World J Microbiol Biotechnol (2010) 26:465–472
DOI 10.1007/s11274-009-0192-2

ORIGINAL PAPER

The potential use of bacterium strain R219 for controlling of the bloom-forming cyanobacteria in freshwater lake

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Received: 12 July 2009 / Accepted: 22 September 2009 / Published online: 3 October 2009
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Abstract Cyanobacterial blooms become a serious environmental threat to the freshwater ecosystem, and several physical and chemical methods have been developed for controlling the blooms. In order to develop a biocontrol agent for controlling the blooms, we isolated a bacterial strain R219 that exhibited strong algicidal activity against the dominant bloom-forming species of *Microcystis aeruginosa* from Lake Tai in China. Based on 16S rDNA sequence analysis we determined the strain R219 to be *Pseudomonas aeruginosa* by the virtue of its sharing about 99.8% similarity with reference strains in the DNA databases. Biochemical and morphological tests were used to support the accurate identification as that of the bacterium *P. aeruginosa*. We also tested culture filtrate and ethyl acetate extract of strain R219 and showed both of them exhibited strong algicidal effect on the growth of *M. aeruginosa* at mid-exponential phase when the R219 filtrate and ethyl acetate extract were applied at various cell densities. Moreover, the *P. aeruginosa* filtrate showed high potency in removal of the mixed species bloom-forming cyanobacteria collected directly from the Lake Tai. When adding the filtrate of the strain R219 to the mixed-species cyanobacteria, the content of chlorophyll-*a* of the algae were reduced by as much as 80–90%. Oral acute toxicity assessment for strain R219 demonstrated that all the mice that received the broth or filtrate in doses of 0.5 or 2.0 g kg^{-1} were alive without any immediate behavioral changes within 14 days of administration of either broth or filtrate. These results indicate that the strain R219 may have potential for a use in controlling the bloom-forming cyanobacteria in freshwater ecosystems.

Keywords *Pseudomonas aeruginosa* R219 · *Microcystis aeruginosa* · Algicidal activity

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Introduction

Microcystis aeruginosa is the dominant species of the bloom-forming cyanobacteria which frequently occur in the eutrophic Lake Tai in China, and impact the drinking water supply and human health due to the production of a range of toxins. One category of the most polluting toxins are the hepatotoxins such as microcystins (MCs) which may be released to the natural environments after the cell lyses (Dai et al. 2008; Ross et al. 2006; Zurawell et al. 2005).

In an effort to control the potentially devastating effects of the harmful algal blooms, several strategies have been applied such as manipulation of physical sedimentation by flocculants, application of chemical algicides and biological agents (Oberholster et al. 2004). Certain species of bacteria, viruses, protozoans, and fungi have been all shown promising as potential algae-bloom suppressors in freshwater and marine ecosystems (Kang et al. 2008; Manage et al. 2001; Mayali and Doucette 2002; Mu et al. 2007; Sigee et al. 1999). In particular, bacteria capable of inhibiting the growth or degrading of the algae (simply inferred as algicidal bacteria) are considered to be one of the key biological agents in control of the phytoplankton blooms observed in freshwater lakes (Caiola and Pellegrini

1984; Yamamoto and Suzuki 1977) or red tides in marine coastal regions (Schoemann et al. 2005; van Rijssel et al. 2000). Most known algicidal bacteria are classified within either *Cytophaga*/*Flavobacterium*/*Bacteroidetes* group or the *Alteromonas*–*Pseudoalteromonas* group, with the former often more prevalent in both marine and freshwater bacterial communities (Roth et al. 2008). However, the control efficiency of algicidal bacteria against the water bloom remains unknown.

In an effort to screen for algicidal bacteria efficient in controlling water bloom, we tried to isolate bacteria strain(s) with growth inhibiting or cell degrading activities to cure severe pollution caused by *M. aeruginosa* in the Lake Tai of China, and to assess the possible application of such a strain as part of procedures that may allow control of *M. aeruginosa* dominant blooms.

Materials and methods

Tested alga, culture medium

The alga tested was axenic strain of *M. aeruginosa* PCC-7820, which was kindly supplied by Dr. Pengfu Li at Nanjing University. The alga was maintained in a growth chamber at 28°C/day and 22°C/night, under an illumination of 55-μmol m^{-2} s^{-1}, 14:10 h (light: dark) cycle. Medium for *M. aeruginosa*'s growth was BG11 as described before (Mu et al. 2007).

Isolation of algicidal bacterium

The strain of R219 with algicidal activity against the growth of *M. aeruginosa* was isolated from heavily occurred blooms at Lake Tai in October 2007. Water samples collected from Lake Tai were filtered through 5 μm Whatmann membrane filters and 200 μl of the filtrate was spread on LB plates at 37°C for 2 days to obtain bacterial strains. All the isolated strains of bacteria were then cultured in liquid LB medium at 37°C for 48 h and filtered through 0.22 μm membrane filters to get filtrate for algicidal activity test. To develop *Cyanobacteria* -lawn, cells of *M. aeruginosa* were cultivated in BG11 medium for 13 days and harvested by centrifugation at 2,900*g* for 20 min. The pellets were mixed with melted BG11 medium (0.5% agar) and poured onto the 9 cm petri dish and incubated at light chamber for 5 days under the algal culture growth conditions mentioned above. About 20 μl of the filtrates of each bacterial strain was added on a sterilized paper disk with 7 mm diameter and randomly placed on the *Cyanobacteria* -lawn, and the plates were incubated in light chamber for 2 days. The algicidal activity was evaluated by observing the formation of clear zones around the paper disk. Strain R219 which showed the greatest algicidal activity against *M. aeruginosa* was stored at −80°C in LB medium containing 50% glycerol.

Identification of strain R219 by phylogenetic analysis

The chromosomal DNA of strain R219 was isolated using a method described by Liu et al. (2007). The primers used for amplification of the bacterial 16S rDNA were 8f (5′AGAGTTTGATCMTGGC-3′; positions 8–23 of *Escherichia coli* chromosome numbering) and 1542r (5′-AAAGGAGGTGATCCA-3′; positions 1,556–1,542) (Saiki et al. 1988). DNA amplification was performed in a GeneAmp PCR system 2400 (Perkin Elmer) with an initial denaturation for 2 min at 94°C, followed by 25 cycles of denaturation (0.5 min at 94°C), annealing (1 min at 55°C), and extension (1 min at 72°C), plus a final extension for 10 min at 72°C. The PCR products were purified with the PCR Purification Kit (Tianwei) and sequenced by automated DNA sequencer (ABI 3100-Avant Genetic Analyzer). The sequence was compared with similar 16S rDNA sequences retrieved from the DNA databases by using the BLAST search program in the National Center for Biotechnology Information (NCBI).

Algicidal activity of the filtrates obtained at different time courses

One flask (1 l) containing 500 ml of LB (Luria–Bertani) medium was inoculated with 1% of the overnight culture broth of strain R219, incubated at 37°C with orbital shaking at 100 rpm, and sampled (20 ml) at the time interval of 24, 48, 72 and 96 h, respectively. The sampled broth were centrifuged at 4,360*g* for 5 min, and filtered through 0.22-μm polycarbonate filters to get the cell-free filtrate. Then, the algicidal activity of the filtrate, at a concentration of 80 μl ml^{-1}, was tested against *M. aeruginosa* at the exponential phase (1×10^7 cells ml^{-1}).

Algicidal effect of the EtOAc extract on the growth of *M. aeruginosa*

In order to determine the active fractions, we used petroleum ether (weak polarity), ethyl acetate (EtOAc, medium polarity) and *n*-butanol (strong polarity) successively to extract the bioactive substances from the culture filtrate of strain R219, and only the EtOAc extract showed algicidal activity against *M. aeruginosa*. For preparation of the EtOAc extract, 1-l flask containing 500 ml of LB medium was inoculated with strain R219 and cultivated at 37°C, 100 rpm for 3 days. After centrifugation at 2,900*g* for 30 min, the cell-free culture filtrate was extracted extensively with EtOAc. The obtained dark oily extract (3.1 mg)

was stored at −20°C until it was used for measurement of algicidal activity.

The algicidal activity of the EtOAc extract of strain R219 was determined against the growth of *M. aeruginosa* at the exponential phase (1×10^7 cells ml^{-1}) using different R219 concentrations as of 0, 5, 10, 20 and 40 μg ml^{-1}. Meanwhile, 40 μg ml^{-1} of EtOAc extract of LB medium was used as additional negative control. This experiment was conducted in a series of 2 ml-tubes containing 990 μl of the alga medium with given cell density. Ten microliter of the acetone–water (3:7 v:v) dissolved EtOAc extract was added into each tube to get the given concentrations.

Similar experiment was carried out to examine the algicidal effect of the EtOAc extract at the concentration of 5 and 10 μg ml^{-1} on the growth of *M. aeruginosa* at the mid-exponential phase at initial densities of 5×10^6, 1×10^7, and 2×10^7 cells ml^{-1}, respectively.

In addition, the thermal stability of the bioactive chemicals in the EtOAc extract of strain R219 was tested. Several 2-ml tubes containing 100 μl of the EtOAc extract at the concentration of 10 μg ml^{-1} were incubated at 25, 45, 65, 85, and 100°C, respectively in water-bath for 30 min. Then, 10 μl of each sample was added into 2-ml tubes containing 990 μl of exponential phase of *M. aeruginosa* (5×10^6 cells ml^{-1}) and maintained in light chamber for 24 h to estimate the algicidal activity using the above method. All experiments were repeated in triplicate.

Control efficiency of the filtrate of strain R219 on the bloom-forming algal species obtained from Lake Tai

The bloom-forming water samples which contain 98% of the *M. aeruginosa* species based on the light microscopic observation were collected from the Lake Tai in June 2008. A series of 50-ml flasks containing 17 ml of the water samples was inoculated with 0.5, 1.0, 2.0, and 3.0 ml of the filtrate of strain R219 (prepared as above). A suitable amount of water was added to make up to a final volume of 20 ml in each flask, while the concentration of the filtrate was 25, 50, 100, and 150 μl ml^{-1}, respectively. The algicidal effects of the filtrate were measured by determining the reduction of chlorophyll-*a* content of the cyanobacteria when they were maintained in growth chamber for 4 days. Chlorophyll-*a* was extracted from the water samples with 95% acetone and measured on the UV-2100 spectrophotometer at $\lambda = 647$ and 665 nm, respectively, and calculated with the equation: $12.63 \times A_{665} - 2.52 \times A_{647}$ (Inskeep and Bloom 1985).

Analysis of algicidal activity

Algal cells were counted after 48 h. The algicidal activity of the filtrate of strain R219 was estimated by the flowing equation (Kim et al. 2007): Algicidal activity (%) = $(1 - T_t/C_0) \times 100$, where C_0 and T_t are the cell numbers (or chlorophyll-*a* content) of *M. aeruginosa* before and after being treated with the filtrate or crude extract of strain for 48 h (or 96 h) (t). The cell numbers of *M. aeruginosa* were calculated with the aid of a hemocytometer using bright-field microscopy. All experiments were repeated in triplicates and data are shown as mean values and standard deviations. Results showed in the figures were obtained from three replicates or independent experiments and One-way ANOVA program was used for mean variance analysis at 95% level with the GraphPad Prism software (version 3.02).

Assessment of acute oral toxicity

Five groups of ten mice each were used in the experiments. The culture broth (containing 10^9 CFU ml^{-1} of the live cells of strain R219) and the filtrate of strain R219 prepared as above, in doses of 0.5 and 2.0 g kg^{-1} body weight, respectively were administered orally after at least 5 days adaptation, using intragastric tubes, to the animals as a single dose. The control group was given an equal volume of saline. Careful observations of animals, including body weight, mortality, and clinical abnormality, were performed for 14 days. A pathologist carried out postmortem examinations of the viscera (liver, heart, spleen and kidney) and the serum biochemical indexes including glutamic oxalacetic transaminase (GOT), uric acid (UA), creatinine (Cr) of the mice. Data that were obtained from the culture broth or filtrate treated and the saline treated mice were represented as of mean value and standard deviation, and analyzed statistically by *T*-test at 95% level with the GraphPad Prism software (version 3.02).

Results

Isolation and identification of algicidal bacteria

In order to search for algicidal bacteria which could be used to control the cyanobacteria, a total of 23 isolates forming different colonies were obtained from the water bloom that occurred in Lake Tai in China. Three of the 23 strains showed high efficiency against *M. aeruginosa* in the plate assay and, amongst these, strain R219 showed the strongest algicidal activity when 20 μl of the filtrate was added to the sterilized paper disk and placed on the medium surface where *M. aeruginosa* culture was incubated and grown in chambers for 2 days (Fig. 1). The strain R219 is gram-negative, rod-shaped with polar flagella, aerobic, non-spore forming, positive catalase test, and producing blue-green pigments in LB medium after shaking at 37°C,

Fig. 1 Algicidal activity of the filtrate of strain R219. *A* 20 μl of the filtrate, *B* 20 μl of the LB medium

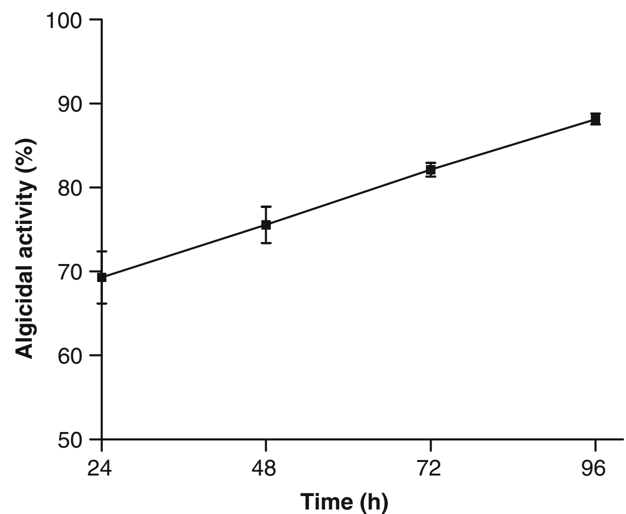

Fig. 2 Algicidal activities of the culture filtrate of strain R219 against *M. aeruginosa* correlated significantly with the incubation times from 24 to 96 h. Mean values from at least three independent assays are plotted ±standard deviation. The initial cell density of *M. aeruginosa* was 1×10^7 cells ml^{-1} at the mid-exponential phase

100 rpm for 4 days. These characteristics indicated that the strain R219 might be *Pseaudomonas aeruginosa* (Ryan and Ray 2004). The partial sequence analysis (705 bp) of 16S rDNA also demonstrated that the strain R219 (Accession No. FJ472952) was most likely to be *P. aeruginosa* and had a 99.8% similarity to *P. aeruginosa* strain TL12 (Accession No. FJ361190.1), ZFJ-1 (Accession No. EU931548.1), D2 (Accession No. EU915713.1), MCCB 102 (Accession No. EF062514.2), BWB21 (Accession No. EU826026.1), NBRAJG92 (Accession No. EU661708.2), PS1 (Accession No. EU741797.1), pke117 (Accession No. EU515133.1), and PBCC1 (Accession No. EU391426.1) retrieved from GenBank. Consequently, the strain R219 was identified as *P. aeruginosa.* We designated it as *P. aeruginosa* R219, and deposited it under this name in the China General Microbiological Culture Collection Center (CGMCC, No2754).

Correlation between the sampling time of the filtrates and their algicidal activity

The algicidal activity of the filtrates against the test alga was strongly correlated with the fermentation time of the R219 strain, from 24 to 96 h (Fig. 2). The correlation coefficient R^2 was 0.9998 ($P < 0.0001$). The filtrate prepared at 96 h after inoculation showed the highest algicidal effect inhibiting the growth/killing almost 90% on the cyanobacterium of *M. aeruginosa.*

Algicidal effect of the EtOAc extract at different concentrations

The algicidal activity of the EtOAc extract from strain R219 was generally dose dependent across the tested concentrations from 5.0 to 40.0 μg ml^{-1}, with the most efficient dose/effect ratio at 10 μg ml^{-1} and the strongest algicidal effect at the maximum concentration, 40 μg ml^{-1} (Fig. 3). In addition, the algicidal activity of the EtOAc extract was significantly different at the tested concentrations ($P < 0.05$), except the activity was about 71.3 and

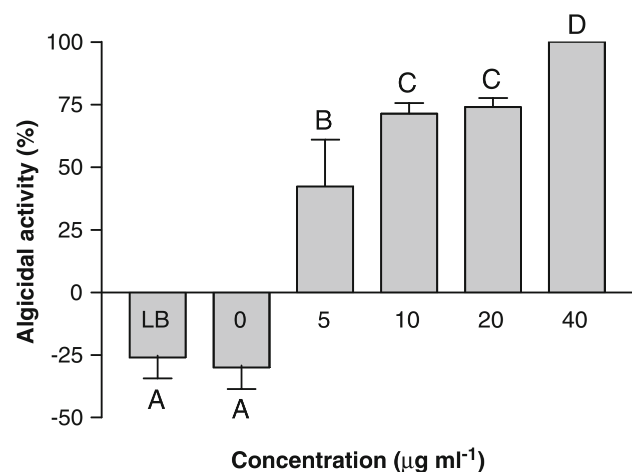

Fig. 3 Algicidal activities of the EtOAc extract of strain R219 against *M. aeruginosa* at different concentrations. LB represents 40 μg ml^{-1} of the EtOAc extract of LB medium that was used as an additional negative control. The *plotted data* are the mean values of at least three independent assays ±standard deviation. The initial cell density of *M. aeruginosa* is 1×10^7 cells ml^{-1} at the mid-exponential phase. *A*, *B*, *C* and *D* represent the significant difference of the algicidal activity of the EtOAc extract at the given concentrations, as revealed by one-way analysis and the Duncan's multiple comparison at 0.05 level

74.0% at the concentrations of 10 and 20 μg ml^{-1}, respectively, which was not significantly different ($P > 0.05$), as revealed by one-way analysis and the Duncan's comparison. Moreover, the algicidal activities of the negative controls treated with the same volume of acetone–water (3:7 v:v) as well as the EtOAc extract of LB medium (40 μg ml^{-1}) were −30 and −26%, respectively, which were not significantly different ($P > 0.05$). The result indicated that the algicidal substance in the filtrate of strain R219 was produced by the bacterium, which could be extracted with the organic solvent of EtOAc. On the contrary, the EtOAc extract of LB medium did not have any algicidal activity against the test algae.

Influence of algal cell densities on the algicidal activity of the EtOAc extract of strain R219

The effect of algal cell density on algicidal activity of strain R219 is shown in Fig. 4. The algicidal action against *M. aeruginosa* on the initial cell densities from 5×10^6 to 2×10^7 cells ml^{-1} was not significantly different ($P > 0.05$) at the given concentrations of EtOAc extract. However, the algicidal activity was increased about twofold at each cell density when the concentration of the extract was increased from 5 to 10 μg ml^{-1}.

Moreover, the algicidal substance in the EtOAc extract of strain R219 was quite thermally stable. After incubated at 25, 45, 65, 85, and 100°C for 30 min, respectively, the EtOAc extract (at concentration of 10 μg ml^{-1}) had 49, 56, 56, 64 and 58% remaining algicidal activities, which were not significantly different from each other ($P > 0.05$).

Algicidal effect of the filtrate of strain R219 on the mixture of bloom-forming species of cyanobacteria at Lake Tai

Based on the reduction of the chlorophyll-*a*, the culture filtrate of strain R219 showed great algicidal activity against the mixed bloom-forming species of cyanobacteria that were collected from Lake Tai. Even when the filtrate was at the lowest tested concentration as of 25 μl ml^{-1}, the algicidal activity was as high as 69.8%. Moreover, the highest activity was found between 82.4 and 89.0% without significant difference ($P > 0.05$) when the concentration of the R219 filtrate was higher than 50 μl ml^{-1} (Fig. 5).

Oral acute toxicity assessment for the culture broth and filtrate of strain R219

Within 14 days of administration of either culture broth or filtrate, all the mice that received 0.5 or 2.0 g kg^{-1} of the broth or filtrate were alive without any immediate behavioral changes. The mice move and fed normally. The body weight, viscera index (heart, liver, kidney and spleen) as well as serum GOT, UA and Creatinine (Cr) in the serum of the mice treated with the culture broth or filtrate did not show significant difference ($P > 0.05$) from that of the saline treated control (Table 1). Moreover, no obviously pathological changes had been found by histological observation of the mice at the end of experiment.

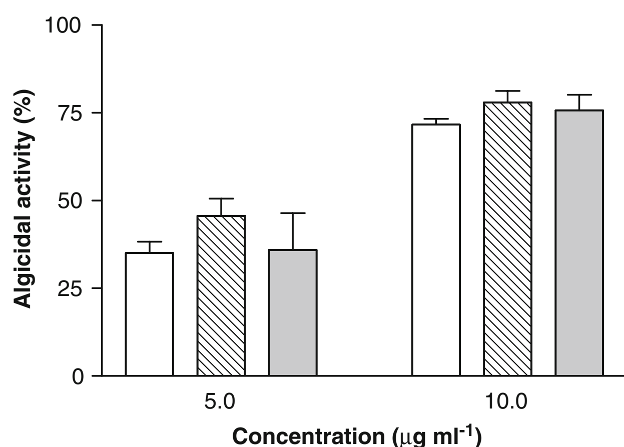

Fig. 4 Algicidal effect of the EtOAc extract of strain R219 on three cell densities of *M. aeruginosa* at concentrations of 5 and 10 μg ml^{-1}, respectively. Clear, slash and grey bars represent that the algal cell densities at 5×10^6, 1×10^7 and 2×10^7 cells ml^{-1}, respectively. *Data* are representative for the mean values of three independent assays ±standard deviation

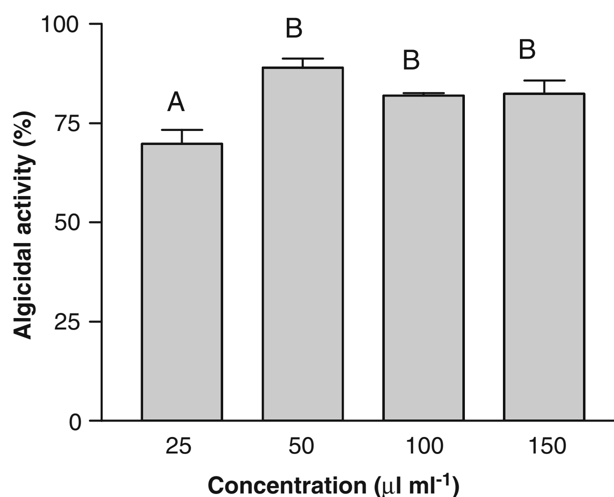

Fig. 5 Algicidal effect of the filtrate of strain R219 on bloom-forming algae at Lake Tai. The plotted data are the mean values of at least three independent assays ±standard deviation. *A* and *B* represent the significant difference of the algicidal activity of the filtrate at the given concentrations, as revealed by one-way analysis and the Duncan's multiple comparison at 0.05 level

Table 1 Assessment of the oral cute toxic effect of strain R219 culture broth and filtrate in mice

Samples	Dose (mg kg^{-1})	Weight (g)	Serum			Viscera index (×100)			
			UA (μmol l^{-1})	GOT (U l^{-1})	Cr (μmol l^{-1})	Heart	Liver	Kidney	Spleen
Saline	0	26.96 ± 0.62	195.4 ± 19.6	183.4 ± 15.5	17.3 ± 0.8	0.46 ± 0.02	5.53 ± 0.29	1.19 ± 0.06	0.70 ± 0.09
Broth	500	25.18 ± 1.55	137.3 ± 18.2			0.47 ± 0.012	5.38 ± 0.16	1.16 ± 0.06	0.70 ± 0.07
	2000	24.91 ± 1.18	116.8 ± 20.1	189.6 ± 18.4	15.2 ± 0.5	0.50 ± 0.02	4.96 ± 0.15	1.30 ± 0.04	0.55 ± 0.05
Filtrate	500	26.71 ± 0.87	153.9 ± 7.8			0.54 ± 0.03	4.92 ± 0.17	1.39 ± 0.05	0.49 ± 0.04
	2000	25.79 ± 1.41	175.3 ± 23.0	155.0 ± 12.0	17.3 ± 0.8	0.49 ± 0.013	5.15 ± 0.11	1.39 ± 0.06	0.53 ± 0.04

Discussion

Because the toxic cyanobacteria exercise great effect on human health or/and wildlife sustainability, many scientists have spent much attentions on isolation and identification of algicidal bacteria that could be used as a potential biocontrol agent for controlling the unicellular algal species, in particular, since the explosion of water-bloom happened in 2007 in the Lake Tai in China (Guan et al. 2008; Li et al. 2007; Wang et al. 2008a). In fact, many strains of bacteria with algal growth inhibition or cells lysis potential have been isolated already for controlling freshwater or marine bloom-forming algae (Choi et al. 2005; Kang et al. 2007, 2008; Mu et al. 2007; Roth et al. 2008). In order to provide more alternative bioagents that could help in the control of water bloom in the freshwater lake in China, we isolated and identified one strain (R219) of *M. aeruginosa*-lysing bacterium in this study, and its algicidal activity was evaluated in our lab.

Recently, many algicidal bacteria such as *Myxobacteria*, *Flavobacterium*, *Cytophaga*, *Sphingomonas*, *Bacillus, Achromobacter*, *Arthrobacter, Pseudoalteromonas, Vibrio* and *Pseudomonas* have been reported (Cui et al. 2005; Su et al. 2007; Kang et al. 2008; Kim et al. 2007; Mu et al. 2007). Generally, algicidal bacteria inhibit algal growth or kill the algal cells through direct or indirect actions. Most of the algicidal bacteria exert their growth inhibition against or lysis of the algae by secreting extracellular compounds that are toxic to the algal cells. These compounds can be proteins (Lee et al. 2000; Mitsutani et al. 2001), peptides (Banin et al. 2001; Imamura et al. 2001; Yamamoto et al. 1998), amino acids (Yoshikawa et al. 2000), antibiotics (Dakhama et al. 1993), biosurfactants (Gustafsson et al. 2009; Wang et al. 2005), bacillamide (Jeong et al. 2003), hydroxylamine (Paul et al. 1979), lipid peroxidation (Wang et al. 2008b) and others. Only a few algicidal bacteria inhibit the growth of algae through direct contact with or penetration into the algal cells (Shi et al. 2006).

Several bacteria species which belong to the genera of *Pseudomonas* showed strong algicidal activities. Among the examples of *Pseudomonas* species with algicidal action are *Pseudomonas* sp. T827/2B (Baker and Herson 1978), *P. fluorescens* (Jung et al. 2008; Kim et al. 2007) and *P. aeruginosa* (Dakhama et al. 1993; Gong et al. 2004; Takenaka and Watanabe 1997). In particular, *P. aeruginosa* has been shown to produce effective algicidal substances such as 1-hydroxyphenazine, oxychlororaphine and biosurfactant against harmful algal blooms in eutrophic lake environments as well as red tide in marine (Dakhama et al. 1993; Gustafsson et al. 2009; Kim et al. 2007; Wang et al. 2005). One strain of *P. aeruginosa* isolated from the surface water has been reported to degrade the toxic compound of microcystin LR of *M. aeruginosa* in vitro test (Takenaka and Watanabe 1997). However, these compounds could not be detected in the EtOAc extract of strain R219 by LC/MS analysis (data not shown), which indicates that the algicidal chemicals produced by strain R219 might be novel or unreported ones.

Our results show that the algicidal activity of strain R219 EtOAc extract is much higher than that of the earlier reported results for *P. aeruginosa* O-2-2(Gong et al. 2004). The ecologically friendly bioagents such as naturally occurring in the same habitat bacteria have shown the greatest promise as potential algal bloom suppressors in freshwater and marine ecosystems (Kang et al. 2008; Mu et al. 2007). However, given full environment and ecosystem consideration, it is necessary to access the effect of such biologically active agents on other algae, protozoa, fish that are integral parts of a sustainable ecosystem. Particularly important is the evaluation of the effect on human health of the bacterial algicides and the compounds they or their targets are releasing. For example, it is well-known that some cyanobacteria can synthesize toxic compounds such as microcystins that will be released into the water after the cell death (Liu et al. 1998), therefore, the algicidal agents that will be used to control algae outbreak should consider the effect by the released toxic constituents. However, it has been documented that *P. aeruginosa* have both algae-lytic activity and microcystin degradation ability (Takenaka and Watanabe 1997; Zhao et al. 2005). This dual action makes *P. aeruginosa* extremely useful for biocontrol of cyanobacteria-forming blooms.

Although there are several studies regarding the algicidal action of *P. aeruginosa*, a few of them carried out real field trial as well as assessment for acute toxicity. In fact, *P. aeruginosa* is a common bacterium which can cause disease in animals and humans. As an opportunistic human pathogen, *P. aeruginosa* can cause chronic opportunistic infections that have become increasingly apparent in immunocompromised patients and the ageing population of industrialized societies (Cornelis 2008). However, based on the assessment of acute toxicity of the culture broth or the filtrate of *P. aeruginosa* R219 in mice, the cells of strain R219 or its metabolites did not show significant side effects on the mice when they were administered orally at the dose of 0.5 g kg^{-1} even higher at 2.0 g kg^{-1} body weight. In addition, the culture filtrate of straom R219 showed about 90% of algicidal activity at concentration of 50 μl ml^{-1} against the mixed species of bloom-forming cyanobacteria collected from Lake Tai. These results indicate that the strain R219 could be used at a low concentration as a safe biocontrol agent in control of the outbreaks of harmful algal blooms in the field. Meanwhile, the algicidal substances of the *P. aeruginosa* R219 should be further assessed in order for a better understanding of their algicidal mechanism and effect on other species in the ecosystem.

Acknowledgments This work was supported by the National Basic Research Program of China (2008CB418004) and the Special Program for Taihu Lake of Jiangsu Natural Science Foundation (BK2007739), for which the authors are thankful. Special thanks for Professor Lubomir Sokolov who made a lot of valuable comments during the manuscript preparation.

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