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Abiotic factors in colony formation: effects of nutrition and light on extracellular polysaccharide production and cell aggregates of *Microcystis aeruginosa**

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Abstract Colony morphology is important for *Microcystis* to sustain a competitive advantage in eutrophic lakes. The mechanism of colony formation in *Microcystis* is currently unclear. Extracellular polysaccharide (EPS) has been reported to play an important role in cell aggregate formation of some phytoplankton. *Microcystis aeruginosa* was cultivated under varied abiotic conditions, including different nutrient, light, and temperature conditions, to investigate their effects on EPS production and morphological change. The results show that nutrient concentration and light intensity have great effects on EPS production in *M. aeruginosa*. There was a considerable increase in EPS production after *M. aeruginosa* was cultivated in adjusted culture conditions similar to those present in the field (28.9 mg C/L, 1.98 mg N/L, 0.65 mg P/L, light intensity: 100 μmol/(m^2·s)). These results indicate that abiotic factors might be one of the triggers for colony formation in *Microcystis*.

Keyword: *Microcystis*; colony formation; abiotic factors; cell aggregates

1 INTRODUCTION

Cyanobacteria blooms are an important environmental issue that has recently plagued several countries. Not only do the blooms upset the aquatic ecosystem (Reynolds and Walsby, 1975), but they also contaminate potable water with undesirable odorous compounds (Zhang et al., 2011) and microcystin (Humpage and Falconer, 1999). *Microcystis* has been reported to overwhelmingly dominate in most eutrophic lakes during a cyanobacteria bloom, especially in the summer (Reynolds and Walsby, 1975; Duan et al., 2009). Under natural conditions, these bacteria often occur as large colonies with thousands of algal cells. Large colonies provide a great competitive advantage for *Microcystis* in phytoplankton populations because they increase buoyancy (Oliver, 1994), photosynthetic efficiency (Wu and Song, 2008), defensive abilities (Yang et al., 2009), and physiological activity (Li and Li, 2012). The colonial algae disaggregate and develop into unicellular algae after *Microcystis* are isolated from the field and cultivated in axenic cultures in the laboratory for multiple generations (Reynolds et al., 1987; Bolch and Blackburn, 1996). Their physico-chemical properties are completely different from those of colonial algae. Although many studies focus on the mechanism of colony formation in *Microcystis*, there are not enough data to explain it completely (Šejnohová and Maršálek, 2012).

As an adhesion component, extracellular polysaccharide (EPS) plays an important role in algal cell aggregates, and its production directly correlates with colony formation in some phytoplankton (De philippis and Vincenzini, 1998; Van Rijssel et al., 2000; Pajdak-stós et al., 2001; Bahat-Samet et al., 2004). Similarly, quantitative analysis and ultrastructural observations showed colonial *M.*

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Table 1 Nutrition concentration of different treatments

Treatments	Nitrogen density (mg/L)	Phosphorus density (mg/L)	Carbon density (mg/L)
Control (BG-11)	198	6.5	2.89
N↓	1.98	6.5	2.89
P↓	198	0.65	2.89
C↑	198	0.65	28.9
C↑P↓	198	0.65	28.9
C↑N↓	1.98	6.5	28.9
NP↓	1.98	0.65	2.89
C↑NP↓	1.98	0.65	28.9

aeruginosa cells had a significantly higher EPS content than those of disaggregated cells (Zhang et al., 2007). *Microcystis* colonies induced by grazing pressure also showed a higher EPS content than unicellular *Microcystis* (Yang et al., 2008). These results indicate the importance of EPS content for colony formation in *Microcystis*.

EPS production by phytoplankton partly depends on the environment in which they grow (Helm and Potts, 2012). During the last three decades, several important factors that control the production of phytoplankton EPS have been identified. Lower nitrogen or phosphorus levels result in more EPS production in *Chlamydomonas mexicana* (Kroen and Raybum, 1984), *Cyanothece* sp. (De Philippis et al., 1993), *Anabaena* sp. (Moreno et al., 1998), *Cylindrotheca closterium* (Staats et al., 2000), and *Microcoleus vaginatus* (Chen et al., 2006). Because excessive carbon is used for polysaccharide synthesis first, the increased concentration of carbon in the culture caused an increase of EPS production in *Cyanospira capsulata* (De Philippis et al., 1991). Light intensity and duration also have been reported to exert significant effects on EPS production in *Prophyridium aerugineum* (Friedman et al., 1991), *Nostoc* sp. (Otero and Vincenzini, 2003), *Gloeocapsa gelatinosa* (Raungsomboon et al., 2006), and *Chlorococcum* sp. (Di Pippo et al., 2012). In addition, EPS production in *Anabaena* sp. is markedly enhanced with an increase in temperature (Moreno et al., 1998).

These findings indicate that a change in EPS production would also contribute to the differences between growing environments in the field and in the laboratory. To test this hypothesis, we cultivated unicellular *Microcystis* under varied abiotic conditions, including different nutrient, light, and temperature conditions, to investigate the effect of these factors on EPS production and colony formation in *Microcystis*.

2 MATRIAL AND METHOD

2.1 Algal strain

Cyanobacteria *Microcystis aeruginosa* (Kützing) Kützing PCC 7806 was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB905) and grown in axenic BG-11 culture medium (Stanier et al., 1971) at 25°C under fluorescent light at an intensity of 40 μmol/(m^2·s) with a light-dark period of 12 h:12 h.

2.2 Experimental design

The flask culture experiments were performed in 250-mL flasks containing 100 mL of BG-11 culture medium. To determine the effect of nutritional conditions on EPS production by *M. aeruginosa*, concentrations of carbon, nitrogen, and phosphorus in culture were adjusted individually (C↑, N↓, P↓) or simultaneously (C↑P↓, C↑N↓, N↓P↓, C↑N↓P↓) to levels that were similar to eutrophic lake conditions (Table 1). Varying light intensities (5, 40, and 100 μmol/(m^2·s)) and temperatures (15, 25, and 30°C) were used to determine the effects of light intensity and temperature on EPS production by *M. aeruginosa* cultivated with the unadjusted BG-11 culture medium. Under sterile conditions, exponentially growing unicellular *M. aeruginosa* were inoculated and grown in different treatments for eight days (the initial number of algae cells was 1.0×10^6 cell/mL) after centrifuging and washing with deionized water. Algae that were cultivated in the BG-11 culture medium at 25°C and under light at an intensity of 40 μmol/(m^2·s) were used as controls. All treatments and controls were run in triplicate, and cultures were shaken manually once every day. Cell concentrations and EPS content were determined by hemocytometer under a light microscope (Axio Scope A1, Zeiss, Germany) and the method described in 2.3 after incubation for eight days. Factors that could increase *M. aeruginosa* EPS production were simultaneously investigated through further studies to expose possible synergistic effects on morphology changes and EPS production.

2.3 Polysaccharide assay

The EPS were extracted according to the methods of DelGallo and Haegi (1990) with slight modifications

and quantified spectrophotometrically using the Anthrone method (Herbert et al., 1971). Samples (10 mL) were adjusted to pH 10 and incubated in 45°C water for 4 h, then centrifuged at 27 476×*g* for 15 min. The supernatants were filtered through 0.45-μm membrane filters and dialyzed against deionized water to remove any interference from the presence of ions in the cultures. The polysaccharide content in the dialyzed supernatant was determined using glucose solutions for calibration.

2.4 Data analyses

The specific growth rates (μ, /d) were calculated by the equation: $\mu=\ln(N_t/N_0)/t$, where N_0 and N_t are the initial cell density and the cell density after incubation for t days. All data were presented as the mean±SD and were analyzed by one-way analysis of variance (ANOVA). Differences between the treatments and controls were compared using *t*-tests. All statistical analyses were performed with SPSS 13.0.

3 RESULT

The adjustment of carbon, nitrogen, and phosphorus concentrations in the culture medium resulted in significant effects on *M. aeruginosa* (Fig.1). The growth rates of *M. aeruginosa* in the nutrient adjusted culture medium were lower than those in unaltered culture. Lower nitrogen concentration showed a positive effect on EPS production by *M. aeruginosa*. The EPS production by *M. aeruginosa* cultivated in C↑N↓P↓ was the highest (2.19±0.18 pg/cell) among the treatments and was significantly increased (P<0.05) over that in the standard BG-11 culture medium (1.76±0.28 pg/cell).

The effect of temperature on *M. aeruginosa* in this study showed only in growth changes (Fig.2). Compared with a temperature of 25°C, the higher (30°C) or lower (15°C) temperatures significantly (P<0.05) suppressed the growth rate of *M. aeruginosa*. However, there was no significant difference (P>0.05) in EPS production by *M. aeruginosa* among the temperature treatments.

Growth rates of *M. aeruginosa* cultivated under the high (100 μmol/(m²·s)) and low (5 μmol/(m²·s)) light intensities were significantly lower (P<0.05) than those under the medium (40 μmol/(m²·s)) light intensity. *M. aeruginosa* produced 2.43±0.34 pg/cell of EPS under 100 μmol/(m²·s), which was significantly higher (P<0.05) than under light intensities below 40 μmol/(m²·s) (Fig.3).

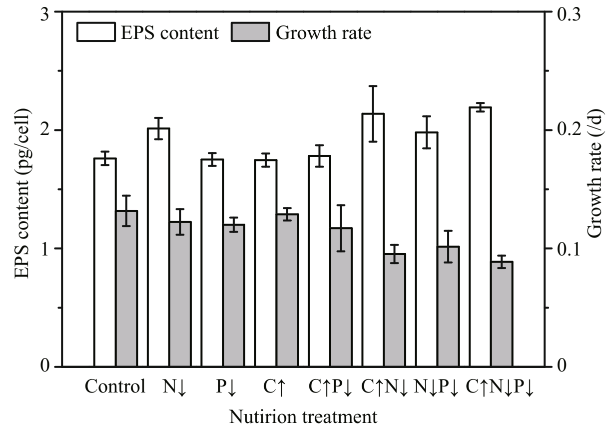

Fig.1 Growth rates and EPS production of *M. aeruginosa* under different nutrition conditions

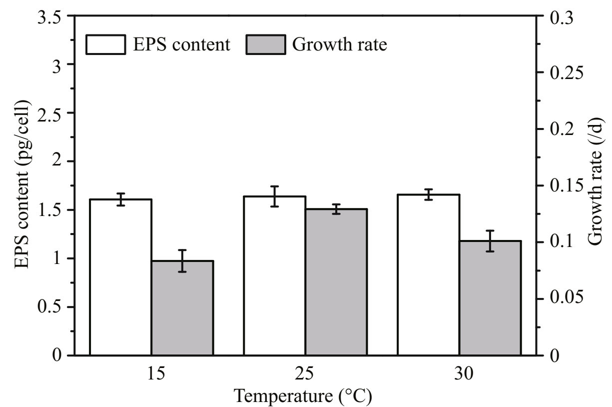

Fig.2 Growth rates and EPS production of *M. aeruginosa* under different temperatures

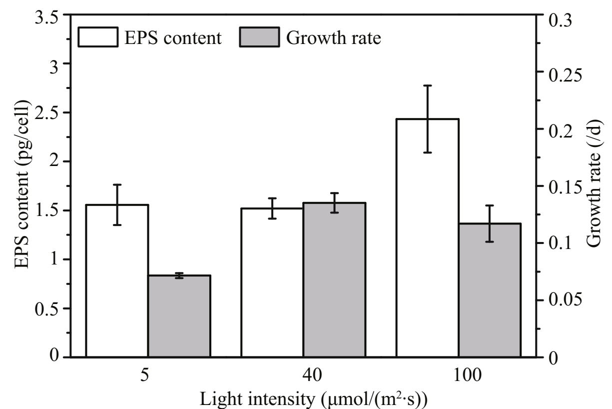

Fig.3 Growth rates and EPS production of *M. aeruginosa* under different light intensities

M. aeruginosa was cultivated under conditions with C↑N↓P↓ and 100 μmol/(m²·s) at 25°C to determine if there were synergistic effects of nutrient concentrations and light intensities on EPS production. After cultivating under these conditions for 4 days,

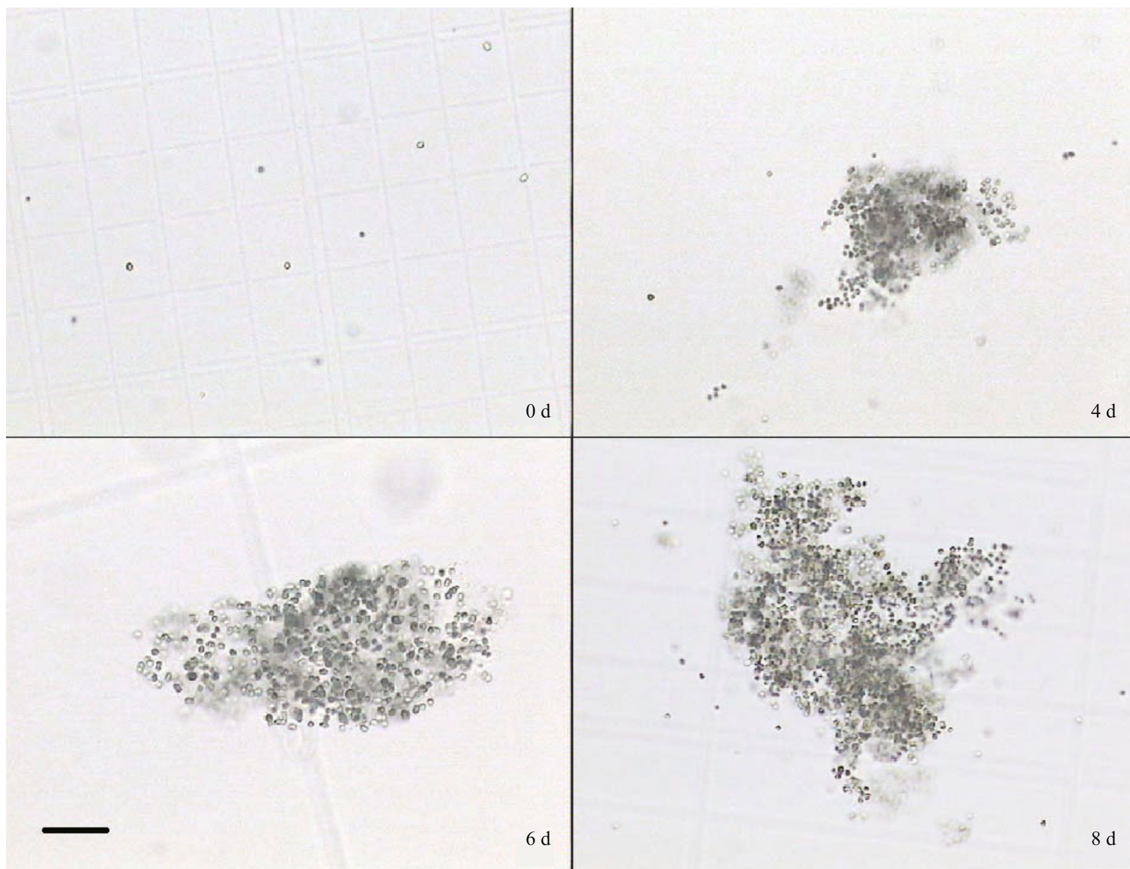

Fig.4 Aggregate formation in *M. aeruginosa* cultivated with adjusted BG-11 medium culture (28.9 mg C/L, 1.98 mg N/L, 0.65 mg P/L) under 100 μmol/(m²·s)

Scale bar = 50 μm

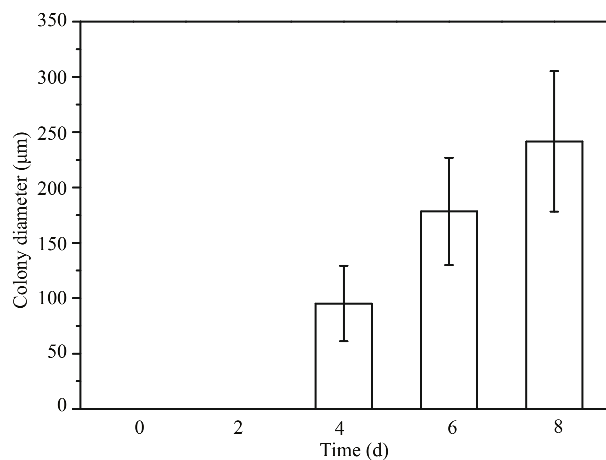

Fig.5 Average diameter of *M. aeruginosa* cell aggregates cultivated with adjusted BG-11 medium culture (28.9 mg C/L, 1.98 mg N/L, 0.65 mg P/L) under 100 μmol/(m²·s)

visible *M. aeruginosa* cell aggregates were observed at the bottom of the flask. The aggregates had an average diameter (by counting at least 50 aggregates from each bottle under a light microscope) of 95.2±34.2, 178.4±48.5, and 211.7±63.4 μm after 4, 6, and 8 days of culture (Figs.4, 5). Although the growth rate was lower than that of the controls, *M. aeruginosa* still showed a growth rate of 0.088/d in culture conditions containing C↑N↓P↓ under an illumination of 100 μmol/(m²·s). Compared with the control conditions (1.61±0.14 pg/cell of EPS), the polysaccharide assay showed a considerable increase (P<0.05) of EPS (3.15±0.15 pg/cell) in cells treated with C↑N↓P↓ and 100 μmol/(m²·s) (Fig.6).

4 DISCUSSION

Colony morphology, an important factor contributing to the dominance of *Microcystis* over other phytoplankton, is often lost after *Microcystis* is cultivated in axenic culture for several generations. In general, nutrient concentrations, especially nitrogen and phosphorus, in the culture medium are excessive for algae growth and are much higher than in natural lakes, while the ratio of C:N in the culture medium is much lower than those found in the field. The most common light intensity used for cyanobacteria culture

in the laboratory is lower than phytoplankton obtain in the upper water column. We adjusted the nutrients concentrations in the culture to 28.9 mg C/L, 1.98 mg N/L, and 0.65 mg P/L, which were closer to those found in eutrophic lakes, such as Chaohu lake, China (Xu et al., 2012). Aggregation formation was observed when unicellular *M. aeruginosa* were cultivated in this adjusted culture medium under a higher light intensity. This finding indicates that differences in nutrient concentration and light intensity between the field and the laboratory might be responsible for colony disaggregation in *M. aeruginosa* cultures.

In this work, we demonstrated that EPS production, a major factor affects the stickiness of the cell surface and cell aggregation in *Microcystis* (Yang et al., 2008; Helm and Potts, 2012), was strongly affected by nutrient concentration and light intensity. Lower nitrogen concentrations exerted a positive influence on EPS production, which might contribute to the C:N ratio increase, thus promoting the incorporation of carbon into polymers (Otero and Vincenzini, 2003). Enhance EPS production by *M. aeruginosa* under phosphorus limitation has been reported previously (Wang et al., 2010a). It may be because the phosphorus concentration was still sufficient for *M. aeruginosa* growth in our study, no significant change was found on EPS production by *M. aeruginosa* after phosphorus concentration in the medium was deceased. Light provides the main source of energy for cellular material synthesis in phytoplankton. The net accumulation of EPS in phytoplankton is controlled by the ratio of carbon fixation and utilization (Reynolds et al., 1987; Reynolds, 2007). The rate of photosynthetic carbon fixation is essentially governed by light intensity and ambient CO_2 density. Protein synthesis predominates in phytoplankton cells under low light intensity because of their low photon saturation (Hawes, 1990). When light intensity exceeds the photon saturation required for protein synthesis, the synthesis of other materials, such as EPS or pigments, will increase (Lancelot et al., 1986). Although no morphological changes were found in the *M. aeruginosa* population when unicellular algae were cultivated in adjusted cultures or at higher light intensity, the synergistic effects of nutrient adjustment and higher light intensity resulted in sufficient EPS production to support cell aggregation in *M. aeruginosa*.

Microcystis aggregates in our experiments had differing morphology from colonies obtained from the field, which were loose and without clear mucilage.

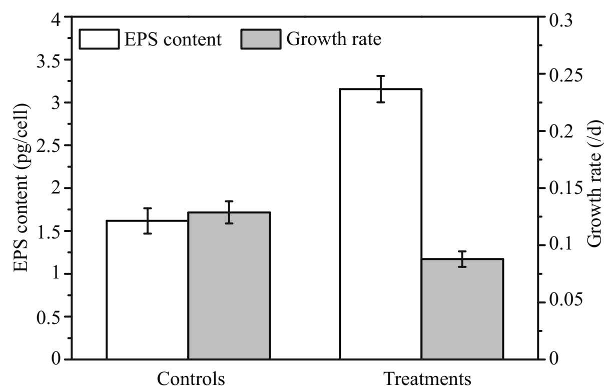

Fig.6 Growth rates and EPS production of *M. aeruginosa* under the control conditions (2.89 mg C/L, 198 mg N/L, 6.5 mg P/L, light intensity: 40 μmol/(m²·s)) and in the treatment (28.9 mg C/L, 1.98 mg N/L, 0.65 mg P/L, light intensity: 100 μmol/(m²·s)) groups

Aggregates are clearly resulted from the adhesion of previously existing single cells because it was impossible to form an aggregate of thousands of cells after cell division over several days. Previous studies have demonstrated that grazing pressure from flagellates could induce colony formation in *Microcystis* (Yang et al., 2008, 2009). These induced colonies were formed from daughter cells of a freshly divided cell that failed to separate during the reproductive process. Colony formation may be affected by the combined effects of nitrogen concentration and flagellate grazing, which exist under laboratory conditions (Wang et al., 2010b). These results demonstrate that both biotic and abiotic factors may be responsible for colony disaggregation and formation in *Microcystis*. The effects of other environmental factors, such as pH, dissolved oxygen, and micronutrients should be covered in future studies.

5 CONCLUSION

Our results show nutrient concentration and light intensity have large effects on EPS production in *M. aeruginosa*. A considerable increase in EPS production and aggregation formation was observed when unicellular *M. aeruginosa* were cultivated in an adjusted culture (28.9 mg C/L, 1.98 mg N/L, 0.65 mg P/L) with nutrient concentrations and light intensity closer to those in eutrophic lakes. This finding indicates that differences in nutrient concentration and light intensity between the field and the laboratory might be responsible for colony disaggregation in *M. aeruginosa* cultures.

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