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Competitive dominance of *Microcystis aeruginosa* against *Raphidiopsis raciborskii* is strain- and temperature-dependent

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Abstract – *Microcystis aeruginosa* and *Raphidiopsis raciborskii* (previously *Cylindrospermopsis raciborskii*) are both common bloom-forming cyanobacteria which can coexist but alternatively dominate in freshwater ecosystems. To predict their blooming dynamics, we need to understand the potential environmental factors determining their succession. In the present study, we examined the pairwise competition of the three *M. aeruginosa* strains (FACHB905, 469 and 915) with one *R. raciborskii* strain (N8) at three temperature levels (16 °C, 24 °C, and 32 °C). We found that the competitive ability of three *Microcystis* strains were highly variable. *M. aeruginosa* FACHB905 was the strongest competitor among them which can finally exclude *R. raciborskii* N8 regardless of initial biovolume ratios and temperature levels. The competitive exclusion of N8 by 915 also was observed at 24 °C, but they coexisted at 16 °C and 32 °C. We observed that *M. aeruginosa* FACHB469 and *R. raciborskii* N8 were able to coexist under all the temperature levels, and *M. aeruginosa* FACHB469 was the weakest competitor among the three *M. aeruginosa* strains. Rates of competitive exclusion (RCE) showed that temperature affects the competition between three *M. aeruginosa* strains and *R. raciborskii* N8. *M. aeruginosa* strains always grew quickly at 24 °C and significantly enlarged its dominance in the co-culture system, while *R. raciborskii* N8 was able to maintain its initial advantages at both 16 and 32 °C. The competitive advantage of *M. aeruginosa* FACHB905 may be explained by allelopathic interactions through its allelochemicals and other secondary metabolites other than microcystin. We concluded that both strain difference and temperature can affect the competition between *M. aeruginosa* and *R. raciborskii*. Our results highlighted the complexity of cyanobacterial dynamics in waterbodies where there exist multiple strains.

Keywords: *Microcystis aeruginosa* / *Raphidiopsis raciborskii* / temperature / allelopathy / competition

Résumé – La domination compétitive de *Microcystis aeruginosa* sur *Raphidiopsis raciborskii* dépend de la souche et de la température. *Microcystis aeruginosa* et *Raphidiopsis raciborskii* (anciennement *Cylindrospermopsis raciborskii*) sont deux cyanobactéries communes formant des fleurs d'eau qui peuvent coexister mais aussi dominer alternativement dans les écosystèmes d'eau douce. Pour prédire la dynamique de leur floraison, nous devons comprendre les facteurs environnementaux potentiels qui déterminent leur succession. Dans la présente étude, nous avons examiné la compétition par paires des trois souches de *M. aeruginosa* (FACHB905, 469 et 915) avec une souche de *R. raciborskii* (N8) à trois niveaux de température (16, 24, et 32°C). Nous avons constaté que la capacité concurrentielle des trois souches de Microcystis était très variable. *M. aeruginosa* FACHB905 était la plus forte concurrente qui peut finalement exclure *R. raciborskii* N8 indépendamment des rapports de biovolume et des niveaux de température initiaux. L'exclusion compétitive de N8 par 915 a également été observée à 24°C, mais elles ont coexisté à 16°C et 32°C. Nous avons observé que *M. aeruginosa* FACHB469 et *R. raciborskii* N8 étaient capables de coexister à tous les niveaux de température, et que *M. aeruginosa* FACHB469 était le plus faible concurrent parmi les trois souches de *M. aeruginosa*. Les taux d'exclusion compétitive (TCE) ont montré que la température affecte la concurrence entre les trois souches de *M. aeruginosa* et *R. raciborskii* N8. Les souches de *M. aeruginosa* ont toujours connu une croissance rapide à 24°C et ont considérablement accru leur domination dans le système de co-culture, tandis que *R. raciborskii* N8 a pu maintenir ses avantages initiaux à la fois à 16 et à 32°C. L'avantage concurrentiel de *M. aeruginosa* FACHB905 peut

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s'expliquer par des interactions allélopathiques par le biais de ses métabolites allélochimiques et d'autres secondaires autres que la microcystine. Nous avons conclu que la différence de souche et la température peuvent toutes deux affecter la concurrence entre *M. aeruginosa* et *R. raciborskii*. Nos résultats ont mis en évidence la complexité de la dynamique des cyanobactéries dans les masses d'eau où il existe de multiples souches.

Mots clés : *Microcystis aeruginosa* / *Raphidiopsis raciborskii* / température / allélopathie / compétition

1 Introduction

Toxic cyanobacterial blooms have widely occurred in freshwater ecosystems around the world, and are being facilitated by global warming (Paerl and Huisman, 2008; El-Shehawy *et al.*, 2012). Bloom-forming cyanobacteria often cause severe problems in the management of water quality due to the release of cyanotoxins (Paerl *et al.*, 2016). *Microcystis* is one of the most notorious genus which causes heavy blooms. Many *Microcystis* strains can produce hepatotoxin microcystins (MCs), and thus their bloom occurrence presents a risk to those who use blooming water for drinking, aquaculture, recreation and agricultural irrigation (Harke *et al.*, 2016; Paerl *et al.*, 2016). Numerous studies including both field investigations and laboratory experiments have been conducted to clarify the potential environmental drivers for the competitive advantages of *Microcystis* (Paerl and Huisman, 2008; El-Shehawy *et al.*, 2012; Harke *et al.*, 2016). The increasing temperature related to global warming is expected to promote its proliferation and dominance.

Previous studies showed that *Microcystis* strains exhibit a large degree of morphological, genetic and chemical diversity (Welker *et al.*, 2004; Haande *et al.*, 2007; Shen *et al.*, 2007). Moreover, a high variability of growth and toxicity has been observed among strains of *Microcystis* (Vézie *et al.*, 2002; Wilson *et al.*, 2006; Xiao *et al.*, 2017a), indicating each strain represent an ecotype able to adapt and to survive in changing environmental conditions (Pimentel and Giani, 2014). The recent comparative proteomic and genomic studies with different *Microcystis* strains have revealed a significant proportion of the identified proteins and that 127 to 911 genes were strain-specific (Alexova *et al.*, 2011; Yang *et al.*, 2015). These unique proteins or genes are assumed to be beneficial for survival and proliferation of *Microcystis*. Due to the existence of a high diversity at the inter-strain level, it is meaningful to investigate multiple *Microcystis* strains to explore the mechanism underlying their competitive advantage. Many previous laboratory studies usually used only one cyanobacterial strain to perform physiological experiments (Wu *et al.*, 2009; Krüger *et al.*, 2012), which are not adequate for our understanding of the effects of morphological, genetic or chemical variation among strains on cyanobacterial dynamics (Wilson *et al.*, 2006; Shen *et al.*, 2007; Willis *et al.*, 2016).

Filamentous *Raphidiopsis raciborskii* (previously *Cylindrospermopsis raciborskii*) is another most successful bloom-forming cyanobacterial species in freshwater ecosystems. Studies of *R. raciborskii* explosively increased in the last decade due to its expansion into temperate region and its toxin-producing potential (Antunes *et al.*, 2015; Burford *et al.*, 2016). In some subtropical and tropical water bodies, co-existence or seasonal succession of *M. aeruginosa* and *R. raciborskii* is commonly observed (Costa *et al.*, 2006; Moustaka-Gouni *et al.*, 2007; Soares *et al.*, 2009; Miller and McMahon, 2011). Changes in their relative dominance have been related to environmental and ecological conditions such as nutrient levels, physical factors, selective grazing and allelopathy (Moustaka-Gouni *et al.*, 2007; Soares *et al.*, 2009; Marinho *et al.*, 2013; Rzymski *et al.*, 2014). Due to this complicated scenario, predicting competition between *M. aeruginosa* and *R. raciborskii* is rather difficult.

Competition is a major factor shaping the phytoplankton structure and succession. Several studies investigated the effects of a variety of factors such as temperature, light, pH, nutrients and secondary metabolites on the growth and competition between *M. aeruginosa* and *R. raciborskii* (Figueredo *et al.*, 2007; Mello *et al.*, 2012; Marinho *et al.*, 2013; Chislock *et al.*, 2014; Rzymski *et al.*, 2014; Thomas and Litchman, 2016; Xiao *et al.*, 2017b; da Silva Brito *et al.*, 2018). However, it has been realized that the effects of these factors remain inconsistent. For example, the relative and absolute biomass of *M. aeruginosa* declined and *R. raciborskii* was found to dominate in all N:P (from 7:1 to 122:1) treatments (Chislock *et al.*, 2014), whereas *R. raciborskii* can either exclude or be displaced by *M. aeruginosa* under phosphorus limitation of 4.5 μmol L^{-1} K_2HPO_4 (Marinho *et al.*, 2013). The morphological, genetic and chemical differences between strains of one species may contribute to the inconsistence (Marinho *et al.*, 2013; Xiao *et al.*, 2017b).

Temperature is among the major determinants which influence phytoplankton growth, nutrient uptake, and spatial-temporal distribution in freshwater systems. Both *M. aeruginosa* and *R. raciborskii* benefit from higher temperatures, which can give the two species a competitive advantage to outcompete other phytoplankton taxa through increased growth rates (Paerl and Huisman, 2008; O'Neil *et al.*, 2012). As a tropical and invasive species, *R. raciborskii* seems to be one of the most likely cyanobacteria to benefit from global warming (Antunes *et al.*, 2015). The persistent dominance of *R. raciborskii* has been observed in some tropical reservoirs, where *M. aeruginosa* occurred during a certain period (Branco and Senna, 1994; Figueredo and Giani, 2009; Soares *et al.*, 2009; Jovanović *et al.*, 2017). A recent study with monocultures reported that *R. raciborskii* strains had higher growth rates than toxic *M. aeruginosa* strains under a temperature above 20 °C, but had no apparent advantage over the non-toxic *M. aeruginosa* from 15 °C to 40 °C (Thomas and Litchman, 2016).

Accordingly, we hypothesize that both temperatures and strain differences may be crucial to for competition between *M. aeruginosa* and *R. raciborskii*. Currently, there are few studies assessing the effects of temperature on the competition of two species, and variation in stain-specific response has

often been ignored. In this study, we examined competition between three strains of *M. aeruginosa* and one strain of *R. raciborskii* when exposed to different temperatures. We also tried to clarify the competitive abilities of *M. aeruginosa* with respect to the role of MC production.

2 Materials and methods

2.1 Strains and culture conditions

Two MC-producing *M. aeruginosa* strains FACHB905 and FACHB915, one non-MC-producing *M. aeruginosa* strain FACHB469 were provided by the culture collection of Chinese Academy of Science in Wuhan. *R. raciborskii* N8 was isolated from Zhenhai Reservoir, Guangdong Province, tropical China. All four strains were non-axenic. *M. aeruginosa* grew as single-cell populations and *R. raciborskii* filaments were straight. A pre-culture of each strain was grown at 25 °C in a 1 L Pyrex Erlenmeyer flasks containing 500 mL BG11 medium with an adjusted pH of 8.2 (Ripka *et al.*, 1979). Erlenmeyer flasks were placed in a culture chamber with 30 $\mu mol\ m^{-2}\ s^{-1}$ light intensity using cool white fluorescent lights. A 12/12 h light/dark cycle was systematically applied.

2.2 Competition experiments

In the pre-experiments, *M.aeruginosa* FACHB905 and *R. raciborskii* N8 were inoculated with different biovolume ratios (1:90, 1:180, 1:270, 1:450, 1:900 and 1:1800) to give *R. raciborskii* an initial advantage, however, we finally found *M. aeruginosa* FACHB905 exerted an exclusive competitive advantage and outcompeted *R. raciborskii* in all the competition experiments, regardless of the initial biovolume ratios (Fig. S1). Therefore, a lower biovolume ratio of 1:30 was applied throughout the competition experiments. Two-week-old cultures (in the exponential growth phase) served as inoculums. The inoculating cell densities of *M. aeruginosa* FACHB905, FACHB915 or FACHB469 and *R. raciborskii* N8 were 0.5×10^5 cell mL^{-1} and 0.5×10^5 filament mL^{-1}, respectively, referred to as 1:30 biovolume ratio. The final volume of mixed cultures was 400 mL and each treatment was run in triplicate. The competition experiment was conducted under three temperature levels (LT = 16 °C, MT = 24 °C, HT = 32 °C). The flasks were gently mixed twice daily and samples of 2 mL were taken every 3−4 days for cell number counting. Cell numbers were counted in a Sedgewick Rafter counting chamber under an Olympus microscope with non-inverted optics at 400× magnification. The average cell size of a specie was obtained from the median volume of at least 100 random selected individuals according to the biovolume calculation methods by Hillebrand *et al.* (1999). Cell abundance of each species is converted to algal biovolume based on the average cell size of the species.

2.3 Effects of MC-LR on *R. raciborskii* N8

An experiment was designed to assess whether MC-LR or other allelopathic chemicals may be responsible for the competition ability of *M. aeruginosa* FACHB905. *R. raciborskii* N8 was harvested at the exponential growth phase and inoculated with initial chlorophyll-a concentration of 20 $\mu g\ L^{-1}$ in 50 mL capped test tubes (25 mm × 150 mm) containing 35 mL of BG11 medium. The purified MC-LR (Alexis Biochemicals, USA) was added to each test tube and the final toxin concentration was as follows: 0, 0.1, 0.5, 1.0, 5.0, 10, 50, 100, 500 and 1000 $\mu g\ L^{-1}$. Each treatment was carried out in triplicate and gently mixed twice daily. Chlorophyll-a (chla) concentrations were measured daily with aTD-700 laboratory Fluorometer (Turner Designs, California, USA). Significant difference ($P < 0.05$) among different treatments was tested by One-way ANOVA and Turkey's test in SPSS 16.0 statistical package.

2.4 Effects of spent medium of *M. aeruginosa* FACHB905 on *R. raciborskii* N8

In this experiment, spent medium of *M. aeruginosa* FACHB905 grown for 3 weeks was collected by separation of cells and medium via centrifugation. Subsequently, the supernatant was filter-sterilized through the injection filter with a nominal pore size of 0.22 μm (MILLEX-GP, Millipore, USA). Spent medium was then added to fresh BG11 medium with different volume ratios: 0%, 2.5%, 5.0%, 10%, 20%, 30%, 40% and 50%. *R. raciborskii* N8 was harvested and inoculated to the modified BG11 media as described in the first experiment. Each treatment was carried out in triplicate and gently mixed twice daily. Chlorophyll-a concentrations were measured daily with aTD-700 laboratory Fluorometer (Turner Designs, California, USA). The statistical analyses as showed in 2.3 were applied.

2.5 Effects of crude extract of *M. aeruginosa* FACHB905 on *R. raciborskii* N8

In this experiment, for the preparation of crude extract, 250 mL *M. aeruginosa* FACHB905 grown for 3 weeks was harvested by centrifugation, and the cell pellet was rinsed with sterile distilled water for three times. The resuspended cells were disrupted by freeze-thaw cycles and then 2 min sonication on ice. The suspension was centrifuged and the supernatant was filter-sterilized before added to fresh BG11 medium with different volume ratios: 0%, 0.33%, 0.67%, 1.67%, 3.33%, and 6.67%. *R. raciborskii* N8 was inoculated to the modified BG11 media as described in the first experiment. Each treatment was carried out in triplicate and gently mixed twice daily. Chlorophyll-a concentrations were measured daily with a TD-700 laboratory Fluorometer (Turner Designs, California, USA). The statistical analyses as showed in 2.3 were applied.

3 Results

3.1 Growth and competition of *M. aeruginosa* and *R. raciborskii* under three temperature levels

In the competition between 905 and N8 under three temperatures, *M. aeruginosa* completely displaced *R. raciborskii,* however, the competitive exclusion of N8 by 905 changed with the experimental temperature levels. *R. raciborskii* N8 showed a slight growth only at the beginning of 4 days at 16 °C, but a longer growth phase and higher biovolume were observed at 24 °C and 32 °C (Fig. 1). Although N8 biovolumes markedly increased

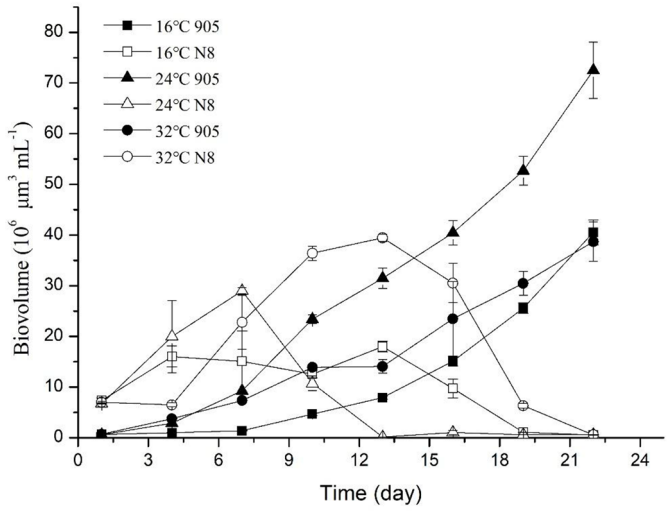

Fig. 1. Time course of the biovolumes of *M. aeruginosa* FACHB905 and *R. raciborskii* N8 in the competition experiments at 16 °C, 24 °C and 32 °C.

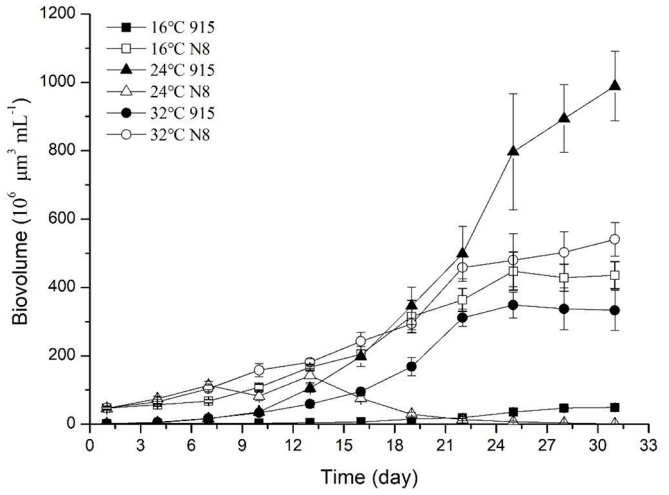

Fig. 2. Time course of the biovolumes of *M. aeruginosa* FACHB915 and *R. raciborskii* N8 in the competition experiments at 16 °C, 24 °C and 32 °C.

under high temperature, they dropped after reaching maximum value on the 14th day and the strain 905 completely dominated at the end of the experiments (Fig. 1).

In the competition experiments between 915 and N8 under three temperature levels, the competitive outcomes between two species were variable. 915 and N8 were able to coexist at 16 °C and 32 °C, but 915 competitively excluded N8 at 24 °C (Fig. 2). *M. aeruginosa* FACHB915 showed a strong competitive ability at 24 °C and displaced *R. raciborskii* N8 from day 14. At 16 °C and 32 °C, the biovolumes of both species increased, but *M. aeruginosa* FACHB915 increased with a higher growth rate in the mixed population (Fig. 2). Hence, we observed a shift of 1:30 initial ratio towards ratio 1:8.8 (16 °C) and 1:1.6 (32 °C) at the end of the experiments.

In the competition experiments between 469 and N8 under three temperature levels, two species coexisted, but

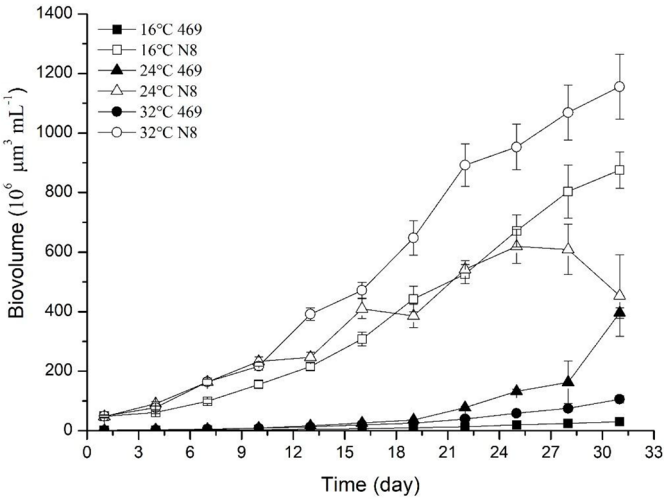

Fig. 3. Time course of the biovolumes of *M. aeruginosa* FACHB469 and *R. raciborskii* N8 in the competition experiments at 16 °C, 24 °C and 32 °C.

their relative dominance was significantly affected by temperature (Fig. 3). At 16 °C, both species increased with a similar growth rate and the biovolume ratio between 469 and N8 was still kept around 1:30 at the end of the experiment (Fig. 3). At 24 °C, *M. aeruginosa* FACHB469 increased with a higher growth rate in the mixed population and its relative contribution in total biovolume showed a significant increase from 3.3% of day 1 to 47.6% of day 31 (Fig. 3). At 32 °C, both species increased with a similar growth rate until the twenty-second day and then *M. aeruginosa* FACHB469 slightly increased its growth rate. Therefore, with an initial advantage, *R. raciborskii* N8 showed a slight decrease (from 96.7% to 91.7%) in its relative proportion and was still able to maintain its dominance at 32 °C (Fig. 3).

For each species pair, we calculated rates of competitive exclusion (RCE) according to Grover (1991), as the slope of the linear regression of ln (*Microcystis* biovolume/*Raphidiopsis* biovolume) with time. One-way ANOVA test showed the RCD values of the same species pair were significantly different at three temperature levels ($P < 0.05$). The RCD values confirmed that *M. aeruginosa* gained the advantage under most of experimental conditions and all three strains excluded *R. raciborskii* N8 at a much faster rate at 24 °C (Tab. 1). Under the same temperature, the RCD was always highest for *Microcystis* FACHB905 and lowest for *Microcystis* FACHB469.

3.2 *R. raciborskii* N8 growth changing with MC-LR concentrations

Compared to the control (0 μg L^{-1} MC-LR), *R. raciborskii* N8 incubated with different MC-LR concentrations increased rapidly and grew well during the whole experimental periods (Fig. 4). Although chla concentrations of *R. raciborskii* at stationary phase slightly changed, the difference between the control and treatments was not significant ($P > 0.05$).

Table 1. Rates of competition exclusion (RCE) for each species pair under three temperature levels.

	RCE		
	Temperature		
Species pair	16 °C	24 °C	32 °C
FACHB905 vs. N8	1.02 ± 0.04^{b}	1.21 ± 0.02^{a}	0.70 ± 0.05^{c}
FACHB915 vs. N8	-0.13 ± 0.03^{c}	1.05 ± 0.05^{a}	0.23 ± 0.03^{b}
FACHB469 vs. N8	-0.05 ± 0.00^{c}	0.37 ± 0.02^{a}	0.11 ± 0.01^{b}

Different letters indicate significant differences ($P < 0.05$).

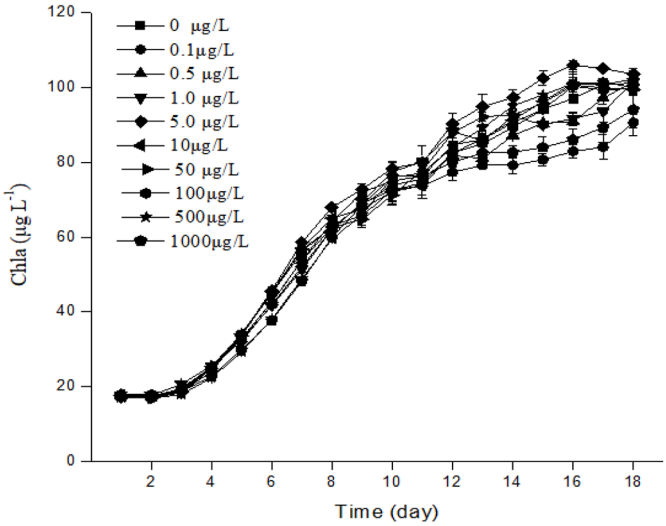

Fig. 4. Growth curves of *R. raciborskii* N8 incubated with MC-LR.

3.3 *R. raciborskii* N8 growth in spent medium of *M. aeruginosa* FACHB905

Spent medium of *M.aeruginosa* FACHB905 had a concentration-related effect on the growth of *R. raciborskii* N8 (Fig. 5). Compared to the control (0% spent medium), 5 ~ 50% spent medium resulted in a rapid decrease in both the chla concentrations ($P < 0.05$) and growth phase of *R. raciborskii* N8. Excellent growth was observed in both the control and 2.5% spent medium and there was no significant difference between them ($P > 0.05$; Fig. 5). In 5% spent medium, the biomass of *R. raciborskii* N8 increased and reached maximal concentrations of 73.4 $\mu g\,L^{-1}$ at 11th day, and a 35% inhibition of *R. raciborskii* growth was observed at the end of experiment. The strongest inhibition (up to 96%) on growth of *R. raciborskii* N8 was observed in the treatments with 40% and 50% spent medium.

3.4 *R. raciborskii* N8 growth in crude extract of *M. aeruginosa* FACHB905

All *R. raciborskii* groups incubated with six ratios of *M. aeruginosa* FACHB905 crude extract grew well during the whole experimental periods (Fig. 6). Although the chla concentrations of *R. raciborskii* at stationary phase slightly changed, the difference between the control and treatments was not significant ($P > 0.05$).

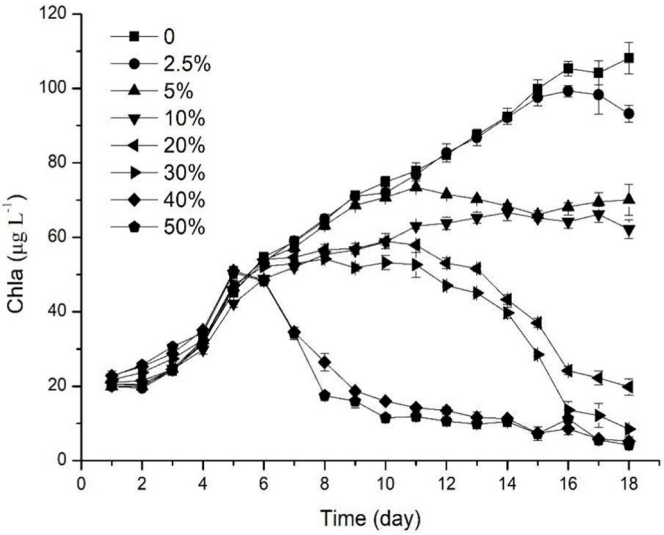

Fig. 5. Growth curves of *R. raciborskii* N8 in BG11 with 0–50% spent medium of *M. aeruginosa* FACHB905.

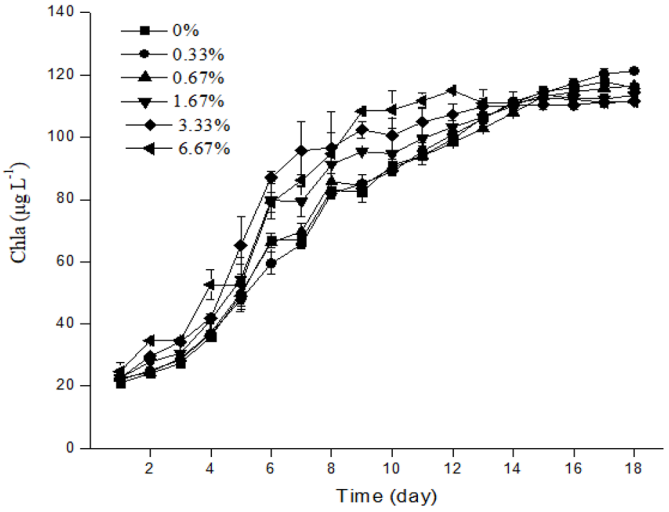

Fig. 6. Growth curves of *R. raciborskii* N8 of in BG11 with 0–6.67% crude extract of *M. aeruginosa* FACHB905.

4 Discussion

Our findings highlight the strong variation in the competitiveness of three *M. aeruginosa* strains. *M. aeruginosa* FACHB905 was the most competitive, and can completely exclude *R. raciborskii* N8 in the competition experiments. The other two strains of *M. aeruginosa* can coexist with *R. raciborskii*, but the strain FACHB915 is more competitive than FACHB469. Our study confirms the previous findings that *Microcystis* isolated from the same or different water bodies are strain-specific and diverse in phenotypes, genotypes and chemotypes (Welker *et al.*, 2004; Haande *et al.*, 2007;

Alexova *et al.*, 2011; Yang *et al.*, 2015; Xiao *et al.*, 2017a). The high plasticity of *Microcystis* strains led to considerable variation in their responses to environmental conditions. Therefore, the competition outcomes between *M. aeruginosa* and *R. raciborskii* were highly uncertain (Marinho *et al.*, 2013; Xiao *et al.*, 2017b; da Silva Brito *et al.*, 2018).

Some studies demonstrated that it was unlikely to generalize the environmental conditions in which one species/strain may dominate because biological differences between cyanobacterial strains significantly affected growth responses to environmental conditions. When exposed to different light or phosphate limitation, *R. raciborskii* can either dominate or be displaced by *M. aeruginosa* (Marinho *et al.*, 2013). da Silva Brito *et al.* (2018) found that *M. aeruginosa* dominated under conditions of high alkalinity and high pH but was overcome by *R. raciborskii* under conditions of low alkalinity and lower pH. When exposed to compounds produced by *R. raciborskii*, all *M. aeruginosa* strains but LEA-04 formed colony, indicating that specificity played a role in the interaction between two species (Mello *et al.*, 2012) These results supported the argument that cyanobacterial competition was highly variable, depending on strains and environmental conditions (Marinho *et al.*, 2013; Xiao *et al.*, 2017b).

Competition between phytoplankton species can include an active process defined as allelopathy (Rice, 1984; Leão *et al.*, 2009). Several previous studies showed that toxic *M. aeruginosa* FACHB905 had a strong inhibitory effects on growth of other phytoplankton species such as non-toxic *M. aeruginosa* FACHB469, *M. wesenbergii*, *Aphanizomenon flos-aquae*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Scenedesmus quadricauda*, and *Cyclotella meneghiniana* (Yang *et al.*, 2014; Lei *et al.*, 2015; Ma *et al.*, 2015a; Ma *et al.*, 2015b; Wang *et al.*, 2017). In this study, we observed a complete exclusion of *M. aeruginosa* FACHB905 against *R. raciborskii* N8 in the competition experiments, even if the initial ratio of their biovolumes was as high as 1:1800. The extreme competitive ability of FACHB905 may significantly attribute to its allelopathic effects on other phytoplankton species (Yang *et al.*, 2014; Lei *et al.*, 2015; Ma *et al.*, 2015a; Ma *et al.*, 2015b; Wang *et al.*, 2017). MCs was considered as allelopathic compounds that allow toxic *Microcystis* strains to inhibit the growth of their competitors in phytoplankton communities (Sedmak and Elersek, 2005; Leão *et al.*, 2009). Yang *et al.* (2014) put forward that both MC and other allelopathic compounds in *M. aeruginosa* FACHB905 had synergistic effects on inhibition of *M. wesenbergii*. However, a bioassay with pure MC-LR with concentrations of 250 $\mu g\,L^{-1}$ and 500 $\mu g\,L^{-1}$ did not inhibit the growth of *A. flos-aquae* (Ma *et al.*, 2015a), and our results were consistent with the above investigation that no significant difference of *R. raciborskii* growth was observed between the control and treatment with 0.1–1000 $\mu g\,L^{-1}$ MC-LR. Thus MC-LR was excluded as a candidate allelopathic compound and there existed other unknown secondary metabolites responsible for the allelopathic effects of *M. aeruginosa* FACHB905 (Yang *et al.*, 2014; Ma *et al.*, 2015a).

Allelopathy has been suggested to be an important explanation for mediating the seasonal dynamics of different cyanobacterial species (Figueredo *et al.*, 2007). Cyanobacteria are known to produce a variety of secondary metabolites with potent biological activities, some of which have been released into the surrounding medium as allelopathic compounds aiding in interspecific competition (Leão *et al.*, 2009, 2010). Cylindrospermosin and an unknown bioactive compound secreted by *R. raciborskii* can inhibit the growth of *M. aeruginosa*, and thus contribute to the stable dominance of *R. raciborskii* (Figueredo *et al.*, 2007; Mello *et al.*, 2012; Rzymski *et al.*, 2014). Conversely, our results showed that the growth of *R. raciborskii* N8 was significantly inhibited by *M. aeruginosa* FACHB905, indicating that the chemically mediated process between *R. raciborskii* and *M. aeruginosa* was mutual and interactive. The findings that the growth inhibition was induced by spent medium of *M. aeruginosa* FACHB905 but not by its crude extracts demonstrated that allelopathic compounds were extracellularly released. Several studies have also confirmed that allelopathic effects of *M. aeruginosa* FACHB905 on other phytoplankton species were from its filtrates or exudates (Yang *et al.*, 2014; Ma *et al.*, 2015a; Wang *et al.*, 2017). Yang *et al.* (2014) tried to characterize allelopathic compounds from this strain with GC-MS and an unknown compound of molecular weight 678.4 was found. However, the exact chemical structure of the compound remains unclear.

Changes in environmental factors can also affect the competition outcomes between *M. aeruginosa* and *R. raciborskii*. Temperature is one of crucial environmental factors regulating bloom development and phytoplankton succession. Many studies have demonstrated that rising temperature can promote the growth and spread of invasive species of *R. raciborskii*, and thus likely favor these cyanobacteria over the native species *M. aeruginosa* (O'Neil *et al.*, 2012; Thomas and Litchman, 2016). In the present study, we found that temperature has a large impact on the competition between three *M. aeruginosa* strains and *R. raciborskii* N8. In 905 & N8 co-cultures, although *M. aeruginosa* was absolutely a winner at the end of the experiment, the growth of *R. raciborskii* obviously increased with elevated temperatures. In the competition experiments for 915 and N8, *M. aeruginosa* almost excluded *R. raciborskii* at 24 °C but coexisted until the end of the experiment at 16 °C and 32 °C. *M. aeruginosa* FACHB469 also showed a strong competitiveness at 24 °C and increased its percentage from 3.3% to 47.6%. Therefore, *M. aeruginosa* species can dominate over or outcompete *R. raciborskii* at 24 °C, while *R. raciborskii* maintained its initial advantage at 16 °C and 32 °C, indicating that rising temperatures can better promote the growth of *R. raciborskii*. Our result was similar to previous study that the dominant strain in cyanobacterial competition always was the species *M. aeruginosa* at 20 °C and *R. raciborskii* at 28 °C (Xiao *et al.*, 2017b).

5 Conclusion

In conclusion, our experiments showed that there existed physiological variation between *M. aeruginosa* strains, and thus the competition outcome between *M. aeruginosa* and *R. raciborskii* were highly variable. *M. aeruginosa* FACHB905 was the strongest competitor and almost excluded *R. raciborskii*, while *M. aeruginosa* FACHB469 was the weakest one and always coexisted with *R. raciborskii*. Temperature has a significant influence on the competition

between the two species. *M. aeruginosa* outcompeted *R. raciborskii* at 24 °C, while *R. raciborskii* maintained its initial advantage at 16 °C and 32 °C. In order to predict the development of cyanobacteria bloom, physiological difference between strains should be considered as well as environmental conditions.

Supplementary Material

Supplementary Figure S1.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2020023/olm.

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