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Occurrence of heterotrophic bacteria causing lysis of cyanobacteria in a eutrophic lake

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192 strains of heterotrophic bacteria were isolated from the eutrophic Lake Suwa and examined for their ability to lyse cyanobacteria. Approximately 40% of the strains were able to lyse at least one strain of cyanobacteria. The ratio of cyanobacteria-lytic to total isolates in each month was highest in September, when *Microcystis* blooms began to disintegrate. The genera *Alcaligenes, Flavobacterium/Cytophaga* group and *Pseudomonas* accounted for the great majority of strains capable of lysing cyanobacteria.

Key Index Words: alga-lysing bacteria—cyanobacteria—lake water

The distribution and production of heterotrophic bacteria in natural aquatic ecosystems depend on the supply of organic matter from primary producers, particularly phytoplankton. For instance, Coveney et al. (1977) and Straskrabova and Komarikova (1976), reported that an increased abundance of heterotrophic bacteria follows the development and decline of phytoplankton in reservoirs. Kuroda and Sakamoto (1986) studied seasonal changes of the heterotrophic bacterial community in the eutrophic Lake Suwa and found changes in the composition of the bacterial and phytoplankton community. *Pseudomonas* spp. accounted for a larger percentage of the isolates during June and July when cyanobacterial blooms were present, whereas, *Alcaligenes* spp. dominated in September when the cyanobacteria bloom disintegrated. They suggested that the change of organic matter supply caused the observed changes in the bacterial flora.

Much information has accumulated on the potential role of cyanophages and other lytic agents for regulating cyanobacterial blooms (Burnham et al.1976, Cannon et al. 1976, Daft and Stewart 1971, Daft et al. 1975, Granhall and Berg 1972, Mitsutani et al. 1988, Rein et al. 1974, Shilo 1969, 1970, Yamamoto 1981, Yamamoto and Suzuki 1977, 1990, Yamamoto et al. 1991).

The present study examines the ability of bacteria from Lake Suwa to lyse cyanobacteria.

Materials and Methods

1. *Study area*

Lake Suwa, located in Nagano Prefecture, central Japan (36°3N, 138°E), is a eutrophic lake in which cyanobacteria, *Microcystis* spp., are dominant every year from May to September. The lake is about 13.3 km^2 in area with a maximum depth of 6.8 m and a mean depth of 4.6 m.

2. *Heterotrophic bacteria*

The heterotrophic bacterial strains used in this study were 192 isolates obtained from the surface water of Lake Suwa in 1981 and 1984 (Table 1). The isolates were maintained on

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Table 1. Number of heterotrophic bacterial strains isolated from Lake Suwa during the study.

Generic group	1981					1984			Total numbers
	May	Jun	Jul	Aug	Sep	Jun	oct	Nov	
Alcaligenes	2		3	10	18	3	1	4	41
Flavobacterium-Cytophaga group	6	5	21	13	4	6	1	3	59
Pseudomonas	4	14	18			1	6	2	45
Enterobacter	3	1		3	1	1	1		10
Xanthomonas	4	4					1		9
Moraxella-Acinetobacter group	3	1					1		5
Acinetobacter		1				1	1		3
Agromobacterium-Alcaligenes		1							1
Micrococcus	2								2
Vibrio							2		2
Unidentified	1	7	2	2	1	1	1		15
Total numbers	25	34	44	28	24	13	15	9	192

basal medium containing 200 mg tryptocase (BBL), 100 mg yeast extract (Difco), 50 mg glucose (Wako), 12 g agar in 1 l of distilled water at 20°C.

3. *Cyanobacteria*

The cyanobacteria employed as test organisms for lytic activity were grown in media (Table 2) under continuous cool-white fluorescent lamps (50–75 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 28°C.

Table 2. Media for algal culture.

Species and strains	Medium***
Microcystis aeruginosa Kützing (NIES 99*)	CT
M. aeruginosa f. aeruginosa Kützing (NIES 44)	CT
M. aeruginosa (M-11)	M-11
M. aeruginosa (F-F)	CT
M. flos-aquae (IAM-M-178**)	CT
M. flos-aquae	CT
M. viridis Lemmermann	MA
M. wesenbergii Komárek (NIES 108)	CB
M. wesenbergii Komárek (NIES 112)	CT
Anabaena affinis Lemmermann (NIES 40)	CT
A. circinalis Rabenhorst (NIES 41)	CT
A. solitaria f. solitaria Klebahn	CT
A. cylindrica Lemmermann (IAM-M-1)	MDM
Anacystis nidulans (IAM-M-6)	MDM

* NIES=Microbial Culture Collection at National Institute for Environmental Studies.

** IAM=Institute of Applied Microbiology, University of Tokyo.

*** CT, MA and CB medium: Ichimura (1979)
M-11 medium: Hagiwara et al. (1984)
MDM medium: Watanabe (1960)

4. *Assay method of lytic activity*

Each strain of bacteria was streaked onto

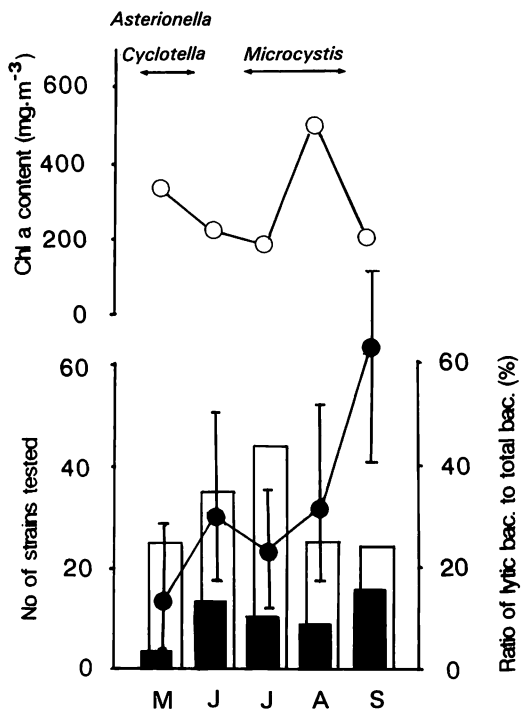

Fig. 1. Seasonal changes in cyanobacteria-lytic heterotrophs, dominant species of phytoplankton and Chl *a* content in 1981. Symbols; □, number of bacterial strains tested; ■, number of lytic strains; ●, percentages of lytic strains; ○, chlorophyll *a* content in the surface water. Bar indicates 95% confidence interval.

1% agar plates of the basal medium and incubated at 25°C. When there was detectable growth, streaks were cut into small blocks and placed on mats of cyanobacterial cells spread onto the surface of Whatman GF/C filters. The results were determined by the appearance of a clear zone formed on the cyanobacterial mats after several days (Yamamoto and Suzuki, 1990). When a tested strain showed lytic ability for at least one strain of cyanobacteria, it was judged to be lytic.

Results

Seasonal changes in the ratios of cyanobacteria-lytic bacteria, the dominant species of phytoplankton, the Chl *a* content (Fig. 1) and environmental parameters were measured in surface water of the lake from May to September in 1981. The pH of the water ranged from 7.1 to 9.6 and the temperature range between 15.5 to 25.6°C. *Cyclotella meneghiniana* Kützing and *Asterionella gracillima* (Hantzsch) Heiberg appeared in April and dominated throughout May. In July *Microcystis* spp. (Aoyama, 1985) were dominant. The chlorophyll *a* content was maximum on August in 1981. The phytoplankton responsible for an increase in the chlorophyll during this period were *M. wesenbergii* and *M. viridis*. These populations declined in September. The contribution of lytic bacteria was low (16%), in May, when the water temperature was low. Subsequently, it increased to 20-25%, but did not show significant variation in the period from June to August. In September when *Microcystis* spp. began to decline, more than 50% of the hetrotrophs were lytic.

The cyanobacteria-lytic spectrum of heterotrophic bacteria and the ratio (as percentage) of the cyanobacteria-lytic heterotrophs to the total number of isolates tested are shown in

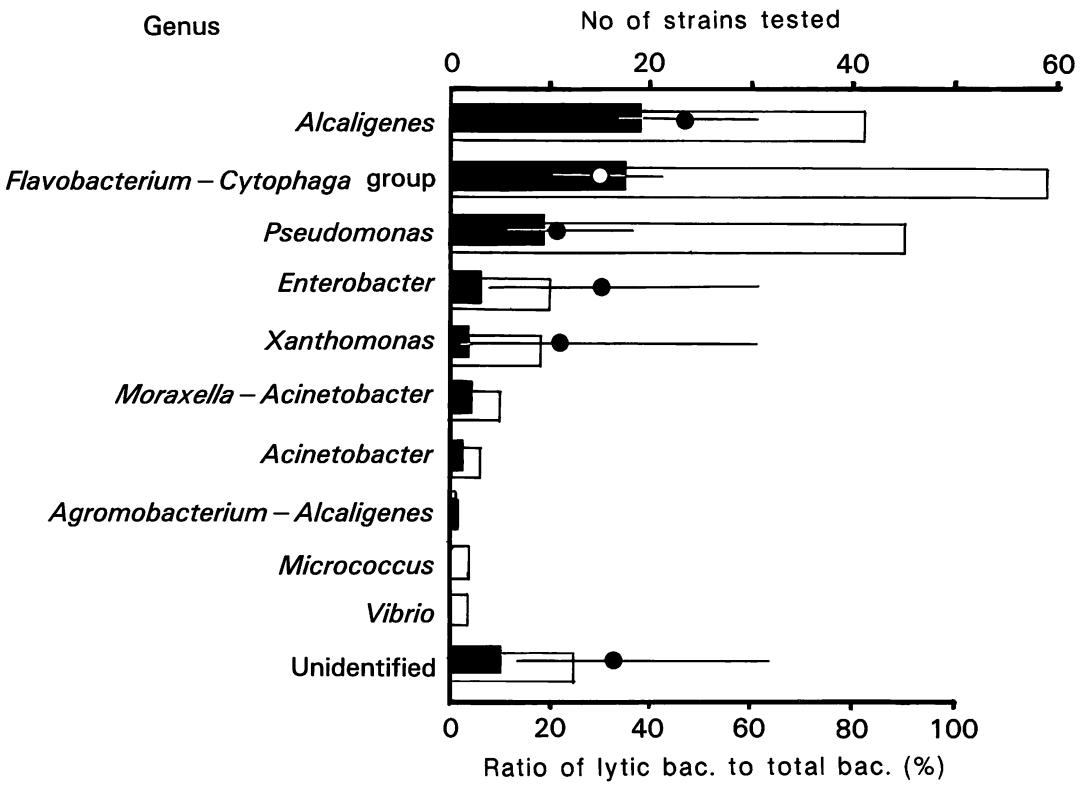

Fig. 2. Occurrence of heterotrophic bacteria lysing cyanobacteria. Symbols; □, number of bacterial strains tested; ■, number of lytic strains; ●, percentages of strains lysing cyanobacteria. Bar indicates 95% confidence interval.

Table 3. Lytic potential of heterotrophic bacteria.

Heterotrophic bacteria	Cyanobacteria strains													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Alcaligenes	[a]41/6[b]	41/2	40/0	37/3	41/3	41/3	41/7	41/1	41/0	41/2	41/6	41/0	40/0	36/0
Flavobacterium-Cytophaga group	50/3	53/0	42/0	48/1	51/1	48/4	50/3	53/0	47/2	50/2	52/1	52/0	52/0	54/0
Pseudomonas	44/0	42/0	42/0	44/0	44/0	41/3	41/3	44/0	42/2	44/0	42/2	42/0	44/0	43/0
Enterobacter	9/0	9/0	9/0	6/2	8/1	8/1	9/0	8/1	9/0	9/0	8/1	9/0	8/0	8/0
Xanthomonas	9/0	9/0	9/0	7/2	9/0	8/1	9/0	9/0	9/0	9/0	9/0	9/0	9/0	9/0
Moraxella-Acinetobacter group	4/1	5/0	5/0	5/0	5/0	5/0	4/1	5/0	5/0	4/1	5/0	5/0	5/0	5/0
Acinetobacter	3/0	3/0	3/0	2/1	2/1	3/0	3/0	3/0	3/0	2/1	3/0	3/0	3/0	3/0
Agromobacterium-Alcaligenes	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0
Micrococcus	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0
Vibrio	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0
Unidentified	12/1	14/0	14/0	10/1	12/0	13/1	11/1	12/0	12/0	8/4	12/0	12/2	11/0	11/0

1) *Microcystis aeruginosa* Kützing (NIES 99); 2) *M. aeruginosa f. aeruginosa* Kützing (NIES 44)=176; 3) *M. aeruginosa* (M-11); 4) *M. aeruginosa* (F-F); 5) *M. elabens* (NIES 42); 6) *M. flos-aquae* (IAM-M-178); 7) *M. flos-aquae*; 8) *M. viridis* (A. Brown) Lemmermann (NIES 102); 9) *M. wesenbergii* Komárek (NIES 108); 10) *M. wesenbergii* Komárek (NIES 112); 11) *Anabaena circinalis* Rabenhorst (NIES 41); 12) *A. solitaria f. solitaria* Klebahn; 13) *A. cylindrica* Lemmermann (IAM-M-1); 14) *Anacystis nidulans* (IAM-M-6).

[a] The total number of heterotrophic bacteria strains tested.

[b] The number of heterotrophic bacteria strains that were active against the test substrate organisms (cyanobacteria).

Figure 2 and Table 3. *Alcaligenes, Flavobacterium/Cytophaga* group and *Pseudomonas* spp. accounted for the great part of strains showing lytic activity against cyanobacteria (Fig. 2). Of 41 strains of *Alcaligenes*, 19 showed lytic ability (46%); 17 strains constituting the 59 *Flavobacterium/Cytophaga* group (29%), and 9 of the 44 *Pseudomonas* spp. (20%) were capable of lysing cyanobacteria. The others did not cause lysis. Although the number of cyanobacteria-lytic heterotrophic bacteria differs from strains to strains, there were almost always at least a few heterotrophic bacteria lytic for *Microcystis* spp. (except strain M-11), *Anabaena circinalis* and *A. solitaria* which occur abundantly in hypereutrophic lakes such as Lake Suwa. No bacterium lytic for soil (*Anabaena cylindrica*) or mesotrophic (*Anacystis nidulans*) isolates was detected.

Discussion

In the present study, a considerable number of bacteria showed lytic activity for cyanobacteria, which occur abundantly in eutrophic lakes, but no bacteria showed it for cyanobacteria originating from the other habitats. Cyanobacteria-lytic heterotrophic bacteria in a eutrophic lake, may perhaps be active only against cyanobacteria occuring in such lakes.

According to Fallon and Brock (1979), the abundance of cyanobacteria-lytic heterotrophic bacteria was correlated positively with cyanobacterial biomass in Lake Mendota, Wisconsin. However, in Lake Suwa surface water and other eutrophic lakes in Japan, cyanobacteria-lytic organisms appear to coincide with the disintegration of cyanobacterial blooms in the fall (Yamamoto 1981, 1988, Yamamoto and Suzuki 1990, Yamamoto et al. 1988). This was also confirmed by the present study; abundant heterotrophs lysing cyanobacteria were also observed when the bloom of *Microcystis* declined (Fig. 1). The cause of the sudden decline of cyanobacterial blooms is not well understood. Increases of cyanobacteria lytic-heterotrophs may play an important role in this process.

As shown in Figure 2, most of the cyanobacteria-lytic bacterial strains consisted of *Alcaligenes*, the *Flavobacterium/Cytophaga* group and *Pseudomonas* spp. These genera are believed to be widely distributed in marine and freshwater environments (Konda 1982, 1985, Starr et al. 1981). *Alcaligenes* spp.

(Day and Withers 1985, Martin et al. 1978) and *Pseudomonas* spp. (Ramasamy and Verachtert 1980) produce exoenzymes such as β-glucosidase, and many *Cytophaga* can produce protease (Christison and Martin 1971) and β-lactam antibiotics (Redhead and Wright 1980) capable of lysing cyanobacteria. β-Glucosidase is one of the broad-specificity enzymes that catalyze the hydrolysis of β-linked glucose carbohydrates and is widely distributed in aquatic environments. Chróst (1989) showed that β-glucosidase activity was low during spring when phytoplankton grew rapidly, began to increase gradually during the bloom break-down and reached the highest values during the late stage of phytoplankton collapse; a similar study has been done in brackish water (Somville, 1984). These enzymes and antibiotics may play a role in the breakdown of organic matter including cyanobacteria.

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山本鎔子*・新妻成一**・黒田伸郎***・坂本　充****：富栄養湖におけるラン藻溶解性細菌について

諏訪湖の表層水から無作為に分離した192株の従属栄養細菌を用いて，ラン藻 *Microcystis* 溶解能の有無を調べた。溶藻能をもつ主な従属栄養細菌は，*Alcaligenes, Flavobacterium/Cytophaga* および *Pseudomonas* 属であった。湖沼中の *Microcystis* ブルームが減少しはじめる9月に分離された株は，その60%が溶藻能を示した。（*214 神奈川県川崎市多摩区東三田　明治大学，**254 神奈川県平塚市東八幡　全農・農業技術センター，***443 愛知県蒲郡市三谷町　愛知県水産試験場，****464 名古屋市千種区　名古屋大学）

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