Korean J. Limnol. 38 (4): 494~502 (2005)

Antialgal Interactions of Biological Control Agents on Cyanobacterium and Diatom Blooms *in vitro*

Kim, Baik-Ho[1], Yoon-Ho Kang[2], Hee-Jin Choi[1], Soon-Kyu Ka[2] and Myung-Soo Han[1,2,*]

([1]Department of Life Science and [2]Environmental Science Hanyang University, Seoul 133-791, Korea)

Antialgal bacteria and ciliates were tested alone and in combination for their abilities to decrease the densities of the warm-weather cyanobacterium, *Microcystis aeruginosa*, and the cold-weather centric diatom, *Stephanodiscus hantzschii*. Our results indicate that the density of *M. aeruginosa* was effectively suppressed by the bacterium, *Streptomyces neyagawensis*, and the heterotrich ciliate, *Stentor roeselii*. However, co-treatment with both bio-agents stimulated the algal density rather than decreasing it, suggesting that *S. neyagawensis* and *S. roeselii* may have an antagonistic relationship. Additional experiments revealed that the density of *S. hantzschii* was effectively suppressed by the bacterium, *Pseudomonas putida*, and by the above mentioned strain of *S. roeselii*. Co-treatment with both bio-agents had a higher antialgal activity than treatment with each alone, indicating that the bio-agents may act synergistically. These results suggest that the anti-algal efficacy of co-treatment with multiple biological control agents is likely to differ depending on the bio-agents and target organisms.

INTRODUCTION

The eutrophication of waterways is an increasingly serious problem worldwide, due to the introduction of domestic and industrial wastes into aquatic environments such as lakes and reservoirs. A direct effect of this is algal blooms, which consist primarily of cyanobacteria, diatoms, dinoflagellates and cryptomonads. Since the 1980s, profligate blooms of cyanobacteria (*Microcystis*, *Anabaena* and *Aphanizomenon*), diatoms (*Stephanodiscus, Aulacoseira* and *Asterionella*), dinoflagellates (*Peridinium*) and cryptomonads (*Cryptomonas*) have been reported in bodies of water throughout Korea (Han *et al*., 1995; Kim, 1996; Han *et al*., 2002; Lee *et al*., 2005). These blooms have been found in sluggish reservoirs used for drinking water, upstream of artificial dams and in small, slow-moving streams near metropolitan areas (Parker *et al*., 1997; Kim *et al*., 2003). Indeed, phytoplankton populations are generally dense throughout the year in Korea except during the deep winter freeze, and are responsible for producing green or brown colored water, bad odors, screen chapering, and eventual sludge production (Lee *et al*., 2001).

Many countries, including Korea, have attempted to control nuisance algal blooms through the direct application of chemicals such as cupric-sulfate, smazine, dichromate and ozone (McGuire *et al*., 1984; Jeffries and Mills, 1990; Sigee *et al*., 1999; Lee *et al*., 2001). However, these agents can cause irreversible damage to the aquatic ecosystem by killing off beneficial plankton and fish (Reynolds, 1984; Reyssac and Pletinistic, 1990).

*Corresponding Author: Tel: +82-2-2220-0956, Fax: +82-2-2296-1741, E-mail: hanms@hanyang.ac.kr

Thus, researchers are currently seeking to develop environment-friendly bio-agents for controlling algal density in a manner that is consistent with the current pan-ecological and environmental approaches to lake water conservation (Redhead and Wright, 1978; Barnet *et al*., 1981; Brabrand *et al*., 1983; Imai *et al*., 1993; Manage *et al*., 2000).

Microorganisms such as viruses, bacteria, actinomycetes, fungi, amoebae, and cyanophages have been shown to kill cyanobacteria (Yamamoto, 1981; Mitsutani *et al*., 1987; Yamamoto *et al*., 1998; Sigee *et al*., 1999). Among these, antagonistic bacteria have the potential to become useful agents for algal control, as they are simple to culture and manipulate (see Sigee *et al*., 1999; Rashidan and Bird, 2001). We have previously investigated a number of aquatic bacteria, zooplanktons and ciliates for their abilities to control algal blooms (Kim *et al*., 2003; Kim *et al*., 2004). Here, we sought to identify bio-agents capable of managing two target algae: the cyanobacterium, *Microcystis*, and the diatom, *Stephanodiscus*. In the Pal'tang Riverine of Korea, *Microcystis* is often found during the warm season (water temperatures above 20°C), while *Stephanodiscus* is more common during the cold season (water temperatures below 20°C) (Kim *et al*., 2003). Together, the two organisms are representative of many naturally occurring blooms.

Several previous reports have identified algicidal bacteria capable of killing cyanobacteria such as *Microcystis aeruginosa* (Yamamoto *et al*., 1998; Manage *et al*., 2001; Kodani *et al*., 2002), but there have been few reports of bio-agents capable of managing the diatom, *Stephanodiscus hantzschii* (Kang *et al*., 2005). Here, we investigated the ability of several bacterial and ciliate bio-agents, alone and in combination, to decrease the density of these two representative algae. This is the first investigation of combined treatment with both bacteria and ciliates to control cyanobacterial and diatomal blooms *in situ*, and our findings provide the first evidence that the ciliate, *Stentor roeselii*, is algicidal to both *Microcystis* and *Stephanodiscus*.

MATERIALS AND METHODS

1. Study organisms and culture conditions

The cyanobacterium, *M. aeruginosa* NIES-44, was obtained from the National Institute for Environmental Studies, Japan (NIES), and cultured in CB medium (MCC-NIES 2005) at a temperature of 27±2°C, with shaking at 120 rpm and a light cycle of 12L : 12D (light intensity = 40 μE m^{-2} s^{-1}). The small centric diatom, *Stephanodiscus hantzschii* UTCC-269, was obtained from the University of Toronto Culture Collection of Algae and Cyanobacteria, Canada (UTCC), and cultured in DM medium (Beakes *et al*., 1988) at 20±2°C with a 12L : 12D photo-cycle (light intensity = 50 μE m^{-2} s^{-1}). Algal cells in the exponential stage were used in all experiments. Biomasses were determined by ultrasonic disintegration of cell colonies (20 kHz for 60s) followed by direct counting of cells using a haemocytometer at ×400 magnification under light microscopy (Zeiss Axioplan; Zeiss, Germany).

2. Preparation of antialgal agents

All anti-algal/algicidal organisms utilized in this study were isolated in August 2002 from the sediment and surface water of a meso-eutrophic Kyungan stream that forms one of the major tributaries of the Pal'tang Riverine in the Kyung-ki prefecture of South Korea.

The bacterium *Streptomyces neyagawensis*, previously reported by Choi *et al*. (2005), was isolated using the plaque method (Safferman and Morris, 1962) with slight modification. Briefly, algal lawns were prepared in Petri dishes containing axenic cultures of *M. aeruginosa* (MCC-NIES 44) on nutrient broth (NB) agar medium (Manage *et al*., 1999). These were inoculated with 1 mL of stream water filtered through a Nucleopore membrane (Millipore). Cultures were incubated at 25°C with a 12L : 12D photo-cycle ane 40-50 μE m^{-2} s^{-1} for 6-8 days. Potential anti-algal or algicidal bacteria were picked from clear zones where the *M. aeruginosa* cells had lysed. These bacteria were purified, isolated, grown to the logarithmic growth phase, and inoculated into an axenic culture of *M. aeruginosa* in NB medium. We then used 16S rDNA sequencing and biochemical reactions to identify the anti-algal/algicidal bacteria as *S. neyagawensis* (Choi *et al*., 2005). The isolates were axenically maintained under optimal growth conditions on nutrient agar (NA) plates, cryopreserved at −76°C in NB medium containing 20% glycerol. The bacterial biomass of *S. neyagawensis* was determined by dry

weight, measured as the weight difference of membrane filters (0.2 μm pore size, 25 mm diameter; Whatman) before and after cultivation with the target alga (APHA, 1998). The dry weight contribution of *Microcystis* was ignored because it was approximately 1000-fold smaller than that of *S. neyagawensis*.

Pseudomonas putida HYK0203-SK02, which is capable of inhibiting the growth of *Stephanodiscus hantzschii*, was isolated by Kang *et al.* (2005) using the above method, except that the algal lawns were prepared with an axenic culture of *S. hantzschii* (strain UTCC 267) on NB medium (Manage *et al.*, 1999). This strain of *P. putida* grows at 20-30°C and pH 5-9, with optimal growth at 30°C and pH 7. The bacterium was axenically maintained in the dark on NA plates containing 1.5% agar, at 30°C and pH 7, or cryopreserved at −76°C in NB medium containing 20% glycerol. For enumeration of bacteria, water samples were fixed with formalin (2% v/v final concentration) immediately after sampling, stained with DAPI (1 mg mL^{-1}), filtered through isopore membrane filters (0.2 μm, Millipore, Ireland) and counted at ×1,000 magnification using an epifluorescence microscope (Zeiss Axioplan; Karl Zeiss, Germany).

For isolation of the heterotrich ciliate, *Stentor roeselii*, the waterway of the Pal'tang Riverine of South Korea, was screened with a plankton net (pore size 40 μm) near the marginal portion of the waterway, where aquatic plants flourished. In the laboratory, the plankton samples were size-fractionated with a net (0.1-4 mm) and *Stentor* cells were directly picked under an inverted microscope. The picked cells were concentrated in a depression glass, rinsed 3 times with filtered water, and inoculated into a multi-well chamber containing different prey species and mixtures thereof. Samples were maintained at 23°C, with a 12L : 12D photo-cycle and 2 μE m^{-2} s^{-1}. To investigate the changes in ciliate density in the presence of algal prey, water samples were fixed immediately with bouin's solution (3% v/v final concentration), filtered with a cellulose nitrate membrane filter (0.45 μm; Sartorius, Germany) and stained with protargol stain. The ciliates were enumerated at ×400 magnification under light microscopy (Zeiss Axioplan; Zeiss, Germany).

3. Single treatment with bio-agents

Microcystis aeruginosa maintained in CB medium (MCC-NIES 2005) was diluted to $2-6\times10^6$ cells mL^{-1} with the same medium, and 95 mL aliquots were inoculated into duplicate 250 mL Erlenmeyer flasks.

To investigate the antialgal effects of the *Microcystis*-lysing bacterium, *Streptomyces neyagawensis*, the bacterium was incubated in liquid NB medium at 37°C for 48 hours. When the cell density reached $1-2\times10^8$ cells mL^{-1}, the culture was centrifuged at 8,000 rpm for 20 min, and the pellet was washed twice with sterilized NB medium. Five mL (5% v/v) aliquots were inoculated into the algal cultures at a final concentration of $1-2\times10^4$ cells mL^{-1}. Duplicate control cultures were prepared by adding 5 mL of *S. neyagawensis* with NB medium to 95 mL of *Microcystis* culture. The flasks were incubated at 27±2°C with a 12L : 12D photo-cycle and 40-50 μE m^{-2} s^{-1}.

To first test the antialgal effect of the *Stephanodiscus*-lysing bacterium, *Pseudomonas putida*, we cultured the bacterium to the logarithmic growth phase and diluted the samples with CB medium to 1.5×10^3 cells mL^{-1} or-10^5 cells mL^{-1}. We inoculated 5 mL of exponentially growing the *P. putida* cells into 95 mL of *Stephanodiscus hantzschii* culture, for a final algal concentration of 1%. The samples were incubated as above. Bacteria-free cultures served as controls. To investigate the algicidal effect of each of these organisms on *Stephanodiscus*, we used the same methods described above for the *Microcystis* assays, except that the culture flasks were incubated at 20°C with a 12L : 12D photo-cycle (light intensity = 40-50 μE m^{-2} s^{-1}). The initial concentration of the diatom (1.5×10^3 cells mL^{-1}) was similar to that encountered in nature during the bloom period. Algal cells were enumerated daily under light microscopy, as described above.

To examine the removal activity of the ciliate, *Stentor roeselli*, on *Microcystis* and/or *Stephanodiscus*, 6-well plates (Falcon, USA) were loaded with 5 cells mL^{-1} 3-day starved ciliates in 5 mL filtered water/well, and while the algae were prepared to be 2×10^6 cells mL^{-1} of *Microcystis* and/or 1.5×10^3 cells mL^{-1} of *Stephanodiscus*, respectively. Algal densities and ciliate numbers were enumerated daily under light microscopy after fixed with glutaraldehyde solution.

4. Combined treatment with bio−agents

To examine the combined algicidal effects of the tested organisms on algal growth, two bio−agents (bacteria or ciliates) were introduced into culture flasks containing *Microcystis* and/or *Stephanodiscus*. The initial concentrations of bacteria and starved ciliates, and the final culture volumes were very similar to those described above. Cultures were maintained at 20°C, with a 12L : 12D photo−cycle and 2 μEm^{-2} s^{-1}. Ciliates and algae were counted under an inverted microscope (Nikon, Japan) and a phase contrast microscope (Nikon, Japan), using a hemocytometer or Sedgwick−Rafter chamber. To further investigate the antialgal relationship between the bacterium and the ciliate, two bio−agents were co−cultured in filtered fresh water at 20±2°C with a 12L : 12D photo−cycle and 2 μEm^{-2} s^{-1}. As the bacteria grew similarly under dark conditions and 2 μEm^{-2} s^{-1}, we were able to ignore the influences of light in our calculations. *Streptomyces neyagawensis*, *Pseudomonas putida* and *Stentor roeselli* populations were measured or enumerated daily for 6−7 days.

5. Calculations

The algicidal activity (AA) of the bacteria or ciliate on *M. aeruginosa* and *S. hantzschii* were calculated as AA = (1−D/Do)×100, where Do and D are the cell densities in the control and treated groups, respectively, at the end of each experiment. The AA increase (indicating an effective algicide) or decrease (indicating an ineffective algicide) was analyzed by the Student's *t* test. The rates of decrease in *M. aeruginosa* (MA) and *S. hantzschii* (SH) were also calculated by the equation, *ln* (C/Co)/t, where Co and C are the algal densities at the beginning and end of the experiment, respectively, and t is the time interval.

RESULTS

1. Antialgal effects of bacteria and ciliates on cyanobacterium *Microcystis aeruginosa*

The bacterium *Streptomyces neyagawensis* effectively suppressed the growth of *Microcystis aeruginosa* cultured with filtered water, without any apparent change in the biomass of *S. neyagawensis* (Fig. 1). The lack of change in the bacterial biomass may be attributed to the differences in optimal temperature and pH ranges between the bacteria and the algae. The ciliate, *Stentor roeselii*, gradually inhibited the growth of *Microcystis* cultured with filtered water, beginning one day after cultivation, while their abundance increased slightly over the first five days of cultivation, and declined thereafter (Fig. 2). Unexpectedly, combined treatment with bacteria and ciliates did not effectively inhibit the growth of *Microcystis* (Fig. 3, Table 1). However, the cell densities and

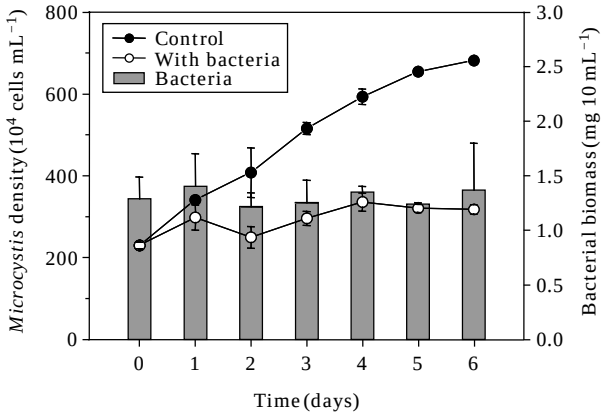

Fig. 1. Abundance of *Streptomyces nagayawensis* and its anti−algal effect on *Microcystis aeruginosa* in filtered water. The densities of *Microcystis* and bacteria remained consistent for 6−12 days.

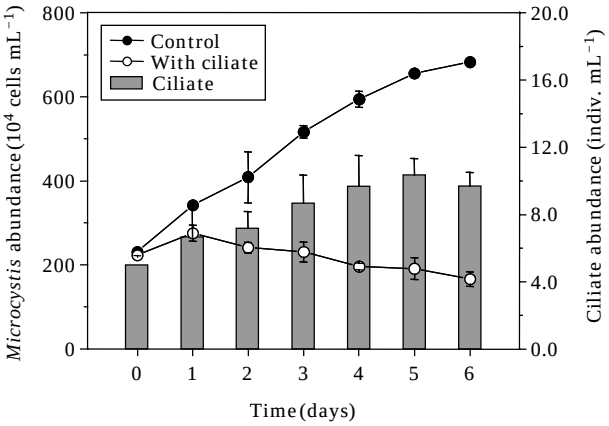

Fig. 2. Abundance of the ciliate, *Stentor roeselii*, and its antialgal effect on *Microcystis aeruginosa* in filtered water. The density of *Microcystis* in the presence of the ciliate did not decrease significantly after 6 days, whereas the ciliate density gradually decreased to 10% of the control level over 12 days.

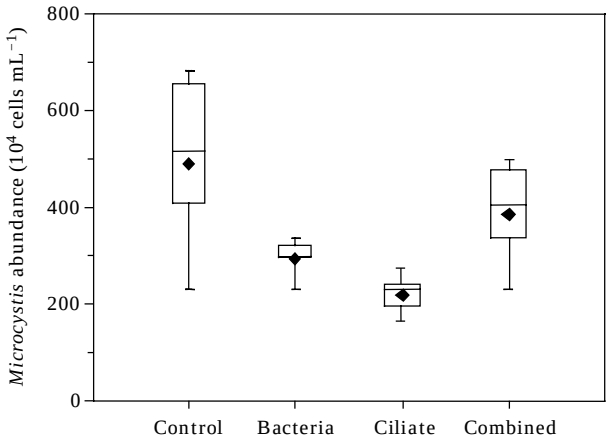

Fig. 3. Comparison of individual and combined antialgal effects of the bacterium and the ciliate on *Microcystis*. The heights of the blank boxes represent the growth of *Microcystis* and its inhibition or stimulation by the treatments. Combined, co-treatment with bacteria plus ciliates. Larger distances between the averages (◆) and the transverse lines in the blank boxes represent greater growth and/or inhibition of *Microcystis* following treatment.

Table 1. Decreases in *Microcystis aeruginosa* (MA) and *Stephanodiscus hantzschii* (SH) biomass following individual or combined treatment with bacteria and ciliates, and their algicidal activities on both algae

Treatments	MA	SH
Streptomyces	0.05**	
Stentor	−0.04*	
Streptomyces + *Stentor*	0.10**	
Pseudomonas		−0.12***
Stentor		−0.06***
Pseudomonas + *Stentor*		−0.42***

Microcystis; Microcystis aeruginosa NIES-44
Stephanodiscus; Stephanodiscus hantzschii UTCC 762
Streptomyces; Streptomyces nagayawensis
Pseudomonas; Pseudomonas putida
Stentor; Stentor roeselli
*P < 0.05, **P < 0.01, ***P < 0.001

growth of *M. aeruginosa* in the combined treatment cultures were lower than those in control cultures. In mixed bacterial/ciliate cultures without nutrient supplementation, the growth of the ciliate was severely limited (decreased to 80% of the control) on the first day, with < 100% mortality by the second day (Fig. 4). Although the cell density and total cell number of *M. aeruginosa* decreased in the presence of *S. neyagawensis*, the bacterium induced the *M. aeruginosa* cells to form

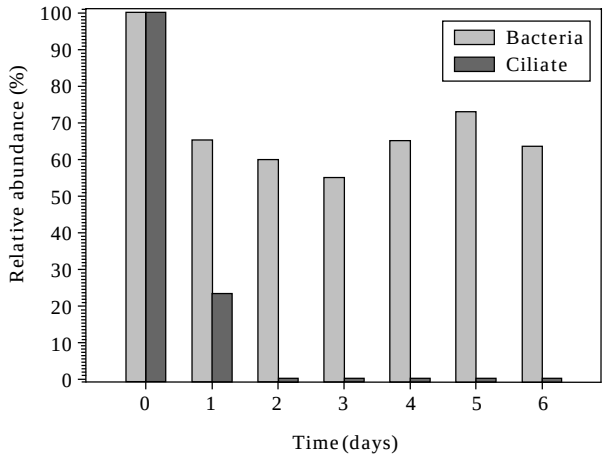

Fig. 4. Time course of variations in the relative abundances of ciliates and bacteria in mixed cultures. All ciliates died within 2 days of cultivation.

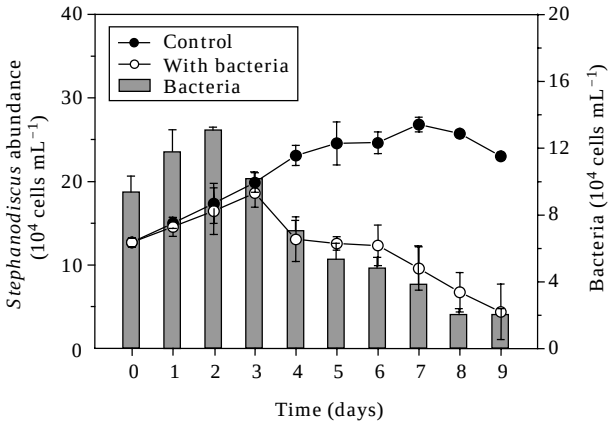

Fig. 5. Antialgal effect of *Pseudomonas putida* on the centric diatom, *Stephanodiscus hantzschii*, in filtered water. The density of *S. hantzschii* in the presence of bacteria remained above zero even after 15 days.

colonies of approximately 6 to 8 cells (data not shown). No colony formation was observed in the presence of the ciliate, *S. roeselli*.

2. Antialgal effects of bacteria and ciliates on diatom *Stephanodiscus hantzschii*

The bacterium *Pseudomonas putida* effectively inhibited the growth of the diatom *Stephanodiscus hantzschii* in filtered water, decreasing its population to 80% of the control levels (Fig. 5). The bacterial population gradually decreased with time, and the density of the diatom declined to −14% of the control level after 8 days of cultivation. The

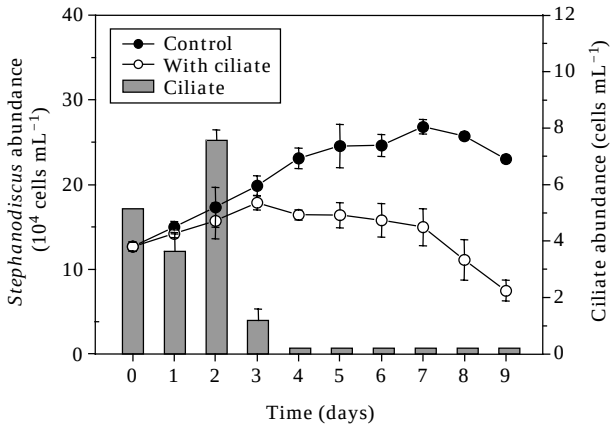

Fig. 6. Abundance and antialgal effect of the ciliate, *Stentor roeselii*, on *Stephanodiscus hantzschii*. The density of *Microcystis* in the presence of bacteria remained above zero even after 15 days.

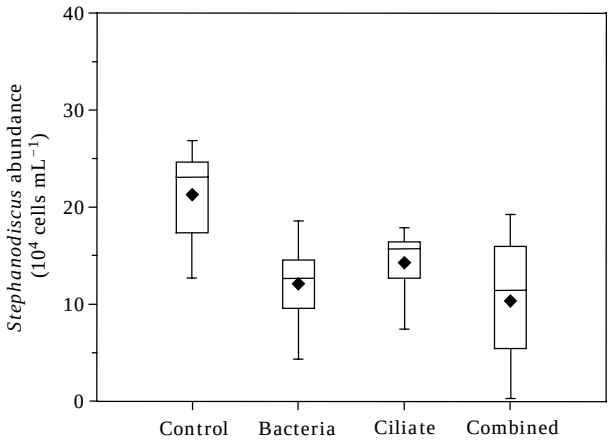

Fig. 7. Comparison of the antialgal effects of bacteria, ciliates, and combined treatments on *S. hantzschii*. The heights of the blank boxes represent the growth of *S. hantzschii* and its inhibition or stimulation by each treatment. Combined, co−treatment with bacteria plus ciliates. Larger distances between the averages (◆) and the transverse lines in the blank boxes represent greater growth and/or inhibition of *S. hantzschii* following treatment with the indicated agent (s).

ciliate *S. roeselii* similarly inhibited growth of *S. hantzschii*, although its effect was weaker than that of *P. putida* (Fig. 6), and all ciliates died within 4 days of cultivation. In contrast to our finding of decreased anti−algal effects following co−treatment of *Microcystis*, combined treatment of *S. hantzschii* with both *P. putida* plus *S. roeselii* was more effective than either of the single treatments alone (Fig. 7, Table 1). Following the combined treatment, *S. hantzschii* levels reached zero within 9 days of cultivation.

3. Antialgal interactions between different agents

Combined treatment of *Stephanodiscus hantzschii* with *Pseudomonas putida* and *Stentor roeselii* decreased the algal cell density, whereas treatment of *Microcystis aeruginosa* with the bacterium *Streptomyces neyagawensis* plus the ciliate did not effectively inhibit algal growth (Table 1).

DISCUSSION

Our results clearly show that the efficacies of bio−agents and their co−treatments vary depending on the target algae and the applied agents. In the presence of the *Microcystis*−lysing bacterium, *Streptomyces neyagawensis* (Choi *et al*., 2005), the density of *Microcystis* fluctuated near the average value, whereas control cultures lacking the lytic bacterium showed a steady increase in the cell density of *Microcystis* (MA = 0.05), indicating that the bacterium had a suppressive effect on the proliferation of *Microcystis*. In the presence of the *Stephanodiscus*−lysing bacterium, *Pseudomonas putida* (Kang *et al*., 2005), the cell density of the diatom, *Stephanodiscus hantzschii*, gradually decreased over time (SH = −0.12). In terms of the bacterial growth patterns, the density of *S. neyagawensis* fluctuated near the initial density in the presence of *M. aeruginosa*, while the density of *P. putida* increased during the first two days of diatom culture and sharply decreased thereafter. Our observation of feeble or nonexistent bacterial growth in the presence of algae is perhaps due to differences between the optimal growth conditions for the algae (used in our experiments) and those preferred by the bacteria. The growth of *Microcystis* and *Stephanodiscus* in the presence of the ciliate, *Stentor*, showed a similar pattern to that observed in the presence of the two bacteria. However, during the co−treatments, the ciliate showed different growth patterns depending on the bacteria; the *Stentor* cell density declined to zero after four days of cultivation with *S. hantzschii*, whereas

the ciliate levels gradually increased in the presence of *M. aeruginosa*.

Surprisingly, we found that co-treatment of *Microcystis*-containing cultures with *S. neyagawensis* and *Stentor* showed a smaller algicidal effect than treatment with either agent alone, perhaps due to some sort of antagonistic effect. Our growth pattern results (Fig. 7) showed that *S. neyagawensis* and ciliates grown in nutrient-free filtered water of the sampling stations decreased simultaneously with each other; however, this observation was insufficient to explain all of the observed differences. In the presence of *S. neyagawensis*, ciliate levels decreased more severely than did *S. neyagawensis* levels, with total ciliate mortality within 4 days of cultivation. This phenomenon might suggest that the bacterium may even have an anti-ciliate property, similar to the anti-nematodal and antibiotic properties seen in other bacteria (Dicklw *et al*., 1993; Esnar *et al*., 1995). Future work will be required to study the relationship between co-treated bio-agents in an aquatic ecosystem.

We also investigated the effect of a second bacterium, *P. putida*, and the ciliate, *Stentor*, on the diatom, *Stephanodiscus*. We observed widespread ciliate death in the presence of *Stephanodiscus*, possibly due to a lack of nutrients. We used the same nutrient conditions as found the lake, and seeded cultures with $<10^5$ diatoms mL^{-1}, which is consistent with the diatom load found in nature. The species used in this study (NIES 44) is not a toxic strain, and does not produce microcystin, in contrast to the previously reported NIES-298 (Yasuno *et al*., 2000). Although the target algae were inhibited by treatment with these bio-agents, the co-culture conditions seemed not to be optimal for growth of the bacteria and/or ciliate. However, our results revealed that co-treatment of *Stephanodiscus* with *Pseudomonas putida* and *Stentor roeselii* more effectively inhibited the diatom than did treatment with each agent alone, suggesting some kind of synergistic interaction.

Collectively, these results indicate that the efficacies of bio-agents and their co-treatments vary depending on the target algae and the applied agents. Future work will be required to investigate the use of bio-agents such as algicidal bacteria and ciliates to control cyanobacterial and diatomaceous blooms. A better understanding of the interactions among bio-agents will allow researchers to select relevant predators and/or bacteria within each freshwater ecosystem. Here, our preliminary data show the possibility of synergistic and antagonistic effects during co-treatments of bio-agents, providing new insight that may be useful for the future bioremediation of diatomaceous and cyanobacterial blooms.

ACKNOWLEDGEMENT

This work was supported by the Korea Research Foundation Grant (KRF-2004-C00018) of K.B. H. We also thank three anonymous reviewers for valuable comments on the original manuscript.

LITERATURE CITED

APHA. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, D.C.

Barnet, Y.M., M.J. Daft and W.D. Stewart. 1981. Cyanobacteria-cyanophage interactions in continuous culture. *J. Appl. Bacteriol*. **51**: 541-552.

Beakes, G., H.M. Canter and G.H.M. Jaworski. 1988. Zoospores ultrastructure of *Zygorhizidium affluens* Canter and *Z. planktonicum* Canter, two chytrids parasitizing the diatom *Asterionella formosa* Hassall. *Canadian J. Bot*. **66**: 1054-1067.

Brabrand, A., B.A. Faafeng, T. Kallquist and J.P. Nilssen. 1983. Biological control of undesirable cyanobacteria in culturally eutrophic lakes. *Oecologia*. **60**: 1-5.

Choi, H.J., B.H. Kim, J.D. Kim and M.-S. Han. 2005. *Streptomyces neyagawaensis* as a control for the harzardous biomass of *Microcystis aeruginosa* (Cyanobacteria) in eutrophic freshwaters. *Biological Control*. **33**: 335-343.

Dicklw, M.B., N. Acosta and B.M. Zuckerman. 1993. A novel *Stresptomyces* species for controlling plant-parastic nematodes. *J. Chem. Ecol*. **19**: 159-173

Esnar, J., T.L. Potter and B.M. Zuckerman. 1995. *Streptomyces costarinus* sp. nov., isolated from nematode-supressive soil. *J. Sys. Bacteriol*. **45**: 775-779.

Han, M.S., Y.Y. Aug and S.S. Hong. 2002. Ecological studies on Pal'tang River-Reservoir system in Korea 4. Dynamics on inorganic nutrients, POM and phytoplankton succession in the lower stream Kyungan. *Korean J. Limnol*. **35**: 1-9.

Han, M.-S., Y.Y. Aug, J.K. Ryu, K.I. Yoo and Y.K. Choi. 1995. Ecological studies on Pal'tang River-Reservoir system in Korea 2. Changes in phytoplankton community structure. *Korean J. Limnol*. **28**: 335-344.

Imai, I., Y. Ishida and Y. Hata. 1993. Killing of marine phytoplankton by a gliding bacterium *Cytophaga* sp., isolated from the coastal sea of Japan. *Marine Biology*. **116**: 527–532.

Jeffries, M. and D. Mills. 1990. Freshwater ecology. Principles and applications. Belhavan Press, London, UK.

Kang, Y.H., J.D. Kim., B.H. Kim., D.S. Kong and M.–S. Han. 2005. Isolation and characterization of a bio–agent antagonistic to diatom, *Stephanodiscus hantzschii. J. Appl. Microbiol*. **98**: 1030–1038.

Kim, B.H. 1996. Ecological studies on the phytoplankton in Lake Chuam. Chonnam University. Ph.D.

Kim, B.H., H.J. Choi and M.–S. Han. 2003. Potential in the application for biological control of harmful algal bloom cause by *Microcystis aeruginosa*. *Korean J. Limnol*. **36**: 64–69.

Kim, B.H., Y.H. Kang and M.–S. Han. 2004. Potential in the application for biological control of winter diatom bloom caused by *Stephanodiscus hantzschii. Korean J. Limnol*. **37**: 236–240.

Kodani, S., A. Imoto, A. Mitsutani and M. Murakami. 2002. Isolation and identification of the antialgal compound, harmane (1–methyl–β–carboline), produced by the algicidal bacterium, *Pseudomonas* sp. K44–1. *J. Appl. Phycol*. **14**: 109–114.

Lee, C.W., C.W. Jung, S.W. Han, L.S. Kang and J.H. Lee. 2001. The removal of algae by oxidation and coagulation process. *J KSEE* **23**: 1527–1536.

Lee, K.W., S.K. Baik and B.H. Kim. 2005. Dynamics of phytoplankton community in Lake Juam, Korea. *Korean J. Limnol*. **38**: 249–260.

Manage, P.M., Z. Kawabata and S. Nakano. 2000. Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp. *Aquat. Microb. Ecol*. **22**: 111–117.

Manage, P.M., Z. Kawabata and S. Nakano. 1999. Seasonal changes in densities of cyanophage infectious to *Microcystis aeruginosa* in hypereutrophic pond. *Hydrobiologia* **411**: 211–216.

Manage, P.M., Z. Kawabata and S. Nakano. 2001. Dynamics of cyanophage–like and algicidal bacteria causing *Microcystis aeruginosa* mortality. *Limnology* **2**: 73–78.

MCC–NIES. 2005. http://www.nies.go.jp/biology/mcc/strainlist_j.htm

McGuire, R.M., J.M. Jones, E.G. Means, G. Lzaquive and A.E. Reston.1984. Controlling attached blue–green algae with copper sulfate. *Res. Technol*. **27**: 60–65.

Mitsutani, A., A. Uchida and Y. Ishida. 1987. Occurrence of blue–green algae algal lytic bacteria in lake Biwa. *Nippon Biseibutsu Gakkaiho*. **2**: 21–28.

Parker, D.L., H.D. Kumar, L.C. Rai and J.B. Singh. 1997. Potassium salts inhibit growth of the Cyanobacteria *Microcystis* spp. in pond water and defined media: implications for control of Microcystin–producing aquatic blooms. *Appl. Environ. Microbiol*. **63**: 2324–2329.

Rashidan, K.K. and D.F. Bird. 2000. Role of predatory bacteria in the termination of a cyanobacterial bloom. *Microbiol. Ecol*. **16**: 97–105.

Redhead, K. and S.J. Wright. 1978. Isolation and properties of fungi that lyse blue–green algae. *Appl. Environ. Microbiol*. **35**: 962–969.

Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University press. Cambridge.

Reyssac, S.J. and M. Pletikosic. 1990. Cyanobacteria in fish–ponds. *Aquaculture*. **88**: 1–20.

Safferman, R.S. and M.E. Morris. 1962. Evaluation of natural products for algicidal properties. *J. Appl. Microbiol*. **10**: 289–292.

Sigee, D.C., R. Glenn, M.J. Andrews, E.G. Bellinger, R.D. Butler, H.A.S. Epton and R.D. Hendry. 1999. Biological control of cyanobacteria: principles and possibilities. *Hydrobiologia*. **395/396**: 161–172.

Yamamoto, Y. 1981. Observations on the occurrence of microbial agents which cause lysis of blue–green algae in Lake Kasumigaura. *Jpn. J. Limnol*. **42**: 20–27.

Yamamoto, Y., T. Kouchiwa, Y. Hodoki, K. Hotta, H. Uchida and K. Harada. 1998. Distribution and identification of actinomycetes lysing cyanobacteria in a eutrophic lake. *J. Appl. Phycol*. **10**: 391–397.

Yasuno, M., Y. Sugaya, K. Kaya and M.M. Watanabe. 2000. Variations in the toxicity of *Microcystis* species to *Moina macrocopa*. *In*: Watanabe, M.M. and K. Kaya (eds.). Advances in microalgal and protozoal studies in Asia. Global Environmental Forum. pp. 43–51.

(Manuscript received 21 November 2005,
Revision accepted 17 December 2005)

＜**국문적요**＞

유해조류 제어를 위한 두 가지 이상의 생물제재 적용 및 효과

김백호[1] · 강윤호[2] · 최희진[2] · 가순규[2] · 한명수[1,2,*]

([1]한양대학교 생명과학과, [2]환경과학과)

녹조대발생의 원인종인 남조 *Microcystis aeruginosa*와 규조 *Stephanodiscus hantzschii*에 대한 살조 및 섭식능을 갖는 박테리아 및 섬모충을 각각 단독 및 혼합처리하고 살조능을 비교하였다. 먼저 남조 *M. aeruginosa*에 대하여 살조세균 *Streptomyces neyagawensis*와 섬모충 *Stentor roeselii*를 단독 및 혼합처리한 결과, 각각의 단독처리가 혼합처리보다 더 높은 살조능을 나타내는 두 생물제재의 길항적 살조효과를 나타냈다. 또한 규조 *S. hantzschii*에 대해 살조능을 갖는 박테리아 *Pseudomonas putida*와 *S. roeselii*를 전과 동일한 방법으로 단독 및 혼합처리하여 결과를 비교하였다. 전 실험과는 다르게 두 생물제재의 혼합처리가 단독처리보다 더 높은 살조능을 보이는 두 생물제재의 상승적 살조효과를 나타냈다. 이러한 결과들은 녹조 제어를 위한 생물제재의 적용시 유용하게 이용될 것이며, 다양한 생물제재를 이용함으로서 수중생태계의 혼란을 최소화시킬 수 있을 것으로 사료되었다.