Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp.

Pathmalal M. Manage[1],*, **Zen'ichiro Kawabata[2]**, **Shin-ichi Nakano[3]**

[1]Department of Life Environment Conservation Sciences, The United Graduate School of Agricultural Sciences, Ehime University, Tarumi 3-5-7, Matsuyama 790-8566, Ehime, Japan
[2]Center for Ecological Research, Kyoto University, Kamitanakami Hirano-cho 509-3, Otsu 520-2113, Japan
[3]Center for Marine Environmental Studies (CMES), Ehime University, 3 Bunkyo-cho, Matsuyama 790-8577, Ehime, Japan

ABSTRACT: A freshwater gliding bacterium, *Alcaligenes denitrificans*, was isolated from a hypereutrophic pond in Japan. This bacterium caused cell lysis and death of some cyanobacterial species, but showed no algicidal effects on the species of chlorophyceae tested. *Microcystis aeruginosa*, *M. viridis* and *M. wesenbergii* were susceptible to the bacterial attack and the growth-inhibiting effect of the bacterium was significant on *M. aeruginosa*, particularly when the alga was in the exponential growth phase. When *A. denitrificans* was 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. *M. aeruginosa* died when *A. denitrificans* was added to the algal culture but not when only the filtrate from the bacterial culture was added. This suggests that extracellular products are not inhibitory to *M. aeruginosa* and that only direct contact between *A. denitrificans* and *M. aeruginosa* was lethal. Thus, we suggest that *A. denitrificans* plays an important role in influencing the growth of *Microcystis* spp. and contributes to the death of *Microcystis* spp. in freshwater environments.

KEY WORDS: Algicidal effect · *Alcaligenes denitrificans* · *Microcystis*

INTRODUCTION

It is now widely accepted that bacteria can determine the physiological state of microalgae. Some bacteria selectively promote bloom formation (Furuki & Kobayashi 1991), while others have algicidal effects and are involved in the termination and decomposition of algal blooms (Fukami et al. 1992). However, the importance of inhibitory bacteria in regulating populations of different algal species has not yet been fully investigated (Fukami et al. 1996).

Gliding bacteria such as *Myxococcus* (Burnham et al. 1981, 1984), *Lysobacter* (Daft & Stewart, 1971, Mitsutani et al. 1987), *Flexibacter* (Gromov et al. 1972), *Myxobacter* (Shilo 1970, Yamamoto & Suzuki 1990), *Alcaligenes* spp., *Flavobacterium* spp., *Pseudomonas* spp. (Yamamoto et al. 1993) and *Cytophaga* (Stewart &

Brown 1969, Yamamoto et al. 1993) all have the ability to lyse blue green algae in freshwater environments. Daft & Stewart (1971) recorded that isolated *Cytophaga* strains have the ability to lyse *Microcystis* spp. and Yamamoto & Suzuki (1990) reported the lysis effect of *Myxococcus fulvus* (S-1-8) on *Microcystis aeruginosa*, *M. wesenbergii* and *M. viridis.* Yamamoto et al. (1993) found that some strains of *Alcaligenes* also lysed *Microcystis* spp., and their studies reported isolation, morphology and the host range of *Alcaligenes.* Thus, the amount of detailed information about the relationship between cyanobacteria and their algicidal bacteria is still limited. We have previously observed the seasonal occurrence of *M. aeruginosa* and of algicidal bacteria which lyse this cyanobacterium in a hypereutrophic pond and suggested the use of algicidal bacteria to suppress blooms of *M. aeruginosa.* While monitoring that situation, we isolated the bacterium *Alcaligenes denitrificans*. In this paper we

---

*E-mail: path@agr.ehime-u.ac.jp

describe some properties of *A. denitrificans* and its growth-inhibiting or algicidal effects on the growth of *M. aeruginosa*, *M. wesenbergii* and *M. viridis*. We also discuss the effect of the bacterium on different growth stages of the 3 *Microcystis* spp., the minimum bacterial density required for rapid lysis, the range of potential target organisms for this bacterium, and its algicidal activity in culture filtrate. As far as we are aware, this is the first report of the algicidal effect of *A. denitrificans* on *Microcystis* spp.

MATERIALS AND METHODS

Organisms. Bacterial strains thought to kill *Microcystis aeruginosa* were isolated from surface water samples collected from the hypereutrophic Furuike Pond in Sancho, Matsuyama City, Ehime Prefecture, Japan, in August 1998. A slightly modified version of the plaque method (Safferman & Morris 1964) was used to isolate the algicidal bacteria. Algal lawns were prepared on MA agar medium (Manage et al. 1999) in petri dishes, using an axenic culture of *M. aeruginosa* (NIES-298). They were inoculated with 1 ml of water sample filtered through a sterilized 0.8 µm Nuclepore membrane filter (Millipore Co.). The *M. aeruginosa* lawns thus treated were incubated at 25°C, under a light intensity of (48.8 to 58.6) µE m^{-2} s^{-1} with a 12 h light:12 h dark photo-cycle for 10 to 12 d, and bacteria were then picked out from a clear plaque, where the *M. aeruginosa* had died out. These bacteria were cultured in modified casitone medium and incubated overnight under dark conditions at 34°C. Each bacterial culture was diluted and 1 ml from each dilution was mixed with 1.5% of modified casitone molten agar medium (38 to 40°C, 20 to 25 ml) and poured onto sterilized petri dishes. The treated petri dishes were incubated in the dark at 34°C for 3 d. Bacteria were re-isolated repeating this spread plate method (Adams 1959, Shilo 1970, Yamamoto & Suzuki 1990). After purification, each isolated strain of bacteria, in the logarithmic growth phase, was inoculated into an axenic culture of *M. aeruginosa* which was previously cultured in MA medium (Ichimura 1978). In this way, 1 strain with a significant inhibitory effect on *M. aeruginosa* was successfully isolated. This bacterium was identified as *Alcaligenes denitrificans* (PROB 94% TYPE GN-OXI[+]) using a Biolog System (Microlog™ Microbial Identification System, Release 4.0, 1990. Biolog, Inc., Hayward, CA, USA). It was maintained on modified casitone medium containing 0.1 g yeast extract, 1 g Difco casitone, 0.1 g MgSO4 7H2O and 1.5 g agar in 100 ml of distilled water (Yamamoto & Suzuki 1990).

Algicidal effects. The algicidal effects of *Alcaligenes denitrificans* on the growth of *Microcystis aeruginosa*

(NIE 298), *M. wesenbergii* (NIE 104) and *M. viridis* (NIE 102) in the lag, exponential and stationary growth phases were investigated. Axenic cultures of these algae were provided by the National Institute of Environmental Studies, Japan. Algal cultures of each species, in MA medium, were appropriately diluted (2 to 6×10^6 cells ml^{-1}) with the same medium and 95 ml aliquots were inoculated into duplicate 250 ml Erlenmeyer flasks. The bacterium *A. denitrificans* was incubated in liquid casitone medium at 34°C overnight. When the cell density reached 1 to 2×10^8 cells ml^{-1}, the culture was then centrifuged (3500 rpm [2200 × *g*] 10 min), washed twice with sterilized MA medium and 5 ml aliquots were inoculated into the algal cultures at final concentrations of 1 to 2×10^4 cells ml^{-1}. Duplicate control cultures were prepared by adding 5 ml of MA medium to 95 ml of the algal culture. The flasks were incubated at 25°C under a light intensity of 48.8 to 58.6 µE m^{-2} s^{-1} with a 12 h light:12 h dark photo-cycle. The cell density of the 3 *Microcystis* spp. was determined using a haemocytometer under a microscope. The decrease rate (*h*) of the cell densities of the 3 *Microcystis* spp. were calculated as $h = \ln(C/C_0)/t$, where C_0 and C are the *Microcystis* cell densities at the beginning and at the end of the time interval *t*, respectively.

Bacterial cell density and algicidal effect. A cell suspension of *Alcaligenes denitrificans* in the logarithmic growth phase was diluted in MA medium, and 90 ml of the bacterial culture, at initial concentrations of 1.5×10^3, 1.5×10^4, 1.5×10^5 and 1.5×10^6 cells ml^{-1} were prepared. We inoculated 10 ml of exponentially growing cultures of each *Microcystis* spp. into these bacterial cultures at a final concentration of 2.0×10^6 cells ml^{-1}. Cultures without the bacterium served as controls. Incubation conditions were the same as described above for algae. The cell densities of *Microcystis* spp. and the bacterium were determined in the haemocytometer and by the spread plate method (Adams 1959), respectively.

Algicidal range of *Alcaligenes denitrificans*. The algicidal range of the bacterium *Alcaligenes denitrificans* was examined by liquid culture and algal lawn assay with 18 species of freshwater phytoplankton. There were 9 species of Cyanophyceae and 9 species of Chlorophyceae (see Table 3). The algal cultures were all axenic except for *A. spiroides*, *A. affinis*, *Aphanizomenon flosaquae*, *Oscillatoria limnetica* and *Anabaenopsis curcularis*. Each algal species was appropriately diluted (2 to 5×10^5 cells ml^{-1}) with the culture medium and 9 ml aliquots were inoculated into duplicate test tubes. The bacterium was grown in the liquid casitone medium described above, and an overnight culture (incubation at 34°C) of 1 to 2×10^8 cells ml^{-1} was diluted, centrifuged and washed twice with sterilized culture solutions. Aliquots of 1 ml were added to the tubes of algae at final

concentrations of 1 to 2×10^4 cells ml^{-1}, and the test tubes were incubated at 25°C under a light intensity of 48.8 to 58.6 µE m^{-2} s^{-1} with a 12 h light:12 h dark photo-cycle. Enumeration of algal cell densities in treated and control tubes was carried out using a haemocytometer and the significant decreases of cell densities in the treated tubes compared to controls were considered to indicate susceptibility to bacterial attack. For the algal lawn assay, the algae were considered to be susceptible when a distinctly clear zone of algal lysis occurred with the addition of the bacterium *A. denitrificans*.

Algicidal activity in culture filtrate. The algicidal effect of dissolved chemical substances derived from *Alcaligenes denitrificans* was examined. We inoculated 2 ml of the bacterium culture, in its logarithmic growth phase, into 300 ml of *Microcystis aeruginosa* culture (2.0×10^5 cells ml^{-1}) in a 500 ml Erlenmeyer flask. After 4 d of incubation the bacterium reached a density of 6.2×10^8 cells ml^{-1} while the cell density of *M. aeruginosa* decreased to 3.6×10^3 cells ml^{-1}. The culture was then filtered through a sterilized 0.1 µm Nuclepore filter, and the filtrate was diluted to final concentrations of 10, 50 and 90 % with autoclaved filtrate, in duplicate, and then inoculated with *M. aeruginosa* (2.0×10^5 cells ml^{-1}). The cell densities of *M. aeruginosa* were determined as described above.

RESULTS

The bacterium isolated from the pond water was identified as *Alcaligenes denitrificans* and showed a significant growth-inhibiting effect on 3 species of *Microcystis* in different growth phases (Fig. 1). When *Microcystis* spp. was in the exponential growth phase, the effect was very pronounced. *M. aeruginosa* was particularly susceptible when *A. denitrificans* was added to the exponential or stationary growth phases and the rate of decrease in the cyanobacterium was significant (Table 1). However, the algicidal effect was not so significant when it was added to the lag growth phase of *M. aeruginosa*, and after an initial slight decrease the algal cell density increased again (Fig. 1a). With

M. viridis, the effects of the bacterium were very similar in all 3 growth phases, but those rates were low when compared with the rate of decrease in *M. aeruginosa* (Table 1, Fig. 1b). In the case of *M. wesenbergii*, the algicidal effect was less than that on the other 2 species and the rates of decrease in each growth phase were lower still (Table 1, Fig. 1c).

Thus, in this study there appeared to be remarkable decreases in cell densities of *Microcystis aeruginosa* at any bacterial inoculum size, after the bacteria cell den-
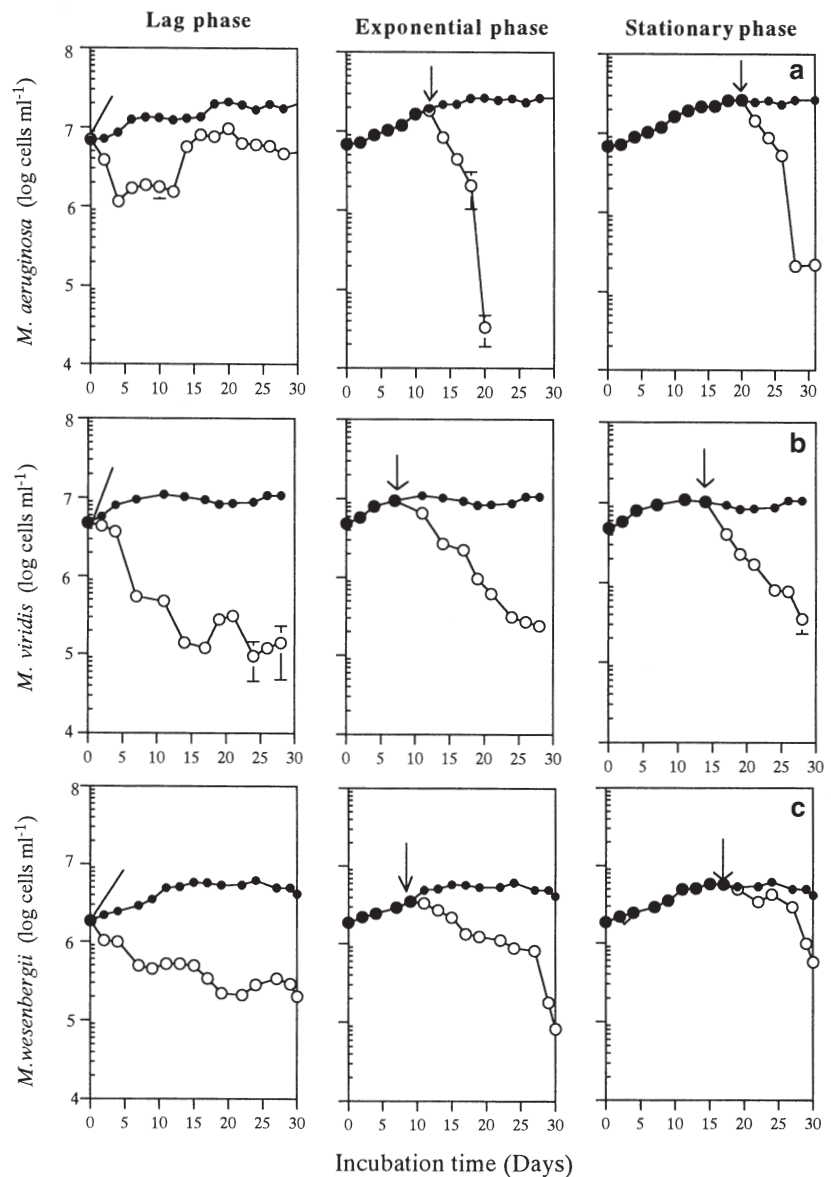

Fig. 1. Effect of the bacterium *Alcaligenes denitrificans* on the growth of (a) *Microcystis aeruginosa*, (b) *M. viridis*, and (c) *M. wesenbergii* in different stages of growth (○). Control without bacterium inoculation (●) is also shown. The time of bacterium inoculation is indicated by an arrow. When error bars are not shown, standard deviation was less than the width of the symbols

Table 1. *Microcystis* spp. Rate of decrease in cell densities in the lag, exponential and stationary growth phases (given as mean values of duplicates)

Growth phase	Days after bacterium inoculation	Decrease rate (d^{-1})	$\pm SD$
M. aeruginosa			
Lag	ND[a]	ND[a]	ND[a]
Exponential	10	0.621	0.030
Stationary	13	0.352	0.020
M. viridis			
Lag	17	0.216	0.009
Exponential	17	0.201	0.006
Stationary	12	0.215	0.008
M. wesenbergii			
Lag	19	0.112	0.007
Exponential	18	0.081	0.019
Stationary	10	0.067	0.005

[a]The decrease rate was not determined, as *M. aeruginosa* cell density increased after a slight decrease

sity reached the order 10^8 cells ml^{-1} (Fig. 2a). The cell densities of *M. aeruginosa* decreased from about 10^6 to 10^4 cells ml^{-1} in 4 d, and continued to decline steeply, except where the initial inoculum concentration of the bacterium was 10^3 cells ml^{-1}. *M. aeruginosa* was undetectable after 10 d of incubation, coincident with a decrease in the cell density of *Alcaligenes denitrificans* (Fig. 2b). The cell density of *M. viridis* also declined at all bacterial inoculum concentrations, although the rates of decrease were lower (Table. 2), and considerable densities of *M. viridis* cells were detected with each bacterial inoculum even after 12 d of incubation (Fig. 2c). In the case of *M. wesenbergii*, after 2 d of incubation the concentrations of each bacterial inoculum increased up to the order of 10^7 cells ml^{-1} (Fig. 2f). The cell densities of *M. wesenbergii* decreased slightly and remained in the order of 10^5 cells ml^{-1} until Day 9, when they decreased more steeply (Fig. 2e) and the bacterial cell density increased to 10^8 cells ml^{-1}. Thus, in this study there was no rapid death of *M. viridis* and *M. wesenbergii* with any inoculum concentration, even after the bacterial cell density reached 10^8 cells ml^{-1} (Fig. 2).

All the Cyanophyceae tested were susceptible to bacterial attack. When the bacterium was placed on a algal lawn of *Microcystis* spp., the test culture produced a gradient of lysis. Under the microscope, we found disintegrating algal cells surrounded by the bacterium, in the center of the lytic zone, where the algal cells were completely destroyed. In an infected liquid culture of algae, we observed gradual decolorization and disintegration of the cells. None of the strains of Chlorophyceae tested were susceptible to bacterial attack, although growth of *Treubaria triappendiculata* and *Tetraedron incus* was suppressed (Table 3).
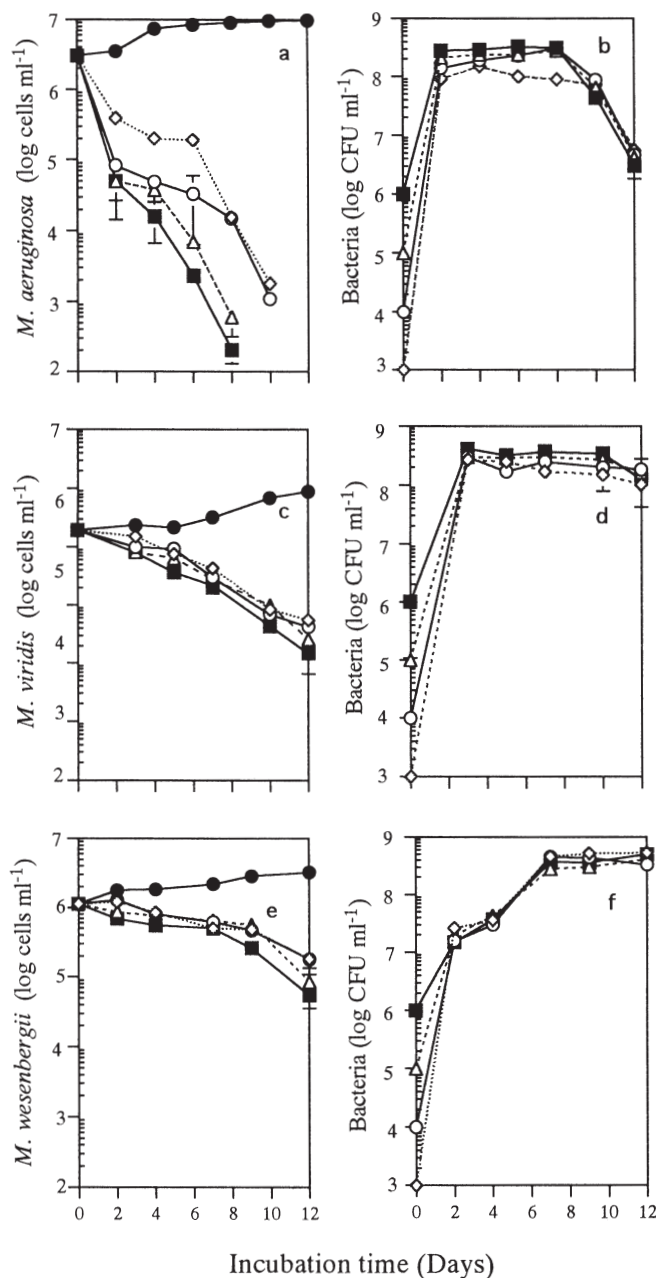

Fig. 2. Changes in cell densities of (a) *Microcystis aeruginosa*, (c) *M. viridis*, (e) *M. wesenbergii* and (b, d, f) the bacterium *Alcaligenes denitrificans* after inoculation with different initial densities (●, control without bacterial inoculation cells ml^{-1}; ◇, 10^3 bacteria CFU ml^{-1}; ○, 10^4 bacteria CFU ml^{-1}; △, 10^5 bacteria CFU ml^{-1}; ■, 10^6 bacteria CFU ml^{-1}). When error bars are not shown, standard deviation was less than the width of the symbols. CFU: colony forming units

The 10 and 50% diluted culture filtrates, in which *Microcystis aeruginosa* had been killed by the bacterium *Alcaligenes denitrificans*, had no inhibitory effect on *M. aeruginosa*. Moreover, *M. aeruginosa* even grew in the presence of 90% culture filtrate without any inhibitory effect being detected (Fig. 3).

Table 2. *Microcystis* spp. Rate of decrease in cell densities (2 d after the bacterium inoculation) in each bacterium inoculum (given as mean values of duplicates)

| Bacterium inoculum size (cells ml^{-1})	——— Decrease rate (d^{-1}) ———		
	M. aeruginosa	*M. viridis*	*M. wesenbergii*
10^3	1.02 ± 0.06	0.08 ± 0.04	0.03 ± 0.006
10^4	1.70 ± 0.66	0.22 ± 0.02	0.06 ± 0.030
10^5	2.05 ± 0.15	0.28 ± 0.08	0.13 ± 0.025
10^6	2.05 ± 0.24	0.28 ± 0.09	0.24 ± 0.046

Table 3. *Alcaligenes denitrificans*. Host susceptibility of the bacterium. +: susceptible; ±: growth affected; −: non-susceptible

Species	Susceptibility
Microcystisa eruginosa (NIES298)	+
Microcystis viridis (NIES102)	+
Microcystis wesenbergii (NIES104)	+
Anabaena flos-aquae (NIES73)	+
Anabaena spiroides (NIES77)	+
Aphanizomenon flos-aquae (NIES81)	+
Anabaena affinis (NIES40)	+
Anabaenopsis circularis (NIES21)	+
Oscillatoria limnetica (NIES36)	+
Treubaria triappendiuculata (NIE394)	±
Tetraedron incus (NIES392)	±
Cateria inversa (NIES422)	−
Chlamydomonas neglecta (NIES439)	−
Pediastrum duplex (NIES210)	−
Scenedesmus acutus (NIES94)	−
Closterium acerosum (NIES124)	−
Micractinium pusillum (NIES151)	−
Staurastrum paradoxum (NIES528)	−

DISCUSSION

Blooms of freshwater cyanobacteria, particularly of the genus *Microcystis*, are a serious indication of freshwater eutrophication and have harmful effects on lake ecology, livestock and, potentially, on human beings (Carmichael et al. 1985, Song et al. 1998). The most direct form of control involves the removal of high nutrient load (Horne & Goldman 1994) and application of chemical algicides, but the latter is potentially damaging to the environment, and there is a risk of accumulation of harmful concentrations in bottom sediments (Mason 1996). An alternative approach to the direct elimination of nuisance algae involves the application of biological control agents that have the ability to destroy or limit the growth of the target algae (Sigee et al. 1999).

Alcaligenes denitrificans is aerobic and is capable of anaerobic growth by denitrification (Holt et al. 1994). The decolorization and disintegration of *Microcystis*
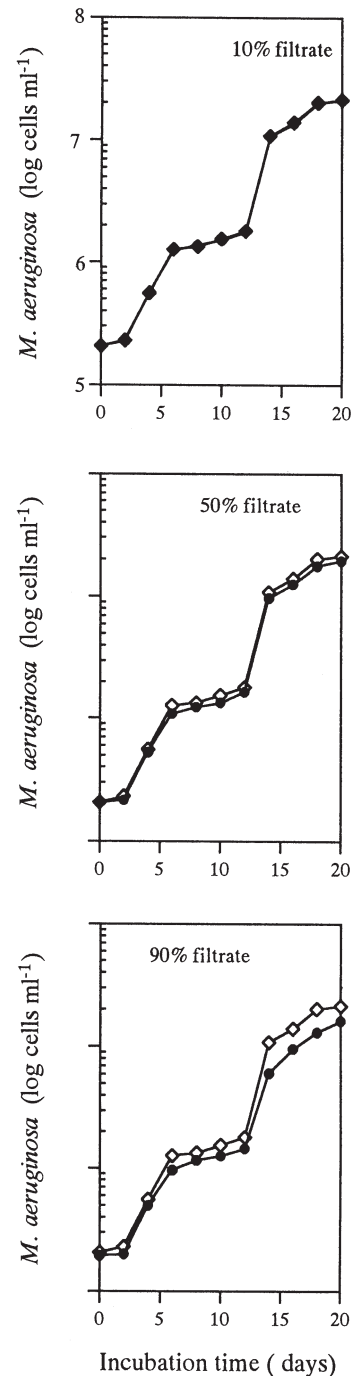
Fig. 3. Effect of filtrate from the culture in which *Microcystis aeruginosa* was killed by the bacterium *Alcaligenes denitrificans* (◇, autoclaved culture filtrate as control; ●, with culture filtrate). When error bars are not shown, standard deviation was less than the width of the symbols

spp. cells after being attacked by the bacterium indicate rapid inhibition of the metabolic activity of the algae. *A. denitrificans* is a Gram-negative rod which moves by slow gliding, has non-pigmented colonies,

does not use carbohydrates on nutrient agar and has an optimum temperature of 20 to 37°C. Thus, most of the characteristic features of *A. denitrificans* are similar to those of other freshwater algicidal bacteria which have been isolated so far (Daft & Stewart 1971, Burnham et al. 1981, 1984, Mitsutani et al. 1987).

The bacterium *Alcaligenes denitrificans* had an algicidal effect on the 3 *Microcystis* species tested (Fig. 1). We detected a significant growth-inhibiting effect on *M. aeruginosa* when in the exponential and stationary growth phases (Fig. 1, Table 1). In contrast, the decrease in cell density in *M. viridis* and *M. wesenbergii* was much less significant, even when they were in the exponential and stationary growth phases. Thus, we suggest that the algicidal effect of the bacterium is stronger for *M. aeruginosa* than for these other *Microcystis* spp. The algicidal effect of the bacterium on *M. aeruginosa* was less significant when it was added to the lag growth phase, and this may suggest that the physiological status of the algae is important for bacterial attack.

The freshwater gliding bacterium *Myxococcus fulvus* (BGO2) could not reduce the densities of the cyanobacteria *Nostoc muscorum* and *Phormidium luridum* when its initial density was lower than 5×10^6 cells ml^{-1} (Fraleigh & Burnham 1988), and at least 10^6 cells ml^{-1} of the bacterium were required to cause significant algal cell lysis (Fraleigh & Burnham 1988). Fallon & Brock (1979) suggested that a decrease in natural algal abundance due to lysis was negligible when lytic bacteria populations were $<10^3$ cells ml^{-1}. In the present study, even when *Alcaligenes denitrificans* was inoculated at low densities (10^3 cells ml^{-1}) it proliferated to 10^8 cells ml^{-1} and caused algal cell lysis in the 3 *Microcystis* spp. *M. aeruginosa* was undetectable 10 d after incubation, while considerable densities of *M. viridis* and *M. wesenbergii* remained with all *A. denitrificans* inoculum concentrations, even after 12 d of incubation. Thus, we suggest that the algicidal effect of *A. denitrificans* is more lethal on *M. aeruginosa* than on *M. viridis* and *M. wesenbergii*.

Rapid lysis of several species of blue-green algae by an isolated *Myxobacter* strain (Shilo 1970), isolated *Cytophage* strains (Daft & Stewart 1971), and an isolated *Flavobacterium* (Yamamoto & Suzuki 1990) have been recorded. In the present study, we also detected that all the tested Cyanophyceae were susceptible to bacterial attack, suggesting that *A. denitrificans* has a wide host range (Table 3).

Two particular kills of phytoplankton by bacteria have been studied in detail with regard to contact (Burnham et al. 1984) and production of inhibitory substances (Day & Withers 1985). Burnham et al. (1981) reported that the cyanobacterium *Phormidium luridum* was lysed by *Myxococcus xanthus* through direct contact. In the present study, death was detected when *Alcaligenes denitrificans* was added to the algal culture but not when only the culture filtrate was added (Figs. 1 & 3). This indicates that *A. denitrificans* did not release extracellular products inhibitory to *Microcystis aeruginosa*, and that the bacterium killed the alga by direct contact. Daft & Stewart (1971) noted that Myxobacteriales lysed cyanobacteria within 20 min of contact, and that there were no detectable extracellular products from the bacterium, suggesting that enzymes on the surfaces of the bacteria might be effective in causing lysis. Shilo (1970), Baker & Herson (1978) and Burnham et al. (1984) suggested that direct contact was ecologically more advantageous for algicidal bacteria, as any chemically mediated substances might easily be diluted by diffusion.

Biological control of cyanobacteria represents a potential short-term measure to reduce or prevent the build-up of nuisance algal populations (Martin et al. 1987). Natural *Microcystis* blooms decreased rapidly when an increase in algicidal bacteria was previously observed in a hypereutrophic pond. Thus, our laboratory studies support the view that application of *Alcaligenes denitrificans* into a natural *Microcystis* bloom may be effective. However, these findings, if extrapolated to natural populations, suggest that, given sufficient lytic bacteria, an algal bloom will not occur unless resistant algae are present.

LITERATURE CITED

Adams MH (1959) Bacteriophage. Inter-Science Publishers, New York, p 450–451

Baker KH, Herson DS (1978) Interactions between the diatom *Thallasiosira pseudonanna* and an associated *Pseudomonad* in a mariculture system. Appl Environ Microbiol 35:791–796

Burnham JC, Collart SA, Highison BW (1981) Entrapment and lysis of the cyanobacterium *Phormidium luridum* by aqueous colonies of *Myxococcus xanthus* PCo2. Arch Microbiol 129:285–294

Burnham JC, Collart SA, Daft MJ (1984) Myxococcal predation of the cyanobacterium *Phormidium luridum* in aqueous environments. Arch Microbiol 137:220–225

Carmichael WW, Tones CLA, Mahmood NA, Theiss WC (1985) Algal toxins and water-based diseases. CPC Crit Rev Environ Control 15:275–283

Daft MJ, Stewart WDP (1971) Bacterial pathogens of freshwa-

ter blue green algae. New Phytol 70:819–829

Day AG, Withers SG (1985) The purification and characterization of a β-glucosidase from *Alcaligenes faecalis*. Biochem Cell Biol 64:914–922

Fallon RD, Brock TD (1979) Lytic organisms and photooxidative effects: influence of blue-green algae (Cyanobacteria) in Lake Mendota, Wisconsin. Appl Environ Microbiol 38:499–505

Fraleigh PC, Burnham JC (1988) Myxococcal predation on cyanobacterial populations: nutrient effects. Limnol Oceanogr 33:476–483

Fukami K, Yuzawa A, Nishijima T, Hata Y (1992) Isolation and properties of a bacterium inhibiting the growth of *Gymnodinium nagasakiense*. Nippon Suisan Gakkaishi 58:1073–1077

Fukami K, Sakaguchi K, Kanou M, Nishijima T (1996) Effect of bacterial assemblage on the succession of the blooming phytoplankton from *Skeletonema costatum* to *Heterosigma akashiwo*. In: Yasumoto TY, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. Intergovernmental Oceanographic Commission of UNESCO, Paris, p 335–338

Furuki K, Kobayashi M (1991) Interaction between *Chattonella* and bacteria and prevention of this red tide. Mar Pollut Bull 23:189–193

Gromov BV, Ivanov OG, Mamkaeva KA, Avilov IV (1972) A flexibacter that lyses blue green algae. Mikrobiologia 41:1074–1079

Holt GJ, Krieg NR, Sneath PHA, Stanley TT, Williams ST (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams & Wilkins, Baltimore, MD

Horne JA, Goldman CR (1994) Limnology, 2nd edn. McGraw-Hill, Inc, New York, p 500–519

Ichimura T (1978) Media for blue-green algae. In: Nishizawa K, Chihara M (eds) Methods in algalogical studies. Kyoritsu, Tokyo, p 294–305 (in Japanese)

Mason CF (1996) Biology of fresh water pollution, 3rd edn. Longman, Essex

Manage PM, Kawabata Z, Nakano S (1999) Seasonal changes in densities of cyanophages infectious to *M. aeruginosa* in a hypereutrophic pond. Hydrobiologia 411:211–216

Martin EL, Leach JE, Kuo KJ (1987) Biological regulation of bloom causing blue-green algae. In: Loutit MW, Miles JAR (eds) Microbial ecology. Springer-Verlag, New York, p 62–67

Mitsutani A, Uchida A, Ishida Y (1987) Occurrence of blue green algae and algal lytic bacteria in Lake Biwa. Bull Jpn Soc Microb Ecol 2:21–28

Safferman SR, Morris ME (1964) Control of algae with viruses. J Am Water Works Assoc 56:1217–1224

Shilo M (1970) Lysis of blue-green algae by *Myxobacter*. J Bact 104:453–461

Sigee DC, Glenn R, Andrews MJ, Bellinger RD, Butlter RD, Epton HAS, Hendry RD (1999) Biological control of cyanobacteria: principles and possibilities. Hydrobiologia 395/396:161–172

Song L, Sano T, Li R, Watanabe MM, Liu Y, Kaya K (1998) Microcystin production of *Microcystis viridis* (Cyanobacteria) under different culture conditions. Jpn J Phycol 46:19–23

Stewart WDP, Brown RM (1969) Ecological studies on algallysing bacteria in fresh waters. Freshw Biol 5:577–596.

Yamamoto Y, Suzuki K (1990) Distribution and algal-lysing activity of fruiting *Myxobacteria* in Lake Suwa. J Phycol 26:457–492

Yamamoto Y, Niizuma S, Kuroda N, Sakamoto M (1993) Occurrence of heterotrophic bacteria causing lysis of cyanobacteria in a eutrophic lake. Jpn J Phycol 41:215–220