Biochemical composition of *Microcystis aeruginosa* related to specific growth rate: insight into the effects of abiotic factors

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Abstract: The content of different *Microcystis* biochemical components (including total polysaccharides (TPS), proteins and RNA) were analyzed at different growth rates obtained under varying light intensities, temperatures and nutrient concentrations. Higher light intensity and temperature yielded a higher specific growth rate of *Microcystis* but the two different culture media did not significantly affect growth of *Microcystis* in the current study. The differences in the cellular content of TPS, protein and RNA at different temperatures (20 and 25°C) were significant under both light intensities. The differences under different light intensities (30 and 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were significant at 25°C but negligible when temperature was 20°C. These phenomena suggested that temperature would be the limiting factor influencing the cellular processes when temperature was 20°C. However, when temperature was 25°C, the limiting factor was observed to be light intensity. The results also demonstrated that the content of TPS and RNA decreased, but the value of proteins increased with an increase in specific growth rate.

Keywords: *Microcystis aeruginosa*, specific growth rate, polysaccharide, carbohydrate transformation

Introduction

Colony formation is an important contributor to the dominance and bloom formation of the harmful cyanobacterium *Microcystis* in lakes and reservoirs (Yamamoto et al., 2011). This is because of its advantages in anti-predator defense (Cyr and Curtis, 1999) and high floating velocities (Wu and Kong, 2009). Extracellular polysaccharides (EPSs) were reported to play an important role in *Microcystis* colony formation (Plude et al., 1991). The increase of EPS content could promote colony formation in *Microcystis* (Yang et al., 2008). EPS is secreted by *Microcystis* cells and derived from intracellular polysaccharide (IPS). Moreover, the EPS content has been reported to be closely related to the IPS content (Yang et al., 2008). It must be noted that the combination of EPS and IPS is the total polysaccharide content (TPS).

Carbohydrates, the photosynthetic products of *Microcystis*, would transform into polysaccharides (same as TPS), proteins, and nucleic acids (mainly be RNA), if they are not used in respiration. Rapid synthesis of protein and RNA would cause the synthetic rate of TPS decrease (Dai et al. 2013). Thus, study on the proportions of different biochemical components in *Microcystis* (including TPS, proteins and RNA) with varying abiotic factors can provide deeper insight into changes in cellular TPS content and colony formation of *Microcystis*.

The synthetic processes of TPS, protein and RNA are affected by abiotic factors such as light intensity, temperature and nutrient (especially nitrogen), which in turn affect the growth rate of *Microcystis*. It has been reported that TPS is negatively correlated with the specific growth rate of *Microcystis* cultured at different light intensity and phosphorus concentration (Wang et al., 2010). Our previous work (Li et al., 2013) also demonstrated a negative correlation between the specific growth rate and cellular polysaccharide content (EPS and IPS) in *Microcystis* with varying light intensity and temperature. Some researchers (Long et al., 2001; Oh et al., 2000) have found that the proportions of protein are different at different specific growth rates. It is important to note that the content of protein and RNA may be higher at a higher specific growth rate with high light intensity and temperature because protein and RNA relate

significantly to cell division (Rhee 1978). Thus, we hypothesized that the content of TPS, protein and RNA relate significantly to specific growth rate under different abiotic conditions.

The aim of this study was to ascertain the effects of light intensity, temperature and nutrient concentration on the biochemical components of *Microcystis*. Specifically, *Microcystis* biochemical components were analyzed to elucidate the variations in proportions of different *Microcystis* biochemical components.

Materials and Methods

Organisms: *Microcystis aeruginosa* (Kützing) Kützing 1846 strain (FACHB 469) was obtained from the Freshwater Alga Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. This unicellular strain was subjected to long-term axenic cultivation in BG-11 medium. Algae, cultured for seven days (late exponential growth phase), were used in our experiments.

Cultivation and adjustment of specific growth rate: The algae were batch-cultured in triplicate under eight different light intensities (25, 30, 35, 40, 45, 50, 55, and 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) at 20°C and 25°C in BG-11 medium and M-11 medium (150 mL medium in a 250 mL conical flask) under a 12:12 hour light-dark cycle, respectively. Two media were used to make sure nutrient concentration would not affect the relationship between biochemical composition and the specific growth rate of *Microcystis*. The initial pH of each medium was adjusted to 8.0 using a 5 μM NaOH solution. The initial cell density of *M. aeruginosa* was $5 \times 10^4 \text{ cells mL}^{-1}$. Flasks were shaken by hand, two to three times daily, to prevent cells from adhering to the inner walls of flasks.

Cell counting and specific growth rate calculation: The culture time was nine days for all cultures and the specific growth rate was determined on day nine by cell counting. To dissociate the colonies into single cells, a centrifuge tube containing 3 mL culture sample was placed in a 100°C water-bath oscillator and shaken at 120 rpm for 5 min. The cells were counted three times in a haemocytometer under an optical

microscope (Olympus CX31, Olympus Corporation) at 400× magnification. If the three counts differed by less than 10%, the average count was designated the final cell density. Otherwise, additional counting was carried out (Li et al., 2013).

The specific growth rate (μ) was calculated from the following equation (1):

$$\mu = \ln(D_t/D_0)/t \quad (1),$$

where D_t is the cell density at time t , D_0 is the initial cell density, and t is the period of the logarithmic growth phase, which is nine in the current study.

Chemical analysis: The cultured samples (20 mL) were filtered through a tared GF/C glass-fiber filter (Whatman, Maidstone, UK), and then dried using a vacuum freeze dryer for 24 h to gain the dry weight. The content of carbon and nitrogen was analyzed via a Vario EL- III element analyzer. The cultured samples (20 mL) were centrifuged at 11,550 g for 15 min, and the supernatants were discarded. The pellets were re-suspended in their centrifuge tubes in 5 mL of 0.5 mol L⁻¹ caustic soda solution (Rausch, 1981). The tubes were placed in a 100°C water-bath oscillator, shaken at 120 rpm for 15 min, and then centrifuged at 11,550 g for 15 min. Supernatants were then separated from pellets. Two repeats of these procedures were carried out. Supernatants were combined, and the polysaccharide content and protein content of the combined supernatants were assayed using the anthrone sulfuric acid method and Coomassie Brilliant Blue method, each in triplicate, and normalized by cell counts.

For RNA content analysis, cultured samples (20 mL) were centrifuged at 11,550 g for 15 min, and the supernatants were discarded. The pellets were re-suspended in 50 mL centrifuge tubes containing 5 mL of 1 mol L⁻¹ HClO₃, placed in a 90°C water-bath oscillator, shaken at 120 rpm for 15 min, and then centrifuged at 11,550 g for 15 min. Supernatants were then separated from pellets. Two repeats of these procedures were carried out. Supernatants were combined and the content of RNA was analyzed following the methods described by Rhee et al. (1978).

Statistical analysis: The specific growth rate was presented as mean± SD. Other data were presented as mean and a Pearson correlation was used to analyze their relations

to specific growth rate. The differences of dry weight, contents of TPS, protein and RNA were analyzed by one-way ANOVA using tukey post hoc test. All statistical analyses were performed using SPSS 10.0.

Results

Growth of *M. aeruginosa* under different culture conditions

Figure 1 shows the specific growth of *M. aeruginosa* under varying light intensities at 20°C and 25°C in BG-11 and M-11 media, respectively. The specific growth rate of *M. aeruginosa* was obviously affected by light intensity and temperature but no significant differences were found between the two different culture media. The maximum specific growth rate, obtained under a light intensity of 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was 0.62 day^{-1} and 0.55 day^{-1} at 25°C and 20°C, respectively. The minimum specific growth rate was 0.55 day^{-1} and 0.32 day^{-1} at 25°C and 20°C, respectively.

[Insert Fig. 1 here]

The variation in cellular dry weight of *M. aeruginosa* along with the increase in specific growth rate, is shown in Fig. 2. The cellular dry weight was 17-23 pg cell^{-1} and decreased slightly with increasing specific growth rate.

Variation of biochemical composition

Figure 3 shows the relationship between specific growth rate and *Microcystis* biochemical components. Cellular TPS and RNA decreased with increasing specific growth rate. The cellular TPS content at a specific growth rate of 0.40 day^{-1} was approximately three times greater than the content at a specific growth rate of 0.60 day^{-1} . However, a significant positive linear relationship was found between cellular protein content and specific growth rate. Cellular protein content increased from 6 to 8 pg cell^{-1} while specific growth rate increased from 0.4 to 0.6 day^{-1} .

[Insert Fig. 3 here]

Content of carbon and nitrogen

Figure 4 shows carbon and nitrogen content of *Microcystis* at different specific growth rate under different culture conditions. Both carbon and nitrogen content increased

with increasing specific growth rate. The carbon content and nitrogen content increased from 22% to 28% and from 4% to 5%, respectively, while the specific growth rate of 0.40 day⁻¹ increased from 0.4 to 0.6 day⁻¹.

[Insert Fig. 4 here]

Differences of biochemical components under different conditions

Figure 5 illustrates the differences of *Microcystis* biochemical components under different conditions. No significant differences were found in the dry weight under different culture conditions. Under the light intensity of 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the cellular TPS content at 20°C was significantly higher than that at 25°C but the relation was inverse under the light intensity of 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. This change pattern of TPS was similar to that of RNA but opposite to that of protein. The differences in the cellular content of TPS, protein and RNA between different light intensity (20 and 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) at 20°C were significant as well.

[Insert Fig. 5 here]

Discussion

It was expected that the cellular TPS content would be higher at higher specific growth rates than that at lower specific growth rate (Li et al. 2013) because the ability of *Microcystis* to produce carbohydrate was higher at higher temperature and light intensity. This assertion was denied by our current results. Although the ability of *Microcystis* to produce carbohydrate was promoted, the cellular TPS content was affected by the process of carbohydrate transformation.

We found that the percentage composition of TPS decreased from 10 to 5%, but protein increased from 30 to 45% with an increase in specific growth rate from 0.3 to 0.6 day⁻¹ (data not shown). The percentage composition of RNA also decreased with increasing specific growth rate. Recently, Gan et al. (2012) reported that the regulation of certain parts of four polysaccharide biosynthesis-related genes would affect the polysaccharide content of *Microcystis*. Light conditions have also been reported to alter genetic expression in *Microcystis* (Straub et al., 2011). Changes in genetic expression may induce variation in the percentage composition of different

Microcystis biochemical components.

Long et al. (2001) found that both the *Microcystis* cellular protein content and the percentage composition of protein were high at a higher specific growth rate under nitrogen-limited conditions, and vice versa. The same phenomenon has been reported by others (Downing et al., 2005; Wiedner et al., 2003). Oh et al. (2000) measured the specific growth rate, protein content (the ratio to dry weight of cells), and microcystin content of *M. aeruginosa* grown in P-limited medium modified from Smith and Wiedeman (1964). A correlation analysis of their data was performed and a significantly positive relationship between specific growth rate and percentage composition of protein ($r^2=0.760$, $n=8$, $P<0.01$) was observed.

These findings have revealed that proportions of different *Microcystis* biochemical components vary along with changes in specific growth rate. Specifically, the proportions of TPS and nucleic acids transformed from carbohydrates decreased at a high specific growth rate but the proportions of protein increased. This process could be termed as a transformation effect, and may be the result of changes in genetic expression triggered by variation in environmental factors.

Protein is the main material of *Microcystis* cell construction and is closely related to the life activities of the cells. It is noteworthy that the correct proteins must be formed before cell division (Reynolds, 2006). Thus, high proportions of protein at high specific growth rates favor the maintenance of high specific growth rates in *Microcystis*. It was surprising that RNA content decreased along with the increase of growth rate of *Microcystis*. However, the reduction was observed to be slow and the RNA content was found to reduce from 20% to 10%. Although, Nagai et al. (2011) established a positive relationship between RNA content and specific growth rate of *Microcystis* which was opposite to the relationship in the current study, it is worth noting that that positive relationship was only significant when the specific growth rate was below 0.2 day^{-1} . In addition, a decreasing trend of RNA content could be found when the specific growth rate was above 0.2 day^{-1} . In the current study, all the values of specific growth rate was larger than 0.3 day^{-1} . Thus, the relationship in previous study (Nagai et al. 2011) and the current study did not conflict to each other.

The mechanisms of the interaction between specific growth rate and RNA content was unclear but the fact that a certain amount of RNA was a requirement for cell division was credible. Thus, the relationship between RNA content and specific growth rate and the underlying mechanism should be deliberated with more data.

Our results also illustrated that both carbon and nitrogen content increased along with the increase of growth rate of *Microcystis* with varying abiotic factors. The increasing carbon content indicated that the ability of carbohydrate synthesis was promoted at high growth rate resulting from high light intensity and temperature. However, *Microcystis* absorbed more nitrogen at higher specific growth rate to synthesize more protein to maintain the high rate of cell division. Thus, the TPS content decreased with the increase of specific growth rate.

In the current study, the selection of different growth medium neither provided different growth rates nor gave rise to differences in the cellular content of TPS, protein and RNA. Thus, nutrient content was high enough that it did not influence the growth and cellular processes.

The differences in the cellular content of TPS, protein and RNA at different temperatures (20 and 25°C) were significant under both light intensities. The differences under different light intensity (30 and 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were significant at 25°C but negligible when temperature was 20°C. These phenomena suggest that temperature was probably the limiting factor influencing the rates of cellular processes when temperature was 20°C. However, when temperature was 25°C, the limiting factor was observed to be light intensity. Robarts and Zohary (1987) suggested the optimal temperature for *Microcystis* growth was 25°C (or greater) and this support the assertion that the growth of *Microcystis* would not be limited by temperature. Yang et al. (2012) studied the combined effect of various environmental factors on the growth and TPS content of *Microcystis*. They reported that temperature had no effect on TPS content at both 25 and 30°C. This finding supports our deduction that the limiting factor influencing the rates of cellular processes was not temperature but light intensity when temperature was 25°C.

In conclusion, higher light intensity and temperature yielded a higher specific growth

rate of *Microcystis* but the two different culture media did not significantly affect growth of *Microcystis* in the current study. The content of TPS and RNA decreased, but proteins increased with an increase in specific growth rate. Our results also suggested that temperature would be the limiting factor influencing the synthetic processes of TPS, protein and RNA when temperature was 20°C. However, when temperature was 25°C, the limiting factor was observed to be light intensity.

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Figure captions:

Fig. 1 The specific growth of *M. aeruginosa* under varying light intensities at 20°C and 25°C in BG-11 medium and M-11 medium, respectively.

Fig. 2 Variation in cellular dry weight of *M. aeruginosa* along with increase in specific growth rate.

Fig. 3 Relationship between specific growth rate and percentage composition of *Microcystis* biochemical components.

Fig. 4 Carbon and nitrogen content of *Microcystis* at different specific growth rate under different culture conditions.

Fig. 5 The differences of *Microcystis* biochemical components under different conditions.

+ Indicates that differences of *Microcystis* biochemical components between different temperatures or different medium under the same light intensity are significant ($P < 0.05$)

* Indicates that differences of *Microcystis* biochemical components between different light intensities at the same temperature with the same medium are

significant ($P < 0.05$)

[Fig. 1]

[Fig. 2]

[Fig. 3]

[Fig. 4]

[Fig. 5]