The Journal of Microbiology, December 1997, p.284-289

Vol. 35, No. 4

# Isolation of a Bacterium That Inhibits the Growth of *Anabaena cylindrica*

**Chul-Ho Kim[1], Mi-Hyea Leem, and Yong-Keel Choi***

*[1]Water Resources Research Institute, Korea Water Resources Corporation, Taejon 305-390*
*Department of Biology, College of Natural Sciences, Hanyang University, Seoul 133-791, and*
*Research Center for Molecular Microbiology, Seoul National University, Seoul 151-742, Korea*

(Received April 30, 1997 / Accepted November 10, 1997)

**A Gram (-), rod-shaped bacterium 2.3~2.8×0.45 μm in size which exhibited growth-inhibiting effects against a cyanobacterium (*Anabaena cylindrica*) was isolated from Daechung Dam Reservoir. This isolate was identified as *Moraxella* sp. and designated *Moraxella* sp. CK-1. Hollow zones formed around bacterial colonies on the cyanobacterial lawn. In a mixed-culture of *A. cylindrica* and the isolate, each microorganism grew inverse-proportionally, and the cyanobacterial vegetative cells completely disappeared within 24 hours. On treatment with *Moraxella* sp. CK-1, cell walls of *A. cylindrica* disappeared, but sheathes remained in a more electron dense form. The unit membrane such as thylakoidal membrane was stable to bacterial lysing activity. This bacterium showed a broad action spectrum against cyanobacteria. The growth-inhibiting activity of *Moraxella* sp. CK-1 against *A. cylindrica* is believed to be performed through the excretion of active substances.**

**Key words: Cyanobacteria, *Moraxella* sp., growth-inhibition**

Blooms of planktonic algae are common in many freshwater lochs and reservoirs around the world (18, 19). The most noticeable blooms are usually of cyanobacteria, a group of gram-negative photosynthetic prokaryotes which create many problems such as formation of surface scums, deoxygenation of the water, production of offensive odours, death of wild and domestic animals, etc. (3, 14, 21). It is also well-known that cyanobacterial toxins may provoke serious troubles in man (3, 11).

The inhibition of the growth of cyanobacteria is very important with regards to water supply and public health (15, 16, 20). The most frequently used algicides are copper sulfate and simazine which block photosynthesis (20). However these algicides are expensive and occasionally exhibit harmful effects on other organisms in water (15, 16, 21, 31).

A more suitable alternative would be the use of biological control agents to regulate cyanobacterial blooms (15, 16). Bacteria have been recommended as effective pathogens against cyanobacteria because they are not only abundant in water columns, but also less specific in their host range (5, 15, 16, 20).

This paper describes a strain of *Moraxella* sp. isolated from Daechung Dam reservoir, which excellently inhibits the growth of cyanobacteria through the excretion of active substances.

* To whom correspondence should be addressed.

## Materials and Methods

### Cyanobacterial cultures

*Anabaena cylindrica* M-1, *Microcystis aeruginosa* M-176, *Microcystis* flos-aquae M-178, *Nostoc carneum* M-35, *Oscillatoria animalis* M-75, and *Synechococcus leopoliensis* (*Anacystis nidulans*) M-6 were obtained from the Institute of Applied Microbiology (IAM) culture collection of Tokyo University. *Synechococcus* sp. (13), *Anabaena flos-aquae, Phormidium* sp., and *Oscillatoria* sp. were isolated in Korea. *Anabaena variabilis* ATCC 29413, *Synechocystis* sp. ATCC 27184 and *Chlorogloea fritschii* CCAP 1411/1A were gifts from Dr. K. S. Lee, Department of biology, College of natural sceinces, Paichai University.

### Culture conditions of cyanobacteria and bacterial isolate

BG-11 culture medium (22) was routinely used for the growth of cyanobacteria except for *Anabaena cylindrica* which was cultured in BG-$11_0$ medium (4), the BG-11 medium without sodium nitrate. The cyanobacteria were cultured under continuous illumination of cool white fluorescent lamps giving an incident light intensity of 25 μmol photon $m^{-2}s^{-1}$ at 25~28°C.

Isolated bacteria were cultured in BG-11C medium (5, 7) consisting of BG-11 medium and 0.2%

casitone (Difco) at 37°C with agitation at 150 rpm on rotary shaker. Solid culture plates were prepared by adding 1.5% agar (Difco).

**Isolation of a cyanobacterial growth-inhibiting bacterium**

The 'cyanobacterial lawn' technique (17, 23, 25) was used for the isolation of active bacteria. To 5 ml of BG-11 medium containing 0.8% agar adjusted to 50°C after autoclaving, 1 ml of *Anabaena cylindrica* culture containing about $10^6$~$10^7$ cells/ml was added. 1 ml of water samples obtained from Deachung Dam reservoirs was then added, after which these mixtures were poured on to pre-made BG-11 agar plates. After incubation for about 5~10 days in the growth chamber, the active bacteria were isolated from plaques which had developed on the cyanobacterial lawns. The isolated colonies were streaked on BG-11C agar plates several times to ensure pure cultures. Among these isolates, one was selected on the basis of its growth-inhibiting activity.

**Identification of the cyanobacterial growth-inhibiting isolate**

General characteristics of the isolate were determined according to Methods for General and Molecular Bacteriology (26) and Bergey's Manual of Systematic Bacteriology (27). Transmission electron microscopy (TEM) followed Stewart and Brown's method (30) using a Hitachi H-600 electron microscope.

**Mixed-culture of *A. cylindrica* and *Moraxella* sp. CK-1**

Broth culture of *A. cylindrica* in the log phase growth was centrifuged (9,000× g, 15°C, 15 min), washed twice with fresh BG-$11_0$ medium, and inoculated in 100 ml of the same medium contained in a 500 ml flask. *Moraxella* sp. CK-1 was inoculated in this culture medium to a density of $5.0\times 10^6$ cells/ml. At intervals during incubation in the growth chamber, of cyanobacterial and bacterial cells were counted. Cyanobacterial cells were counted using a hemocytometer under a light microscope in which 1 mm of cyanobacterial filament was assumed to contain 200 cells. Bacterial cells were counted using colony forming units (CFUs) on BG-11C agar plates. The growth of *A. cylindrica* in the same medium free of lytic bacteria was measured as a negative control.

**Action spectrum of *Moraxella* sp. CK-1**

The cyanobacterial host range of the *Moraxella* sp. CK-1 was determined by the agar-spot method (9, 28) and mixed culture in BG-11 broth media (1). Agar-spots were made by dropping 25 μl of bacteria-suspended BG-11C media containg 0.75 % agar on the cyanobacterial lawns formed on BG-11 agar plates.

**Electron microscopic observation of lytic activity**

The lytic mechanism of *Moraxella* sp. CK-1 against *A. cylindrica* was observed by transmission electron microscopy (TEM) following the method of Stewart and Brown (30) using Hitachi H-600 electron microscope at 75 kV.

**Subcellular localization of the active substance(s)**

Subcellular fractions of *Moraxella* sp. CK-1 were prepared by the method of Watt and Clarke (32). The extracellular fraction was prepared as follows: 300 ml of bacterial culture medium, in which *Moraxella* sp. CK-1 was in log phase growth, was centrifuged (6,000× g, 4°C, 15 min). The supernatant was then saturated 80% with ammonium sulfate (Sigma), and centrifuged (20,000× g, 4°C, 20 min). The pellet was suspended in 5 ml of 20 mM HEPES buffer (pH 8.0).

Each fraction was dialyzed against 20 mM HEPES buffer (pH 8.0), and the volumes were adjusted to be 15 ml with same buffer solution. After drying and resuspending in 2 ml of the same buffer, 0.5 ml of each fraction was added to BG-$11_0$ medium in which *A. cylindrica* was growing exponentially. The changes in the turbidity of culture media containing each subcellular fraction were determined spectrophotometically and the growth-inhibiting activities were compared.

## Results

**Isolation and characteristics of *Moraxella* sp. CK-1**

Some plaques began to form on cyanobacteiral lawns about 5 days after incubation (Fig. 1). From these plaques, a bacterium which efficiently inhibits the growth of *A. cylindrica* was selected and characterized. This isolate was a Gram-negative, rod-shaped bacterium 2.3~2.8× 0.45 μm in size (Fig. 2). This bacterium produced oxidase, catalase, amylase, protease and nitrate reductase. It was sensitive to the antibiotics of penicillin, chloramphenicol, ampicillin, kanamycin and tetracycline, but was resistant to carbenicillin and streptomycin. The isolate was so sensitive to penicillin that it could not grow in the medium containing 0.1 unit/ml of penicillin, which is the typical characteristic of *Moraxella* species (27). The bacterium did not

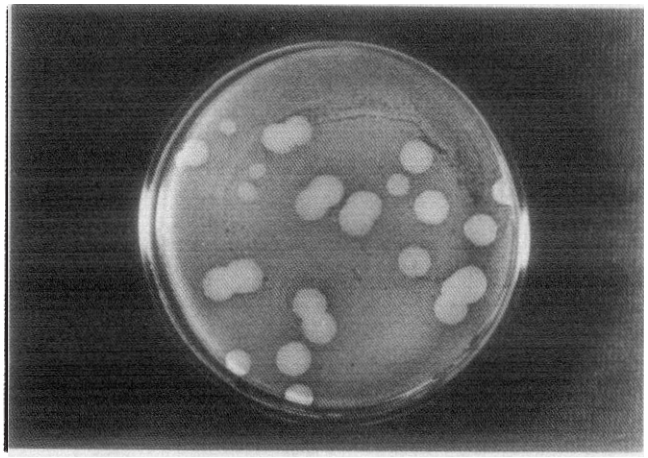

**Fig. 1.** Hollow zones formed on cyanobacterial lawn by the growth-inhibiting activities of bacteria present in Deachung Dam reservoir. *Moraxella* sp. CK-1 was isolated from these zones.

grow in such a hypertonic condition as BG-11C medium supplemented with 1.0% of NaCl. The bacterium grew in BG-11 medium containing casitone, tryptone or peptone, but almost did not grow in commonly used bacterial culture media such as trypticase soy, McConkey and nutrient media even when supplemented with ethanol, casitone, tryptone, peptone, casein or glucose. The optimal growth temperature and pH of the isolate were 37°C and pH 8.0, respectively (Table 1). From these results, the isolate was identified as a *Moraxella* sp. and designated *Moraxella* sp. CK-1 in this report.

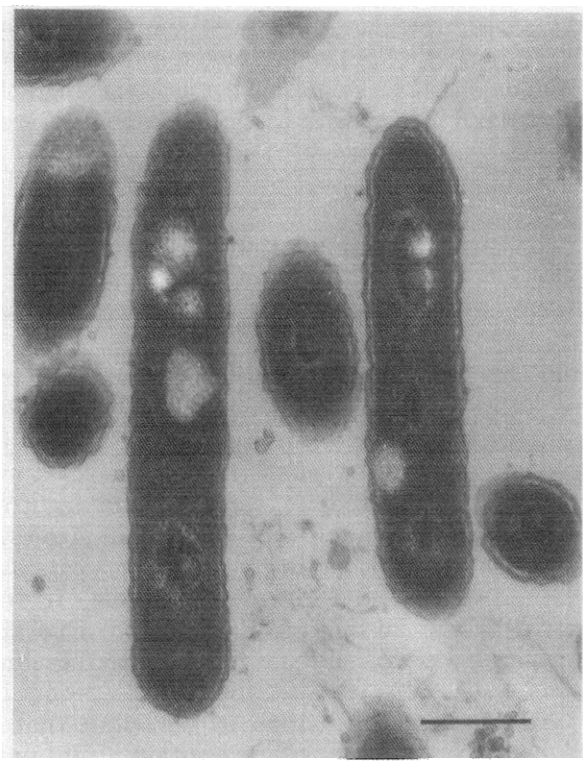

**Fig. 2.** Transmission electron micrograph of *Moraxella* sp. CK-1. Bar represents 0.5 μm.

## Growth-inhibiting activity of *Moraxella* sp. CK-1 against *A. cylindrica*

The growth of *Moraxella* sp. CK-1 was inversely proportional to that of *A. cylindrica* in a mixed-

**Table 1.** Physicochemical characteristics of *Moraxella* sp. CK-1[a]

| Items | Result[b] |
|---|---|
| Optimal growth temperature | 37°C |
| Optimal growth pH | 8.0 |
| Gram staining | − |
| Cell morphology | Rod |
| Cell size | 2.3~2.8× 0.45 μm |
| Colony morphology | |
| Form | Round |
| Margin | Irregular |
| Elevation | Raised |
| Pigment | Yellow |
| Oxidase test | + |
| Catalase test | + |
| Phenylalanine deaminase | − |
| Urease | − |
| Cellulose hydrolysis | − |
| Starch hydrolysis | + |
| Gelatin hydrolysis | + |
| Hemolysis | + |
| Nitrate reduction | + |
| Sensitive to antibiotics | |
| Penicillin (0.1 U/ml, 0.6 μg/ml) | + |
| Chloramphenicol (20 μg/ml) | + |
| Ampicillin (20 μg/ml) | + |
| Carbenicillin (20 μg/ml) | − |
| Streptomycin (20 μg/ml) | − |
| Kanamycin (20 μg/ml) | + |
| Tetracycline (20 μg/ml) | + |
| Maximum NaCl tolerance (%) | 1.0 |
| Growth at 4°C | − |
| Growth on | |
| BG-11 (Casitone) | + |
| BG-11 (Tryptone) | + |
| BG-11 (Peptone) | + |
| NA (Et-OH) | − |
| NA (Casitone) | ± |
| NA (Tryptone) | ± |
| NA (Peptone) | − |
| NA (Casein) | − |
| NA (Glucose) | − |
| Trypticase soy agar | − |
| McConkey | − |
| Blood agar | + |

[a] Each test was performed following the methods in Methods for General and Molecular Bacteriology. (26)

[b] +, positive result; ±, nearly positive result; −, negative result.

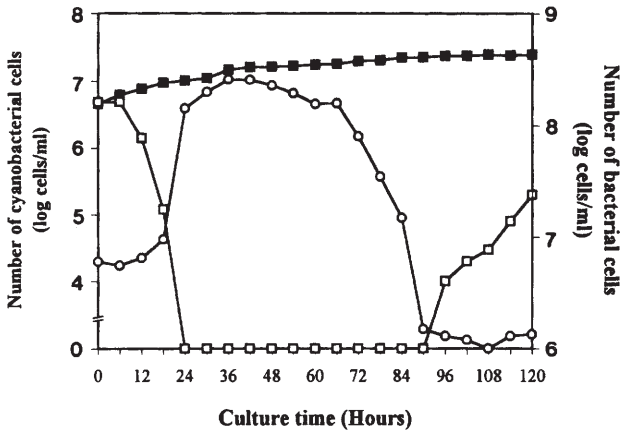


**Fig. 3.** Growth pattern of *A. cylindrica* (□) and *Moraxella* sp. CK-1 (○) in a mixed-culture. *A. cylindrica* and *Moraxella* sp. CK-1 were inoculated in BG-$11_0$ medium to a final concentration of $3.0\times10^6$ cells/ml and $5.0\times10^6$ cells/ml, respectively. The numbers of each microorganism were then counted at intervals during incubation. The growth of *A. cylindrica* in a medium without *Moraxella* sp. CK-1 was used as negative control (■).

culture (Fig. 3). In this condition, the cyanobacterial vegetative cells began to diminish after 6~9 h of incubation and completely disappeared after 18~24 h. This growth-inhibiting activity continued for about 3~5 days and was followed by the redevelopment of cyanobacterial vegetative cells accompanying the decrease in the size of the bacterial population size.

## Action spectrum of *Moraxella* sp. CK-1

*Moraxella* sp. CK-1 showed a broad action spectrum against cyanobacteria (Table 2). This bacterium inhibited the growth of *A. cylindirca*, *A. variabilis*, *A. flos-aquae*, *N. carneum*, *O. animalis* and *Chlorogloea fritschii* completely, and that of *M. aeruginosa*, *M. flos-aquae* and an *Oscillatoria* sp. slightly. However, *Phormidium* sp., *Synechococcus* sp., *S. leopoliensis* and *Synechocystis* sp. were resistant to the the bacterium.

**Table 2.** Action spectrum of *Moraxella* sp. CK-1 against cyanobacteria

| Strains of cyanobacteria | Lytic activity[a] |
|---|---|
| *Anabaena variabilis* (ATCC 29413) | + |
| *Anabaena flos-aquae* | + |
| *Anabaena cylindrica* (IAM M-1) | + |
| *Chlorogloea fritschii* | + |
| *Microcystis aeruginosa* (IAM M-176) | ± |
| *Microcystis flos-aquae* (IAM M-178) | ± |
| *Nostoc carneum* (IAM M-35) | + |
| *Oscillatoria animalis* (IAM M-75) | + |
| *Oscillatoria* sp. | ± |
| *Phormidium* sp. | − |
| *Synechococcus leopoliensis* (*Anacystis nidulans*) (IAM M-6) | − |
| *Synechococcus* sp. | − |
| *Synechocystis* sp. (ATCC 27184) | − |

[a] The lytic activities were determined throuth the agar-spot method and mixed-culture in BG-$11_0$ medium.
+, predominantly active; ±, slightly active; −, inactive.

## Electron microscopic observation of lytic activity

Typical vegetative cells of *A. cylindrica* have a regular appearance, being surrounded by a smooth cell wall and containing various cellular inclusions (Fig. 4A). On treatment with *Moraxella* sp. CK-1, cell walls of A. cylindrica disappeared, but sheathes re-

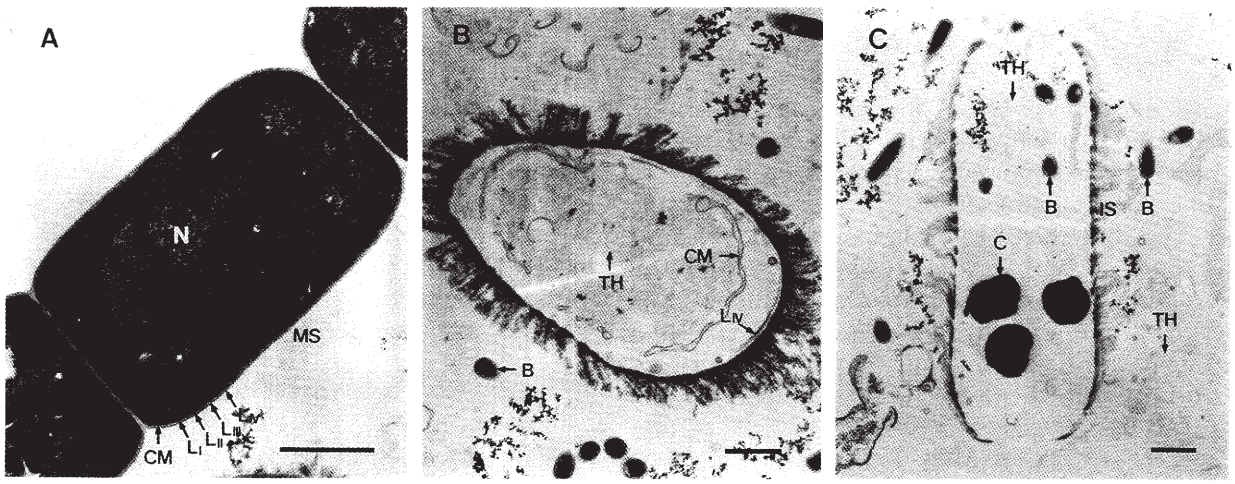


**Fig. 4.** Transmission electron micrographs showing the lysis of *Anabaena cylindrica* cells by *Moraxella* sp. CK-1. Panels: A, Normal cell; B and C, Lysed cell. $L_I$~$L_{IV}$, Cell wall layers; CM, Cell membrane; MS, Mucillageous sheath; IS, Irregular sheath; TH, Thylakoid; C, Carboxysome (polyhedral body); N, Nucleoplasm; B, *Moraxella* sp. CK-1 cell. Bar represents 1.0 μm.

**Table 3.** Growth-inhibiting activities of subcellular fractions of *Moraxella* sp. CK-1 against *Anabaena cylindrica*[a]

| Subcellular fractions | Activity[b] |
|---|---|
| Supernatant | ++ |
| Cell homogenate | + |
| Cytoplasmic fraction | + |
| Periplasmic fraction | − |
| Outer membrane fraction | − |
| Cytoplasmic membrane fraction | − |
| Peptidoglycan associated fraction | − |

[a] Subcellular fractions were prepared following the method of Watt and Clarke (1994). Each fraction was dialyzed against 20 mM HEPES buffer (pH 8.0) and added to BG-$11_0$ mdeium containing viable *Anabaena cylindrica* cells. The activities were determined by the change in the turbidity of culture media. However, the relative activity between each fraction was not measured because the culture media showed different OD values.

[b] ++, predominantly active; +, slightly active; −, non-active

mained in a more electron dense form (Fig. 4B and C). Fig. 4C shows that the polar regions of the cyanobacterial cell are most sensitive to lysis. There were some bacterial cells attached to the cyanobacterial cell (Fig. 4C). The bacteria inside a lysed cyanobacterial cell (Fig. 4C) seem to have entered after the cell ruptured. Disorganized cell contents due to loss of cell wall appeared in the medium leaving a mass of membranous material.

### Subcellular localization of active substances

The extracellular fraction predominantly inhibited the cyanobacterial growth. There were some activities in cytoplasmic and cell homogenate fractions. However, the growth-inhibiting activity did not appear in the periplasm, outer membrane, cytoplasmic membrane and peptidoglycan (Table 3).

## Discussion

We isolated one strain of *Moraxella* sp. which effectively inhibits the growth of a cyanobacterium *A. cylindrica*, and designated it as *Moraxella* sp. CK-1. This bacterium requires specific nitrogen sources such as casitone, tryptone or peptone for the growth.

It is known that bacteria grow proportionally to cyanobacteria in natural aqueous environments (6, 16, 31), but the isolate, *Moraxella* sp. CK-1, and the cyanobacterium A. cylindrica grew in reverse-proportion in mixed-culture. When the growthinhibiting activity was observed under a light microscope, it was noticed that the vegetative cells of cyanobacteria lysed, whereas heterocysts and akinetes dit not. Also the resistant akinetes germinated when bacteria began to diminish. This phenomenon explains the redevelopment of the cyanobacterial population 3~5 days after the complete disappearence of vegetative cells in the mixed-culture. The resistance of heterocysts and akinetes to the lytic activity of *Moraxella* sp. CK-1 seems to be a result of the chemistry of their cell wall compositions (10). Physiological recovery of cyanobacterial vegetative cells, which was observed in *Anabaena inaequalis* treated with *Cellvibrio* sp. (12), was not shown in this study.

Thylakoidal skeletons were stable against lytic activity; no bacteria were seen in the thylakoidal skeletons. There was some contact between bacteria and cyanobacteria viewed in other study (8) when observed under an electron microscope.

Inhibition of cyanobacterial growth by bacteria may be brought about by the excretion of active products (1, 2, 5, 24, 29), by contact mechanisms (7, 8, 25), or by entrapment (1). *Moraxella* sp. CK-1 seems to lyse cyanobacteria through the production of extracelluar products such as enzyme(s). Our results support this view in that clear zones formed around bacterial colonies, that the extracellular fraction predominantly inhibited the algal growth, and that cyanobacterial growth inhibition occured when mixed culture medium was incubated with vigorous shaking as well as in standing culture. *Myxococcus* groups that have been described previously have not lysed cyanobacteria in agitated liquid conditions (7, 8, 25).

*Moraxella* sp. CK-1 showed a wide action spectrum against cyanobacteria as seen in other bacterial pathogens (5, 15, 16, 20). The different sensitivities of cyanobacterial species to the growth-inhibiting activity of *Moraxella* sp. CK-1 seems to be a result of the different cell wall compositions of the cyanobacterial hosts.

*Morarella* sp. CK-1's characteristics such as optimal growth condition of pH 8.0 (this bacteria grew even at pH 10.0 although the exponential growth was delayed compared to that in the optimal condition), inversely-proportional growth with cyanobacteria in mixed-culture, and wide action spectrum indicate the possibility of using this bacterial species to control cyanobacterial blooms. This could be done by introducing bacteraial cultures into water columns or by adding their filtrates to the sites where cyanobacterial blooms are present.

## Acknowledgment

This work was supported by the Korea Science and Engineering Foundation (KOSEF) research grant for the Research Center for Molecular Microbiology, Seoul National University.

## References


1. **Burnham, J.C., S.A. Collart, and B.W. Highison.** 1981. Entrapment and lysis of the cyanobacterium *Phormidium luridum* by aqueous colonies of *Myxococcus xanthus* PCO2. *Arch. Microbiol.* **129**, 285-294.
2. **Burnham, J.C., T. Stetak, and G. Locher.** 1976. Extracellular lysis of the blue-green algae *Phormidium luridum* by *Bdellovibrio bacteriovorus. J. Phycol.* **12**, 306-313.
3. **Carmichael, W.W.** 1994. The toxins of cyanobacteria. *Sci. Am.* **270**, 64-72.
4. **Castenholz, R.W.** 1988. Culturing methods for cyanobacteria. *Methods Enzymol.* **167**, 68-92.
5. **Daft, M.J., S. McCord, and W.D.P. Stewart.** 1973. The occurrence of blue-green algae and lytic bacteria at a waterworks in Scotland. *Water Treat. Exam.* **22**, 114-124.
6. **Daft, M.J., S.B. McCord, and W.D.P. Stewart.** 1975. Ecological studies on algal-lysing bacteria in fresh waters. *Freshwater Biol.* **5**, 577-596.
7. **Daft, M.J. and W.D.P. Stewart.** 1971. Bacterial pathogens of freshwater blue-green algae. *New Phytol.* **70**, 819-829.
8. **Daft, M.J. and W.D.P. Stewart.** 1973. Light and electron microscope observations on algal lysis by bacterium CP-1. *New Phytol.* **72**, 799-808.
9. **Dakhama, A., J. de la Noüe, and M.C. Lavoie.** 1993. Isolation and identification of antialgal substances produced by *Pseudomonas aeruginosa. J. Appl. Phycol.* **5**, 297-306.
10. **Dunn, J.H. and C.P. Wolk.** 1970. Composition of the cellular envelopes of *Anabaena cylindrica. J. Bacteriol.* **103**, 153-158.
11. **Gorham, P.R. and W.W. Carmichael.** 1980. Toxic substances from freshwater algae. *Prog. Water Technol.* **12**, 189-198.
12. **Granhall, V. and B. Berg.** 1972. Antimicrobial effects of *Cellvibrio* on blue-green algae. *Arch. Mikrobiol.* **84**, 234-242.
13. **Kim, M. and Y.K. Choi.** 1994. A new *Synechococcus* cyanophage from a reservoir in Korea. *Viology* **204**, 338-342.
14. **Kiviranta, J., K. Sivonen, K. Lahti, R. Luukkainen, and S.I.N. Helsinki.** 1991. Production and biodegradation of cyanobacterial toxins-a laboratory study. *Arch. Hydrobiol.* **121**, 281-294.
15. **Lee, R.E.** 1980. Phycology, 1st ed., p. 32-65. Cambridge University Press, New York, New York.
16. **Lee, R.E.** 1989. Phycology, 2nd ed., p. 55-101. Cambridge University Press, New York, New York.
17. **Mitsutani, A., A. Uchida, and Y. Ishida.** 1987. Occurrence of blue-green algae and algal lytic bacteria in lake Biwa. *Bull. JSME* **2**, 21-28.
18. **Ohtake, A., M. Shirai, T. Aida, N. Mori, K. Harada, K. Matsuura, M. Suzuki, and M. Nakano.** 1989. Toxicity of *Microcystis species* isolated from natural blooms and purification of the toxin. *Appl. Environ. Microbiol.* **55**, 3202-3207.
19. **Reynolds, C.S. and A.E. Walsby.** 1975. Water-blooms. *Biol. Rev.* **50**, 437-481.
20. **Reyssac, S.J. and M. Pletikosic.** 1990. Cyanobacteria in fish ponds. *Aquaculture* **88**, 1-20.
21. **Rinehart, K.L., M. Namikoshi, and B.W. Choi.** 1994. Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *J. Appl. Phycol.* **6**, 159-176.
22. **Rippka, R.** 1988. Isolation and purification of cyanobacteria. *Methods Enzymol.* **167**, 3-154.
23. **Safferman, R.S. and M.E. Morris.** 1963. The antagonistic effects of actinomycetes on algae found in waste stabilization ponds. *Bacteriol. Proc.* p. 14.
24. **Sallal, A.K.J.** 1994. Lysis of cyanobacteria with *Flexibacter* spp. isolated from domestic sewage. *Microbios* **77**, 57-67.
25. **Shilo, M.** 1970. Lysis of blue-green algae by *Myxobacter. J. Bacteriol.* **104**, 453-461.
26. **Smibert, R.T. and N.R. Krieg.** 1994. Phenotypic characterization, p. 607-654. *In* P. Garhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (eds.), Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
27. **Sneath, P.A.A., N.S. Mair, M.E. Sharpe, and J.G. Holt.** 1989. Bergey' Manual of Systematic Bacteriology, Vol. 2. Willams and Wilkins, Baltimore, Maryland.
28. **Spelhaug, S.R. and S.A. Harlander.** 1989. Inhibition of food-borne bacterial pathogens by bacteriocins from *Lactococcus lactis* and *Pediococcus pentosaceus. J. Food Protec.* **52**, 856-862.
29. **Stewart, J.R. and R.M. Brown.** 1969. Cytophage that kills or lyses algae. *Science* **164**, 1523-1524.
30. **Stewart, J.R. and R.M. Brown.** 1971. Algicidal nonfruiting myxobacteria with high G+C ratios. *Arch. Mikrobiol.* **80**, 176-190.
31. **Stewart, W.D.P. and M.J. Daft.** 1977. Microbial pathogens of cyanophycean blooms, p. 177-218. *In* M.R. Droop and H.W. Kannasch (eds.), Advances in aquatic microbiology. Academic Press, London.
32. **Watt, S.R. and A.J. Clarke.** 1994. Initial characterization of two extracellular autolysins from *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* **176**, 4784-4789.