Physiological comparison between colonial and unicellular forms of *Microcystis aeruginosa* Kütz. (Cyanobacteria)

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In order to gain insight into the bloom sustainment of colonial *Microcystis aeruginosa* Kütz., physiological characterizations were undertaken in this study. Compared with unicellular *Microcystis*, colonial *Microcystis* phenotypes exhibited a higher maximum photosynthetic rate (P_m), a higher maximum electron transfer rate (ETR_{max}), higher phycocyanin content, and a higher affinity for inorganic carbon ($K_{0.5}$ DIC $\leq 8.4 \pm 0.7$ μM) during the growth period monitored in this study. This suggests that photosynthetic efficiency is a dominant physiological adaptation found in colonial *Microcystis*, thus promoting bloom sustainment. In addition, the high content of soluble and total carbohydrates in colonial *Microcystis* suggests that this phenotype may possess a higher ability to tolerate enhanced stress conditions when compared to unicellular (noncolonial) phenotypes. Therefore, high photosynthetic activities and high tolerance abilities may explain the bloom sustainment of colonial *Microcystis* in eutrophic lakes.

KEY WORDS: Dissolved inorganic carbon (DIC), Electron transport rate (ETR), Photosynthetic rate, Unicellular and colonial *Microcystis aeruginosa*

INTRODUCTION

The tendency for cyanobacteria to dominate the microbial flora for considerable lengths of time has been previously reported in many eutrophic lakes and reservoirs (Reynolds *et al.* 1987). The widespread occurrence of cyanobacteria has been attributed to their ecological and physiological advantages over other algae (Oliver & Ganf 2000). However, with the exception of a few species, much of the biology of the cyanobacteria involved in bloom sustainment remains unknown (Yamamoto & Nakahara 2005a).

Microcystis aeruginosa Kütz is a cyanobacterium found globally in freshwater (Reynolds & Walsby 1975). In China, *Microcystis* blooms often accumulate and proliferate as serious surface scum during active growth periods in many lakes, including lakes Taihu and Dianchi. Because of water management problems associated with its blooms (Eloff 1981) and toxins (Carmichael 1994; Sivonen 1996), *M. aeruginosa* has received considerable attention over the past few decades. It has been hypothesized that certain physiological strategies may be involved in *Microcystis* bloom development and sustainment such as buoyancy regulation (Reynolds *et al.* 1987), dissolved inorganic carbon uptake (Yamamoto & Nakahara 2005b), and light intensity adaptation (Raps *et al.* 1983). However, none of the hypotheses has been given confirmation, and the problem persists (Shapiro 1972). Thus, the physiological mechanism and strategies involved in *Microcystis* blooms still need to be further studied.

Compared with noncolonial phenotypes that grow predominantly in culture, *Microcystis* occurs mainly as a colonial form under natural conditions (Reynolds *et al.* 1981). Because of the lack of comparisons, however, the role of the colonial form in competition remains largely unknown. In addition, many studies on physiological strategies have focused mainly on the unicellular *Microcystis*. Therefore, considering the predominance of colonial *Microcystis* in the field, the present study compared physiological strategies utilized by unicellular and colonial *Microcystis* phenotypes.

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MATERIALS AND METHODS

Strains and culture conditions

The strains of colonial and unicellular *M. aeruginosa* Kütz used in this study are listed in Table 1. All axenic strains were obtained from the Culture Collections of the Freshwater Algae of the Institute Hydrobiology (FACHB-Collection, Wuhan, China). The strains were grown in BG11 medium (Rippka *et al.* 1979) under constant white light intensity at 25 μmol photons m^{-2} s^{-1}, on a 12 : 12 L : D cycle and at a temperature of 25 ± 1°C.

Photosynthetic activities measurements

The photosynthetic oxygen evolution of *M. aeruginosa* during log phase of growth was measured using a Clark-type oxygen electrode at 25°C. Samples were harvested by centrifugation and resuspended in fresh BG11 medium. Illumination was provided by a halogen lamp and ranged from 45 to 1200 μmol photons m^{-2} s^{-1}. Irradiance was measured with a quantum sensor LI-185B (LI-COR Biosciences, Lincoln, NE). Oxygen evolution was measured

Table 1. The strains of *Microcystis aeruginosa* used in this study.

Code	Origin	Sampling stations	Size	Form
924	NSW[1]	Australia	2–3 μm	unicellular
905	FACHB	Dianchi, China	2–3 μm	unicellular
942	FACHB	Dianchi, China	2–3 μm	unicellular
7806	PCC[2]	The Netherlands	2–3 μm	unicellular
907	FACHB	Dianchi, China	62.5–100 μm	intermediate colony
938	FACHB	Tuanshang, China	>650 μm	large colony
909	FACHB	Bao'anhu, China	>205 μm	large colony
910	FACHB	Xinyan, China	25–50 μm	small colony
975	FACHB	Wudalianchi, China	37.5–50 μm	small colony

for at least 5 min at each irradiance value. The parameters for the photosynthetic responses to irradiance curves (photosynthesis–irradiance [P-I] curves) were analyzed according to Henley (1993):

$$P = Pm \tan h(\alpha I / Pm) + Rd, Ik$$
$$= Pm /\alpha, Ic = - Rd / \alpha$$

where I represents irradiance, P the photosynthetic rate at irradiance I, P_m the maximum photosynthesis rate, I_k the saturating irradiance for photosynthesis, I_c the light compensation point α the slope of the light-limited part of the P-I curve, and R_d the dark respiration rate. The nonlinear curve fitting of the data was performed with Microcal Origin (Version 6.1; Microcal Software Northampton, MA).

Photosynthetic oxygen evolution responses to dissolved inorganic carbon (DIC) concentration were measured at 25°C and 700 μmol photons m^{-2} s^{-1}. DIC-free medium was prepared according to Qiu & Gao (2002). Fresh samples were washed four times with reaction medium for each measurement. Different concentrations of DIC (0–300 μmol l^{-1}) were obtained by adding a known concentration of $NaHCO_3$ to the DIC-free medium. The parameters of photosynthetic responses to DIC were obtained by fitting net photosynthetic rates at various DIC concentrations with the Michaelis–Menten formula: $v = V_{max}\cdot[S]/(K_{0.5}\,(DIC) + [S])$, where v is the photosynthetic rate, V_{max} the DIC-saturated photosynthetic rate, [S] the concentration of DIC, and $K_{0.5}$ (DIC) the DIC concentration for which photosynthetic activity is half the maximum value.

Light response curve and electrons transfer rate determinations

The light response curve was measured according to Wu *et al.* (2007). The electron transport rate of PSII (ETR) was calculated as follows: relative ETR $= [((F_m' - Ft)/F_m') \times 0.84 \times 0.5 \times PAR\ (\ m^{-2}\ s^{-1}\ )]$ and F_t are the maximum and steady state fluorescence in light, respectively (Maxwell & Johnson 2000).

Photosynthetic pigments and products measurements

Chlorophyll *a* (Chl *a*) was extracted in 80% acetone. The contents of phycobilisomes were measured according to Abelson & Simon (1988). Soluble carbohydrates (EPS) and total carbohydrates were quantified spectrophotometrically by the phenol-sulfuric acid method using glucose as a standard (Dubois *et al.* 1956).

Statistical analysis

All experiments were performed with three replicates. Data are presented as the means ± standard deviation (*s*). Significance analysis was performed by analysis of variance with Microcal Origin Version 6.1. Differences were considered to be significant at $P < 0.05$.

RESULTS

Photosynthetic characteristics

The photosynthetic responses to irradiance in nine strains of *Microcystis* are shown in Fig. 1. No apparent photoinhibition was observed at irradiances up to 1200 μmol photons m^{-2} s^{-1} in either unicellular or colonial *Microcystis* (Fig. 1a, b). The photosynthetic parameters are shown in Table 2. The light-saturated photosynthetic rates (P_m) of colonial *Microcystis* were significantly higher than those of unicellular *Microcystis* ($F = 7.878$, $P = 0.000$). Saturating irradiances (I_k) for photosynthesis in colonial and unicellular *Microcystis* were 357–487 and 237–366 μmol photons m^{-2} s^{-1}, respectively ($F = 5.48$, $P = 0.052$). However, the difference in light compensation points (I_c), dark respiratory rates (R_d), and photosynthetic efficiencies (α) among the nine strains did not differ significantly.

The results of photosynthetic responses to DIC concentrations in unicellular and colonial *Microcystis* are shown in Fig. 2. Compared with unicellular *Microcystis*, the values of $K_{0.5}$ (DIC) in colonial *Microcystis* decreased significantly ($F = 66.905$, $P = 0.000$). The values of $K_{0.5}$ (DIC) in unicellular and colonial *Microcystis* were 10.8–33.6 and 1.1–8.4 μM, respectively.

The results of a comparison of maximal electrons transfer rates (ETR_{max}) between unicellular and colonial *Microcystis* are shown in Fig. 3. The results indicate that the ETR_{max} in the colonial *Microcystis* were significantly higher than those in the unicellular *Microcystis* ($F = 66.905$, $P = 0.000$). ETR_{max} values in the unicellular *Microcystis* ranged from 17.8 to 31.7 μmol electrons m^{-2} s^{-1}, whereas those in the colonial *Microcystis* ranged from 61.9 to 86.5 μmol electrons m^{-2} s^{-1}.

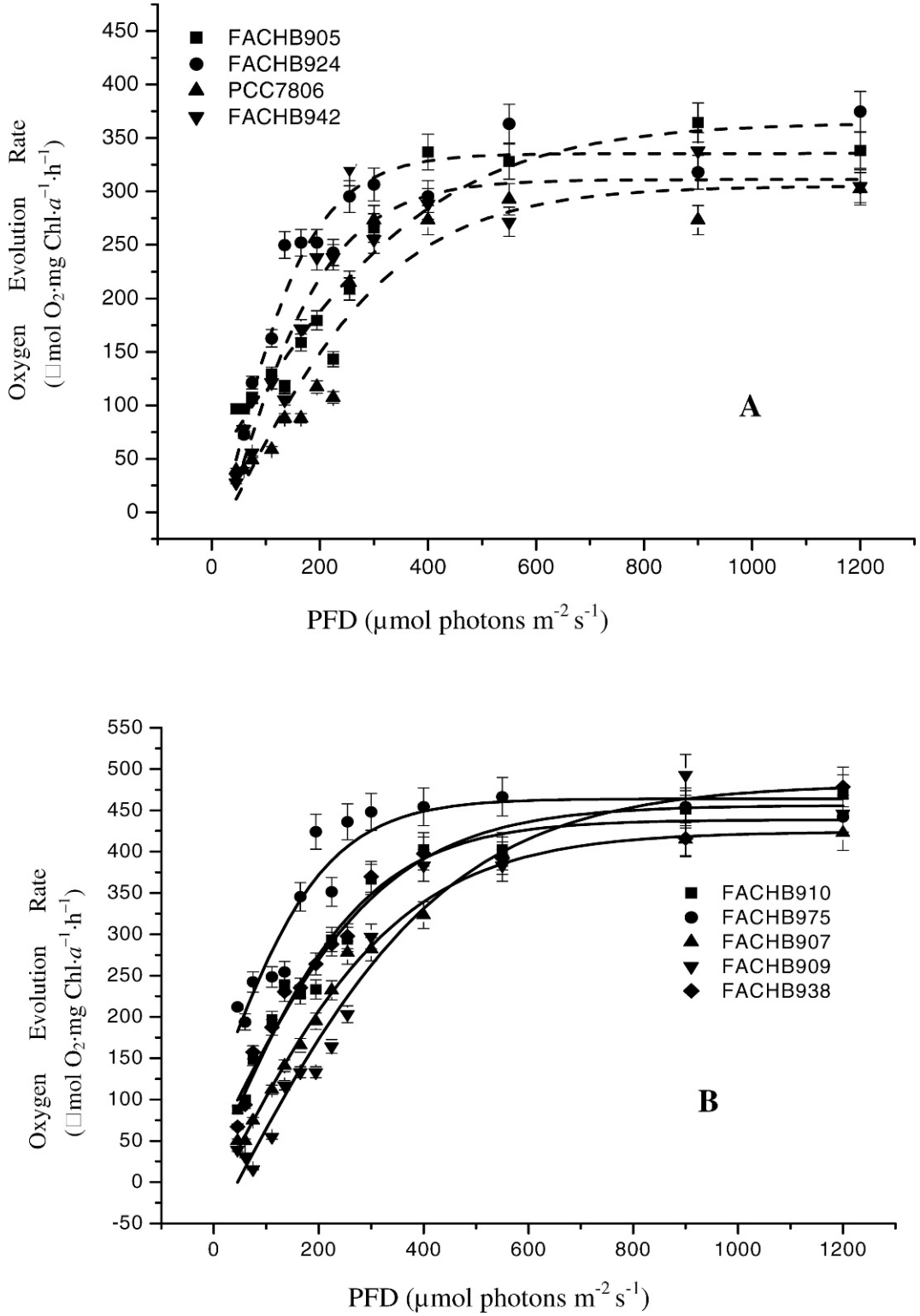

Fig. 1. Photosynthetic O_2 evolution as a function of incident photon flux density (PFD) in unicellular and colonial *M. aeruginosa* at 25°C. Values are presented as the means ± *s.* A, Unicellular *M. aeruginosa*; B, Colonial *M. aeruginosa.*

Pigment composition and content

Compared with the unicellular *Microcystis*, the colonial *Microcystis* contained lower Chl *a* contents (Fig. 4). However, total phycocyanin content in the colonial *Microcystis* was higher than in unicellular *Microcystis* ($F = 0.657$, $P = 0.444$) (Fig. 5), and the ratios of allophycocyanin to phycoerythrin in colonial *Microcystis* (1.7 : 1) are more constant than those in the unicellular *Microcystis* (1.7–2.1 : 1) (data not shown).

Table 2. Parameters of photosynthesis–irradiance (P-I) curves for unicellular and colonial *Microcystis aeruginosa*.[1]

Code	P_m	α	R_d	I_k	I_c	r^2
905U	324.16 ± 26.42	1.29 ± 0.15	−40.44 ± 21.72	282.56 ± 6.25	31.34 ± 2.82	0.93
924U	385.94 ± 13.24	1.26 ± 0.49	−50.41 ± 18.05	346.44 ± 18.25	40.02 ± 9.59	0.94
7806U	338.17 ± 32.57	1.01 ± 0.23	−33.07 ± 29.24	366.02 ± 20.62	32.61 ± 9.75	0.91
942U	366.27 ± 26.50	1.77 ± 0.41	−55.07 ± 37.35	237.89 ± 14.57	31.09 ± 7.37	0.92
910C	413.89 ± 20.39*	1.27 ± 0.15	−42.12 ± 18.48	357.13 ± 25.08	33.05 ± 2.41	0.98
975C	417.73 ± 36.81*	1.21 ± 0.41	−36.34 ± 17.57	376.58 ± 33.73	30.14 ± 7.04	0.92
907C	440.83 ± 10.29*	1.23 ± 0.07	−26.77 ± 8.99	378.41 ± 1.22	21.70 ± 0.57	0.99
909C	536.60 ± 30.24*	1.21 ± 0.16	−54.77 ± 23.91	487.04 ± 8.41	45.11 ± 3.71	0.97
938U	415.90 ± 23.01*	1.16 ± 0.19	−22.60 ± 11.79	376.93 ± 9.18	19.43 ± 4.47	0.97

[1] Values are the means ± *s* derived from the P-I curve. P_m, α, R_d, and I_c (μmol O_2 mg Chl a^{-1} h^{-1}), I_k (μmol photons m^{-2} s^{-1}). U = unicellular *Microcystis*, C = colonial *Microcystis*.
* Significantly different at $P = 0.05$.

Soluble and total carbohydrate contents

The results of the determinations of soluble and total carbohydrate contents in unicellular and colonial *Microcystis* (Table 3) indicate that both carbohydrates in colonial *Microcystis* were significantly higher than those in unicellular *Microcystis* ($F = 29.13$, $P = 0.001$; $F = 10.94$, $P = 0.013$). After 18 d, the soluble and total carbohydrate contents in colonial *Microcystis* were 2.9–7.9 and 16.9–159.5 μg mg^{-1} DW, respectively. In contrast, the soluble and total carbohydrate contents in unicellular *Microcystis* were 0.6–1.0 and 2.2–3.4 μg mg^{-1} DW, respectively.

DISCUSSION

A variety of hypotheses have been proposed and a number of experiments performed in order to explain the proliferation of cyanobacteria during freshwater blooms. Tilzer (1987) considered that cyanobacterial dominance in eutrophic lakes was due to the light dependence of photosynthesis and growth in cyanobacteria. In the present study, P-I curve measurements were performed in unicellular and colonial *Microcystis*. The results demonstrated that both unicellular and colonial *Microcystis* could tolerate high light intensity (1200 μmol photons m^{-2} s^{-1}) (Fig. 1). This supported the results that effective prevention of damage by excessive light levels near the lake surface might enable cyanobacteria in surface scums not only to survive (Zohary 1985) but also to maintain active growth (Paerl & Ustach 1982). The results also revealed that colonial *Microcystis* exhibited a higher P_m than unicellular *Microcystis* (Table 2). It is suggested that colonial *Microcystis* exhibit higher photosynthetic activities than the unicellular forms. The results support our previous findings that photosynthetic and growth parameters were related to phenotypes of *M. viridis* (Song *et al.* 2004). However, the observed changes of P_m in the unicellular and colonial *Microcystis* are inconsistent with the results of Li & Gao (2004) obtained from different *Nostoc sphaeriodes* colonies. Previous study has shown that the formation of colonial *Microcystis* was a mucilaginous matrix, unlike *Nostoc* colonies as a sheath or capsule form (Forni *et al.* 1997). Thus, the effect of packaging and self-shading may be not significant features in *Microcystis* in comparison with *Nostoc*.

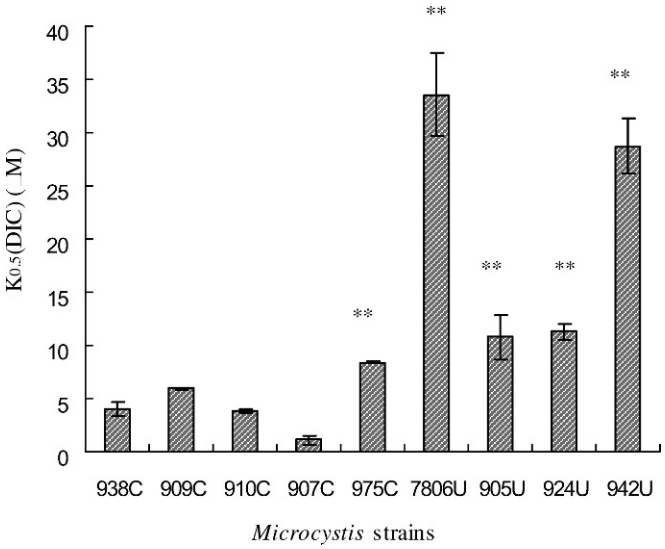

Fig. 2. HCO_3^- uptake values {$K_{0.5}$ (DIC)} for unicellular and colonial strains of *M. aeruginosa*. ** indicates significantly different at $P = 0.01$. U = unicellular *Microcystis*, C = colonial *Microcystis*.

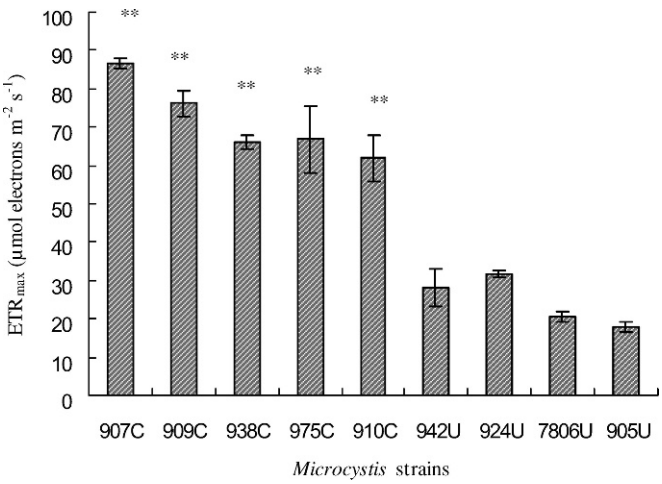

Fig. 3. The maximal electron transfer rates (ETR_{max}) in unicellular and colonial strains of *M. aeruginosa*. ** indicates significantly different at $P = 0.01$. U = unicellular *Microcystis*, C = colonial *Microcystis*.

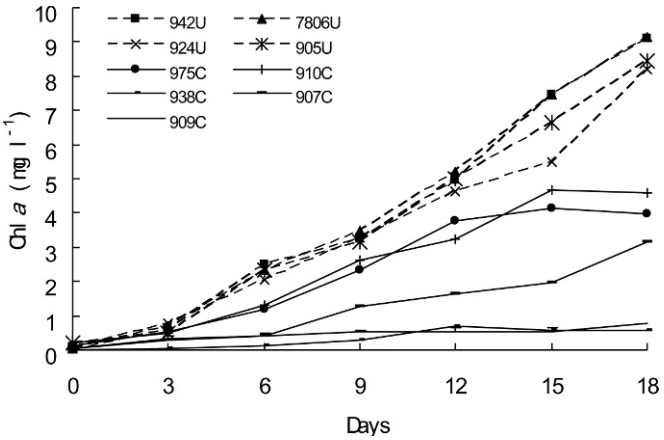

Fig. 4. Comparison of Chl *a* concentration in unicellular and colonial strains of *M. aeruginosa* during the growth phase. U = unicellular *Microcystis*, C = colonial *Microcystis*.

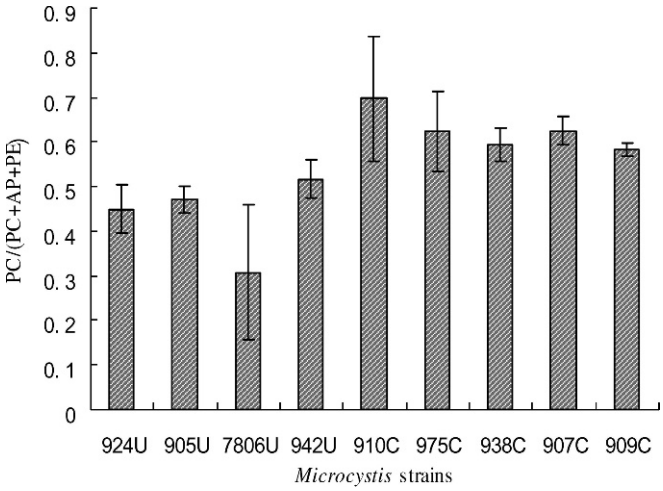

Fig. 5. Comparison on the contents of phycocyanin in unicellular and colonial strains of *M. aeruginosa* during the log phase. AP = allophycocyanin, PC = phycocyanin, PE = phycoerythrin, U = unicellular *Microcystis*, C = colonial *Microcystis*.

King (1970) suggested that cyanobacteria are more efficient in obtaining CO_2 at low concentrations than are green algae. Shapiro (1972) found that the addition of free CO_2 stimulated a shift from blue-green to green algae. Yamamoto & Nakahara (2005b) demonstrated that advantageous DIC uptake system appeared to be responsible for the competitive dominance of *Microcystis aeruginosa* in nutrient-rich culture conditions. Cermeñ *et al.* (2005) found that larger phytoplankton attained higher C-specific photosynthesis rates than those of smaller sizes. In the present study, lower $K_{0.5}$ (DIC) values were observed in colonial *Microcystis* compared to unicellular *Microcystis* (Fig. 2). It is suggested that colonial *Microcystis* possess a high affinity for DIC and that they are affinity-adapted strategists. The results are consistent with the observation that the surface scum of *Microcystis* might be conducive to preferential DIC uptake (Paerl & Ustach 1982; Paerl 1983). In addition, the results provide an explanation for the existence of a colonial morphology under natural conditions.

ETR can be attributed mainly to the presence of the secondary oxygen-consuming processes such as photorespiration and the Mehler reaction and provide supplementary information about the status of the photosynthetic apparatus at the level of PSII-dependent electron transport (Masojídek *et al.* 2001). In the present study, the results demonstrated that colonial *Microcystis* exhibited higher ETR_{max} than unicellular *Microcystis* (Fig. 3). This indicated that colonial *Microcystis* possess a more efficient photosynthetic electron transport system compared to unicellular *Microcystis*. Genty *et al.* (1989) demonstrated a high correlation between ETR and photosynthetic CO_2 fixation rates. Our results also demonstrated that both DIC uptake and ETR_{max} exhibit similar trends in colonial and unicellular *Microcystis*, suggesting that photosynthetic efficiency may be physiologically dominant in colonial *Microcystis*, thus enabling them to form and sustain blooms.

In the present study, the pigment contents were measured in order to assess light-harvesting ability. The results indicated that high diversities of light-harvesting pigments are present in *Microcystis*. Compared with the unicellular *Microcystis*, colonial *Microcystis* possessed lower Chl *a* concentration (Fig. 4). However, higher phycocyanin content was found in colonial *Microcystis* (Fig. 5). Sathyendranathe *et al.* (1987) considered that other pigments could contribute significantly to light absorption, although Chl *a* is commonly used as a measure of pigment content. Sedmak and Elerše (2006) showed that a significant

Table 3. Comparison of soluble carbohydrate (EPS) and total carbohydrate in unicellular and colonial *Microcystis aeruginosa* over an 18-d growth study.[1]

Code	EPS yield (μg mg^{-1} DW d^{-1})	EPS content (μg mg^{-1} DW)	Total carbohydrate yield (μg mg^{-1} DW d^{-1})	Total carbohydrate content (μg mg^{-1} DW)
942U	0.03 ± 0.00	0.71 ± 0.05	0.17 ± 0.02	2.49 ± 0.07
7806U	0.03 ± 0.02	0.62 ± 0.03	0.15 ± 0.08	2.17 ± 0.07
924U	0.05 ± 0.01	0.98 ± 0.07	0.23 ± 0.012	3.42 ± 0.86
905U	0.04 ± 0.01	0.91 ± 0.04	0.21 ± 0.07	3.19 ± 0.08
975C	0.13 ± 0.05**	2.86 ± 0.36**	1.13 ± 0.07*	16.88 ± 1.11*
910C	0.23 ± 0.09**	4.27 ± 0.09**	2.14 ± 0.09*	32.05 ± 0.30*
938C	0.33 ± 0.07**	7.94 ± 0.19**	9.63 ± 0.16*	159.47 ± 3.47*
907C	0.23 ± 0.06**	4.95 ± 0.14**	4.05 ± 0.29*	60.75 ± 0.46*
909C	0.23 ± 0.01**	7.15 ± 0.02**	6.05 ± 0.14*	90.72 ± 1.02*

[1] U = unicellular *Microcystis*, C = colonial *Microcystis*.
* Significantly different at $P = 0.05$.
** Significantly different at $P = 0.01$.

increase in phycocyanin content was found in cyanobacteria with the presence of microcystin. In addition, cyanobacteria are able to regulate their phycobilisome contents according to light intensity and light quality (Aráoz & Häder 1997). Therefore, we consider that the high phycocyanin content in colonial *Microcystis* may be an adaptive mechanism promoting the formation of blooms in eutrophic water bodies.

In addition to its effects on the physiological characterization and pigment content, colony size affects soluble and total carbohydrate contents. In the present study, the contents of soluble and total carbohydrate in unicellular *Microcystis* were similar to those reported by Forni *et al.* (1997). However, compared with the unicellular *Microcystis*, colonial *Microcystis* possessed higher soluble and total carbohydrate contents. Otero & Vincenzini (2003) indicated that the total carbohydrate contents were approximately 2.5–3.5 times higher than the EPS contents in *Nostoc* PCC8113, 7936, and 7413 cultured in BG11 medium. However, in the present study, we found that total carbohydrate contents were significantly higher than the soluble contents in colonial *Microcystis* (Table 3). Tien *et al.* (2002) demonstrated that the highest mucus contents were found in a bloom of *Microcystis* in Rostherne Mere. This suggests that EPS was used to form or maintain the mucus in colonial *Microcystis* rather than being released into the medium. The important roles of EPS are as follows: (1) the storage of water (Li & Gao, 2004); (2) as a carbon source (Lancelot *et al.* 1986); and (3) as a strong metal chelator (Amemiya & Nakayama 1984). Our previous study also found that colonial *Microcystis* could tolerate higher Cu^{2+} stress than unicellular *Microcystis* (Wu *et al.* 2007). In addition, Shen & Song (2007) also found that colonial *Microcystis* had higher affinity for low levels of P and had an advantage with regard to dominance and persistence in fluctuating P conditions. These observations suggest that colonial *Microcystis* could survive longer than unicellular forms under stress conditions because of their higher carbohydrate contents.

CONCLUSIONS

Our results indicated that higher photosynthetic activities, higher phycocyanin contents, and higher EPS and total carbohydrate contents were found in colonial *Microcystis* in comparison with unicellular *Microcystis*. Thus, it is considered that the dominant physiological selection in colonial *Microcystis* may be an adaptive mechanism promoting the formation, sustainment and longevity of blooms in eutrophic water bodies.

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