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Growth Responses of Harmful Algal Species *Microcystis* (Cyanophyceae) under Various Environmental Conditions

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Abstract—*Microcystis* is a flagrant cyanobacterial genus forming heavy blooms in aquatic environments and often cause serious problems in the management of water quality. In Japan, *Micorcystis aeruginosa* and *Microcytis wesenbergii* are commonly found in natural eutrophic water bodies. Although they frequently occur in the same habitat, the mechanisms determining the dominance of the two *Microcystis* species are not yet clearly understood. To clarify the difference of growth characteristics between *M. aeruginosa* and *M. wesenbergii*, we examined their growth rates, using their isolated strains, under various environmental conditions such as water temperature, light intensity and nutrient concentrations. We also examined the effect of grazing on their growth. Growth rate of *M. aeruginosa* was significantly higher than that of *M. wesenbergii* under high temperatures (30 and 35°C). However, growth rates of the two species were similar at lower temperatures (20 and 25°C). No significant difference in growth rates between the two species was found under varying light intensities, nutrient concentrations or the presence of grazers. We suggest that, among the environmental factors examined in the present study, water temperature is the most important factor determining the dominance of the two *Microcystis* species.

Keywords: *Microcystis aeruginosa*, *M. wesenbergii*, harmful algae, water temperature

INTRODUCTION

Microcystis blooms are frequently found at eutrophic ponds. *Microcystis* blooms often consist of multiple species, and dominant species during a bloom frequently changes (Watanabe *et al*., 1986). Some *Microcystis* species produce toxins (Park *et al*., 1998), which have harmful effects not only on domestic animals but also on human (Carmichael, 1992). Various physico-chemical and biological factors may be responsible for the existence of a dominant *Microcystis* species in an environment (Takamura, 1988). *Microcystis* blooms in Japan are usually composed

of *Microcystis aeruginosa* and *M. wesenbergii* (Watanabe *et al.*, 1986). Generally, it has been known that *M. aeruginosa* is toxic, while *M. wesenbergii* is nontoxic (Watanabe *et al.*, 1988; Ozawa *et al.*, 2005). The ecology of *M. wesenbergii* has not yet been fully understood, while *M. aeruginosa* is one of the well studied species (Robarts and Zohary, 1987; Yamamoto and Nakahara, 2005). In natural habitats, dominant *Microcystis* species varies depending on season and/or location. Thus, favorable environmental condition for growth is different for each *Microcystis* species. It is therefore important to know growth characteristics of each *Microcystis* species to understand their succession patterns in relation to environmental conditions. We tested the growth of isolated *Microcystis* strains under various water temperatures, light intensities and nutrient concentrations. We also examined the effect of grazing on their growth.

MATERIALS AND METHODS

The strains of *M. aeruginosa* and *M. wesenbergii* were isolated from Furuike and Ohike Pond (Matsuyama and Toon city, Ehime Pref., Japan). Colonies of *M. aeruginosa* and of *M. wesenbergii* were picked out and incubated at 20°C under a 12:12 L/D cycle at a photon flux density of about 60 μmol m^{-2} s^{-1}. When we had sufficient growth of a *Microcystis* strain, the culture was moved to a 300 ml conical flask containing 200 ml CB medium (Watanabe, 1997). After acclimation of both strains for three weeks, initial colony numbers of both *Microcystis* strains at the beginning of the experiment were adjusted as approximately 15 colonies ml^{-1}. Growths of the two *Microcystis* species were compared under various physico-chemical conditions as follows; light intensity (60, 30 and 0 μmol m^{-2} s^{-1}) and nutrient concentration (CB medium (CB), CB medium without nitrogen (-N), CB medium without phosphorus (-P) and CB medium without nitrogen and phosphorus (-NP)). The results on growth rates of the two *Microcystis* species under changes in water temperature (20, 25, 30 and 35°C) and presence of grazer (without *Daphnia pulex* (control, addition of one individual of *D. pulex* (D1) and addition of three individuals of *D. pulex* (D3) were given in the Imai *et al.* (2009) and Imai (unpublished data), respectively. The zooplankton, *Daphania* species were frequently used in the grazing experiments on *Microcystis* sp. in previous studies (Fulton and Paerl, 1988; Chen and Xie, 2004, Wilson *et al.*, 2006).

All experiments were conducted in triplicate for two weeks. Subsamples were taken every two days, and the cells of *Microcystis* were counted using a haematocytometer after dispersing the *Microcystis* cells by sonication (50W, 30 sec). Growth rates (μ, day^{-1}) of two *Microcystis* species were calculated during the exponential growth phase using following formula:

$$\mu = \ln(N_{t+1}/N_t)/t$$

where N_t and N_{t+1}; the initial and final cell densities (cells ml^{-1}); t; time (hours) of exponential growth phase of *Microcystis*.

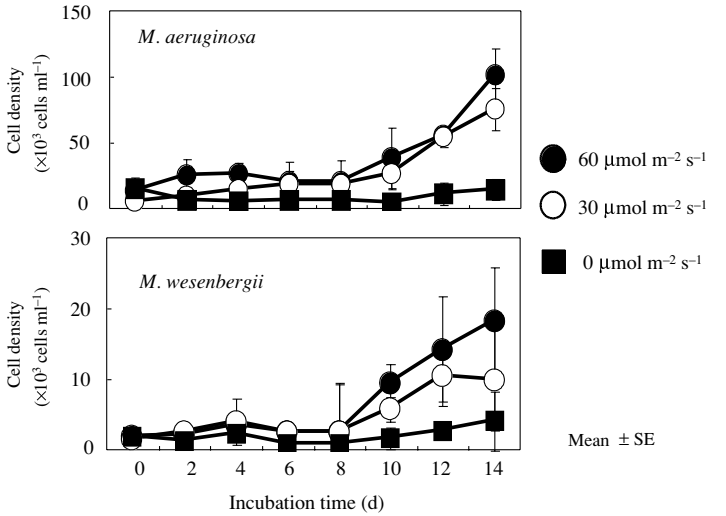

Fig. 1. Changes in abundance of cell density of *M. aeruginosa* (upper) and *M. wesenbergii* (bottom) in laboratory experiment carried out at different light intensities: 60 (black circle), 30 (open circle) and 0 (black square) μmol m^{-2} s^{-1}, respectively. Error bars indicate standard errors.

We performed one-way analysis of variance (ANOVA) to test the differences between growth rates at each environmental factor. When the ANOVA results showed that the variations were significant ($p < 0.05$), we used Tukey's multiple comparison tests to compare the value of each treatment. After Tukey's multiple comparison tests to determine the effect of specific factors, we also used Student's *t*-tests to examine differences between *M. aeruginosa* and *M. wesenbergii*. All statistical analyses were performed with the softwares Microsoft office Excel 2003 and Statcel2.

RESULTS

Cell densities of *M. aeruginosa* and *M. wesenbergii* were higher at 60 and 30 μmol m^{-2} s^{-1} compared to 0 μmol m^{-2} s^{-1} at the experiments of changes in light intensity (Fig. 1). In the changes in nutrient conditions, cell densities of both *Microcystis* species at CB medium were significantly higher than those at -N, -P and -NP medium (Fig. 2). No clear change in cell density between *M. aeruginosa* and *M. wesenbergii* was observed with changes in the conditions of light intensity and nutrient concentrations.

When *M. aeruginosa* was incubated in higher water temperature, mean growth rates at 30 and 35°C were high (30°C; μ = 0.47, 35°C; μ = 0.45, respectively) (Imai *et al.*, 2009, Table 1). We found significant differences in the growth rate of *M. aeruginosa* ($p < 0.05$) between 20 and 30°C with Tukey's multiple comparison tests. On the other hand we could not detect significant differences in the growth rates either between 25 and 30°C or between 30 and

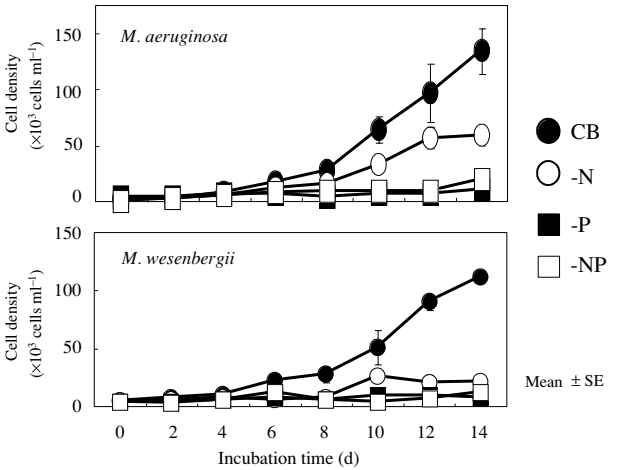

Fig. 2. Changes in abundance of cell density of *M. aeruginosa* (upper) and *M. wesenbergii* (bottom) in laboratory experiment carried out at different nutrient concentrations. CB (black circle), -N (open circle), -P (black square) and -NP (open square) indicates CB medium, CB medium without nitrogen, CB medium without phosphorus and CB medium without nitrogen and phosphorus, respectively. Error bars indicate standard errors.

35°C (Table 2). For *M. wesenbergii*, no significant difference was found in growth rates among the incubation temperatures. The growth rates of *M. aeruginosa* at 30 and 35°C were significantly higher than those of *M. wesenbergii* ($p < 0.05$) at similar temperatures (Student's *t*-test - Table 2). However, we could not find significant difference between *M. aeruginosa* and *M. wesenbergii* at 20°C ($p = 0.348$) and 25°C ($p = 0.127$).

Mean growth rates of *M. aeruginosa* under the light intensities over 60 and 30 μmol m^{-2} s^{-1} were almost identical, though growth rate at 0 μmol m^{-2} s^{-1} was low (Table 1). With regard to the effect of nutrient levels on *Microcystis* growth, it was found that the growth rate of *M. aeruginosa* in CB medium was the highest among the experimental systems (Table 1). For *M. wesenbergii*, the results were similar to those of *M. aeruginosa*. For the effect of grazing on *Microcystis* growth, growth rates of *M. aeruginosa* and *M. wesenbergii* were almost the same in control, D1 and D3, respectively (Imai *et al.*, unpublished data; Table 1). No statistically significant difference in growth rates between the two species was found under varying light intensities, nutrient concentrations and the presence of grazer (Table 2).

DISCUSSION

In natural habitats, *M. aeruginosa* often predominated in higher water temperature compared to *M. wesenbergii* (Ohkubo *et al.*, 1993; Imai *et al.*, 2009). Previous studies demonstrated that optimal temperature of *M. aeruginosa* growth

Table 1. Growth rates of *M. aeruginosa* and *M. wesenbergii* in the laboratory experiment under different environmental factors. Values within parentheses indicate standard error (mean ± SE). Growth rates of two species of *Microcystis* under different water temperatures and presence of grazer were cited from Imai *et al.* (2009) and Imai *et al.* (unpublished data), respectively.

Environmental factors	Treatments	Growth rate of *M. aeruginosa*	Growth rate of *M. wesenbergii*	Literature
Water temperature	20°C	0.20 (0.04)	0.17 (0.09)	Imai *et al.* (2009)
	25°C	0.31 (0.04)	0.16 (0.01)	
	30°C	0.47 (0.02)	0.20 (0.05)	
	35°C	0.45 (0.03)	0.24 (0.01)	
Light intensity	60 μmol m^{-2} s^{-1}	0.20 (0.04)	0.17 (0.09)	This study
	30 μmol m^{-2} s^{-1}	0.21 (0.08)	0.15 (0.08)	
	0 μmol m^{-2} s^{-1}	0.12 (0.01)	0.06 (0.04)	
Nutrient concentration	CB	0.27 (0.01)	0.24 (0.02)	This study
	-N	0.23 (0.05)	0.19 (0.06)	
	-P	0.16 (0.08)	0.09 (0.03)	
	-NP	0.15 (0.08)	0.18 (0.09)	
Presence of grazer	Control	0.29 (0.07)	0.19 (0.05)	Imai *et al.* (unpublished data)
	D1	0.34 (0.06)	0.17 (0.06)	
	D3	0.34 (0.01)	0.20 (0.07)	

Table 2. Statistical analysis on the growth rates of *M. aeruginosa* and *M. wesenbergii*. P-values of *M. aeruginosa* and *M. wesenbergii* indicate the results of ANOVA among each treatment. Asterisk within *P*-value indicates significant differences ($p < 0.05$). Significant differences between *M. aeruginosa* and *M. wesenbergii* show the name of treatments which have significant difference using Student's *t*-tests. N.D. indicates no significant difference ($p > 0.05$).

Environmental factors	Number of treatments	*P*-value of *M. aeruginosa*	*P*-value of *M. wesenbergii*	Significant differences between *M. aeruginosa* and *M. wesenbergii*
Water temperature	4	0.03*	0.24	30°C ($p < 0.05$) 35°C ($p < 0.05$)
Light intensity	3	0.60	0.64	N.D.
Nutrient concentration	4	0.66	0.52	N.D.
Presence of grazer	3	0.72	0.92	N.D.

ranged between 30 and 35°C (Krüger and Eloff, 1978; Van der Westhuizen and Eloff, 1985; Watanabe and Oishi, 1985), and this temperature range overlaps well with our observation. Consequently, it can be suggested that favorable temperature for the growth of *M. aeruginosa* is higher than those of *M. wesenbergii*.

Although we also conducted laboratory experiments under various light intensities, nutrient concentrations and presence of grazer, we did not observe

any remarkable difference between *M. aeruginosa* and *M. wesenbergii*. Honma and Park (2005) reported that nitrate concentration affected the composition of populations containing *M. aeruginosa* and *M. viridis*, while *M. ichthyoblabe* dominated under low phosphate conditions. Moreover, Amemiya *et al.* (1990) reported that *M. aeruginosa* and *M. wesenbergii* showed higher growths at a high light intensity than *M. viridis* in the laboratory experiments. Hence, growth of *Microcystis* may depend on multiple factors and the dominant factor may differ depending on species. However, differences in the growth rates under various light intensities and nutrient concentrations between *M. aeruginosa* and *M. wesenbergii* were insignificant in the present study. We suggest that water temperature is the most important factor determining the dominance either *M. aeruginosa* or *M. wesenbergii*, among the environmental factors examined in the present study.

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