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Effects of Ca and Mg levels on colony formation and EPS content of cultured M. aeruginosa

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Abstract

Colony formation of Microcystis plays a significant role in Microcystis blooms. Calcium (Ca) and magnesium (Mg) are important nutrient elements during algae growth. In this study, the influences of Ca and Mg concentrations on extracellular polysaccharides (EPS) content and colony formation of Microcystis were investigated, and then the effects of EPS content and specific growth rate on colony formation were discussed. The results showed that Ca had a direct observable influence on the colony formation of Microcystis; however, Mg was not obvious. More specifically, when Ca concentration was lower than 20 mg•L-1, the Microcystis was dominated by single cells, when the concentration was over 20 mg•L-1, Microcystis colonies were found and took up more than 50%, and the size of colony was increased with the increasing Ca concentration. Moreover, it showed that the mean size of Microcystis had significant correlation with EPS content, and lower specific growth rate was in favor of colony formation.

Keywords: calcium, magnesium, Microcystis aeruginosa, colony formation, extracellular polysaccharides, specific rate of growth

1. . Introduction

Cyanobacterial blooms caused by Lake Eutrophication have resulted in serious environmental problems in many lakes and other water systems around the world. Microcystis is one species of bloom-forming cyanobacteria, which is widely distributed, large in scale and has long duration [1]. When water bloom occurring, Microcystis was aggregate largely on the surface of water, and could be visible. On the one hand, the colonial formed can effectively deter zooplankton grazing and increase the survival of Microcystis population [2]. On the other hand colonial formed is helpful to Microcystis vertical migration, and can absorb enough light and nutrients. Thus, colonial form plays a vital role in the occurrence of Microcystis blooms; however the mechanism on colony formation of Microcystis is still unclear.

The previous studies showed that the extracellular polysaccharides (EPS) affected the stickiness of the cell surface and contribute to cell aggregation in some algal species, which was release from Microcystis [3,4]. The macroscopical colonies of Microcystis are surrounded by mucilaginous matrix under natural conditions, however, after several generations in the laboratory the characteristics of colonies disappear, existing as single cells [5], and could not formed colonies again under laboratory conditions [6].

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Moreover, the colony formation was found when researchers investigated the interactions between Microcystis and the potential grazer [4,7]. Protozoa grazer and microorganism could promote Microcystis to form colonies, in the same time, the amount of EPS was increased [4,8]. Additionally, the limited of nitrogen and phosphorus could enhance the carbohydrate, especially the nitrogen-limited of culture [9].

Mention above showed that zooplankton, microorganism and nutrients could influence colony formation through the EPS content, which produced by Microcystis. Also, some attention had been paid on the ions, such as Ca and Mg. Both of them are important nutrient elements of plant, could affect carbohydrate formation and transformation. And Ca could enhance the activity of lectins, which was likely an important factor for colony formation [10], Mg was one component of chlorophyll, could activate enzymes in the courses of fermentation and respiration. Van [11] reported that Ca and Mg were essential for the gelling of phaeocystis colony mucus, which was an important factor for colony formation of algae, due to both of them could bind up with colony formation of Microcystis. Although, Wang [12] reported that high-Ca medium could enhance the colonial strain to aggregate, few studies have been done on the influences of Ca and Mg levels on colony formation of Microcystis. Based on the water quality of Taihu Lake, we found Ca and Mg were dominant ions, over 50% of total cations. So, in this study, the effects of calcium and magnesium levels on Microcystis growth colony formation and EPS content were investigate.

2. Materials and methods

A unicellular Microcystis aeruginosa strain (FACHB-469) was obtained from the Culture Collections of Freshwater Algae of the Institute of Hydrobiology, Wuhan, China. The strain was batch cultured in liquid BG11 medium in 1.0L erlenmeyer flasks at 25oC and under a fluorescent light intensity of 40μEm-2s-1 with a light: dark period of 12:12 h. In this study, CaCl2 and MgSO4 were used as sources of Ca and Mg in M11. The alga was cultured with Ca and Mg concentrations of 0, 10, 20, 50, 100 mg•L-1 and 0, 2, 7, 10, 20 mg•L-1, respectively. In addition, pre-culture of M. Aeruginosa was starved without Ca and Mg for 5 days and the alga in its late exponential growth phase was used in the experiments with concentration of 5×104cells•mL-1. The experiments run in triplicate for 11 days, flasks with algal cultures in were shaken three times a day.

The density of algae was measured by UV, according to the Significant linear correlation existed between density of algae and absorbance of algae suspension [7]. Based on the Planner's [13] research, the size of colony or cell was analyzed by laser particle size. Also, according to Yang's method [4], the sEPS (soluble extracellular polysaccharides) and bEPS (bound extracellular polysaccharides) were determined by the anthrone method [14], and the total EPS was equated to the sum of concentrations of sEPS, bEPS. Additionally, specific growth rate was calculated by following formula:

$\mu = (\ln N2 - N1)/(t2 - t1)$

which t2, t1 meant incubation time and N2, N1 represented cell densities at t2, t1 incubation time.

3. Results

3.1 *Effects of calcium and magnesium levels on the growth of M. aeruginosa*

The growth of M. aeruginosa under different Ca and Mg concentrations is showed in Fig. 1. It showed that when Ca concentration was over 50 mg•L-1, the cell densities was decreased, and no significant difference among concentration ranging from 0 to 20 mg•L-1. Moreover, the Cell density of magnesium-

free treatment was obviously less than Mg concentrations treatments from the seventh day of the culturing cycle.

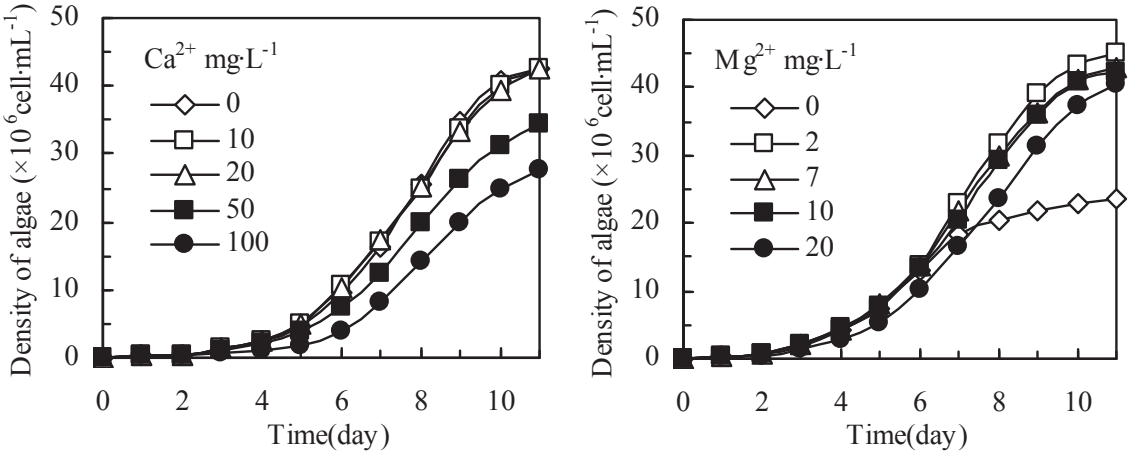

Fig. 1 Growth curves of *M. aeruginosa* under different Ca and Mg concentrations in culture

3.2 Effects of calcium and magnesium levels on colony formation of M. aeruginosa

In the study, M. aeruginosa cells were divided into three fractionations according to particle size: 0-6μm, 6-75μm and >75μm, which represented unicell, small colony (S.colony) and lager colony (L.colony), respectively, which were showed in Fig. 2.

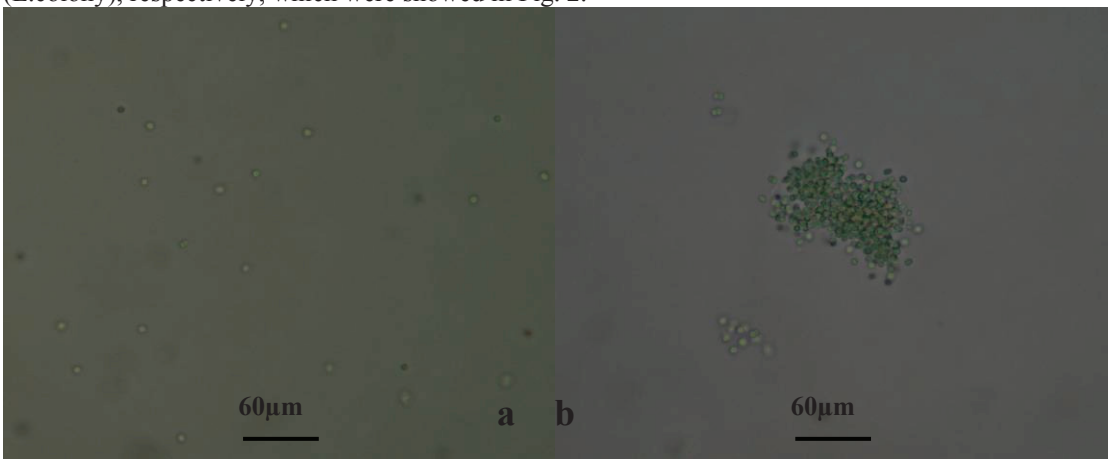

Fig. 2 Microscopic observation of M. aeruginosa colonies in different treatments. a: unicell; b: L.colony.

From Fig. 3a, it displayed that M. aeruginosa populations contained about 58% unicells, with some S.colony and few L.colonies with Ca concentration of 0-10 mg•L-1, while S.colony increased to 73% when Ca concentration reached 20 mg•L-1. Moreover, with increased Ca concentration, more and more

L.colony be found, which could occupied more than 35% with Ca concentration of 100 mg•L-1. Contrarily, few L.colony was observed in the Mg cultures (Fig. 3b).

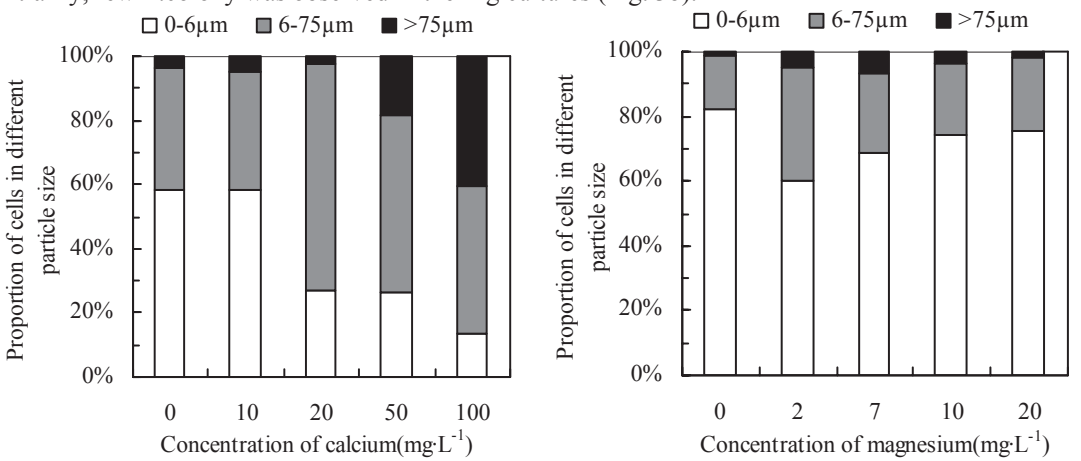

Fig. 2 Changes in proportion of cells in different particles in Ca and Mg concentrations treatments

3.3 Effects of calcium and magnesium levels on EPS content of M. aeruginosa

Fig. 4 shows the EPS contents of M. aeruginosa with different treatments. The sEPS contents of M. aeruginosa increased gradually with increased Ca concentration, while no significant differences appeared on the bEPS contents. Moreover, the sEPS contents of M. aeruginosa decreased firstly and then increased with increased Mg concentration, whereas the bEPS content in with Mg treatment was higher than Mg-free treatment, and was no significant differences with the treatment.

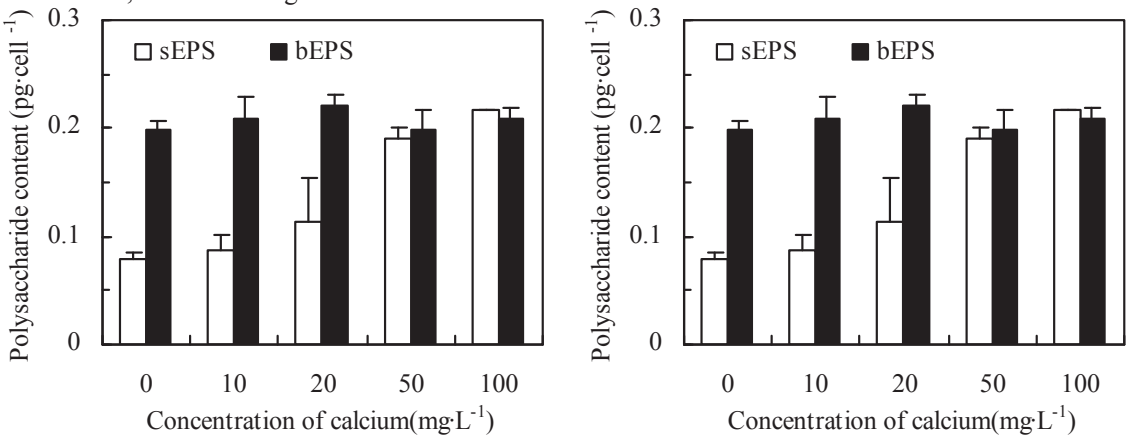

Fig. 4 Production of EPS of *M. aeruginosa* cultured in different Ca and Mg concentrations in culture

4. Discussion

4.1 The relationship between EPS content and colony formation of M. aeruginosa

The fact of macroscopic colonies formed under the high-Ca culture medium, was in accordance with Wang's results [12], who suggested that colony formation in M. aeruginosa may due to adhesion of single cells when an increase in the production of EPS occurred with the increase of Ca levels. While, there are some difference from Wang's results, which may caused by the different experimental conditions and algae strain or others unknown factors. The significant correlation between mean particle size of M. aeruginosa and the production of unicellular EPS with Mg (P=0.038, Fig. 5b) and Ca (P=0.002, Fig. 5a) levels. It indicated that the higher concentration of Ca and Mg could improve the algal carbohydrate formation and transformation, which may resulted in the M. aeruginosa colony formed. Moreover, the lots of L.colony found in high-Ca culture and few with Mg culture suggested that the colony formation of M. aeruginosa not only depended on EPS content, but also was associated with others factor. Thus, further investigations are necessary in future study, such as the Ca flocculation effecting and higher Mg concentration, etc.

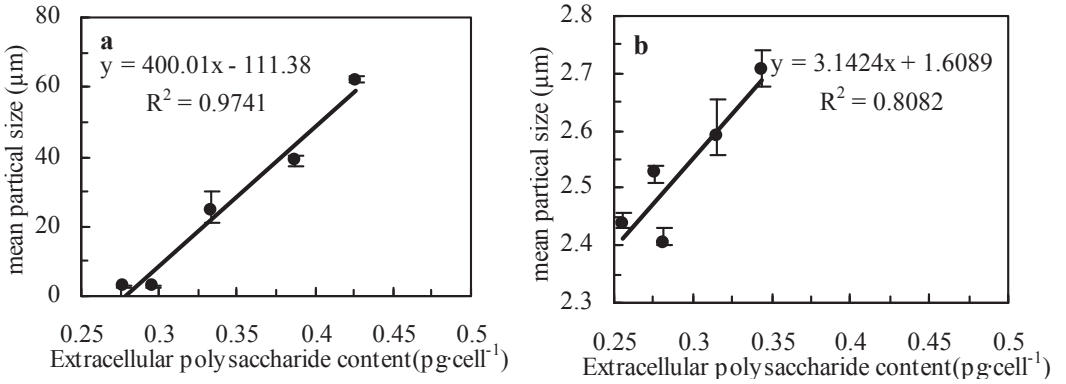

Fig. 5 Relationship between mean particle size of *M. aeruginosa* and production of extracellular polysaccharides per cell

4.2 The relationship between specific growth rate and colony formation of M. aeruginosa

The result showed that colonial proportion was significantly higher than other treatments, while specific growth rate of M. aeruginosa express as decreased to a certain extent (Fig. 6a). Because L.colony may be weaker than unicell in respect of obtaining resources such as soluble nutrients, light. So there was lower specific growth rate in the aspect of M. aeruginosa growth [15], which was identified with lower growth rate caused by bigger colony size, obtaining by Wilson [16]. Moreover, formation and transforming of algal carbohydrate contributed to the cell division, which acquired a higher specific growth rate. At the same time, EPS content might drop, thus there existed negative correlation between intracellular polysaccharides content and specific growth rate of M. aeruginosa [17]. The supposition of that the EPS content could be affected by specific growth rate attributing to the lower EPS production, which influenced alga colony forming. Here, further meaning is that specific growth rate may influence colony formation of M. aeruginosa and the lower specific growth rate was beneficial to colony formation. Additionally, the growth of M. aeruginosa was restricted in the Mg-free culture (Fig. 6b), which may due to the photosynthesis of M. aeruginosa was restricted. And no significant difference was observed on the specific growth rate with Mg levels, which indicated that Mg ion has fewer influences on colony formation of M. aeruginosa.

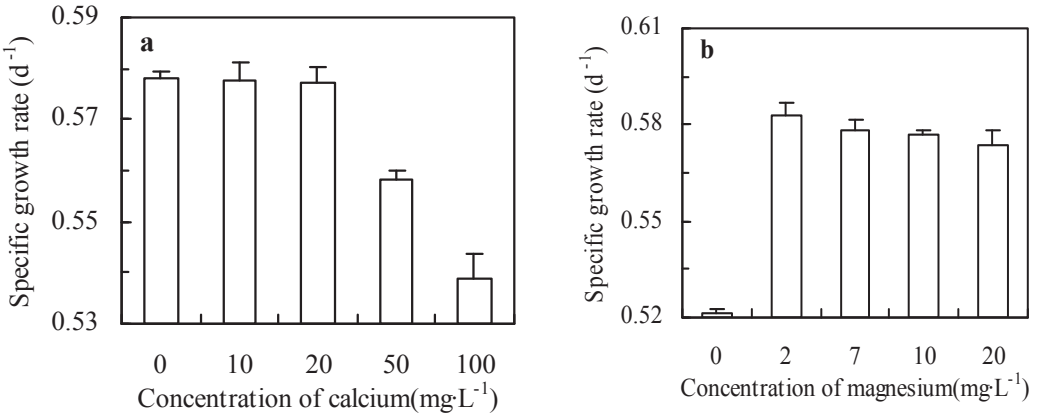

Fig. 6 Relationship between specific growth rate of *M. aeruginosa* and different Ca , Mg concentrations in the culture

5. Conclusions

In this study, there were very significant correlation between the mean particle size of M. aeruginosa and the production of unicellular EPS, thus the increase of EPS contents were helpful for M. aeruginosa colony formation. High-Ca and Mg-free concentration exhibited lower specific growth rate, while no

significant difference was observed among other treatments. Moreover, lower specific growth rate was beneficial to colony formation. The fact that L.colony formation was found in high-Ca treatment, whereas M. aeruginosa populations in Mg treatments were dominated by unicellular, which indicated that the colony formation of M. aeruginosa not only depended on EPS contents, but also was associated with others factor. Furthermore, an increase in Ca concentration may be favor to Microcystis blooms occurring in a eutrophic lake.

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