of *Microcystis* and *Synechococcus* to control cyanobacterial blooms in Lake Taihu.

Unquestionably, nutrient input reductions are very necessary in Lake Taihu to reduce bloom intensity and frequency (Hai et al., 2010; Paerl et al., 2011). However, as interesting supplementary approaches to bloom control, some studies have also investigated various methods for controlling algal and cyanobacterial blooms through physical, chemical or biological means (Sigee et al., 1999; Jeong et al., 2000). Some researchers have suggested that algicidal bacteria could be used to control algal and cyanobacterial blooms, since these bacteria kill the organisms that comprise blooms in lab studies and their increase coincides with the ebb of a bloom (Shilo, 1970; Redhead and Wright, 1980; Yamamoto and Suzuki, 1999). Therefore, algicidal bacteria could potentially be useful in the control of
of cyanobacterial blooms.

In this study, we examined (1) the isolation and identification of an effective algicidal bacterium against *M. aeruginosa*, (2) the algicidal activity of the isolated bacterial strain against *M. aeruginosa*, (3) the algicidal range of the isolated strain against other algal and cyanobacterial species commonly seen in cyanobacterial blooms, and (4) the chemical compounds extracted from the bacterial culture of strain A27 with the ability to inhibit the growth of *M. aeruginosa*.

1 Materials and methods

1.1 Cyanobacterial culture

*Microcystis aeruginosa* PCC7806 was purchased from the Freshwater Algae Culture Bank (FACHB) in China and maintained as a unialgal axenic culture at 25°C under 40 μmol photons/(m²·sec) and a 12 hr:12 hr (light: dark) cycle. The cyanobacterial cells were incubated in BG11 medium (100 mL) adjusted to pH 7.0 containing NaNO₃ (150 mg), K₂HPO₄ (4 mg), MgSO₄·7H₂O (7.5 mg), CaCl₂·2H₂O (3.6 mg), citric acid (0.6 mg), ferric ammonium citrate (0.6 mg), EDTA (0.1 mg), Na₃CO₃ (2 mg), and A5 solution (0.1 mL) (A5 solution: H₂BO₃ (286 mg), MnCl₂·4H₂O (181 mg), ZnSO₄·7H₂O (22.2 mg), CuSO₄·5H₂O (7.9 mg), Na₂MoO₄·2H₂O (3.9 mg), Co(NO₃)₂·6H₂O, distilled water (100 mL)). The cyanobacterial cells were transferred to fresh BG11 medium once every two weeks.

1.2 Isolation and identification of algicidal bacteria

Bacteria to be screened for algicidal activity were isolated during a cyanobacterial bloom in Meiliang Bay, which is located at the northeast part of Lake Taihu. Water samples were collected at the Taihu Ecosystem Research Station (31°24′N, 120°13′E) in Meiliang Bay from 0.5 m below the surface from August 2007 to November 2007 using a sterile sampler. Water samples were collected in sterile bottles and then transported to the laboratory in a minicryobox within four hours.

Using the screening approach described by Imamura et al. (2001), bacterial strain A27, which showed significant algicidal activity against *M. aeruginosa* PCC7806, was successfully isolated from the water samples. After being purified as previously described by Yamamoto and Suzuki (1990), strain A27 was cryopreserved at –20°C in LB medium for 24 hr, harvested by centrifugation (12,000 ×g for 20 min), washed twice with fresh LB medium and re-suspended into an equal amount of LB, after which a suitable volume of this culture was inoculated into *M. aeruginosa* PCC7806 culture (in BG11 medium). In the control, an equal volume of the fresh LB was inoculated into *M. aeruginosa* PCC7806 culture. All experiments were conducted in triplicate and the results are given by the mean and standard deviation of the raw data.

1.3 Analysis of algicidal activity

Cells of *M. aeruginosa* PCC7806 were quantified using a haemocytometer under a light microscope (magnification: ×400) and bacterial cell densities of strain A27 were determined by the CFU method performed on LB agar plates. The algicidal activity (A, %) of strain A27 was measured by the following equation:

\[
A = \left(1 - \frac{D_t\text{-treatment}}{D_t\text{-control}}\right) \times 100% 
\]

where, \(D_t\text{-treatment}\) (cells/mL) and \(D_t\text{-control}\) (cells/mL) are the cell densities of treatment and control (or chlorophyll-α concentrations when the cyanobacterial cells are not countable) of *M. aeruginosa* PCC7806 with and without strain A27, respectively; \(t\) (day) stands for the inoculation time. Strain A27 was incubated at 37°C under 200 r/min in LB medium for 24 hr, harvested by centrifugation (12,000 ×g for 20 min), washed twice with fresh LB medium and re-suspended into an equal amount of LB, after which a suitable volume of this culture was inoculated into *M. aeruginosa* PCC7806 culture (in BG11 medium). The bacterial suspensions (10 mL) were inoculated into test flasks containing 90 mL of exponential-phase cultures of the following test-cultures of *M. aeruginosa* PCC7806, *Microcystis viridis* FACHB-979, *Microcystis wessenbergii* FACHB-908, *Chroococcus* sp. FACHB-191, *Oscillatoria planctonica* FACHB-708, *Nostoc* sp. FACHB-953, *Aphanizomenon flos-aquae* FACHB-943, *Oscillatoria* sp. BN34, *Synechococcus* sp. BN39 and BN60, *Chlorophyta* sp. M7, *Chlamydomonas* sp. BS3 and *Microcystis aeruginosa* 9110. Among these strains, the first seven were purchased from the FACHB, whereas the other six were isolated from Meiliang Bay of Lake Taihu (data not shown). All algal and cyanobacterial strains were cultured under the growth conditions mentioned above. The chlorophyll-α (chl-α) concentrations of all cultures were evaluated daily for up to six days and the algicidal activity was calculated. Three replicates were used for each treatment and control.
1.5 Correlations between bacterial density and algicidal activity

To elucidate the algicidal activity of strain A27 with different cell densities against *M. aeruginosa* PCC7806, the bacterial culture was incubated, harvested and washed as described above and then serially diluted with LB to densities of 1.5 \times 10^3, 1.5 \times 10^4, 1.5 \times 10^5, and 1.5 \times 10^6 CFU/mL. Next, 10 mL aliquots of these five suspensions with different cell densities were added to 90 mL of *M. aeruginosa* PCC7806 culture (1.0 \times 10^7 cells/mL) at the mid-exponential phase. For the control, an equal volume of fresh LB (10 mL) was inoculated into 90 mL of *M. aeruginosa* PCC7806 culture. The cell density of *M. aeruginosa* PCC7806 was measured daily for ten days. The dissolved organic carbon concentrations (DOC) were also measured with a TOC-500 total organic carbon analyzer (Shimadzu, Japan) after all the bacterial and cyanobacterial cells were removed from the culture by centrifugation (12,000 \times g for 20 min). Three replicates were used for each dilution and control.

1.6 Effects of cyanobacterial and bacterial growth phases on the observed algicidal activity

The algicidal activity of strain A27 against cells of *M. aeruginosa* PCC7806 was investigated during different growth phases of the cyanobacterium. The bacterial culture was prepared (incubated for 12 hr; stationary phase), after growth phases of the cyanobacterium. The bacterial culture was centrifuged at 12,000 \times g, harvested by centrifugation at 12,000 \times g, and the supernatant was passed through a 0.2-m pore size filter (diameter 45 mm; Millipore, USA) to obtain cell-free supernatant. The supernatant was then concentrated tenfold after which 100 \mu L of the concentrated supernatant was added to a sterilized paper disk and placed on the surface of a cyanobacterial-lawn of *M. aeruginosa* PCC7806 as described below. Additionally, after the sediment of the culture was washed twice with fresh LB and re-suspended in 100 mL LB medium, the bacterial cells in the suspension were subjected to ultrasonication (400 W, every cycle contained 3 sec of ultrasonication and 3 sec of pause) for 20 min to obtain a mixture of cell fragments. This mixture was also concentrated tenfold, after which 100 \mu L of the concentrated mixture was added to the cyanobacterial-lawn. For the control, 100 \mu L of tenfold concentrated LB was added to the cyanobacterial-lawn. All samples were then incubated for two days under cyanobacterial growth conditions. Three replicates were used for each treatment and control.

To develop the cyanobacterial-lawn, axenic cultures of *M. aeruginosa* PCC7806 were grown in BG11 medium for one week under the conditions described above. After which 40 mL of the culture were harvested by centrifugation at 12,000 \times g for 20 min and added into 20 mL of molten BG11 soft agar (1.0% agar, equilibrated to 53°C). The mixture was immediately poured onto a BG11 agar plate (1.5% agar), allowed to solidify and then incubated for two days under the cyanobacterial growth conditions described above. Algicidal activity was evaluated by observing the formation of clear zones around the paper discs.

1.7 Evaluation of the algicidal mode

To determine if the algicidal activity of strain A27 occurred via the production of bioactive compounds, the strain was incubated at 37°C and 200 t/min for 24 hr in a 250-mL flask containing 100 mL LB medium. Next, the culture was centrifuged at 12,000 \times g for 20 min, after which the supernatant was passed through a 0.2-\mu m pore size filter (diameter 45 mm; Millipore, USA) to obtain cell-free supernatant. The supernatant was then concentrated tenfold using a centrifugal dryer, after which 100 \mu L of the concentrated supernatant was added to a sterilized paper disk and placed on the surface of a cyanobacterial-lawn of *M. aeruginosa* PCC7806 as described below. Additionally, after the sediment of the culture was washed twice with fresh LB and re-suspended in 100 mL LB medium, the bacterial cells in the suspension were subjected to ultrasonication (400 W, every cycle contained 3 sec of ultrasonication and 3 sec of pause) for 20 min to obtain a mixture of cell fragments. This mixture was also concentrated tenfold, after which 100 \mu L of the concentrated mixture was added to the cyanobacterial-lawn. For the control, 100 \mu L of tenfold concentrated LB was added to the cyanobacterial-lawn. All samples were then incubated for two days under cyanobacterial growth conditions. Three replicates were used for each treatment and control.

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1.8 Extraction and purification of the algicidal compounds

The supernatant of LB-grown (24 hr) cultures of strain A27 was obtained as described above. The supernatant was evaporated and extracted with MeOH. After concentration, the tenfold-concentrated MeOH extract was subjected to column chromatography (commercial silica gel, Qingdao Haiyang Chemical Group Co., China; 200–300 mesh; 1×50 cm) at a flow rate of 1 mL/min with a gradually increasing ratio of MeOH/CHCl₃ (0%–30% MeOH) and monitored at 254 nm. The 6%–8% MeOH fraction was then collected and further purified by reversed-phase semi-preparation HPLC (Spursil C18-EP, 5 \mu m, 10 mm×250 mm, Dikma, China; mobile phase: 100% H₂O; flow rate: 4.5 mL/min; UV detection at 210 nm). These collected fractions were evaporated and the residuals were collected respectively.

In the column chromatography and HPLC experiments, the cyanobacterial-lawns were used to test the algicidal activities of all the fractions collected and only the ones with algicidal activities were further analyzed and purified.

1.9 Evaluation of the algicidal activities of the fractions collected in HPLC experiments

To further confirm the algicidal ability of the collected fractions against *M. aeruginosa* PCC7806 and other primary cyanobacterial species in the blooms of Lake Taihu, three cyanobacterial lawns were prepared using cultures of *M. aeruginosa* PCC7806, *M. aeruginosa* 9110 and Synechococcus sp. BN60. Briefly, 0.6 mg of the residual of each algicidal fraction was dissolved in distilled water.
and equally added to three sterilized paper discs, which were placed on the surface of three cyanobacterial lawns. The plates were then incubated for four days under the cyanobacterial growth conditions described above, after which the inhibition of cyanobacterial growth was assessed by observing the clear inhibition zone around the paper discs. Three replicates were used for each treatment and control.

2 Results

2.1 Isolation and identification of algicidal bacteria

Forty-four bacterial strains were isolated from the surface water samples collected from Meiliang Bay of Lake Taihu from August 2007 to November 2007. Among these strains, only ten exhibited significant algicidal activity toward M. aeruginosa PCC7806, and strain A27 appeared to exert the strongest algicidal activity.

The 16S rRNA gene sequence of strain A27 was analyzed by the RDP classifier software (version 2.2) and it was classified as an Exiguobacterium sequence with a 100% confidence threshold. Comparison of the 16S rRNA gene of this strain with those available in the GenBank database (http://www.ncbi.nlm.nih.gov/blast) indicated that it was most closely related to Exiguobacterium sp. AT1b (97% homology, GenBank database accession number CP001615.1) (Vishnivetskaya et al., 2011). The 16S rRNA gene sequence of strain A27 has been deposited in the GenBank database under accession number FJ751911, and this bacterial culture on an LB agar plate was deposited in the China General Microbiological Collection Center (CGMCC) under accession number CGMCC-4114.

2.2 Algicidal range of strain A27

The algicidal activities (t = 2 days) of strain A27 against several algal and cyanobacterial species are shown in Table 1. Strain A27 exhibited algicidal effects toward most of the test cyanobacterial species and the algicidal effects ranged from 44.7% (against Aphanizomenon flos-aquae FACHB-943, t = 2 days) to 82.9% (against Oscillatoria sp. BN34). However, this bacterial strain showed little or no algicidal activity against two eukaryotic algal strains, Chlorophyta sp. M7 (0.7%) and Chlamydomonas sp. BS3 (27.2%). Interestingly, the growth of cyanobacterial strain Microcystis wesenbergii FACHB-908 was not inhibited, and was instead stimulated by strain A27 (–10.4%).

2.3 Correlations between bacterial density and algicidal effect

As shown in Fig. 1a, there was a distinct difference in the algicidal effect exhibited by strain A27 between two groups: the two treatments with initial bacterial densities of 1.5×10^4 and 1.5×10^5 CFU/mL (group A) and the other three treatments with bacterial densities of 1.5×10^6, 1.5×10^7, and 1.5×10^8 CFU/mL (group B). In group A, no significant algicidal activity was observed at the first two days after A27 inoculation, whereas the algicidal effect started to manifest on the first day after inoculation in group B (average algicidal effect 59.0%–68.9%, t = 2 days). These findings suggested that 1.5×10^6→1.5×10^8 CFU/mL was needed to exert the strongest algicidal activity.

### Table 1 Algicidal effect of strain A27 against several algal and cyanobacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Algicidal effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystis viridis FACHB-979</td>
<td>54.3 ± 4.0</td>
</tr>
<tr>
<td>Microcystis wesenbergii FACHB-908</td>
<td>–10.4 ± 2.5</td>
</tr>
<tr>
<td>Chroococcus sp. FACHB-191</td>
<td>75.6 ± 9.7</td>
</tr>
<tr>
<td>Oscillatoria planctonica FACHB-708</td>
<td>59.2 ± 5.1</td>
</tr>
<tr>
<td>Nostoc sp. FACHB-953</td>
<td>69.8 ± 8.8</td>
</tr>
<tr>
<td>Aphanizomenon flos-aquae FACHB-943</td>
<td>44.7 ± 2.2</td>
</tr>
<tr>
<td>Oscillatoria sp. BN34*</td>
<td>82.9 ± 6.7</td>
</tr>
<tr>
<td>Synechococcus sp. BN39*</td>
<td>73.3 ± 3.4</td>
</tr>
<tr>
<td>Synechococcus sp. BN60*</td>
<td>78.7 ± 6.6</td>
</tr>
<tr>
<td>Chlorophyta sp. M7*</td>
<td>0.7 ± 2.1</td>
</tr>
<tr>
<td>Chlamydomonas sp. BS3*</td>
<td>27.2 ± 3.9</td>
</tr>
<tr>
<td>Microcystis aeruginosa 9110*</td>
<td>58.3 ± 8.2</td>
</tr>
</tbody>
</table>

*Oscillatoria sp. BN34, Synechococcus sp. BN39 and BN60, Chlorophyta sp. M7, Chlamydomonas sp. BS3 and Microcystis aeruginosa 9110 were isolated from Meiliang Bay of Lake Taihu.

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Fig. 1 Dynamics of cell density of M. aeruginosa PCC7806 (a), and DOC level (b) in cultures inoculated with various initial concentrations of algicidal strain A27. The arrows indicate the time of inoculation. Data are the mean ± SD from three independent assays.
CFU/mL might be the effective algicidal threshold density of strain A27 against *M. aeruginosa* PCC7806. In group A, the bacterial density of strain A27 was lower than this threshold density after A27 inoculation, so no significant algicidal activity was observed in the first two days. But after two days of incubation, the bacterial density of strain A27 may reach the level above this threshold density and the algicidal activity would begin to manifest (Fig. 1a).

The DOC levels (Fig. 1b) did not show distinct difference between the two groups after inoculation, which suggested that organic nutrient concentration was not the cause of the distinct difference in algicidal effects between group A and B.

### 2.4 Effect of algal and bacterial growth phases on the observed algicidal ability

Strain A27 effectively inhibited the growth of *M. aeruginosa* PCC7806 during two growth phases of the cyanobacterium, showing average algicidal effects of 61.9% during the lag phase (*t* = 2 days) and 62.6% during the exponential phase (*t* = 2 days). However, this bacterial strain did not show very strong algicidal activity against early stationary phase cells of *M. aeruginosa* PCC7806 (average algicidal effect: 22.4%, *t* = 2 days) (Fig. 2a). At the fourth day after inoculation, the algicidal activity of strain A27 against early stationary phase cells of *M. aeruginosa* PCC7806 (68.2%) was also the lowest (the algicidal effects against lag phase and exponential phase cells were 96.2% and 94.4%, respectively).

In addition, strain A27 at different growth phases consistently inhibited mid-exponential *M. aeruginosa* PCC7806, yielding average inhibitions of 52.4% (lag, *t* = 2 days), 56.4% (exponential, *t* = 2 days) and 62.6% (stationary, *t* = 2 days) (Fig. 2b).

### 2.5 Evaluation of the algicidal mode

To determine whether the algicidal activity of strain A27 occurred via the production of bioactive compounds, we examined the algicidal activity of tenfold-concentrated supernatant using a cyanobacterial lawn. The results (Fig. 3) showed that the concentrated supernatant exhibited a strong algicidal effect and a clear zone was formed around the paper disk, while the concentrated cell fragments mixture and LB medium showed no algicidal activities. These results suggested that the algicidal ability of stain A27 occurred via the production of one or more extracellular algicidal compounds.

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**Fig. 2**  (a) Dynamics of cell density of *M. aeruginosa* PCC7806 in cultures inoculated with algicidal strain A27 at three different growth phases of the cyanobacteria and without algicidal bacteria. (b) Dynamics of cell density of *M. aeruginosa* PCC7806 in cultures inoculated with algicidal strain A27 at three different growth phases of the bacterial strain and without algicidal bacteria. The arrows indicate the time of inoculation. Data are the mean ± SD from three independent assays.

**Fig. 3**  Algicidal activity of the cell-free filtrate of strain A27. (a) 100 µL of the tenfold-concentrated cell-free filtrate; (b) 100 µL of the tenfold-concentrated cell fragments of strain A27; (c) 100 µL of the tenfold-concentrated LB medium.
2.6 Extraction and purification of the algicidal compounds

Only the 6%–8% MeOH fraction from the silica gel column exhibited algicidal activity, and two collected fractions (fraction B and D with retention time values of 13.5–14.2 min and 8–8.7 min respectively, Fig. 4) in HPLC experiments were found to have algicidal activities against Microcystis aeruginosa PCC7806. The existence of these two algicidal fractions suggested that there were at least two different kinds of algicidal compounds present in the supernatant of strain A27.

2.7 Evaluation of the algicidal activities of the fractions collected in HPLC experiments

The residual of fraction B collected from the bacterial culture of strain A27 showed algicidal activity against Microcystis aeruginosa PCC7806 (Fig. 5a) as well as two other cyanobacterial species, M. aeruginosa 9110 (Fig. 5b) and Synechococcus sp. BN60 (Fig. 5c), which were the two dominant cyanobacteria in Lake Taihu and were isolated from Meiliang Bay. The residual of fraction D also showed similar algicidal activities against these three cyanobacterial species (Fig. 5d, e and f).

3 Discussion

Among ten algicidal bacterial strains we selected in our initial screen, strain A27 showed the highest algicidal activity and was selected for further study. This strain belonged to the genus Exiguobacterium (phylum Firmicutes) and was Gram-positive. This is the first report of an algicidal bacterial strain belonging to the genus Exiguobacterium. Most of the reported algicidal bacteria fall into either Cytophaga-Flavobacterium-Bacteroides (CFB) or γ-Proteobacteria groups, including members of genera Cytophaga, Saprospira, Pseudoalteromonas and Alteromonas (Mayali and Azam, 2004; Su et al., 2007). Based on several reviews, about 50% of the algicidal bacterial strains belong to the CFB group, while about 45% are members of γ-Proteobacteria; only the remaining strains are Gram-positive (Fukuyo et al., 2002; Mayali and Azam, 2004; Hare et al., 2005; Kim et al., 2009).

Strain A27 exhibited algicidal activity against a broad range of cyanobacteria, while it elicited little or no algicidal activity against the two green algal strains tested (Table 1). It is interesting to observe that strain A27 facilitated the growth of Microcystis wesenbergii, which is phylogenetically very similar to Microcystis aeruginosa and Microcystis viridis (Table 1). Fukami et al. (1992) reported that bacteria may have selectively beneficial and/or harmful effects against specific algae. Other studies have reported that algicidal bacteria have species- and/or strain-specific activity (Kang et al., 2005).

It appeared that the algicidal activity of strain A27 was bacterial cell density-dependent, since algicidal activity occurred only at higher bacterial cell densities. Doucette et al. (1999) suggested that the initial algicidal bacteria density influenced the manifestation of algicidal activity, and Fraleigh and Burnham (1988) postulated that algicidal effects will occur above the threshold density of algicidal bacteria, with little dependence on inorganic nutrient concentrations or host density. Kang et al. (2005) also reported an initial threshold density of 1×10⁶ CFU/mL. Our data showed that the threshold density above which strain A27 had algicidal effects against M. aeruginosa PCC7806 was 1.5×10⁶ CFU/mL.

The results of this study showed that the algicidal activity of strain A27 depended on different growth stages of M. aeruginosa PCC7806 (exponential = lag phase > early stationary) (Table 2a). In contrast, the algicidal activity of this strain did not differ greatly among the growth stages of the bacteria itself (Fig. 2b). It has been reported that the physiological status of the host algae might play a crucial role in the success of bacterial attack (Manage et al., 2001). Toncheva-Panova and Ivanova (2000) found that the lysis effect of bacteria was accompanied by changes in algal physiology. Thus, our results indicated that the physiological status of the cyanobacterium, not the bacterium, was a crucial factor in the algicidal activity of strain A27 against M. aeruginosa PCC7806. A similar result was reported for another algicidal bacterium, HYK0203-SK02, which also suggested that the algicidal effect is more closely related to the physiological status of the test algae than that of the algicidal bacterium (Kang et al., 2005). However, their findings differed from ours in that bacterium HYK0203-SK02 showed a significant algicidal effect against stationary-phase algae.

In general, bacteria that inhibit the growth of algae and/or cyanobacteria exert their effects through direct (Imai et al., 1993; Mayali and Doucette, 2002; Kang et al., 2005) or indirect attack (Amaro et al., 2005; Su et al., 2007; Ren et al., 2010). Indirect attacks are usually chemically mediated (Kang et al., 2005). In the present study, algicidal activity was detected in the supernatants, while the cell fragments showed no significant algicidal activity (Fig. 3b). These findings revealed that the algicidal activity of strain A27 may occur via extracellular compounds. Alteromonas sp. K and D (Imai, 1995), Pseudoalteromonas sp. A28 (Lee et al., 2000) and Pseudoalteromonas sp. SP48 (Su et al., 2007) were also reported to have algicidal effects that occurred against test algal species through indirect attack.

Great difficulties exist in the extraction, purification,
and characterization of algicidal compounds owing to their apparent variation in characteristics across species of algicidal bacteria (Doucette and Powell, 1998; Skerratt et al., 2002). Indeed, only a few algicidal compounds have been successfully extracted, purified and characterized (Dakhama et al., 1993; Imamura et al., 2000; Lee et al., 2002; Ahn et al., 2003; Wang et al., 2005). These compounds comprise many chemicals usually seen in bacterial metabolites, such as proteases (Lee et al., 2002), peptides (Imamura et al., 2000), biosurfactants (Ahn et al., 2003; Wang et al., 2005) and antibiotic-like substances (Dakhama et al., 1993). In this study, we found that there was more than one kind of algicidal compound in the culture of strain A27 that had algicidal effect against *M. aeruginosa* PCC7806 since there were two algicidal fractions found in HPLC experiments. These findings indicate that the algicidal activity of strain A27 might be a complex process involving multiple algicidal mechanisms. However, we were unable to extract the pure algicidal compounds from fractions B and D so far because there were several other compounds with similar chemical properties existing in each fraction. Other better separation methods might be needed to solve this problem.

It has been reported that *Microcystis* and *Synechococcus* are the two main species comprising cyanobacterial blooms in Lake Taihu (Ye et al., 2011). Strain A27 not only showed strong algicidal activity against *M. aeruginosa* PCC7806, but also significantly inhibited the growth of several other cyanobacterial species isolated from cyanobacterial blooms in Lake Taihu, including one strain of *Microcystis* and two strains of *Synechococcus* (Table 1). These results indicated that strain A27 has great potential in the control of outbreaks of cyanobacterial blooms in Lake Taihu. However, *Exiguobacterium* sp. A27 is far from directly being practical for use in controlling cyanobacterial blooms and there are still problems to be solved. Specifically, (1) the chemical structures of the algicidal compounds have not been determined due to the limitations of the separation methods used in this study; (2) the algicidal effect of strain A27 and the algicidal compounds it produces in natural freshwater environments remains unclear; and (3) the algicidal effect mechanism of strain A27 requires further study.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20877055), the National Basic Research Program (973) of China (No. 2012CB720802), and the National High Technology Research and Development Program (863) of China (No. 2011AA100901).

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