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Effect of nitrate, ammonium and phosphate on the growth and microcystin production of Korean *Microcystis* species

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Abstract

Aim: This study aimed to investigate the effects of nitrogen (NO₃-N, NH₃-N) and phosphorus (PO₄-P) on the growth and microcystin production of two bloom-forming *Microcystis* species (toxic *M. aeruginosa* MAHC160824 and non-toxic *M. viridis* MVHC160824).

Methodology: The two *Microcystis* species were isolated from the lower reaches of the Nakdong River, South Korea. In the culture experiments, the average nutrient concentrations (NH₃-N, NO₃-N and PO₄-P) at which *Microcystis* appeared (> 15°C) was used as control medium. Different concentrations of NH₃-N, NO₃-N and PO₄-P were then employed in nutrient testing (control, vs. 4 times and 16 times higher than the control). Microcystin levels were measured using a UPLCTM (LC MS/MS) system.

Results: Both toxic and non-toxic *Microcystis* strains exhibited a maximum cell density at 30°C and a maximum growth rate at 25-30°C. In the nutrient addition assays, the maximum growth of two *Microcystis* species were found at nutrient concentrations 4 to 16 times higher than the control (NH₃-N: 0.468 mg l⁻¹, PO₄-P: 0.100 mg l⁻¹, NO₃-N: 32.5 mg l⁻¹). The highest microcystin production levels were found under optimal growth conditions. The microcystin levels of toxic *M. aeruginosa* MAHC160824 were below the detection limit despite a higher number of cells (> 300,000 cells ml⁻¹) at the same nutrients concentrations as those found in raw water from the Nakdong River.

Interpretation: Higher production of microcystin occurs when there is an increase in NH₃-N and PO₄-P within a restricted range in toxic species *M. aeruginosa* MAHC160824, else the production is low.

Keywords: Microcystins, *Microcystis* species, Nakdong River, Nitrogen, Phosphorus

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Introduction

Climate change, global warming, urbanization and industrialization has numerous deleterious impacts on the environment globally, one of them include higher occurrence of toxic cyanobacteria in freshwater ecosystems (Sabour *et al.*, 2002; Kemp and John, 2006). Cyanobacterial blooms depletes dissolved oxygen and produces toxins and odor leading to direct and indirect damage to aquatic organisms and humans. Toxic cyanobacteria produces cyanotoxins such as microcystin, anatoxin-a and cylindrospermopsins that can interact with environmental conditions and pose a negative effect on human body, causing diarrhea, vomiting, headaches and skin disease. As such, to ensure safety of drinking water, the World Health Organization has proposed a permissible limit of $1 \mu\text{g l}^{-1}$ for most toxic microcystin, microcystin-LR (WHO, 2017). *Microcystis* is a common toxic cyanobacterium that produce microcystins, and causes aquatic blooms during summers in South Korea (Lee *et al.*, 2008; Lee *et al.*, 2018). Generally, the production of microcystins is determined by environmental factors (Hesse *et al.*, 2001; Long *et al.*, 2001), and *Microcystis* is reported to exhibit species and strain specific growth and toxin production based on environmental factors such as temperature, light intensity and nutrient concentration (Graham *et al.*, 2004; Giani *et al.*, 2005). As a result, microcystin levels can be detected depending on the composition of *Microcystis* species in an ecosystem based on their physiological and ecological responses to various environmental factors (Via-Ordorika *et al.*, 2004; Lee *et al.*, 2008; Lee *et al.*, 2018). Particularly, in temperate regions, the ratio of toxic and non-toxic *Microcystis* species vary with water temperature and nutrient concentration, which affects the temporal and spatial distribution of microcystins (Graham *et al.*, 2004; Giani *et al.*, 2005; Davis *et al.*, 2009). For example, it has been reported that species that can potentially produce microcystins exhibit an increase in frequency and composition in water bodies subject to eutrophication (Rantala *et al.*, 2006). Of particular concern in this regard is nitrogen and phosphorus, which can enter water bodies from point or non-point pollutants, and facilitate *Microcystis* blooms leading to higher production of microcystins (Harke *et al.*, 2012). Indeed, phosphorus and various forms of nitrogen have been identified as main factors affecting the growth of *Microcystis* and microcystin production (Paerl *et al.*, 2011). Although previous studies on microcystin production have been conducted, species or strain specific research related to growth and microcystin production of *Microcystis* are meagre. In Korea, the Nakdong River supplies drinking water to about 13 million people in major cities such as Daegu and Busan. Eight multipurpose weirs have been built along the Nakdong River, increasing the water retention time and consequently promoting *Microcystis* blooms and anthropogenic eutrophication in the middle and lower reaches of the river. Two bloom-forming *Microcystis* species (*M. aeruginosa* and *M. viridis*) responsible for these blooms in the Nakdong River were isolated

in the present study. Culture experiments were conducted to evaluate the characteristics of microcystin production in relation to growth response to variation in water temperature and nitrogen and phosphorus concentration.

Materials and Methods

Sampling, identification and isolation: The samples were collected at the Hapcheon-Changnyeong weir section of the Nakdong River ($35^{\circ}36'45.15''\text{N}$, $128^{\circ}21'25.43''\text{E}$). Using Pasteur pipettes, a single culture could be obtained by re-isolating species from the sample several times. Species were identified using a light microscope (Axio Imager M2, Zeiss, Germany), based on Komárek and Anagnostidis (2008). The *mcyA* gene was identified to distinguish the toxic and non-toxic species. The primers used were MSF (5'-ATCCAGCAGTTGAGC AAGC-3') and MSR (5'-TGCAGATAACT CCGCAGTTG-3') (Tillett *et al.*, 2001).

Experimental conditions: All culture experiments were conducted using CB medium in 250 ml flasks. Unialgal stock cultures were maintained in CB medium (adjusted to pH 9.0 with a carbon free NaOH buffer) at $25 \pm 1^{\circ}\text{C}$ and a light intensity of ca. $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ under a 16:8 light/dark cycle. Cells of two species in their exponential growth phase were inoculated into each test medium to produce a density of approximately $2,000 \text{ cells ml}^{-1}$. For culture experiments, clones were selected from the stock cultures during the exponential growth phase. Incubation experiment was conducted at 15°C , 20°C , 25°C and 30°C to identify differences in growth and microcystin production according to temperature. For nutrient testing, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were adjusted based on the composition of CB medium, and the average nutrient concentration during *Microcystis* appeared ($> 15^{\circ}\text{C}$, three-year average nutrients concentrations is $\text{NO}_3\text{-N } 2.033 \text{ mg l}^{-1}$, $\text{NH}_3\text{-N } 0.117 \text{ mg l}^{-1}$ and $\text{PO}_4\text{-P } 0.025 \text{ mg l}^{-1}$) was used as control medium (Table 1). Different concentrations of $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were then employed for testing (control, vs. 4 times and 16 times higher than the control) $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ + KNO_3 , NH_4Cl and $\beta\text{-Na}_2$ glycerophosphate were used as source for $\text{NO}_3\text{-N}$, $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$.

Nutrient testing lasted for three weeks under the following conditions: pH 9.0, $30 \pm 1^{\circ}\text{C}$ and ca. $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a 16:8 light/dark cycle. All experiments were carried out in triplicate and cell numbers were determined using a Sedgewick Rafter Chamber following sonication (30 W, 10 sec; ULH-700S, Jeiotech, Korea). The growth rate was calculated by the equation $\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$, where N_2 and N_1 are the number of cells during growth period at time t_2 and t_1 .

Microcystin analysis: After incubating toxic *Microcystis aeruginosa* MAHC160824, cell samples were harvested from the lag phase (6 day), exponential phase (12 day) and stationary phase (18 day) and used for toxin analysis. For total microcystin analysis, 40 ml of samples were harvested from each growth

Table 1 : Composition of CB medium and control medium used in this study

Component	CB medium	Control medium
Ca(NO ₃) ₂ · 4H ₂ O (mg l ⁻¹)	150	12.16
KNO ₃ (mg l ⁻¹)	100	6.57
MgSO ₄ · 7H ₂ O (mg l ⁻¹)	40	40
⊖-Na ₂ glycerophosphate (mg l ⁻¹)	50	0.25
Vitamin B ₁ (mg l ⁻¹)	0.01	0.01
Vitamin B ₁₂ (mg l ⁻¹)	0.0001	0.0001
Biotin (mg l ⁻¹)	0.0001	0.0001
Bicine (mg l ⁻¹)	500	500
NH ₄ Cl (mg l ⁻¹)	0	0.38
PIV metal mixture (ml)	3	3
pH	9.0	9.0
PIV metal mixture (mg l ⁻¹)		
FeCl ₃ · 6H ₂ O	196	
MnCl ₂ · 4H ₂ O	36	
ZnSO ₄ · 7H ₂ O	22	
CoCl ₂ · 6H ₂ O	4	
Na ₂ MoO ₄ · 2H ₂ O	2.5	
Na ₂ EDTA · 2H ₂ O	1000	

phase. For total microcystin analysis, 20 ml of samples containing cell pellets were homogenized using sonication (30 W, 10 sec; ULH-700S, Jeiotech, Korea) and the homogenized samples were then filtered through a GF/C filter (1.2 µm). For extracellular microcystin analysis, 20 ml of culture samples were directly filtered through a GF/C filter (1.2 µm) without sonication. Following this, total and extracellular microcystin levels were measured using an ultra performance liquid chromatography (UPLC™) system (ACQUITY UPLC I-Class, Waters Corporation, MA, USA) after filtering through a syringe filter (0.2 µm). Microcystin standards (-LA, -LR, -RR, -YR) were used to analyze the four types of microcystin present in the samples. These were assessed using the UPLC™ system, with 10 µl samples injected into the Waters BEH C18 column of a 0.2 µm precolumn filter. The column was maintained at 40°C and flow rate of 400 µl min⁻¹. The mobile phase consisted of water (A) and acetonitrile (B), and was set to 0% B for 0.5 min, 30% B for 2.5 min, 65% B for 4.5 min, 40% B for 5 min, 80% B for 6 min, 0% B for 6.5 min and 0% B for 8 min. Microcystins were qualitatively and quantitatively analyzed using a Xevo TQ-S detector system (Waters Corporation, Manchester, UK) with electrospray ionization (ESI) connected to the UPLC™ system. At positive mode (ES+), multiple action monitoring (MRM) was monitored using collision of argon gas with the samples. The flow rate of argon gas was 150 hr⁻¹ at 150°C. The flow rate of nitrogen gas, which was used as a de-solvent, was 800 hr⁻¹ at 400°C. MassLynx software (Waters Corporation) was used to analyze the results.

Statistical analyses: Statistical significance among the explanatory variables was confirmed by one-way ANOVA and post hoc Tukey's honestly significant difference (HSD) tests using SPSS 12.0 software.

Results and Discussion

The genus *Microcystis* was dominant in 78 of the 240 phytoplankton samples collected from the lower reaches of Nakdong River (water.nier.go.kr). At this point, the cell density was 309,800 cells ml⁻¹ at water temperature of 30.6°C. In the present study, the density of two bloom-forming *Microcystis* strains were 17,500 cells ml⁻¹ at 15°C and 207,500 cells ml⁻¹ at 30°C in the control medium which maintained the same nutrient concentrations as those found in field water. These results indicate that a higher water temperature can cause *Microcystis* blooms involving both toxic and non-toxic species. The cell density of toxic *M. aeruginosa* MAHC160824 and non-toxic *M. viridis* MVHC160824 increased with temperature over the tested range, and the maximum cell density 207,500 cells ml⁻¹ and 107,000 cells ml⁻¹ was observed at 30°C (Fig. 1, A1, B1). The maximum growth rates of both species were observed at 25°-30°C (Fig. 1, A2, B2).

The following nutrient experiments were conducted at 30°C. Both toxic *M. aeruginosa* MAHC160824 and non-toxic *M. viridis* MAHC160824 attained the growth threshold at PO₄-P concentration, four times higher than that of the control (Fig. 2, A1-A4). For the given NH₃-N range, the two strains attained the maximum growth at NH₃-N concentration four times higher than that of the control (Fig. 2, B1-B4). The two *Microcystis* strains exhibited a higher cell density with increasing NO₃-N concentration, and also showed a maximum cell density and growth rate at a concentration 16 times higher than that of the control (Fig. 2, C1-C4).

In the nutrient testing, the two *Microcystis* strains reached the growth threshold four times higher the concentration than that

Fig. 1 : Growth characteristics and growth rates of toxic *M. aeruginosa* MAHC160824 (A1, A2) and non-toxic *M. viridis* MVHC160824 (B1, B2) according to temperature. All values are given as the mean $\pm$ standard deviation ($n=3$). Differences between the treatments are shown based on Tukey's HSD tests ($a < b, p < 0.05$).

of the control ($\text{NH}_3\text{-N}$: 0.468 mg l^{-1} , $\text{PO}_4\text{-P}$: 0.1 mg l^{-1}) and a significantly higher cell density was achieved at nitrate concentration 16 times higher than that of the control ($\text{NO}_3\text{-N}$: 32.5 mg l^{-1} ; Fig. 2). These results suggest that $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ in the lower reaches of the Nakdong River have a stronger influence on the formation of toxic and non-toxic *Microcystis* blooms than $\text{NO}_3\text{-N}$ at higher water temperature. Moreover, they indicated that $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ may be found near the threshold concentrations required for *Microcystis* growth in the lower reaches of the Nakdong River. Harke et al. (2012) suggested that *Microcystis* exhibits high PO_4^{3-} uptake rate due to increased phosphatase activity at low phosphorus levels due to high phosphorus affinity. In the $\text{PO}_4\text{-P}$ culture testing, the two *Microcystis* strains maintained a similar cell density at all concentrations during stationary phase (Fig. 2, A1-A4). This demonstrates that *Microcystis* has growth potential within a limited phosphorus range in the Nakdong River. In other words, our findings suggest that reducing the levels of phosphorus would not control *Microcystis* blooms in the Nakdong River. In fact, the standards for total phosphorus effluent discharge in large rivers like the Nakdong River is 0.2 mg l^{-1} in South Korea (Kim et al., 2010), which far exceeds the phosphorus range required to control *Microcystis* blooms.

Recent studies have suggested that along with phosphorus various forms of nitrogen affect the growth of *Microcystis* (Baldia et al., 2007; Pearl et al., 2011; Li et al., 2016). Nitrogen availability during summers is an important factor limiting the growth of *Microcystis* (Šejnohová and Maršálek, 2012). $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ in particular are the most important nitrogen sources affecting *Microcystis* growth. According to Li et al. (2016), *M. aeruginosa* exhibits a steady increase in proportion to the concentration of $\text{NO}_3\text{-N}$ ($1.2\text{-}6.0 \text{ mg l}^{-1}$), while growth was lower at $1.2 \text{ mg l}^{-1} \text{NH}_3\text{-N}$. This means that $\text{NH}_3\text{-N}$ uptake is faster than $\text{NO}_3\text{-N}$ and it can grow faster in $\text{NH}_3\text{-N}$, which is more limiting than $\text{NO}_3\text{-N}$ (Li et al., 2016). Baldia et al. (2007) reported that *M. aeruginosa* had a low rate of uptake for $\text{NO}_3\text{-N}$ but reached growth saturation at a relatively high concentration of 8.71 mg l^{-1} , indicating high $\text{NO}_3\text{-N}$ requirements. In the present study, growth saturation was not observed in the given $\text{NO}_3\text{-N}$ range ($2.033\text{-}32.53 \text{ mg l}^{-1}$) in the two *Microcystis* strains (Fig. 2, C1-C4). This suggests that both strains isolated from the Nakdong River may reach growth saturation, maximum growth at much higher $\text{NO}_3\text{-N}$ levels than those present in field water. Generally, cyanobacteria, including *Microcystis*, prefer $\text{NH}_3\text{-N}$ to $\text{NO}_3\text{-N}$. Unlike $\text{NH}_3\text{-N}$, which is directly available to cells, $\text{NO}_3\text{-N}$ is reduced to intermediate compounds such as ammonium and amino acids

Fig. 2 : Cell density of toxic *M. aeruginosa* MAHC160824 and non-toxic *M. viridis* MVHC160824 according to phosphate ($\text{PO}_4\text{-P}$: A1, A3), ammonium ($\text{NH}_3\text{-N}$: B1, B3) and nitrate ($\text{NO}_3\text{-N}$: C1, C3) concentration and the growth rate of toxic *M. aeruginosa* MAHC160824 and non-toxic *M. viridis* MVHC160824 according to phosphate ($\text{PO}_4\text{-P}$: A2, A4), ammonium ($\text{NH}_3\text{-N}$: B2, B4) and nitrate ($\text{NO}_3\text{-N}$: C2, C4) concentration. All values are given as the mean $\pm$ standard deviation. Differences between the treatments are shown based on Tukey's HSD tests ($a < b < c$, $p < 0.05$).

(Von Rückert and Giani, 2004). Therefore, *Microcystis* can reach growth saturation more rapidly using $\text{NH}_3\text{-N}$, even in limiting conditions. In the growth experiments, based on nutrient concentrations, the present study confirmed that the two *Microcystis* strains could reach growth saturation within an $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ concentration range that was similar to raw water. Given the results of our research and previous studies, $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ can be classified as strongly synergistic limiting factors for *Microcystis* growth in the Nakdong River during summer.

M. aeruginosa MAHC160824 was tested to determine microcystin production at different temperatures and nutrient levels ($\text{NO}_3\text{-N}$, $\text{NH}_3\text{-N}$, $\text{PO}_4\text{-P}$). The concentration of microcystins in the control medium was below the limit of detection (LOD), as was the case for field water. Dissolved, extracellular microcystins were also below the LOD in all experimental conditions. Of the four microcystin variants (-LA, -LR, -RR, -YR), microcystin-LA was not detected. Lee et al. (2018) reported that microcystin-LA was not detected. Total intracellular microcystins and intracellular microcystin-LR in the culture medium reached their maximum

levels for *M. aeruginosa* MAHC160824 at a $\text{PO}_4\text{-P}$ concentration four times higher than that of the control group during stationary phase (18 days; Fig. 3, A1, A2). Total intracellular microcystins per cell were also highest when the $\text{PO}_4\text{-P}$ concentration was four times higher than that of the control group during stationary phase (18 days), while intracellular microcystin-LR per cell was highest at $\text{PO}_4\text{-P}$ concentration four times higher than that of control during lag phase (6 days). For $\text{NH}_3\text{-N}$, the concentration of total intracellular microcystins and intracellular microcystin-LR was highest at $\text{NH}_3\text{-N}$ concentration four times that of the control during stationary phase (18 days; Fig. 3, B1, B2). Total intracellular microcystin and intracellular microcystin-LR per cell was also highest at four times the $\text{NH}_3\text{-N}$ concentration of the control during stationary phase (18 days). For $\text{NO}_3\text{-N}$, total intracellular microcystin and intracellular microcystin-LR levels increased in a manner similar to the growth response to $\text{NO}_3\text{-N}$ concentration, demonstrating the highest levels during stationary phase (18 days) at $\text{NO}_3\text{-N}$ concentration 16 times higher than that of the control (Fig. 3, C1, C2). Total intracellular microcystin and intracellular microcystin-LR per cell was also highest under these conditions (Fig. 3).

Fig. 3 : Total microcystin (A1, B1, C1) and microcystin-LR (A2, B2, C2) for *M. aeruginosa* MAHC160824 according to phosphate (PO₄-P : A1, A2), ammonium (NH₃-N : B1, B2) and nitrate (NO₃-N : C1, C2) concentration. All values are given as the mean $\pm$ standard deviation. Differences between the treatments are shown based on Tukey's HSD tests ($a < b < c < d < e$, $p < 0.05$). The red line indicates WHO guidelines for microcystin-LR in safe drinking water.

In contrast, in the present study, no microcystins were detected from the toxic *M. aeruginosa* MAHC16082 under any of the temperature conditions (15, 20, 25, 30°C), despite relatively high cell numbers in the control medium (Fig. 3), because the temperature experiment was conducted based on the nutrient concentrations (NO₃-N, NH₃-N, PO₄-P) present in the field water of Nakdong River. This suggests that microcystin

production in the *Microcystis* strains isolated from the Nakdong River is primarily affected by nutrient levels. Interestingly, despite regular occurrence of *Microcystis* blooms, the detection frequency and concentration of microcystins are low in the Nakdong River (water.nier.go.kr). Therefore, it is hypothesized that the concentration of nutrients (NO₃-N, NH₃-N and PO₄-P) in the Nakdong River may not be at a level high

enough to affect microcystin production.

In the nutrient culture experiments, *M. aeruginosa* MAHC160824 exhibited markedly high microcystin production when growth reached its stationary phase, on adding reported $\text{NO}_3\text{-N}$, $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$. This is similar to the results reported by Long *et al.* (2001), who reported that microcystin production per cell was maximum when the growth rate of *M. aeruginosa* was zero. According to Lyck (2004), the intracellular microcystin level of *M. aeruginosa* are higher at early stages of growth, but decrease during exponential growth stage, suggesting the possible release of extracellular microcystin. In the present study, extracellular microcystins were below the LOD. Our results show that $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ simultaneously affected the growth and microcystin production of toxic *M. aeruginosa* MAHC160824 within a restricted nutrient range. Within the limited range of $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations observed in the field, *M. aeruginosa* can produce blooms and maximize toxin production (Dai *et al.*, 2008; Gobler *et al.*, 2016). In this study, microcystin production was below the LOD regardless of cell density at all growth stages when nutrient concentrations ($\text{NO}_3\text{-N}$, $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$) were same as those in the water sampled from the Nakdong River (Fig. 3). Although these results are based on the culture of a specific toxic strain, it is suggested that *M. aeruginosa* had low microcystin production under the nutrient concentrations present in the Nakdong River at the time of sampling, i.e., during blooming season ($\text{NO}_3\text{-N}$: 2.033 mg l⁻¹, $\text{NH}_3\text{-N}$: 0.117 mg l⁻¹, $\text{PO}_4\text{-P}$: 0.025 mg l⁻¹). Interestingly, microcystin detection was infrequent during *Microcystis* blooms in the Nakdong River in 2016, and the concentration of nutrients were similar to or lower than those in the control medium.

In the case of $\text{PO}_4\text{-P}$, microcystin production was highest at $\text{PO}_4\text{-P}$ concentrations 4 times higher than of the control (Fig. 3 A1, A2). Peng *et al.* (2018) suggested that the inorganic phosphorus source was more useful for *Microcystis* and results in the highest growth when the N:P ratio was 16:30. Although in this study, using dissolved organic phosphorus as a source, the N:P ratio of the experiment that increased $\text{PO}_4\text{-P}$ 4 times was about 20:1 (16 times that of the control was 5:1). Increasing phosphorus did not significantly affect cell growth, but it was demonstrated that the appropriate N:P ratio affected microcystin production. When the phosphorus was increase four times the N : P ratio (1 : 4), the oxidative stress and lipid peroxidation of cell membrane and photosynthetic activity decreased consequently. Field and culture research have demonstrated that biologically available nitrogen has a direct impact on microcystin production (Downing *et al.*, 2005; Scott *et al.*, 2013; Horst *et al.*, 2014). In particular, $\text{NH}_3\text{-N}$ directly affects the microcystin production in the form of dissolved inorganic nitrogen, which can be used directly by the members of this genus (Dai *et al.*, 2008; Gobler *et al.*, 2016). In oligotrophic lakes, microcystin-producing species are uncommon, however, eutrophic lakes, which contain higher nitrogen levels, are more likely to contain these species (Rantala

et al., 2006). Horst *et al.* (2014) also suggested that $\text{NO}_3\text{-N}$ availability greatly influences microcystin production. In accordance with these studies, addition of $\text{NO}_3\text{-N}$ and $\text{NH}_3\text{-N}$ increased microcystin production in the present study. When $\text{NO}_3\text{-N}$ concentration increased from 4 to 16 times higher than that of the control, the cell density and microcystin concentration increased simultaneously (Fig. 2, C1, C2; Fig. 3, C1, C2). However, saturation of microcystin production with increasing $\text{NO}_3\text{-N}$ was not observed. The present study, thus, revealed that toxic *M. aeruginosa* MAHC160824 produces higher microcystin concentration more efficiently with $\text{NH}_3\text{-N}$ than with $\text{NO}_3\text{-N}$ within a restricted nutrient range. Our results found that microcystins levels were lower than LOD, even though the growth of toxic *M. aeruginosa* MAHC160824 was relatively high under control conditions that corresponded to those of field water from the Nakdong River.

The results, toxic *M. aeruginosa* in the Nakdong River exhibited relatively low microcystin production. However, if nitrogen and phosphorus level rises above the average concentration during *Microcystis* blooming seasons, it is expected to produce higher concentration of microcystins. Further research is required to determine whether these characteristics are specific to toxic *Microcystis* strains present in the Nakdong River.

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