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RESEARCH ARTICLE

Effects of toxic cyanobacterium *Microcystis aeruginosa* on the morphology of green alga *Chlorella vulgaris*

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Abstract – In eutrophic freshwater lakes, cyanobacteria and green algae are in succession due to abiotic factors. Allelochemical interaction also exists. In this study, we indicated that filtrates of *Microcystis aeruginosa* can influence the growth rate and photosynthetic pigments of the target alga named *Chlorella vulgaris*. We also determined the morphological appearance and colony formation of green alga *C. vulgaris* that were induced by chemicals associated with a competitor cyanobacterium named *M. aeruginosa*. However, microcystins (MCs) were not the active substances in this study. The morphology changes and growth of *C. vulgaris* affected by *M. aeruginosa* filtrates were dependent on the initial cultivation density of the target algae and the cultivation phase of *M. aeruginosa*. We also assumed that the morphology changes were defensive strategies utilised by *C. vulgaris* to resist *M. aeruginosa*. The temporary stress of *M. aeruginosa* was favourable to the growth of *C. vulgaris*. By contrast, the continuous induction of colony formation by *M. aeruginosa* in the field inhibited the growth of *C. vulgaris*. The present results provided new insights into the interaction between algae and theoretical basis for algae succession in the field.

Keywords: *Microcystis aeruginosa* / *Chlorella vulgaris* / allelopathy / colony formation / initial cultivation density / cultivation phase / microcystins

1 Introduction

Given the eutrophication and climate warming, cyanobacterial blooms occurred frequently in freshwater lakes, rivers, reservoirs and ponds worldwide (Paerl and Huisman, 2008; Li *et al.*, 2012). In these areas, *Microcystis* is the major contributor (Davis *et al.*, 2009; Paerl *et al.*, 2010; Zhang *et al.*, 2010); for example, *Microcystis* sp. dominated Lake Taihu (Chen *et al.*, 2003; Cai *et al.*, 2012; Ma *et al.*, 2015) and Dianchi in China (Dong *et al.*, 2015). *Microcystis* blooms have also received increasing attention and posed considerable threats to the survival of aquatic organisms and humans (Chen *et al.*, 2003; Dittmann and Wiegand, 2006; Dong *et al.*, 2015).

The underlying mechanisms of this *Microcystis* dominance and severe reduction of phytoplankton diversity have been widely studied but still not completely understood. Several studies have been conducted to evaluate the role of abiotic factors (mainly hydrodynamics, water temperature, light and nutrients) on the dominance of certain algae. However, recent studies reported that biotic factors underlie the succession between *Microcystis novacekii* and *Scenedesmus quadricauda* or between *Skeletonema costatum* and *Prorocentrum dentatum* (Kuwata and Miyazaki, 2000). Therefore, many researchers proposed that allelopathy may also be an important mechanism for certain algae to obtain a competitive advantage in freshwater ecosystem (Gross, 2003; Jin and Dong, 2003; Leão *et al.*, 2009; Rzymski *et al.*, 2014; Yang *et al.*, 2014). Allelopathy is the release of secondary metabolites, which influence the growth of other organisms. Allelopathic inhibition has been observed in various interactions between cyanobacteria and green algae, and intraspecies competition within cyanobacteria, and green algae (Tab. 1).

Cyanobacteria are one of the most important producers of allelochemicals and toxins in freshwaters (Pflugmacher, 2002; El-Sheekh *et al.*, 2010; Ma *et al.*, 2015). Therefore, cyanobacteria may influence the outcome of competition and dominance of algal communities in aquatic environments by selectively suppressing other growth (Legrand *et al.*, 2003; Gantar *et al.*, 2008; Graneli *et al.*, 2008; Ma *et al.*, 2015). Phytoplankton composition in freshwater ecosystems (especially for eutrophic water region) varies, and it often includes cyanobacteria and green microalgae as major components (Chen *et al.*, 2003; Okello *et al.*, 2010; Cai *et al.*, 2012). In eutrophic lakes, such as Lake Taihu, cyanobacteria and green

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Table 1. Allelopathic interactions between algae.

Taxa	Influencing algae	Target algae	References
Green algae	*Chlorella vulgaris*	*Pseudokirchneriella subcapitata*	Fergola *et al.*, 2007
	Chlamydomonas reinhardtii	*Haematococcus pluvialis*	McCracken *et al.*, 1980
	Olisthodiscus luteus	*Skeletonema costatum*	Pratt, 1966
Cyanobacteria-green algae	*Microcystis aeruginosa*	*Scenedesmus obliquus*	Zheng *et al.*, 2008
	Quadrigula chodatii	*Microcystis aeruginosa*	Zhang *et al.*, 2013
	Chlamydomonas reinhardtii	*Anabaena flos-aquae*	Kearns and Hunter, 2002
	Microcystis aeruginosa	*Oocystis marsonii*	Dunker *et al.*, 2013
Cyanobacteria-Cyanobacteria	*Microcystis* sp.	*Nostoc* sp.	Valdor and Aboal, 2007
	Cylindrospermopsis raciborskii	*Microcystis aeruginosa*	Mello *et al.*, 2012
	Microcystis aeruginosa	*Microcystis wesenbergii*	Yang *et al.*, 2014

algae demonstrate seasonal succession (Cai *et al.*, 2012). Our previous study also suggested that seasonal dynamics occur between cyanobacteria and green algae in Lake Dianchi, China (Dong *et al.*, 2015).

Nevertheless, despite the recent studies on the allelopathic interactions between cyanobacteria and green algae (Tab. 1), the effects of cyanobacteria, (1) allelopathic inhibition or stimulation on target algae and (2) the role of cultivation phase of *cyanobacteria* and initial biomass of target algae between these interactions, remain unknown. To date, related studies are rare. Thus, we need to improve our understanding of the inhibitory and stimulatory interactions amongst different cultivation biomass of the target algae to elucidate the role of allelopathy. In addition, previous studies mainly focused on the growth effects caused by allelopathic interaction. In recent years, some studies found that direct competition between phytoplankton can also influence the morphology of the target algae (Leflaive *et al.*, 2008; Mello *et al.*, 2012). However, the existing relative studies are limited, and many issues must be addressed. The ecological meaning of these morphological changes has also not been studied.

In the present study, we use toxic cyanobacterium *M. aeruginosa* and green alga *Chlorella vulgaris* to determine (1) whether cell-free filtrates associated with *M. aeruginosa* can affect the growth, the underlying mechanism of this effect and the morphology of green alga *C. vulgaris*. We also ascertained (2) whether *C. vulgaris* with different initial inoculation biomass demonstrated different responses to cell-free filtrates associated with *M. aeruginosa* and (3) the ecological significance of morphological changes in *C. vulgaris* when it encountered *M. aeruginosa*. (4) Finally, we discussed whether microcystins (MCs) played roles in the effects of *M. aeruginosa* on *C. vulgaris* in our study. Previous studies suggested that MCs possibly act as allelopathic substances in interspecific phytoplankton interactions (Sedmak and Kosi, 1998; Kaebernick and Neilan, 2001).

2 Materials and methods

2.1 Strains and algal culture

A strain of cyanobacteria *M. aeruginosa* (FACHB-905), which was isolated from Dianchi Lake, China, and green alga *C. vulgaris* (FACHB-8) were provided by Freshwater Algae Culture Collection of Institute of Hydrobiology (FACHB), Chinese Academy of Sciences. Prior to experimentation, both of the strains were cultivated routinely using the sterile BG11 medium (Rippka *et al.*, 1979) at 25 °C, with continuous illumination of 25 μmol photons s^{-1} m^{-2} under a 12-h light: 12-h dark cycle. Both the cultures were shaken three times within a day to prevent algal sedimentation.

2.2 Experimental design

2.2.1 Experiment I: this experiment determined whether cell-free filtrates of *M. aeruginosa* can influence the growth and morphology of target green algae *C. vulgaris* and whether the growth phase of *M. aeruginosa* or the initial cultivation biomass of *C. vulgaris* plays any role

2.2.1.1 Preparation of cell-free filtrates associated with M. aeruginosa

The initial cultivation biomass of *M. aeruginosa* was determined by its optical density (OD) at 665 nm (blue-green algae) under ultraviolet/visible spectrophotometer, and the initial OD_{665} was 0.2 (with cell density 1.86×10^9 cells/L). The cell-free filtrate of *M. aeruginosa* was obtained when *M. aeruginosa* grew to the exponential stationary phase (EP) (cultured for 5 days, when $OD_{665} = 0.56$) and stationary phase (SP) (cultivating for 30 days, when $OD_{665} = 1.56$) (Wang *et al.*, 2017). *M. aeruginosa* cultures at different growth phases were centrifuged at 8000 rpm for 10 min and filtered through Whatman GF/C filters (Whatman International Ltd., Maidstone, England) for subsequent experimentation (Mello *et al.*, 2012).

2.2.1.2 Effects of cell-free filtrates associated with M. aeruginosa *on* C. vulgaris

All experiments were carried out in 250 mL Erlenmeyer flasks with 150 mL volume. The experimentation showed three gradients: control (filtrate BG11 medium) and EP and SP filtrates of *M. aeruginosa* (nutrients were added similar to that of BG11 medium to compensate for nutrient decrease during the preliminary cultivation period). Correspondingly, the initial inoculation OD values of tested green alga *C. vulgaris* were 0.05, 0.1 and 0.2, respectively (Tab. 2). All other

Table 2. Experimental design of the present study.

Initial cultivation biomass (OD)	Control	Treatment	
	Filtrate of BG11 medium	Filtrate of EP	Filtrate of SP
OD = 0.05	Parallel 1–3	Parallel 4–6	Parallel 7–9
OD = 0.1	Parallel 1–3	Parallel 4–6	Parallel 7–9
OD = 0.2	Parallel 1–3	Parallel 4–6	Parallel 7–9

cultivation conditions were the same as described above. The experiments lasted for 7 days.

2.2.1.3 Parameter measurement

To evaluate the effects of *M. aeruginosa* on the morphology of *C. vulgaris*, samples (2 mL) of *C. vulgaris* were viewed under an inverted microscope at 400× magnification. The cell density was determined using Utermöhl method (Lund *et al.*, 1958; Paxinos and Mitchell, 2000). The number of cells per colony and the colony proportion (minimum of three cells) were calculated according to the method of Lürling and Van Donk (1997). Inhibition (I) or enhancement (E) of colony formation to the control was calculated on the basis of the number per colony and colony proportion as follows (Wang *et al.*, 2017):

$$I/E(\text{-fold}) = T/C - 1, \quad (1)$$

where C represents the control data and T represents the filtrate treatment data.

Furthermore, to evaluate the allelopathic effects of *M. aeruginosa* on the growth of *C. vulgaris*, the chlorophyll *a* (Chl*a*) of target green alga *C. vulgaris* was measured and calculated according to the method of Lichtenthaler and Buschmann (2001). The slope of the regression between ln-transformed Chl*a* and time (the lasting experimental period) was used as an estimate of the population growth rate (Lürling, 2006; Mello *et al.*, 2012).

2.2.2 Experiment II: role of MCs in the interaction between *M. aeruginosa* and *C. vulgaris*

In the present study, we selected strain *M. aeruginosa* (FACHB-905), which was isolated from Dianchi Lake (Kunming, China) for study. This strain produces MC-LR of approximately 0.61 μg per 10^7 viable cells (Sun *et al.*, 2012). Thus, to detect whether MC-LR played roles in effects on *C. vulgaris*, we also resorted to purified MC-LR (Taiwan Algal Science Inc., Taiwan R.O.C.) experiments. Four MC-LR concentrations (0.25, 1, 10, 100 μg L^{-1}, the nutrition was added the same as that of BG11) (Gan *et al.*, 2012) were used to investigate the influences of MC-LR on *C. vulgaris*. The control was prepared without MCs. During the whole experiments, the growth and morphology changes of *C. vulgaris* were monitored every day. The experiments lasted for 7 days. The Chl*a* content, growth rate, colony proportion and cell number per colony of *C. vulgaris* were calculated as that in Section 2.2.1.

2.3 Statistical analysis

Mean values and standard deviations were calculated for different replicates ($n = 3$). The effect of different inoculation biomasses of *C. vulgaris* and the growth phase of *M. aeruginosa* on the growth rate of *C. vulgaris* were assessed using a two-way ANOVA with biomass and growth phase as independent factors and subsequent separate *post hoc* tests (Fisher LSD) (Wang *et al.*, 2017).

The photosynthetic pigment, enhancement or inhibition of percentage of cells in colonies and the number of cells per colony of each treatment during the experiment were compared using a repeated measure ANOVA ($\alpha = 0.05$). Both tests were followed with Tukey's *post hoc* tests to detect the differences between treatments ($\alpha = 0.05$). A value of $P < 0.05$ was considered significant in all analyses. Mauchly's sphericity was evaluated. Data were log-transformed when necessary. All these tests were performed in SPSS 13.0 for windows (Mello *et al.*, 2012).

3 Results

3.1 Effects of *M. aeruginosa* filtrates on the cell number per colony and colony proportion of *C. vulgaris*

M. aeruginosa filtrates can influence the morphology of *C. vulgaris*. During the cultivation, particles were significantly observed in the treatment with *M. aeruginosa* filtrate (Fig. 1). According to microscopic observation and data analysis, it was also suggested that the largest colony formed on the second day under *M. aeruginosa* filtrate treatment and the cell number per colony gradually reduced, and, till the end of the experiment, no significant differences were detected between the control and the treatment (Figs. 2 and 3).

According to repeated measure ANOVA analysis, the morphology response of *C. vulgaris* to *M. aeruginosa* filtrates was significantly influenced by its initial cultivation density and phase of *M. aeruginosa* filtrate treatment. The day and the interaction amongst density, day and phase also exerted significant effects on the cell number per colony and colony proportion of *C. vulgaris* (Tab. 3). Further Tukey's *post hoc* test indicated that significant differences were detected in cell number per colony amongst the three initial target algal cultivation densities ($P = 0.000$). The SP and EP *M. aeruginosa* filtrates both exerted significant influences on the cell number per colony in *C. vulgaris* ($P = 0.000$) compared with those of control. Significant differences were detected in the cell number per colony of *C. vulgaris* between SP and EP *M. aeruginosa* filtrate treatments ($P = 0.000$).

The Tukey's *post hoc* test results also suggested that significant differences existed in the colony proportion of *C. vulgaris* between SP and EP *M. aeruginosa* filtrate treatments ($P = 0.000$). The different initial cultivation ODs of the target algae also played significant roles in the response of *C. vulgaris* colony proportion ($P = 0.000$).

3.2 Responses of photosynthetic pigments (Chl*a*) of *C. vulgaris* to *M. aeruginosa* filtrates

There is no difference in the Chl*a* contents of *C. vulgaris* between control and experiments (*M. aeruginosa* filtrates

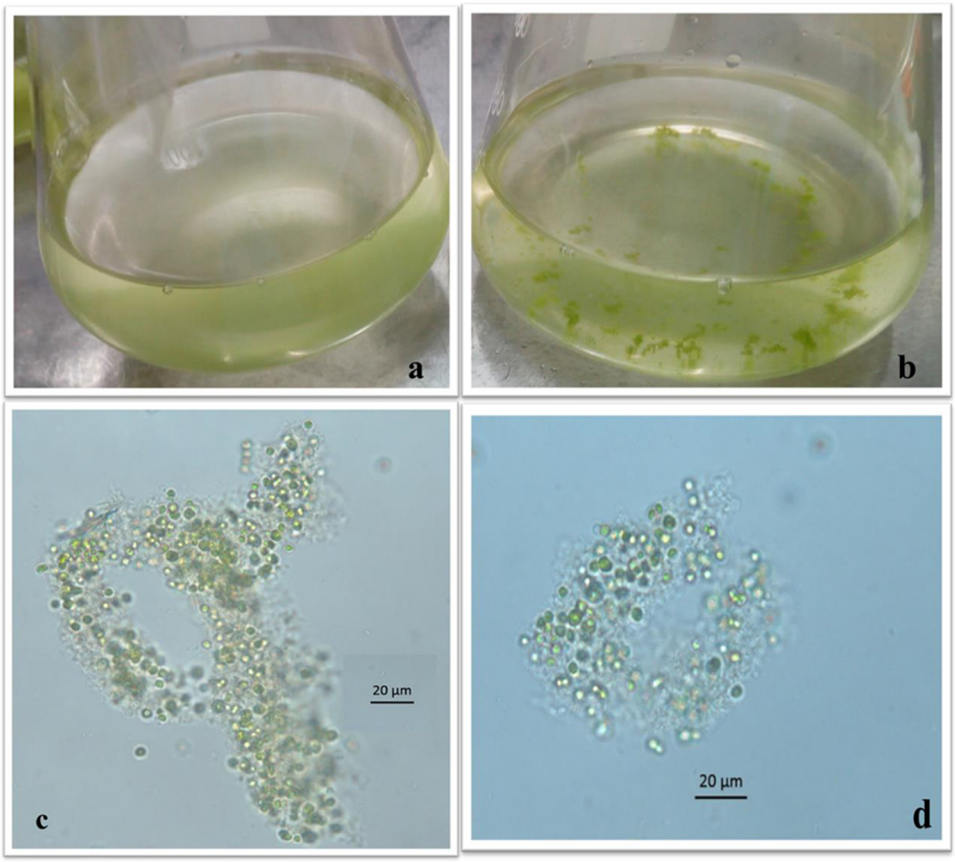

Fig. 1. Photographs of *C. vulgaris* cultivation in control (a) and when it was exposed to *M. aeruginosa* filtrates (b), and the microscopic photos of colonial *C. vulgaris* in the filtrates treatment (c, d).

under the SP and EP) during the first 3 days. However, on the fourth day, the photosynthetic pigments of *C. vulgaris* were significantly higher in SP and EP *M. aeruginosa* filtrate treatments than those of the control (Fig. 4).

According to repeated measure ANOVA analysis, the Chl*a* contents in *C. vulgaris* were significantly influenced with its initial cultivation biomass and the phase of *M. aeruginosa* filtrate treatment. The day and the interaction amongst density, day and phase also exerted significant effects on the Chl*a* contents of *C. vulgaris* (Tab. 2). Tukey's *post hoc* test indicated that SP and EP *M. aeruginosa* filtrates exhibited significant influences on Chl*a* contents in *C. vulgaris* compared with those of control ($P = 0.000$). Significant differences were also detected in Chl*a* contents of *C. vulgaris* between SP and EP *M. aeruginosa* filtrate treatments ($P = 0.002$).

In addition, according to Tukey's *post hoc* test, the initial cultivation density of *C. vulgaris* also played important roles in the target algal response to *M. aeruginosa* filtrates. The significant differences detected in the Chl*a* contents of *C. vulgaris* between the initial cultivation OD were 0.05 and 0.1 ($P = 0.000$), 0.05 and 0.2 ($P = 0.000$) and 0.1 and 0.2 ($P = 0.004$).

3.3 Effects of *M. aeruginosa* filtrates on the growth rate of *C. vulgaris*

The two-way ANOVA detected significant differences in the growth rate of *C. vulgaris* between control and treatments. The growth rate of *C. vulgaris* was significantly affected with its initial cultivation density ($df = 2$; $F = 3650.708$; $P = 0.000$). The Tukey's *post hoc* test revealed significance in three groups. The growth rate of *C. vulgaris* with low cultivation density (OD = 0.05) was significantly higher than those with OD of 0.1 and 0.2 (Fig. 5).

The growth rate of *C. vulgaris* also demonstrated significant differences amongst control and treatment with different phases of *M. aeruginosa* filtrates ($df = 2$; $F = 140.263$; $P = 0.000$). Further, Tukey's *post hoc* test showed that the growth rate of *C. vulgaris* in control was significantly lower than those in SP and EP *M. aeruginosa* treatments ($P = 0.000$). Significant difference was also observed in the growth rate of *C. vulgaris* between *M. aeruginosa* filtrate treatments in SP and EP ($P = 0.000$).

3.4 Effects of MC-LR on *C. vulgaris*

Different from our expectations, the MC-LR with three concentrations exerted no significant influences on the growth and morphology of *C. vulgaris* compared with that of control (data are not shown).

4 Discussion

Allelopathic inhibition is more frequently reported between algal interactions than that of promotion effects.

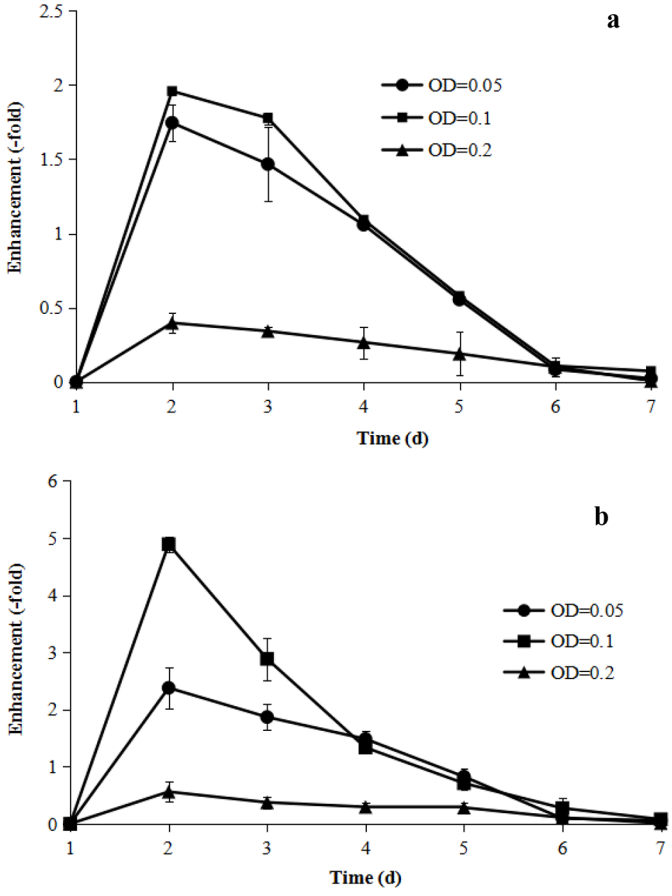

Fig. 2. The enhancement (-fold) of cell number per colony of *C. vulgaris* with different initial cultivation density in *M. aeruginosa* filtrates of EP (a) and SP (b), compared with control.

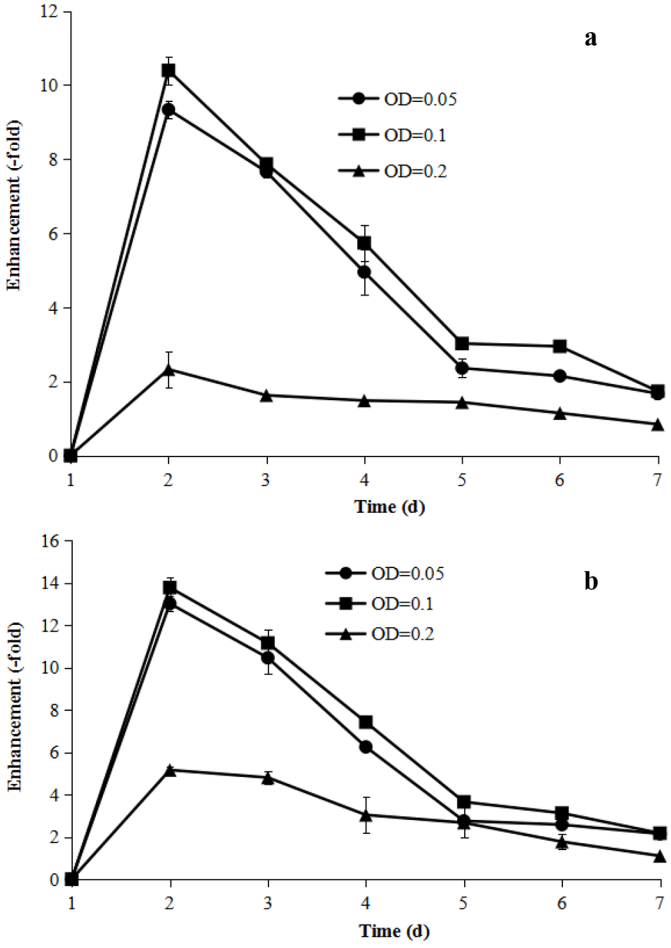

Fig. 3. The enhancement (-fold) of proportion of algae cells in colonies for *C. vulgaris* in *M. aeruginosa* filtrates of EP (a) and SP (b), compared with control.

Cyanobacterin is a biochemical between algal interactions. The cyanobacterin secreted by *Scytonema hofmannii* can inhibit the growth of cyanobacteria (Gleason and Paulson, 1984), eukaryotic algae (Gleason and Baxa, 1986) and higher plants (Gleason and Case, 1986). Moreover, a bloom-forming cyanobacterium *Anabaena flos-aquae* can significantly inhibit the growth of *Chlamydomonas reinhardtii* (Kearns and Hunter, 2001). The exudates from *Microcystis* also inhibit the growth of *Peridium gatunnse* (Sukenik *et al.*, 2002). Considerable studies indicated that MCs or other allelochemcals associated with cyanobacteria are crucial in inhibiting competitive phytoplankton (Suikkanen *et al.*, 2004; B-Béres *et al.*, 2015; Cirés and Ballot, 2016; Harke *et al.*, 2016) or macrophytes (Zheng *et al.*, 2013; Xu *et al.*, 2015; Xu *et al.*, 2016). The most common reported modes of action of allelochemicals towards the target algae include the inhibition of photosynthesis by affecting photosystem II activity (Bagchi *et al.*, 1990; Bagchi, 1995; Srivastava *et al.*, 1998, 2001; Bagchi and Ray, 2001), inhibiting RNA synthesis of other organisms (Doan *et al.*, 2000), interfering with its internal carbonic anhydrase activity (Sukenik *et al.*, 2002), influencing the hormone concentration or disrupting the amino acid metabolism (Weir *et al.*, 2004). The present study proposed that *M. aeruginosa* may influence the growth by affecting the morphology of target algae.

The present study proved the hypothesis that the cell-free filtrate of toxic *M. aeruginosa* can affect the morphology of target algae by inducing large colonies in *C. vulgaris*. The study also provided new insights in allelopathically induced colony formation between algae. To our knowledge, to date, only two previous studies reported about this induced morphological change in alga caused by another alga. Leflaive *et al.* (2008) demonstrated that the allelochemicals released by the filamentous alga *Uronema confervicolum* triggered the colony formation in *Desmodesmus quadrispina*. The other study of Mello *et al.* (2012) indicated that allelochemicals secreted by *Cylindrospermopsis raciborskii* can induce the colony formation of *M. aeruginosa*.

We also indicated that the cell-free filtrates of *M. aeruginosa* in EP and SP treatments significantly reduced the growth of *C. vulgaris* at the first 3 days during the cultivation periods. Such reduction was also accompanied with the colony formation of *C. vulgaris*. This was in accordance with that of Zhu *et al.* (2016) who reported that colony formation requires excessive energy investment, which inhibits the algal growth. Our previous study suggested that

Table 3. Repeated measures analysis for Chl*a* contents in *C. vulgaris* and cell number per colony and colony proportion of *C. vulgaris* in control versus *M. aeruginosa* filtrates treatment, and amongst three initial cultivation densities during days 1–7.

Variable	RM test	*df*	*F*-value	*P*-value
Chl*a* contents in *C. vulgaris*	Phase of *M. aeruginosa* filtrates (phase)	2	143.400	0.000
	Initial cultivation density of target algae (density)	2	1605.443	0.000
	Time	2.742	7535.784	0.000
	Time × phase	5.484	38.117	0.000
	Time × density	5.484	255.406	0.000
	Phase × density	4	17.255	0.000
	Time × phase × density	10.968	3.311	0.002
Cell number per colony (-fold)	Phase of *M. aeruginosa* filtrates (phase)	1	189.605	0.000
	Initial cultivation density of target algae (density)	2	544.047	0.000
	Time	2.491	735.450	0.000
	Time × phase	2.491	61.130	0.000
	Time × density	4.981	139.765	0.000
	Phase × density	2	59.308	0.000
	Time × phase × density	5.641	32.443	0.000
Colony proportion (-fold)	Phase of *M. aeruginosa* filtrates (phase)	1	231.100	0.000
	Initial cultivation density of target algae (density)	2	515.789	0.000
	Time	1.765	772.467	0.000
	Time × phase	1.765	33.352	0.000
	Time × density	3.529	75.843	0.000
	Phase × density	2	0.091	0.913
	Time × phase × density	3.529	0.542	0.686

the colony formation in *C. vulgaris* is due to the accumulation of intracellular carbohydrate (Dong *et al.*, 2018) or carbohydrate secretion. For the target algae, this colony formation may be a defensive strategy to counter adverse conditions (such as competitor toxic *Microcystis*). Mello *et al.* (2012) also suggested *M. aeruginosa* forms colonies, which is potentially a defence mechanism against growth-inhibiting allelochemicals produced by *C. raciborskii*. This hypothesis was supported by Park *et al.* (2009) who also showed that, when exposed to an allelopathic compound isolated from rice (*Oryza sativa*), the growth inhibition in *M. aeruginosa* is stronger in single cells than in colonies. In the field, the large colonies prevent zooplankton from eating them (Van Donk *et al.*, 2011), which is also accompanied with disadvantageous effects, such as increased sedimentation (Dong *et al.*, 2013), and reduced growth rate. Thus, if the *Microcystis* stress is temporary, then the preliminary carbohydrates stored in colonies may provide sufficient energy for them to grow and develop (Dong *et al.*, 2018). This hypothesis was in agreement with our results. In the present study, the fresh cell-free filtrate of *M. aeruginosa* was only added once at the beginning of the experiment (the concentration was maximal). It was suggested that the growth of *C. vulgaris* was reduced, which was accompanied with colony formation at the beginning of the experiment (Figs. 2 and 3). Afterwards, with the degradation of active compounds in the filtrate with the increased experimental time (Wang *et al.*, 2017; Dong *et al.*, 2018), the colonies gradually broke into small colonies and single cells. Until the end of the experiment (seventh day), no differences were detected between the control and filtrate treatments. The final growth rate of *C. vulgaris* in the filtrate treatment was significantly enhanced (Fig. 5). Thus, we concluded that in laboratory experiment, adding filtrates only once was advantageous for the growth and development of *C. vulgaris*. This result was in agreement with that of Żak *et al.* (2012) who demonstrated the stimulated growth of *C. vulgaris* induced by cyanobacteria *Nodularia spumigena*. In the field, the continuous secretion of allelochemicals from *Microcystis* will inhibit the growth of these target green algae, where morphology changes may be crucial.

In the present study, two key issues were also addressed. The first issue is whether filtrates from different culture periods of *M. aeruginosa* exerted different effects on *C. vulgaris*. Previously, limited number of studies have investigated and compared the effects of extracellular metabolites from different growth stages of algae. However, considerable inconsistency exists amongst research results regarding the question of which growth phase of cyanobacteria exerts the strongest allelopathic effects. Arzul *et al.* (1999) demonstrated a stronger allelopathic influence of three *Alexandrium* species in SP than that in EP. Suikkanen *et al.* (2004) indicated that the toxicity of *N. spumigena* in SP is higher than that in EP. Nevertheless, other authors suggested a completely opposite effect: filtrates obtained from cultures of the SP exert less effect on target species than that of filtrates obtained from the same monoculture in the EP (Suikkanen *et al.*, 2004; Wang *et al.*, 2017). The present study proved the hypothesis that the effects of *M. aeruginosa* on target alga *C. vulgaris* depended on cyanobacterial growth phase. The *M. aeruginosa* filtrate treatment of SP exerted more significant influences on the morphology and growth of *C. vulgaris* than that under EP treatment.

The second question is whether *C. vulgaris* with different initial inoculation biomass demonstrated different responses to cell-free filtrates associated with *M. aeruginosa*. During the cultivation, the final growth rate of *C. vulgaris* with low initial

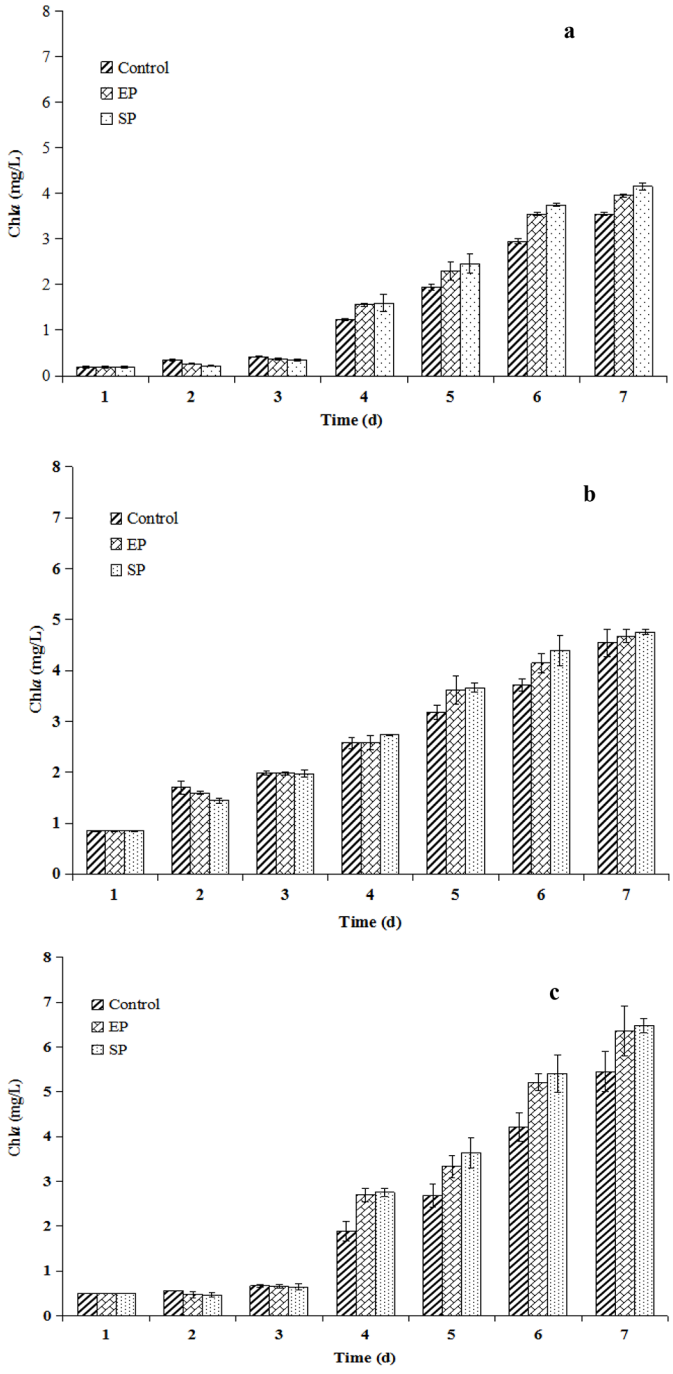

Fig. 4. Effects of *M. aeruginosa* filtrates on Chl*a* contents of *C. vulgaris* with initial cultivation density, OD = 0.05 (a), OD = 0.1 (b) and OD = 0.2 (c).

cultivation biomass was significantly higher than that of higher cultivation density. In addition, more large colonies were detected in initial cultivation of *C. vulgaris* (OD = 0.1) than that of OD of 0.05 and 0.2. The present result was consistent with that of Jiang *et al.* (2015) who indicated that the allelopathic effects of *Hydrilla verticillata* on phytoplankton depended on phytoplankton density. Therefore, the high initial cultivation density of the target algae may resist remarkably against adverse conditions.

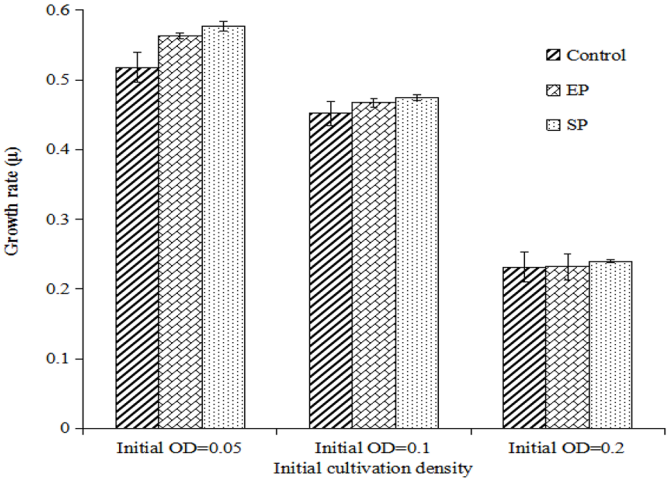

Fig. 5. Growth rate of *C. vulgaris* in control and when it was exposed to *M. aeruginosa* filtrates (EP or SP) with different initial cultivation density (OD = 0.05, 0.1 and 0.2).

Finally, the present study also showed that the biochemicals associated with *M. aeruginosa* may play vital roles in affecting the growth and inducing colony formation of green-algae *C. vulgaris*, because no significant differences were observed in final nutrition content between the control and treatment groups. Bittencourt-Oliveira *et al.* (2016) speculated that the biochemical MCs may play roles in the colony formation of *C. vulgaris*. Although Sedmak and Elersek (2005) and Gan *et al.* (2012) suggested that *M. aeruginosa* can exhibit large cell volumes when exposed to MCs, our simulated study suggested that MC-LR was not the key factor that influenced the morphology of *C. vulgaris*. This result was in agreement with that of Perreault *et al.* (2011) who reported that saxitoxins (released by *C. raciborskii*) induce no toxic effects on algal cells (*C. reinhardtii*). Thus, further separation and identification experiments should be conducted to analyse the active substances in the cell-free filtrates of *M. aeruginosa*, which influences the morphology and growth of *C. vulgaris* in our study.

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