*Desalination and Water Treatment*
www.deswater.com
47 (2012) 205–210
September


doi: 10.1080/19443994.2012.696805

Taylor & Francis
Taylor & Francis Group

# Removal of cyanobacteria by an *Aeromonas* sp.

Yu-Mei Liu[a], Meng-Hui Wang[a], Rui-Bao Jia[b], Li Li[a,*]

[a]*Shandong Provincial Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, No. 27 Shanda Nan Road, Jinan, Shandong 250100, China*
*Fax: +86 531 88364513; email: lili@sdu.edu.cn*
[b]*Jinan Water and Waste Water Monitoring Center, Jinan, China*

Received 30 November 2011; Accepted 6 March 2012

ABSTRACT

In the present study, the biocontrol of harmful algae by an algicidal bacterium *Aeromonas* sp. strain FM was examined. The threshold concentration of FM for its algicidal activity against the exponentially growing *Microcystis aeruginosa* was $2.1 \times 10^8$ cfu mL$^{-1}$. The influence of temperature on algae removal was tested, and the result indicated that the summer temperature 30°C favored the algicidal activity of the bacterium strain when compared to 20°C, the representative temperature of spring or autumn. A strong algicidal effect of strain FM was observed against *M. aeruginosa*, *M. flos-aquae*, *Anabaena cylindrica*, *A. flos-aquae*, and *Nodularia spumigena*. Based on these tests, the removal of *M. aeruginosa* treated by *Aeromonas* sp. strain FM harboring in Aquamats was carried out. After 7 d treatment, *M. aeruginosa* at $7.5 \times 10^5$ cells mL$^{-1}$ with a total volume of 10 L decreased 85% of its biomass, following with a decay of the inoculated algicidal strain FM. The electron microscope images demonstrated that the strain severely damaged the cell wall of *M. aeruginosa*. The results of the present study indicated the application potential of the biocontrol of harmful algae by algicidal bacteria.

*Keywords:* Biocontrol; Harmful algae; Cyanobacteria; Algicidal bacteria; *Aeromonas*

## 1. Introduction

Algal blooms occur widespread in eutrophic water bodies, which intensely affect aquatic ecosystems, resulting in a light-level reduction, water deoxygenation, odor production, and water-quality deterioration. Many species of cyanobacteria produce cyanotoxins [1–4], and as a result, such blooms create serious threats to animal and human health. In particular, *Microcystis aeruginosa* is one of the most common freshwater bloom-forming species of cyanobacteria. It attracts the concern for its ability to produce microcystins and an odour, which significantly impair the water quality.

A variety of approaches have been performed to control the harmful algal blooms, among which, much attention has been devoted to the biological control method owing to its ecological benefits in nature. As reported, microorganisms, such as algal viruses [5–7], algicidal bacteria [8–12], protozoan grazers [13,14], and eukaryotic parasites including fungi [15] and certain flagellates [16,17], all have the potential to exert controlling effects on the species of blooming cyanobacteria. Biological controls using bacteria against harmful algal blooms are of particular interest in

*Corresponding author.

freshwater and marine systems. Up to the present time, many algicidal bacteria have been isolated to control algae-blooming [18–21], and most of the studies on freshwater have targeted the toxin-synthesis cyanobacteria [22–26].

In the present study, the removal of cyanobacteria by an *Aeromonas* sp. harboring in Aquamats was studied. The influence of algicidal bacteria density, temperature, and algal species on algal removal was tested, and the changes of algal cells during treatment were confirmed by an electron microscope observation. The purpose of the present study was to explore the biocontrol of harmful algae by an algicidal bacterium.

## 2. Materials and methods

### 2.1. Cyanobacteria strains and algicidal bacterium

The axenic strains of cyanobacteria used in this study were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB-Collection; Wuhan, China), including *M. aeruginosa* FACHB 927, *M. aeruginosa* FACHB 975, *M. flos-aquae* FACHB 1,028, *Anabaena cylindrica* FACHB 170, *A. cylindrica* FACHB 243, *A. flos-aquae* FACHB 245, and *Nodularia spumigena* FACHB 377. The algicidal bacterium strain FM used in the present study was isolated from a eutrophic reservoir (Jinan, China), which was obtained by screening on the algal plate, as described by Sakata [27]. The algicidal bacterium was identified as *Aeromonas* sp. based on its 16S rRNA gene sequence available under the GenBank Accession No HM560619.

### 2.2. The algae removal tests for the effects of bacteria density, temperature, and algae species

To test algae removal with a bacterial strain FM, a colony of strain FM was inoculated into a nutrient broth and cultured for 20 h at 30°C, shaking at 150 rpm reciprocally and then spreading onto the Nutrient Agar plates to form lawns for 2 d. The cells of lawns were collected and suspended with sterilized BG11 medium and mixed with cyanobacteria of exponential growth in 250 mL flasks. The mixed systems were incubated at 30°C under 20 μmol (photons) $m^{-2} s^{-1}$ in a 12:12 light–dark cycle.

The cell concentration of cyanobacteria was monitored by direct count under a light microscope. The algicidal activity of strain FM was evaluated by the following equation:

$$\text{Algicidal activity } (\%) = (1 - T_t/C_t) \times 100,$$

where $T$ (treatment) and $C$ (control) are the cell densities of cyanobacteria with and without inoculation of strain FM, respectively, and $t$ is the inoculation time. All the tests were carried out in triplicate and were followed with FM-uninoculated controls.

### 2.3. Algicidal tests with strain FM harboring in Aquamats

Algicidal tests against *M. aeruginosa* were conducted in 12 L tanks ($20 \times 20 \times 30$ cm) at a natural temperature (about 18–22°C) with a working volume of 10 L. The bacterium strain FM was cultured in a nutrient broth to its early stationary phase (about 36 h) and incubated with Aquamats (Meridian Applied Technology Systems, Calverton, MD, USA) for 24 h to facilitate strain colonization. The Aquamats ($20 \times 30$ cm) with colonized FM was wrapped with nonwoven (polyester material, mean pore size 40 μm) and was placed vertically into a glass tank. The experimental trials were divided into three groups. Each group was performed in duplicate. Group A was the Aquamats-nonwoven without colonized FM submerged into the *M. aeruginosa* culture in BG11 medium, serving as one of the controls. Group B was FM colonized and nonwoven wrapped Aquamats submerged into a *M. aeruginosa* culture in a BG11 medium, serving as the treatment group. Group C was the *M. aeruginosa* culture in a BG11 medium without inoculation of strain FM and served as the second control (Fig. 1). Artificial light (10 μmol (photons) $m^{-2} s^{-1}$) was supplied to compensate for insufficient room light with a 12:12 light-dark cycle. The initial algal concentration was about $7.5 \times 10^5$ cells $mL^{-1}$. The samples from each tank were monitored for biomass fluctuation of *M. aeruginosa* by a direct count, and the biomass of freely suspended FM was detected by a plate count.

### 2.4. Electron microscopy observation

The cells of *M. aeruginosa* treated by strain FM were observed by a scanning electron microscope (SEM). The samples for SEM the observation were prepared as follows: the samples were washed twice with 0.1 M PBS buffer (pH 7.2) and fixed in 2.5% glutaraldehyde at 4°C for 24 h, and post-fixed in 1% osmium tetroxide at 4°C for 60 min, followed by rinsing with 0.1 M PBS buffer (pH 7.2) after fixation. The specimens were then dehydrated by an acetonitrile series (50, 75, 95, and 100%) and dried with Critical-Point Dryer (HCP-2, Hitachi Electronic Instruments, Tokyo, Japan). Finally, the specimens were sputter-


Fig. 1. The reactor used in the algicidal tests with the strain FM harboring in Aquamats.

coated with platinum and examined under an H-8010 SEM (Hitachi, Japan).

## 3. Results

### 3.1. The effect of algicidal bacteria density on algal removal

To determine the effective alga-lysis threshold concentration of FM against *M. aeruginosa* FACHB 927, the alga culture of about $4.6 \times 10^6$ cells $mL^{-1}$ in an early exponential phase was inoculated with FM at concentrations ranging from $7 \times 10^7$ to $2.1 \times 10^9$ cfu $mL^{-1}$. Significant inhibition of the algal growth took place when more than $2.1 \times 10^8$ cfu $mL^{-1}$ FM was inoculated. When the FM was inoculated at a concentration of $2.1 \times 10^9$ cfu $mL^{-1}$, the algal cell was completely lysed after 4 d (Fig. 2). While the FM inoculated at a concentration below $7 \times 10^7$ cfu $mL^{-1}$ did not inhibit the growth of *M. aeruginosa* significantly. *M. aeruginosa* with increasing concentration could also be lysed effectively with an extension of time. More than 85% of $1.4 \times 10^7$ cells $mL^{-1}$ *M. aeruginosa* was lysed by FM at a concentration of $2.1 \times 10^9$ cfu $mL^{-1}$ within 4 d (Fig. 3).

### 3.2. The effect of temperature on algal removal

To test the effect of temperature on the algae removal by algicidal bacteria, algicidal tests to *M. aeruginosa* by strain FM were carried out at 20 and 30°C, respectively, the former of which represents the temperatures of spring and autumn, and the latter of which represents that of summer. As shown in Fig. 4, the cells of *M. aeruginosa* were completely removed during the 5-d treatment by the strain FM at 30°C, and the removal rate was relatively slow for the treatment by FM at 20°C. The result indicated that a high temperature favored the algicidal activity of bacteria.

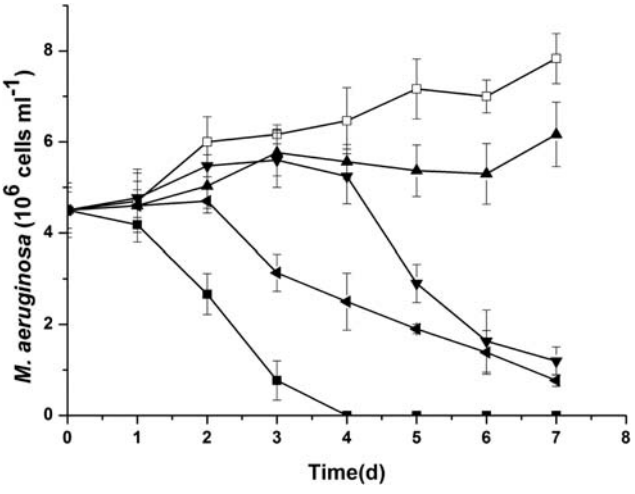


Fig. 2. Growth of *Microcystis aeruginosa* at initial concentrations of $4.6 \times 10^6$ cells $mL^{-1}$ when inoculated FM at the concentration of 0 (□), $7 \times 10^7$(▲), $2.1 \times 10^8$ (▼), $7 \times 10^8$ (◀), and $2.1 \times 10^9$ (■) cfu $mL^{-1}$. Data are means ± standard deviation from at least three independent assays.

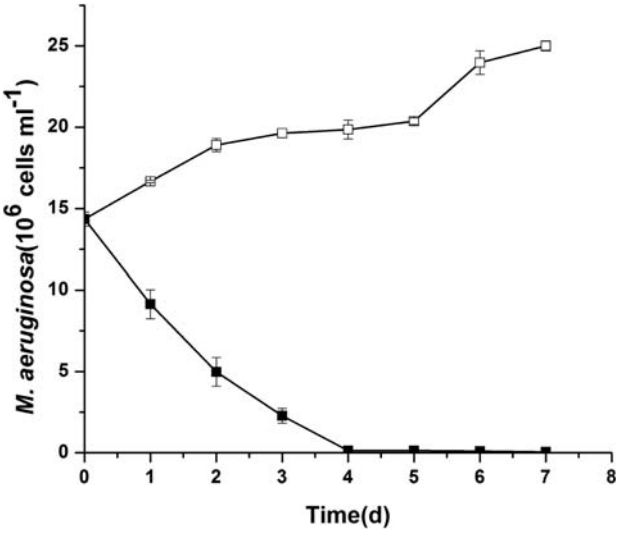


Fig. 3. The growth of *Microcystis aeruginosa* at an initial concentration of $1.4 \times 10^7$ cells $mL^{-1}$. (□) represents the density of *M. aeruginosa* in untreatment control; (■) represents the density of *M. aeruginosa* treated with *Aeromonas* sp. strain FM. Data are means ± standard deviation from at least three independent assays.

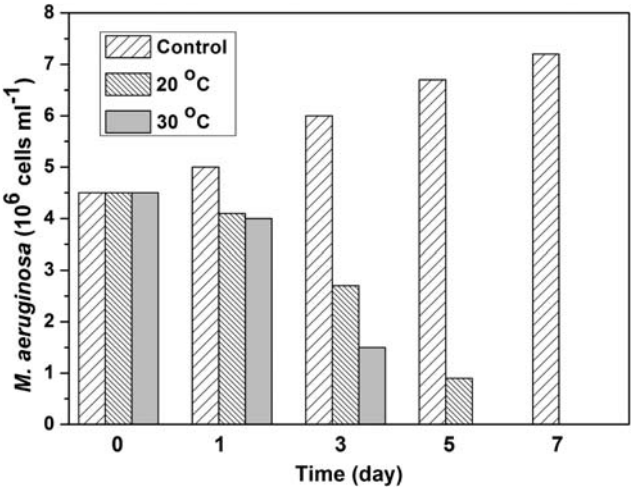


Fig. 4. The effect of temperature on the algicidal activity.

### 3.3. Cyanobactericidal range of strain FM

To test whether the antialgal activity of strain FM was specific to *M. aeruginosa* FACHB 927, the antialgal activity of strain FM against other cyanobacterial strains was tested. As shown in Table 1, after co-culture for 7 d, the growth of all the tested cyanobacteria strains was significantly inhibited.

Table 1
Cyanobactericidal activity of strain FM on different cyanobacteria

| Tested cyanobacteria | Final cell density of FM-uninoculated control ($\times 10^6$ cells mL$^{-1}$) | Algicidal activity after FM treatment for 6d (%) |
|---|---|---|
| *A. flos-aquae* FACHB 245 | 3.13 | 100 |
| *A. cylindrica* FACHB 170 | 3.65 | 100 |
| *N. spumigena* FACHB 377 | 0.58 | 87.9 |
| *A. cylindrica* FACHB 243 | 2.53 | 100 |
| *M. flos-aquae* FACHB 1,028 | 4.23 | 100 |
| *M. aeruginosa* FACHB 975 | 5.88 | 91.2 |

### 3.4. The removal of M. aeruginosa by the strain FM harboring in Aquamats

To explore the field application of algicidal bacteria, artificial substrate Aquamats was used for bacterium harboring, and algicidal tests against *M. aeruginosa* with strain FM harboring in Aquamats were performed. As shown in Fig. 5, the biomass of *M. aeruginosa* with a total volume of 10 L and the concentration of $7.5 \times 10^5$ cells mL$^{-1}$ decreased about 85% after 7 d treatment. During the treatment, the FM cells harboring in Aquamats gradually released to be freely suspended cells and attained the highest concentration of about $6.6 \times 10^6$ cfu mL$^{-1}$ in the tank at the second day (Fig. 6). Then as expected, freely suspended FM cells gradually decreased to be $<10^4$ cfu mL$^{-1}$ with the decrease of algal biomass. Two kinds of controls designed as *M. aeruginosa* cultured in BG11 medium or with uninoculated Aquamats-nonwoven showed an increase in *M. aeruginosa* density, indicating that the algicidal effect was exerted by strain FM and unrelated to the harboring substrate Aquamats.

### 3.5. Morphological changes in M. aeruginosa cells during treatment

The cells of *M. aeruginosa* varied morphologically during the treatment with FM were observed by SEM. Typical scanning electron micrographs are shown in Fig. 7, Fig. 7(A) shows that the untreated cells were smooth and regular, and Fig. 7(B) shows that cells of

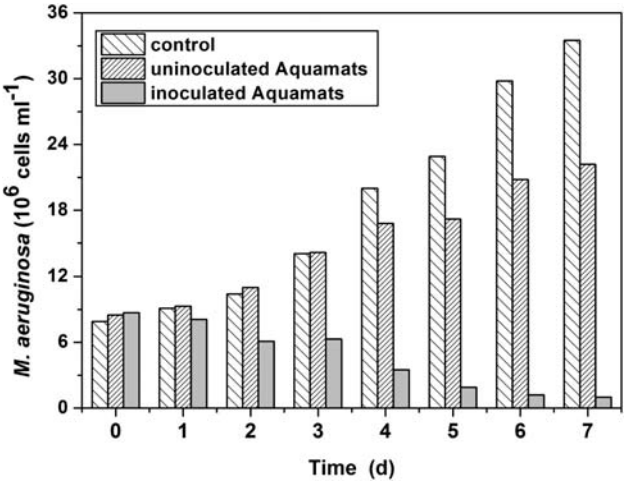


Fig. 5. Algicidal tests against *Microcystis aeruginosa* with strain FM harboring in Aquamats. Bars represent the density of *M. aeruginosa* in an untreatment control, density of *M. aeruginosa* with uninoculated Aquamats, and density of *M. aeruginosa* with inoculated Aquamats, respectively.

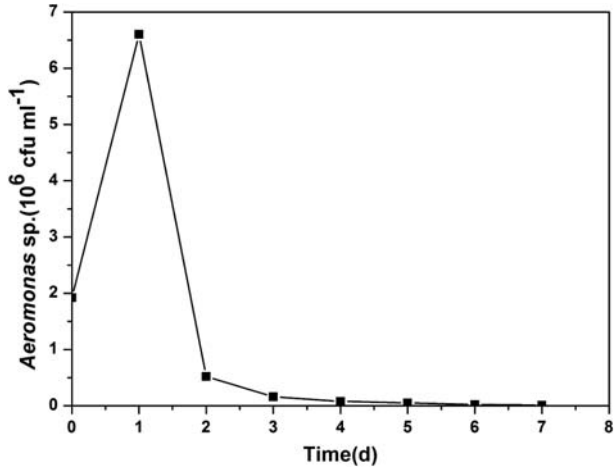


Fig. 6. The biomass of suspended *Aeromonas* sp. strain FM (■) in the reactor during algicidal tests against *M. aeruginosa*.

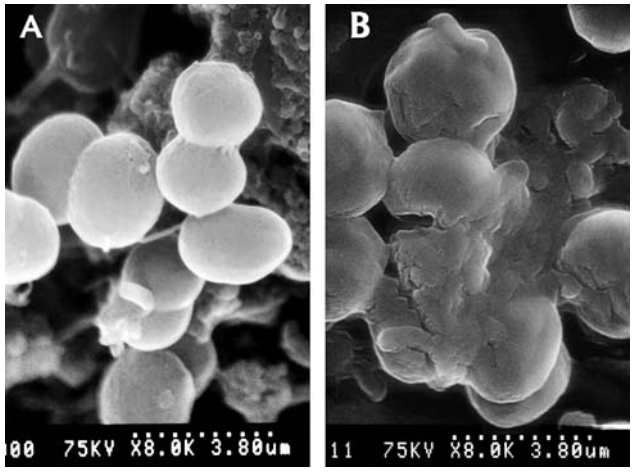


Fig. 7. Scanning electron micrographs of *Microcystis aeruginosa* treated with *Aeromonas* sp. strain FM. The untreated cells of *M. aeruginosa* control are shown in A and treated cells within 96 h are shown in B.

*M. aeruginosa* under treatment presented irregular outlines, deflated surface, and distinct holes, and were wrapped and stuck together.

## 4. Discussion

The biocontrol of harmful algae is a promising approach in aquatic environment, especially, using algicidal bacteria. Up to the present time, the algae removal by some algicidal bacterial strains has been reported, including *Xanthobacter autotrophicus* [26], *Pseudomonas fluorescens* [28], *Pseudoalteromonas* [18,19], *Sinorhizobium kostiense* [29], *Acidovorax delafieldii* [20], *Bacillus*, *Dietzia*, *Janibacter*, and *Micrococcus* [21]. As we have known, the population dynamics of algicidal bacteria have a close relationship with the phytoplankton [30]. For an application purpose, it is important to test the threshold cell concentration of an algicidal strain for its effect on algal-lysis. In the present study, the isolated algicidal bacterium *Aeromonas* sp. FM had a threshold density at $2.1 \times 10^8$ cfu mL$^{-1}$ to inhibit *M. aeruginosa*, which was at an exponential phase and about $4.6 \times 10^6$ cells mL$^{-1}$ initially. The algicidal effect increased with an increase in the FM concentration. The threshold concentration of SM02 for maximal algicidal activity against a natural bloom of *M. aeruginosa* ($1.5 \times 10^6$ cells mL$^{-1}$) was $10^7$ cfu mL$^{-1}$ [27], and very similarly, *Stephanodiscus*-killing bacteria, such as HYY0510-SK04, HYY0511-SK09, and HYK0512-SK12, showed algicidal activity with bacteria at a final concentration higher than the $10^7$ cells mL$^{-1}$ [20]. Considering the difference in algal strains and their concentrations, the algicidal activity of these bacteria was comparable. In these tests, the growth condition was optimal to the algal population, but not to the algicidal heterotrophic bacteria, which where close to the natural condition. In such a case, the algicidal bacteria did not benefit from the decay of algae. This phenomenon was previously proved by the coculture of SM02 and *M. aeruginosa* [27]. In the present study, the algicidal tests in 12 L tanks showed the decline of algicidal bacteria coupled with the decay of *M. aeruginosa*. The result suggested the fates of algicidal bacteria when they were served as biological agents to control the *M. aeruginosa* blooms in the field. It should be noted that the water quality would worsen owing to massive cells' decay. In contrast, when *A. denitrificans* were inoculated at low densities ($10^3$ cells mL$^{-1}$) together with *Microcystis* species, the bacterium proliferated to $10^8$ cells mL$^{-1}$ and caused algal cell lysis [22], implying that the algicidal bacterium proliferation took the place of algae bloom during the period of treatment. Thus, how to use all kinds of algicidal bacteria as biological agents to control the algal blooms in the field remains to be speculated.

## Acknowledgments

This research was supported by National Major Projects on Control and Management of Water Body Pollution (Grant No. 2008ZX07422-002).

## References

[1] V.M. Vasconcelos, E. Pereira, Cyanobacteria diversity and toxicity in a wastewater treatment plant (portugal), Water Res. 35 (2001) 1354–1357.

[2] P.P. Shen, Q. Shi, Z.C. Hua, F.X. Kong, Z.G. Wang, S.X. Zhuang, D.C. Chen, Analysis of microcystins in cyanobacteria blooms and surface water samples from Meiliang Bay, Lake Taihu China. Environ. Int. 29 (2003) 641–647.
[3] Y.H. Kang, J.D. Kim, B.H. Kim, D.S. Kong, M.S. Han, Isolation and characterization of a bio-agent antagonistic to diatom, *Stephanodiscus hantzschii*, J. Appl. Microbiol. 98 (2005) 1030–1038.
[4] E.M. Lehman, Seasonal occurrence and toxicity of *Microcystis* in impoundments of the Huron River, Michigan, USA, Water Res. 41 (2007) 795–802.
[5] R.T. Garry, P. Hearing, E.M. Cosper, Characterization of a lytic virus infectious to the bloom-forming microalga *Aureococcus ano-phagefferens* (Pelagophyceae), J. Phycol. 34 (1998) 616–621.
[6] C.P.D. Brussaard, R. Thyrhaug, D. Marie, G. Bratbak, Flow cytometric analyses of viral infection in two marine phytoplankton species, *Micromonas pusilla* (Prasinophyceae) and *Phaeocystis pouchetii* (Prymnesiophyceae), J. Phycol. 35 (1999) 941–948.
[7] K. Tarutani, K. Nagasaki, M. Yamaguchi, Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo*, Appl. Environ. Microbiol. 66 (2000) 4916–4920.
[8] I. Imai, Y. Ishida, K. Sakaguchi, Y. Hata, Algicidal marine-bacteria Isolated from Northern Hiroshima Bay, Japan. Fish. Sci. 61 (1995) 628–636.
[9] Y.T. Park, J.B. Park, S.Y. Chung, B.C. Song, W.A. Lim, C.H. Kim, W.J. Lee, Isolation and algicidal properties of Micrococcus sp. LG-1 possessing killing activity for harmful dinofiagellate Cochlodinium polykrikoides, J. Kor. Fish. Soc. 31 (1998) 767–773.
[10] G.J. Doucette, E.R. McGovern, J.A. Babinchak, Algicidal bacteria active against *Gymnodinium breve* (Dinophy-ceae). I. Bacterial isolation and characterization of killing activity, J. Phycol. 35 (1999) 1447–1454.
[11] X. Wang, Z.J. Li, J.Q. Su, Y. Tian, X.R. Nin, H.S. Hong, T.L. Zheng, Lysis of a red-tide causing alga, *Alexandrium tamarense*, caused by bacteria from its phycosphere, Biol. Control 52 (2010) 123–130.
[12] J.Q. Su, X.R. Yang, Y.Y. Zhou, T.L. Zheng, Marine bacteria antagonistic to the harmful algal bloom species *Alexandrium tamarense* (Dinophyceae), Biol. Control 56 (2011) 132–138.
[13] T. Kamiyama, Growth and grazing responses of tintinnid ciliates feeding on the toxic dinoflagellate *Heterocapsa cir-cularisquama*, Mar. Biol. 128 (1997) 509–515.
[14] D.C. Sigee, R. Glenn, M.J. Andrews, E.G. Bellinger, R.D. Butler, H.A.S. Epton, R.D. Hendry, Biological control of cyanobacteria: Principles and possibilities, Hydrobiologia 396 (1999) 161–172.
[15] K. Bruning, R. Lingeman, J. Ringelberg, Estimating the impact of fungal parasites on phytoplankton populations, Limnol. Oceanogr. 37 (1992) 252–260.
[16] F.J.R. Taylor, Parasitism of the toxin-producing dinofla-gellate *Gonyaulax catenella* by the endoparasitic dinofla-gellate *Amoebophrya ceratii*, J. Fish. Res. Board. Can. 25 (1968) 2241–2245.
[17] L. Erard, E. Denn, M.J. Chrétiennot-Dinet, I. Probert, First report of parasitism on the toxic dinoflagellate *Alexandrium minutum* Halim, Estuar. Coast. Shelf. Sci. 50 (2000) 109–113.
[18] C. Lovejoy, J.P. Bowman, G. Hallegraeff, Algicidal effects of a novel marine *pseudoalteromonas* isolate (Class Proteobacteria, Gamma Subdivision) on harmful algal bloom species of the genera *Chattonella*, *Gymnodinium*, and *Heterosigma*, Appl. Environ. Microbiol. 64 (1998) 2806–2813.
[19] J.H. Skerratt, J.P. Bowman, G. Hallegraeff, S. James, P.D. Nichols, Algicidal bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary, Mar. Ecol. Prog. Ser. 244 (2002) 1–15.
[20] Y.K. Kang, S.Y. Cho, Y.H. Kang, T. Katano, E.S. Jin, D.S. Kong, M.S. Han, Isolation, identification and characterization of algicidal bacteria against *Stephanodiscus hantzschii* and *Peridinium bipes* for the control of freshwater winter algal blooms, J. Appl. Phycol. 9 (2008) 383–388.
[21] M.J. Kim, S.Y. Jeong, S.J. Lee, Isolation, identification and algicidal activity of marine bacteria against *Cochlodinium polykrikoides*, J. Appl. Phycol. 20 (2008) 1069–1078.
[22] M.P. Manage, Z. Kawabata, S. Nakano, Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp, Aquat. Microb. Ecol. 22 (2000) 111–117.
[23] H.L. Walker, L.R. Higginbotham, An aquatic bacterium that lyses cyanobacteria associated with off-flavor of channel catfish (*Ictalurus punctatus*), Biol. Control 18 (2000) 71–78.
[24] J.H. Paul, H. Lee, G. Dale, S. Terri, G. Mike, R. Bill, S. Karen, A filterable lytic agent obtained from a red tide bloom that caused lysis of *Karenia brevis* (*Gymnodinum breve*) cultures, Aquat. Microb. Ecol. 27 (2002) 21–27.
[25] S.Y. Shi, Y.D. Liu, Y.W. Shen, G.B. Li, D.H. Li, Lysis of *Aphanizomenon Xos-aquae* (Cyanobacterium) by a bacterium *Bacillus cereus*, Biol. Control 39 (2006) 345–351.
[26] B.H. Kim, M. Sang, S.J. Hwang, M.S. Han, In situ bacterial mitigation of the toxic cyanobacterium *Microcystis aeruginosa*: Implications for biological bloom control, Limnol. Oceanogr. Methods 6 (2008) 513–522.
[27] T. Sakata, Y. Fujita, H. Yasumoto, Plaque formation by algicidal *Saprospira* sp. on a lawn of *Chaetoceros ceratosporum*, Nippon Suisan Gakk 57 (1991) 1147–1152.
[28] J.D. Kim, B. Kim, C.G. Lee, Alga-lytic activity of *Pseudomonas fluorescens* against the red tide causing marine alga *Heterosigma*, Biol. Control 41 (2007) 296–303.
[29] J.D. Kim, G.L. Choul, Antialgal effect of a novel polysaccharolytic *Sinorhizobium kostiense* on *Anabaena flosaquae* causing water bloom, J. Microbiol. Biotechnol. 16 (2006) 1613–1621.
[30] M.C. Kim, I. Yoshinaga, I. Imai, K. Nagasaki, S. Itakura, A. Uchida, Y. Ishida, A close relationship between algicidal bacteria and termination of *Heterosigma akashiwo* (Raphidophyceae) bloom in Hiroshima Bay, Japan. Mar. Ecol. Prog. Ser. 170 (1998) 25–32.