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Effect of different concentrations and ratios of ammonium, nitrate, and phosphate on growth of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* isolated from the Nakdong River, Korea

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Microcystis aeruginosa causes harmful algal blooms in the Nakdong River of Korea. We studied the effect of different concentrations and ratios of ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) on growth of this species in BG-11 medium: each nutrient alone, NO_3^- : NH_4^+ ratio, the N : P ratio with fixed total N (TN), and the N : P ratio with fixed total P (TP). The single nutrient experiments indicated that *M. aeruginosa* had the highest growth rate at NH_4^+ and NO_3^- concentrations of 500 μM, and at a PO_4^{3-} concentration of 5 μM. The NO_3^- : NH_4^+ ratio experiments showed that *M. aeruginosa* had the highest growth rate at a ratio of 1 : 1 when TN was 100 μM and 250 μM, and the lowest growth rate at a ratio of 1 : 1 when the TN was 500 μM. The N : P ratio with fixed TN experiments indicated that *M. aeruginosa* had the highest growth rates at 50 : 1, 20 : 1, and 100 : 1 ratios when the TN was 100, 250, and 500 μM, respectively. In contrast, the N : P ratio with fixed TP experiments showed that *M. aeruginosa* had the highest growth rates at 200 : 1 ratio at all tested TP concentrations. In conclusion, our results imply that the NO_3^- : NH_4^+ ratio and the PO_4^{3-} concentration affect the early stage of growth of *M. aeruginosa*. In particular, our results suggest that the maximum growth of *M. aeruginosa* is not simply affected by the NO_3^- : NH_4^+ ratio and the N : P ratio, but is determined by the TN concentration if a certain minimum PO_4^{3-} concentration is present.

Key Words: ammonium; culture study; *Microcystis aeruginosa*; nitrate; phosphate

INTRODUCTION

The Nakdong River is the longest river in the Republic of Korea, and it supplies drinking water for 13 million people. In recent years, summer blooms of *Microcystis aeruginosa* in this river have occurred more frequently and had longer durations. The Korean government has designated *M. aeruginosa* as a hazardous cyanobacterium that must be controlled because it produces the toxin, microcystin as well as the compounds with unpleasant taste and odor, and because its blooms have caused fish and livestock mortality (Lee et al. 2013, National Institute of Environmental Research 2013, Ahn et al. 2015).

A high P concentration is considered the main cause of *Microcystis* blooms (Kim and Kang 1993, Lee et al. 1998). Schindler et al. (2008) and Schindler (2012) emphasized that N is unlikely to be the limiting factor for blooms because of the presence of N_2-fixing cyanobacterium in water bodies. Moreover, when phosphate (PO_4^{3-}) is released from the sediment during summer, *Microcystis* absorbs and stores it in bottom layer (Jacobson and Halmann 1982, Jung and Cho 2003*a*, 2003*b*), then moves toward the

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Fig. 1. Light microscopy image of a *Microcystis aeruginosa* colony. Scale bar represents: 100 μm.

high-intensity light at the surface, using its gas vacuole, and thereby generates blooms (Reynolds et al. 1981, Conley et al. 2009, Ahn et al. 2015).

Other studies have focused on the importance on N in cyanobacterial blooms (Conley et al. 2009, Dolman et al. 2012, Paerl et al. 2014, Hammed et al. 2016). During summer, the ammonium (NH_4^+) concentration increases from the sediment (Jung and Cho 2003*a*, 2003*b*). Lee and Cho (2006) reported that NH_4^+ affects the size of *Microcystis* cells. Brookes and Ganf (2001) reported that *Microcystis* recovers its buoyancy more quickly when the nitrate (NO_3^-) concentration is higher. Several studies reported that a low NO_3^- : NH_4^+ ratio may promote *Microcystis* blooms (Liu et al. 2011, Dai et al. 2012). Thus, many studies have examined the effect of different concentrations and ratios of N and P on *Microcystis* proliferation and long-term growth (Park et al. 1993, Lee et al. 1998, Nalewajko and Murphy 2001, Vézie et al. 2002, Kim and Hwang 2004, Lee and Cho 2006, Baldia et al. 2007, Chen et al. 2009).

However, most these studies simply examined the effect of NO_3^- and PO_4^{3-}, and did not consider NH_4^+ together (Lee et al. 1998, Brookes and Ganf 2001, Baldia et al. 2007). Furthermore, there are disagreements regarding the importance of the N : P ratio on cyanobacterial blooms (Scheffer et al. 1997, Xie et al. 2003, Kim and Hwang 2004) and about whether N or P has a more significant effect on growth of *Microcystis* (Conley et al. 2009, Schindler 2012, Kim et al. 2013). In particular, the P concentration in the Nakdong River has decreased significantly since 2012 due to the efforts of the Four Rivers Restoration Project to improve water quality. Nevertheless, *Microcystis* blooms have become more serious in recent years and have even begun to occur during winter. Therefore, the studies of other nutrients rather than P have been required (Yu et al. 2014, 2015).

In this study, we aimed to identify the effect of NO_3^-, NH_4^+, and PO_4^{3-} on the growth of *M. aeruginosa*. We examined the effect of different concentrations of each nutrient alone, different NO_3^- : NH_4^+ ratios, and different N : P ratios to clarify the effects of N and P and the role of the N : P ratio on *Microcystis* growth. Finally, we analyzed our results in light of recent data from the Nakdong River to suggest a strategy that may help to control *Microcystis* blooms.

MATERIALS AND METHODS

Strain

We used a *Microcystis aeruginosa* strain that was collected from the Gangjeong-Goryeong weir in Dalseong-gun in Daegu, Republic of Korea on Oct 3, 2013 (Fig. 1). A colony was isolated using the capillary method (Guillard 1973). Identification was confirmed by morphological and molecular analysis, and the strain has been maintained at Kyungpook National University, Korea.

Culture conditions

M. aeruginosa cells were cultured in BG-11 medium (Stanier et al. 1971) (Table 1), but $FeCl_3 \cdot 6H_2O$ was substituted for ferric ammonium citrate. $NaNO_3$, K_2HPO_4, and NH_4Cl were used to regulate the concentrations of NO_3^-, PO_4^{3-}, and NH_4^+, respectively, and other nutrients of BG-11 were controlled. Before each experiment, cells were adapted to a medium without N or P for a week. In each experiment, three 125-mL Erlenmeyer flasks with 100 mL of medium were autoclaved, and *M. aeruginosa* was inoculated at an initial cell density of 5,000 cells mL^{-1}. All experiments were performed at a temperature of 30°C, light intensity of 67 ± 2 μmol photons m^{-2} s^{-1} on 16 : 8 h light-dark cycle, and at pH 8.0. The effects of NH_4^+, NO_3^-, and PO_4^{3-} were tested in four sets of experiments: (1) different concentrations of each nutrient alone; (2) different NO_3^- : NH_4^+ ratios; (3) different N : P ratios with fixed total N (TN) concentration and variable P concentration ("N : P ratio with fixed TN"); and (4) different N : P ratios with fixed total P (TP) concentration and variable N concentration ("N : P ratio with fixed TP"). Furthermore, the NO_3^- : NH_4^+ ratio experiments and the N : P ratio with fixed TN experiments were performed at three levels of TN (100, 250, and 500 μM), and the N : P ratio with fixed TP experiments were performed at three levels of TP (1, 5, and 10 μM). In the all experiments for PO_4^{3-} concentrations and N : P ratios, the NO_3^- : NH_4^+ ratio was 10 : 1. Table 2 summarizes the experimental conditions.

Cell counting and calculation of growth rate

M. aeruginosa cells were counted every 3 days using a light microscope (Axio Imager A1, Zeiss, Jena, Germany) and a hemocytometer (Marienfeld-Superior, Lauda-Königshofen, Germany) at a magnification of 200×. Each experiment lasted 24 days, at which the cells were in the stationary phase or death phase. After cell counting, the number of cells per unit volume and the growth rate were calculated. The maximum growth rate (μ) was calculated as: $\mu = \ln (N_2 / N_1) / (t_2 - t_1)$, where N_2 and N_1 indicate the cell density per unit volume at times t_2 and t_1 during the exponential growth phase (Levasseur et al. 1993).

Statistical analysis

All statistical analyses were conducted using the PASW (SPSS) statistics 18 software (SPSS Inc., Chicago, IL, USA). The results were analyzed by one-way ANOVA, two-way ANOVA, and Duncan's *post-hoc* analysis. The results of all tests were considered significant for a p-value below 0.05.

Table 1. Concentrations of main components and trace metal solution in modified BG-11 medium

Main component	Concentration (g L^{-1})	Trace metal solution	Concentration (g L^{-1})
Citric acid	0.006	H_3BO_3	0.00286
$FeCl_3 \cdot 6H_2O$	0.0029	$MnCl_2 \cdot 4H_2O$	0.00181
$NaNO_3$	1.5	$ZnSO_4 \cdot 7H_2O$	0.00022
K_2HPO_4	0.04	$CuSO_4 \cdot 5H_2O$	0.00008
$MgSO_4 \cdot 7H_2O$	0.075	$Na_2MoO_4 \cdot 2H_2O$	0.00039
$CaCl_2 \cdot 2H_2O$	0.036	$Co(NO_3)_2 \cdot 6H_2O$	0.00005
Na_2CO_3	0.02	-	-
Na_2EDTA	0.001	-	-

Table 2. Experimental conditions used to study growth of *Microcystis aeruginosa*

Experiment		Concentration or ratio	Controlled factor
Each nutrient alone			
NO_3^- and NH_4^+		1, 5, 10, 50, 100, 250, 500 μM	PO_4^{3-}, 230 μM
PO_4^{3-}		0.1, 0.5, 1, 5, 10, 20, 50, 100 μM	N, 17.65 mM, NO_3^- : NH_4^+ = 10 : 1
NO_3^- : NH_4^+ ratio	TN 100 μM	1 : 1, 10 : 1, 50 : 1, 100 : 1, 200 : 1	PO_4^{3-}, 230 μM
	TN 250 μM		
	TN 500 μM		
N : P ratio with fixed TN	TN 100 μM	5 : 1, 10 : 1, 20 : 1, 50 : 1, 100 : 1, 200 : 1	NO_3^- : NH_4^+ = 10 : 1
	TN 250 μM		
	TN 500 μM		
N : P ratio with fixed TP	TP 1 μM	5 : 1, 10 : 1, 20 : 1, 50 : 1, 100 : 1, 200 : 1	NO_3^- : NH_4^+ = 10 : 1
	TP 5 μM		
	TP 10 μM		

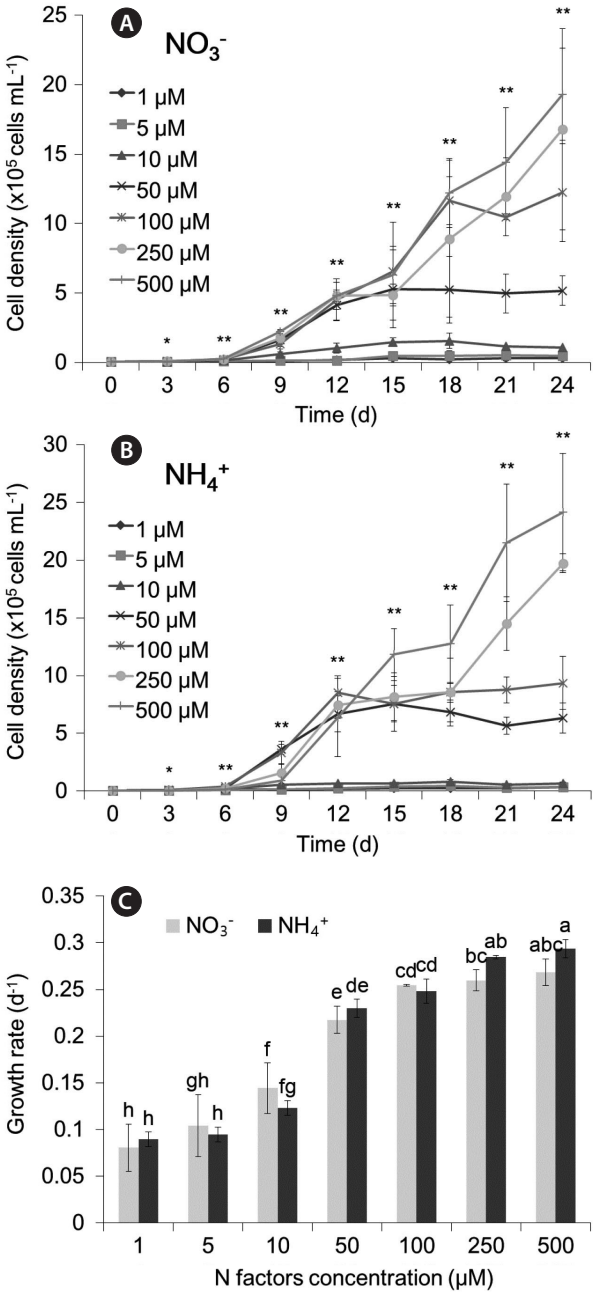

Fig. 2. Growth of *Microcystis aeruginosa* at different NO_3^- concentrations (A) and NH_4^+ concentrations (B), and maximum growth rates under all conditions (C). The PO_4^{3-} concentration was controlled as 230 μM in these experiments. Asterisks above graphs of (A) and (B) denote significant differences in cell density among treatments for the indicated day based on one-way ANOVA (*$p < 0.05$ and **$p < 0.01$). Different letters above bars of (C) denote differences in maximum growth rate based on Duncan's *post-hoc* analysis after an ANOVA revealed difference among conditions ($p < 0.01$). Here and below, error bars denote standard deviations of triplicate samples.

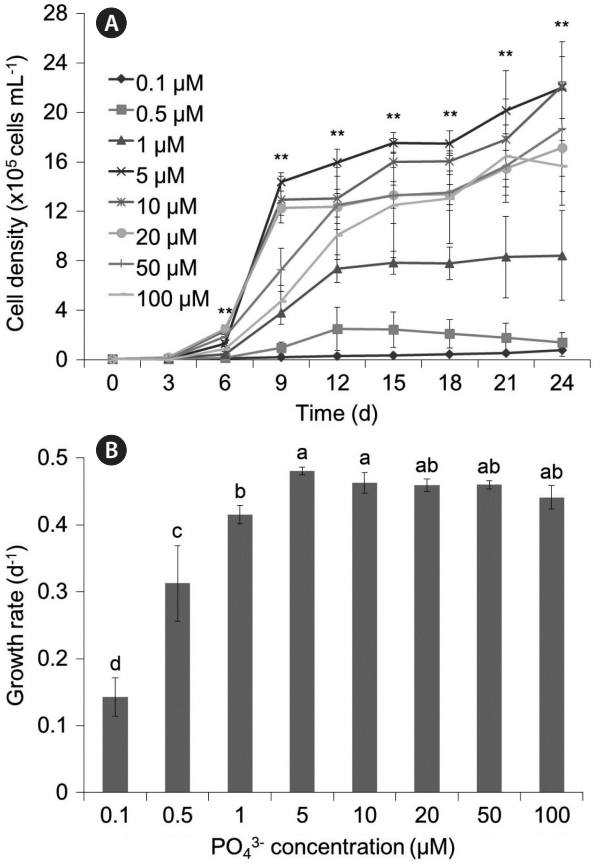

Fig. 3. Growth of *Microcystis aeruginosa* at different PO_4^{3-} concentrations (A) and maximum growth rates at different PO_4^{3-} concentrations (B). The N concentration was controlled as 17.65 mM, and NO_3^- : NH_4^+ ratio was 10 : 1 in these experiments. Asterisks above graphs of (A) denote significant differences in cell density among treatments for the indicated day based on one-way ANOVA (**$p < 0.01$). Different letters above bars of (B) denote differences in maximum growth rate based on Duncan's *post-hoc* analysis after an ANOVA revealed difference among conditions ($p < 0.01$).

RESULTS

Effect of NO_3^- and NH_4^+ concentration

The results for single condition of NO_3^- and NH_4^+ are shown in Fig. 2. The maximum growth rate of *M. aeruginosa* occurred at 500 μM NO_3^- ($\mu = 0.268$ d^{-1}) and 500 μM NH_4^+ ($\mu = 0.294$ d^{-1}) ($p < 0.01$ for each). Although NO_3^- and NH_4^+ concentrations significantly affected the growth of *M. aeruginosa* ($p < 0.01$), but the different forms of N had similar effects on that of this species ($p = 0.388$). Moreover, the results showed that a minimum concentration of 100 μM NH_4^+ or NO_3^- was necessary to grow at least 1,000,000 cells mL^{-1}, a criterion for algal blooms established by the

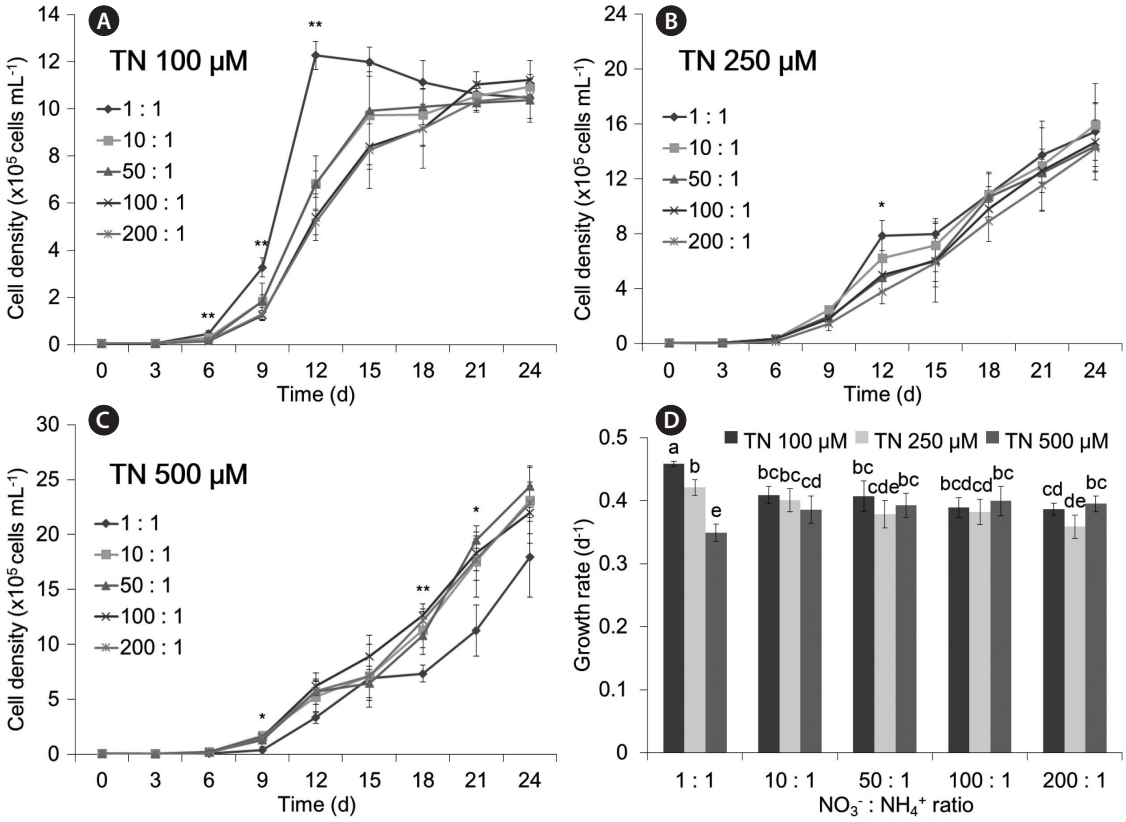

Fig. 4. Growth of *Microcystis aeruginosa* at different NO_3^- : NH_4^+ ratios with a total N (TN) concentration of 100 μM (A), 250 μM (B), and 500 μM (C), and maximum growth rates under all conditions (D). The PO_4^{3-} concentration was controlled as 230 μM in these experiments. Asterisks above graphs of (A), (B), and (C) denote significant differences in cell density among treatments for the indicated day based on one-way ANOVA (*$p < 0.05$ and **$p < 0.01$). Different letters above bars of (D) denote differences in maximum growth rate based on Duncan's *post-hoc* analysis after an ANOVA revealed difference among conditions ($p < 0.01$).

Korean algal-bloom warning system (National Institute of Environmental Research 2013).

Effect of PO_4^{3-} concentration

The results for single condition of PO_4^{3-} are shown in Fig. 3. The maximum growth rate of *M. aeruginosa* was at 5 μM PO_4^{3-} ($\mu = 0.480$ d^{-1}), and growth rates at higher concentrations than 5 μM PO_4^{3-} were also high but slightly lower ($p < 0.01$). In addition, our results showed that a minimum of 1 μM PO_4^{3-} was necessary to grow at least 1,000,000 cells mL^{-1}.

Effect of NO_3^- : NH_4^+ ratio

The results for NO_3^- : NH_4^+ ratio of each TN level are shown in Fig. 4. At a TN concentration of 500 μM, the growth rate of *M. aeruginosa* was the lowest when the NO_3^- : NH_4^+ ratio was 1 : 1 ($\mu = 0.349$ d^{-1}) and the highest when this ratio was 100 : 1 ($\mu = 0.400$ d^{-1}). In contrast, the other experiments showed the highest growth rates for a NO_3^- : NH_4^+ ratio of 1 : 1 for a TN concentration of 100 μM ($\mu = 0.459$ d^{-1}), and 250 μM ($\mu = 0.421$ d^{-1}) ($p < 0.01$ for each comparison). Overall, the TN concentration had a significant effect on the growth of *M. aeruginosa* ($p < 0.05$), but the NO_3^- : NH_4^+ ratio had no such impact ($p = 0.226$). After 24 days, the cell density was not significantly different for the diverse ratios at each TN concentration ($p = 0.411$ for 100 μM TN; $p = 0.880$ for 250 μM TN; $p = 0.204$ for 500 μM TN). In addition, the cell density under each ratio became similar about 1,000,000 cells mL^{-1} when the TN was 100 μM, about 1,800,000 cells mL^{-1} when the TN was 250 μM, and about 2,500,000 cells mL^{-1} when the TN was 500 μM.

Effect of N : P ratio with fixed TN

The results for N : P ratio of each TN level are shown in Fig. 5. The maximum growth rate was at an N : P ratio of

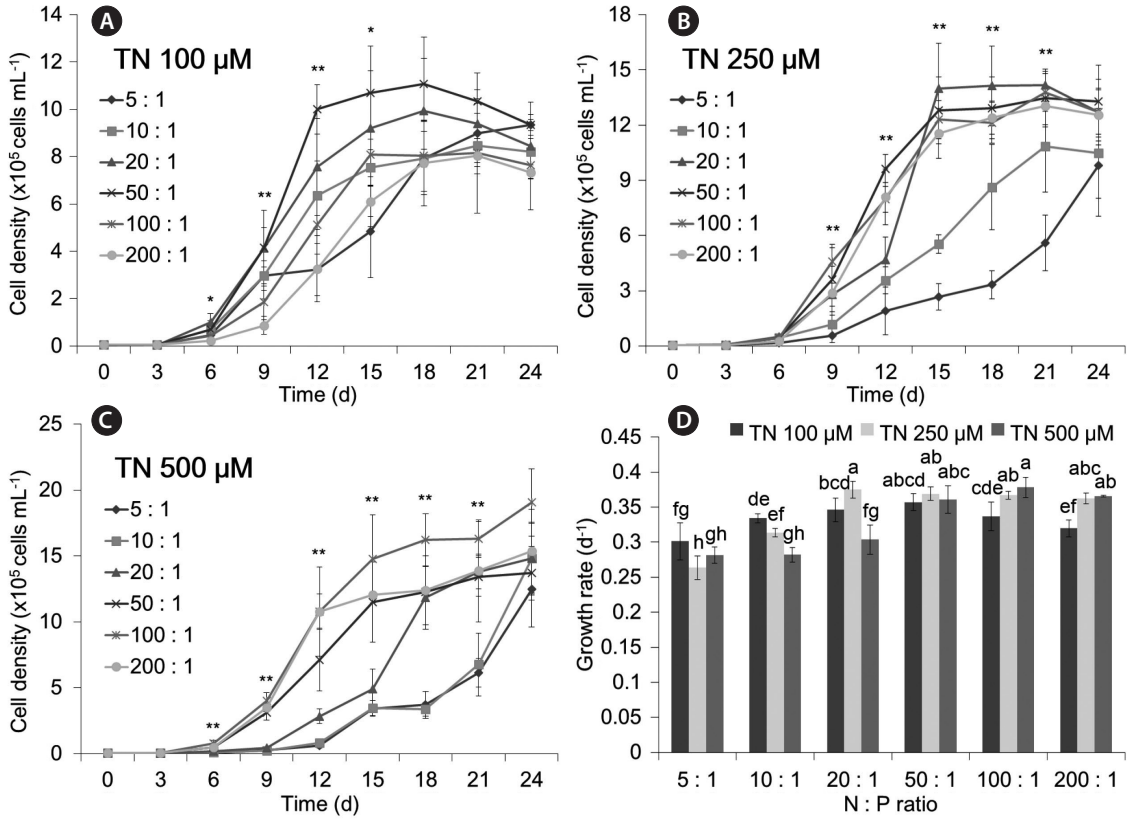

Fig. 5. Growth of *Microcystis aeruginosa* at different N : P ratios with a fixed total N (TN) concentration of 100 μM (A), 250 μM (B), and 500 μM (C), and maximum growth rates under all conditions (D). The NO_3^- : NH_4^+ ratio was 10 : 1 in these experiments. Asterisks above graphs of (A), (B), and (C) denote significant differences in cell density among treatments for the indicated day based on one-way ANOVA (*$p < 0.05$ and **$p < 0.01$). Different letters above bars of (D) denote differences in maximum growth rate based on Duncan's *post-hoc* analysis after an ANOVA revealed difference among conditions ($p < 0.01$).

50 : 1 when the TN was 100 μM ($\mu = 0.357$ d^{-1}), at an N : P ratio of 20 : 1 when the TN was 250 μM ($\mu = 0.375$ d^{-1}), and at an N : P ratio of 100 : 1 when the TN was 500 μM ($\mu = 0.378$ d^{-1}) ($p < 0.01$ for each comparison). Overall, the TN concentration ($p < 0.05$) and the N : P ratio ($p < 0.01$) each had effects on the growth of *M. aeruginosa*. However, after 24 days, the cell density was not statistically different for the diverse N : P ratios ($p = 0.133$ for 100 μM TN; $p = 0.255$ for 250 μM TN; $p = 0.143$ for 500 μM TN). The cell density under each ratio also became similar about 800,000 cells mL^{-1} when the TN was 100 μM, about 1,500,000 cells mL^{-1} when the TN was 250 μM, and about 2,000,000 cells mL^{-1} when the TN was 500 μM.

Effect of N : P ratio with fixed TP

The results for N : P ratio of each TP level are shown in Fig. 6. At all 3 tested P concentrations, the highest population growth and growth rate were at an N : P ratio of 200 : 1 ($\mu = 0.433$ d^{-1} for 1 μM TP; $\mu = 0.447$ d^{-1} for 5 μM TP; $\mu = 0.475$ d^{-1} for 10 μM TP) ($p < 0.01$ for each comparison). Thus, the TP concentration ($p < 0.01$) and the N : P ratio ($p < 0.01$) significantly affected the growth of *M. aeruginosa*.

DISCUSSION

Our experiments indicated that the growth of *M. aeruginosa* increased as the NO_3^- and NH_4^+ concentration increased, in agreement previous studies (Vézie et al. 2002, Lee and Cho 2006, Chen et al. 2009). Rücker and Giani (2004) reported that NH_4^+ had a greater effect than NO_3^- on the early growth of *Microcystis*, and that the growth rate was greater for NO_3^- than NH_4^+. Our results also showed that NH_4^+ promoted slightly faster cell growth initially, but the difference of NO_3^- and NH_4^+ did not differently affected the growth of *M. aeruginosa*.

The results of our experiments on the effect of the PO_4^{3-}

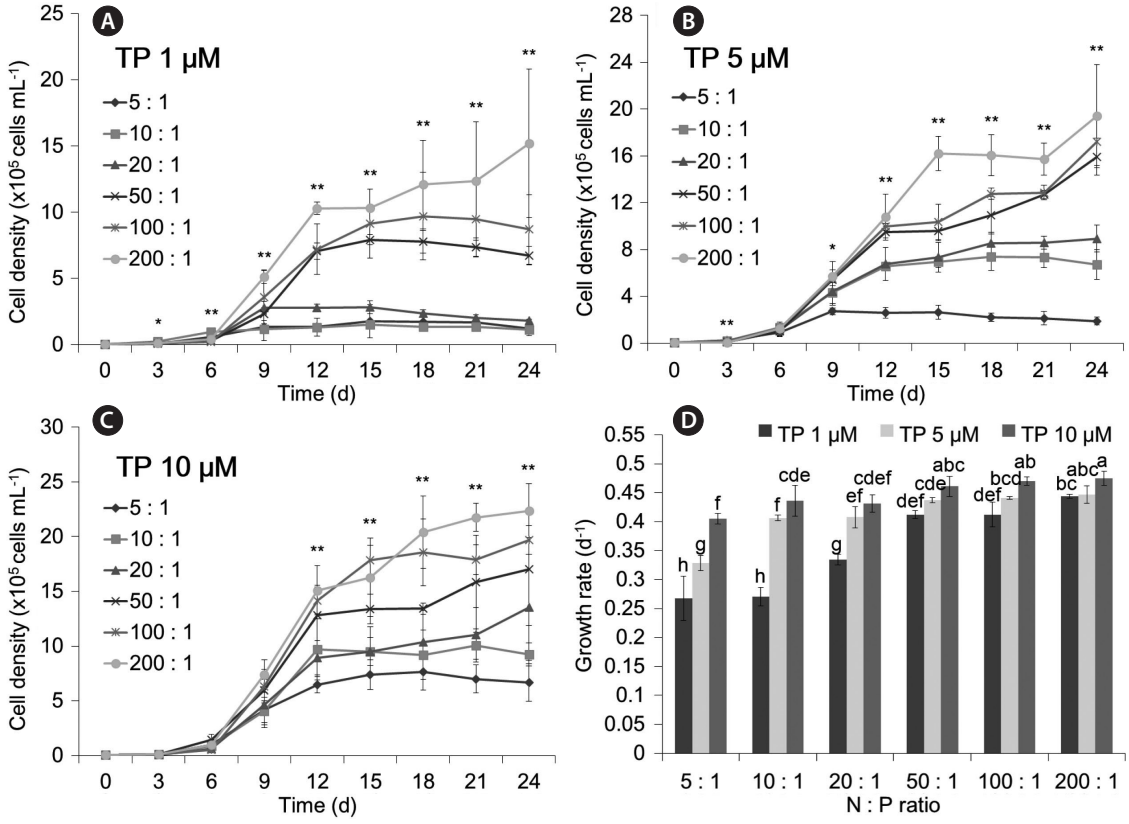

Fig. 6. Growth of *Microcystis aeruginosa* at different N : P ratios with a fixed total P (TP) concentration of 1 μM (A), 5 μM (B), and 10 μM (C), and maximum growth rates under all conditions (D). The NO_3^- : NH_4^+ ratio was 10 : 1 in these experiments. Asterisks above graphs of (A), (B), and (C) denote significant differences in cell density among treatments for the indicated day based on one-way ANOVA (*$p < 0.05$ and **$p < 0.01$). Different letters above bars of (D) denote differences in maximum growth rate based on Duncan's *post-hoc* analysis after an ANOVA revealed difference among conditions ($p < 0.01$).

concentration are in agreement with previous studies (Park et al. 1993, Lee et al. 1998), which reported that a minimum of 0.05 mg L^{-1} (1.5 μM PO_4^{3-}) is necessary for *M. aeruginosa* growth, and 0.3-0.8 mg L^{-1} (10-30 μM PO_4^{3-}) is needed for a high growth rate. Baldia et al. (2007) reported that the growth rate of *M. aeruginosa* increased with N concentrations up to of 620 μM (8.7 mg L^{-1}) and with P concentrations up to 7 μM (0.22 mg L^{-1}). Our results also indicated that maximum growth of *M. aeruginosa* occurred at a relatively high N concentration, but at a relatively low P concentration.

When algae absorb NH_4^+, they immediately incorporate it into amino acids; however, algae can only use NO_3^- after enzymatic reduction to NO_2^- and NH_4^+, and these enzymatic reactions require cellular energy and thereby affect cell growth (Flynn et al. 1997, Flores et al. 2005). Therefore, algae that use NH_4^+ before NO_3^- (Takamura et al. 1987, Liu et al. 2011) may experience inhibition of NO_3^- uptake (Dortch 1990, Dugdale et al. 2007). Similarly, our results showed that *M. aeruginosa* had a lower growth rate at a TN concentration of 500 μM, indicating that decreased NO_3^- absorption in the presence of NH_4^+ seemed to hinder the growth of *M. aeruginosa*. However, Dortch (1990) reported that inhibition of NO_3^- uptake by NH_4^+ and the preference for NH_4^+ uptake vary according to environmental conditions and species. We observed a similar effect for a NH_4^+ concentration below 250 μM, suggesting that NH_4^+ might inhibit NO_3^- uptake at concentrations above 250 μM. However, this effect only occurred during the initial growth phase, and cell densities at 24 days were similar for different TN concentrations. Therefore, our results suggest that the TN concentration has a significant role in the growth of *M. aeruginosa* than the form of N.

We performed two sets of experiments to determine the effect of the N : P ratio on growth of *M. aeruginosa*. The first set of experiments used different N : P ratios with fixed TN, and indicated that the highest growth rate was at an N : P ratio of 20 : 1 when the TN was 250 μM, similar

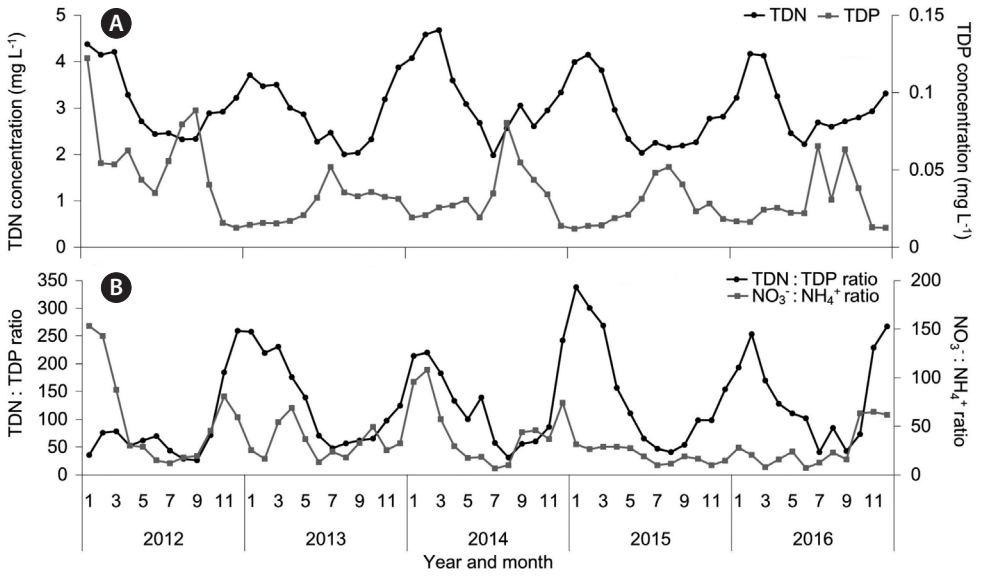

Fig. 7. Average nutrient levels in 5 sites (Changnyeong-Haman Weir, Dalseong Weir, Dodongseowon, Gangjeong-Goryeong Weir, and Hapcheon-Changnyeong Weir) of the Nakdong River (Korea) over the past 5 years. Total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) (A), and the TDN : TDP ratio and the NO_3^- : NH_4^+ ratio (B). Data are from the Water information system (Water Information System, National Institute of Environmental Research, Korea 2016).

to the results of Kim and Hwang (2004) and Lee and Cho (2006), and the highest growth rate was at an N : P ratio of 100 : 1 when the TN was 500 μM, similar to the results of Nalewajko and Murphy (2001). However, the highest population growth rate was at an N : P ratio of 50 : 1 when the TN was 100 μM, in contrast to the results of previous studies (Nalewajko and Murphy 2001, Kim and Hwang 2004, Lee and Cho 2006). Although our results were different from these previous results, their P concentrations were in the range of 2 to 12.5 μM, and the results are similar to the results of our experiments in which PO_4^{3-} alone was varied (Fig. 3). Therefore, it seems that the PO_4^{3-} concentration affects the growth of *M. aeruginosa* rather than the N : P ratio. However, this effect was limited to the initial growth phase and each cell density became similar for different TN concentrations after 24 days.

Our second set of N : P ratio experiments used different N : P ratios with fixed TP, and showed that the highest growth rates occurred at the N : P ratio of 200 : 1 in all case. Kim et al. (2013) reported that there was no significant relationship between growth of *M. aeruginosa* and N : P ratio. Likewise, our results suggested that the N : P ratio itself did not determine the growth of *M. aeruginosa* in that different results were obtained in the two sets of experiments. In addition, some studies reported that differences in the growth of *Microcystis* at different N : P ratios are due to difference in the TP concentration (Scheffer et al. 1997, Kim and Hwang 2004). However, our results suggest that increasing the PO_4^{3-} concentration above 1 μM had no clear effect on growth of *M. aeruginosa*, and only the TN concentration affected cell growth when a minimum PO_4^{3-} concentration was present. In other words, it seems that the absolute amount of N and P, rather than the N : P ratio, affects the growth of *M. aeruginosa*, and the N concentration is more critical than the P concentration.

Unlike other cyanobacterium, *Microcystis* cannot fix atmospheric N_2 and relies on N in the water. However, this species can store extra P within its cells (Reynolds et al. 1981, Xie et al. 2003, Kim and Hwang 2004). Moreover, P-limited conditions have a less effect on small size organisms, such as *Microcystis*, than larger organisms because of the advantage of diffusion through an aqueous boundary layer into cell (Chisholm 1992, Lin et al. 2016). Choi and Kim (2000) also reported that *Microcystis* can produce organophosphate-degrading enzymes, therefore, it can use other forms of P. As a consequence, the P concentration seems less important than the N concentration for promotion of the higher growth of *Microcystis*.

Most of the N and P in the Nakdong River are in the forms of NH_4^+, NO_3^-, and PO_4^{3-}. Moreover, over the past 5 years, this river has had an average the total dissolved N about 215 μM (3.02 mg L^{-1}), and an average total dissolved P of about 1.1 μM (0.035 mg L^{-1}) (Water Information System, National Institute of Environmental Research, Korea

2016). Owing to the efforts of the Four Rivers Restoration Project, the P concentration has remained at about 0.4 μM (0.012 mg L^{-1}) in winter, spring, and late fall. However, the P concentration has increased to about 1.5 μM (0.048 mg L^{-1}) every summer and early fall, when most *Microcystis* blooms have occurred. Moreover, the amount of N, which has a greater effect on growth of *Microcystis* as shown in our results, has remained at 142 μM (1.98 mg L^{-1}) or more in every season (Fig. 7A). Therefore, the concentrations of N and P in the Nakdong River are likely to be sufficient to support summer blooms of *Microcystis*. Furthermore, the Nakdong River has had trends of gradual decrease in the N : P ratio and the NO_3^- : NH_4^+ ratio from winter to summer of each year (Fig. 7B). However, as shown in our results, the change of N : P ratio in this river might not play a vital role in *Microcystis* blooms. Instead, the change of N : P ratio may be just a result from the that of the P concentration and the N concentration. In addition, the change of the NO_3^- : NH_4^+ ratio and the increased level of NH_4^+ in summer may favor the initial growth of *Microcystis* and contribute to explosive its blooms.

In conclusion, we suggest that the PO_4^{3-} concentration in the Nakdong River should be reduced to below 1 μM during summer and early autumn to prevent the formation of *Microcystis* blooms. Alternatively, the N concentration should be regulated to reduce the growth of *Microcystis* while maintaining the P concentration at its current level. However, the physiology of *Microcystis* is incompletely understood, and the N and P cycles are complicated in Nakdong River than in controlled laboratory experiments. Therefore, further studies are required to figure out physiological characteristics of *Microcystis*, to identify exact cause of the change of NH_4^+, NO_3^- and PO_4^{3-}, and to develop effective strategies for control of *Microcystis* blooms.

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