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PRIMARY RESEARCH PAPER

Comparative studies on physiological responses to phosphorus in two phenotypes of bloom-forming *Microcystis*

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Abstract Toxic *Microcystis* blooms frequently occur in eutrophic water bodies and exist in the form of colonial and unicellular cells. In order to understand the mechanism of *Microcystis* dominance in freshwater bodies, the physiological and biochemical responses of unicellular (4 strains) and colonial (4 strains) *Microcystis* strains to phosphorus (P) were comparatively studied. The two phenotype strains exhibit physiological differences mainly in terms of their response to low P concentrations. The growth of four unicellular and one small colonial *Microcystis* strain was significantly inhibited at a P concentration of 0.2 mg l^{-1}; however, that of the large colonial *Microcystis* strains was not inhibited. The results of phosphate uptake experiments conducted using P-starved cells indicated that the colonial strains had a higher affinity for low levels of P. The unicellular strains consumed more P than the colonial strains. Alkaline phosphatase activity in the unicellular strains was significantly induced by low P concentrations. Under P-limited conditions, the oxygen evolution rate, F_v/F_m, and ETR_{max} were lower in unicellular strains than in colonial strains. These findings may shed light on the mechanism by which colonial *Microcystis* strains have an advantage with regard to dominance and persistence in fluctuating P conditions.

Keywords Unicellular and colonial *Microcystis* · Alkaline phosphatase activity · P_i uptake · Oxygen evolution activity · F_v/F_m · Maximum electron transport rate (ETR_{max})

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Introduction

During the last decades, the frequency and intensity of harmful algal blooms have increased (Hallegraeff, 1993). *Microcystis* dominance in freshwater bodies has received substantial attention worldwide due to its harmful effect on animals and its potential hazard to human health (Carmichael, 1992; Song et al., 1998). The cause of cyanobacterial dominance is very complicated. *Microcystis* species possess a variety of adaptive mechanisms, including effective protection against inhibition by sunlight, and various mechanisms for migration in water columns that promote their successful growth in water

(Kolmakov, 2006). Moreover, some environmental factors such as temperature (McQueen & Lean, 1987), low TN/TP ratio (Smith, 1983), and low exchange rate of water (Kuwata & Miyazaki, 2000; Takeya et al., 2004) promote the mass growth of *Microcystis* in water. Among these factors, phosphorus (P) is considered the principal nutrient limiting algal biomass and growth in most freshwater habitats, and the oversupply of this nutrient from human sources underlies the eutrophication of ecosystems worldwide (McCormick et al., 2001). Despite a high nutrient input, the rate of supply of nutrients or light energy can limit the growth rate in eutrophic systems with a high biomass; hence, most nutrients are retained in the algae (Kromkamp et al., 1989). For example, algal growth in the Taihu Lake is mainly limited by light and/or P (Dokulil et al., 2000). Krivtsov et al. (2005) found that *Microcystis aeruginosa* colonies were initially observed when the P concentration in the lake had decreased to minimal levels and that the P cell quota was high.

Cyanobacteria have developed many adaptive strategies to cope with P-limited conditions. They are capable of luxury uptake and storing it as polyphosphates, which allows them to survive for long periods of time under P_i-deficient conditions. Studies conducted on P uptake in P-limited cyanobacterial cultures revealed that these organisms could increase P_i affinity in response to persistent P deficiency. Although P uptake is extremely slow in P-sufficient *Synechococcus* cells, P-starved cells can opportunistically take up P approximately 100 times faster (Ritchie et al., 2001). Most cyanobacteria can synthesize phosphatase and secrete it into the extracellular space when P_i is depleted; this is another important way in which algae enhance P availability by the liberation of P_i from dissolved organic P compounds (Mateo et al., 2006; Lee et al., 2005; Whitton et al., 1991). Moreover, the concentration of the ambient nutrient affects the algal cell size. Kruskopf & Plessis (2006) found that the concentrations of Nitrogen (N) and P could affect the filament length of *Oscillatoria simplicissima* cells. Kilham et al. (1997) reported that the size and volume of *Ankistrodesmus falcatus* cells changed as a function of the type of resource limitation; P-limited cells were larger and denser than non-limited cells. Smith & Kalff (1982) studied eight species obtained from a P-limited lake and revealed that smaller phytoplankton cells had a greater P-uptake rate than larger phytoplankton cells.

It is obvious that P plays an important role in algal bloom. A considerable number of research efforts have focused on the effect of P on the growth of *Microcystis* species. Nalewajko & Murphy (2001) found that *Microcystis* growth declined under P-limited conditions. Under these conditions, the *Microcystis* biomass was restricted to some extent and the protein content per cell in vivo decreased (Ou et al., 2005). The initial specific uptake rate of P_i in P-starved *Microcystis* cells was 10 times higher than that in the cells from P-rich precultures (Okada et al., 1982). Due to high V_{max} and low P content, *Microcystis* outcompeted *Oscillatoria* in P-limited conditions that restricted the growth rate (Kromkamp et al., 1989). Furthermore, the effect of P on the growth of toxic and nontoxic *Microcystis* strains differed. Under low nutrient concentrations, nontoxic *Microcystis* strains grew better than toxic strains (Vézie et al., 2002). Nevertheless, due to the difficulty in collecting both unicellular and colonial *Microcystis* strains, few studies have considered with regard to the relationship between physiological functions and phenotypes in *Microcystis*. Reynolds (2007) reviewed the role and function of the mucilage provision in phytoplankton and found that they include density reduction, dynamic streamlining, sequestration and storage of nutrients, defense against oxygen, metal poisoning, grazing, etc. Since most results for *Microcystis* studies conducted in the laboratory have been obtained with unicellular *Microcystis* strains, it is quite difficult to extrapolate them to explain the field phenomenon in which *Microcystis species* exclusively exist in the colonial form. Our previous studies illustrated that the unicellular and colonial strains differed in growth, toxin production (Lei et al., 2001), inorganic carbon affinity (Xu & Song, 2007), and response to Copper (Cu) stress (Wu et al., 2007). We questioned whether the P response differed between the unicellular and colonial strains. To date, this aspect of study has not been reported. Thus, a

comparative study was proposed with regard to the physiological and biochemical responses of the two *Microcystis* phenotypes to P.

Material and methods

Organisms and culture condition

The *Microcystis* spp. examined in this study were obtained from the Culture Collections of the Freshwater Algae of the Institute Hydrobiology, Wuhan, China (Table 1). Stock cultures of these stains were grown in BG11 medium (Rippka et al., 1979) under cool white fluorescent light at an irradiance of 50 μE m^{-2} s^{-1} with a 12:12-h light:dark cycle at a temperature of 25 ± 1°C, and they were manually shaken 3 or 4 times every day during incubation. All the glassware was soaked in 0.1 M HCl for 24 h and then rinsed with ultrapure water before use in order to prevent P contamination. Solutions were prepared using analytical reagents of the highest available purity and ultrapure water.

Algal cells in the exponential phase were collected by centrifugation, washed 3 times with sterile P-free BG11 medium (the reduced amount of K_2HPO_4 was replaced by equimolar KCl), and then exposed to incubated in P-free BG11 medium for 7–10 days in order to deplete the intracellular polyphosphate stores. After starvation, the algal cells were initially inoculated into 1-L Erlenmeyer flasks containing 400 ml fresh BG11 medium with the following 3 P concentration treatments: P-replete BG11 medium (normal BG11 medium, 5.4 mg l^{-1} P), P-moderate BG11 medium (2.0 mg l^{-1} P), and P-deficient BG11 medium (0.2 mg l^{-1} P). P_i was added in the form of orthophosphate (K_2HPO_4). The lowest concentration of P in our experiments corresponded to the mean value of the total P concentration in the eutrophic lake. The greatest P concentration corresponded to the BG11 medium. All the strains and concentrations were prepared in triplicates, and all the cultures were incubated under identical conditions mentioned above.

Determination of growth rates

The specific growth rate in the exponential growth phase of the experiment was determined using the following exponential growth equation (Pirt, 1975).

$$\mu = \ln(B_{t2} - B_{t1})/\Delta t,$$

where μ is the specific growth rate (d^{-1}); B, the chlorophyll *a* (chla) concentration (μg l^{-1}); and t, time in days. Chla was extracted overnight in 80% acetone at 4°C in the dark. The debris was then removed and the supernatant was analyzed on an ultraviolet-visible spectrophotometer at 663 nm.

Determination of alkaline phosphatase activity

The extracellular alkaline phosphatase activity (APA) was routinely determined using the colorimetric method with *p*-nitrophenyl phosphate (*p*NPP; Sigma) as the substrate (Ray et al., 1991). We added 200 μl 3.6 mM *p*NPP with 2 mM $MgCl_2$ in 0.2 M Tris-HCl buffer (pH 8.5) to 500 μl of culture. The final volume was 3 ml. The vials were incubated at 37°C for 2 h, and the incubation was then terminated by the addition of 300 μl 4 M NaOH. The samples were centrifuged (7000*g*,

Table 1 Strains of *Microcystis* spp. used in this study

Code	Origins	Sampling stations	Size (μm)	Shape
7806	PCC	Braakman Reservoir, The Netherlands	2–3	Unicell
7820	PCC	Loch Balgavies, Scotland	2–3	Unicell
942	FACHB	Dianchi Lake, China	2–3	Unicell
905	FACHB	Dianchi Lake, China	2–3	Unicell
975	FACHB	Wudalianchi Lake, China	37.5–50	Small colony
909	FACHB	Baoanhu Lake, China	>205	Large colony
938	FACHB	Tuanshang Lake, China	>650	Large colony
939	FACHB	Tuanshang Lake, China	>650	Large colony

4 min), and the supernatant was used to determine the extracellular APA. Buffer without a sample was used as the control. Absorbance was read at 405 nm in a spectrophotometer and compared to the standard absorbance curve for *p*-nitrophenol (*p*NP). The enzyme activity was expressed in terms of μM of *p*NP released μg chla h^{-1}.

Determination of oxygen evolution activity and chlorophyll fluorescence parameters

Oxygen evolution activity was measured using an oxygen electrode chamber fitted with a water jacket thermostated at 25°C at the light saturation point (Preliminary experiments showed that the light saturation point of the two phenotype strains was approximately 500 μE m^{-2} s^{-1} PAR). Irradiance was measured using a quantum sensor (LI-185B, Li-Cor Inc.).

A pulse-amplitude-modulated fluorescence monitoring system (PAM, Walz, Effeltrich, Germany) was used to measured the in vivo chlorophyll fluorescence at room temperature, as described by Schreiber et al. (1986). Before the measurements, the algae were dark-adapted for 15 min to guarantee an oxidized electron transport chain. When a saturating pulse of white light was applied, causing the photosystem II reaction centers to close, fluorescence increased to a maximal value (F_m). The change in fluorescence from F_0 to F_m (ΔF) denotes the variable fluorescence F_v. The ratio of F_v to F_m in a dark-adapted sample correlated to the quantum yield of photosynthesis and a convenient measure of the maximum potential quantum yield ($F_v/F_m = (F_mF_0)/F_m = \Delta F/F_m$) (Jones et al., 1999). The rapid light curves (RLCs) based on measurement of the relative electron transport rates (*ETRs*) were derived from estimates of $\Delta F_v/F_m'$ (the operational quantum yield of PSII, Φ_{PSIIe}). The numerical values of chlorophyll fluorescence of samples exposed to 12 intensities of actinic light increasing from 0 to 1,265 μmol photons PAR m^{-2} s^{-1} were recorded during a 3-min time series. The photosynthetic *ETR* may be calculated using the following formula: $ETR = ((F_m' - F_t)/F_m') \times 0.84 \times 0.5 \times$ PAR (m^{-2} s^{-1}), where F_m' and F_t denote the maximum and steady state fluorescence in light, respectively. The approximate amount of incident light absorbed by the algae was 0.84, of which approximately 0.5 was transferred to PSII (Wu et al., 2007).

Phosphate uptake

The phosphate uptake rate was calculated by measuring its disappearance from the medium in the P-starved cells during each sampling interval. The culture solution contained P_i at initial concentrations of 2, 5, 10, 20, 50, 100, and 200 μM. All the samples were incubated under identical conditions mentioned above. The algae in each flask were collected at various times during the experiments and filtered through CF/G filter papers (Whatman, Maidstone, UK) in order to estimate the decrease in P_i concentration in the modified BG11 medium. Sample measurements were obtained at 1, 2, and 3 h for the short-term experiment and at 12, 24, 36, 60, 84, and 120 h for the long-term experiment. The P concentration was colorimetrically determined according to Murphy and Riley (1962). Samples of total and total dissolved P were digested in 5% potassium persulphate solution. Cellular P was calculated as the difference between the two concentrations (Isvánovics et al., 2000).

Statistics

Origin 7.0 (Microcal Software Inc.) was used for graphical plotting and statistical analyses. The data are presented as mean ± standard deviation (SD). Growth rate, APA, oxygen evolution activity, F_v/F_m, ETR_{max}, and Q_p of the 8 strains were examined by one-way analysis of variance (ANOVA). ANOVA effects and treatment differences were considered significant at $P < 0.05$. Regression analyses were used to quantify relationships between the algal phosphate uptake rates and the external phosphate concentration.

Results

Effects of P_i on the growth of the unicellular and colonial Microcystis strains

Algal growth was monitored by measuring the chla concentration at various times during the experiment. Under routine culture conditions,

the lag growth phases of the unicellular *Microcystis* strains were shorter than those of colonial *Microcystis* strains; hence, the logarithmic growth phase in unicellular strains was initiated earlier than that in the colonial ones. At a P concentration of 0.2 mg l^{-1}, the specific growth rates in four unicellular and one small colonial strain were significantly inhibited (51.35%, PCC7806; 57.43%, PCC7820; 79.55%, FACHB942; 79.62%, FACHB905; and 60.34%, FACHB975) ($P < 0.05$), whereas those of large colonial *Microcystis* samples were only inhibited slightly (6.13%, FACHB909; 29.40%, FACHB938; and 36.60%, FACHB939) (Fig. 1). There was no significant difference ($P > 0.05$) in the specific growth rates between cultures with initial P_i concentrations of 5.4 and 2 mg l^{-1}.

Phosphate uptake and storage in unicellular and colonial Microcystis strains

In order to test the phosphate affinity of the different *Microcystis* phenotypes, the phosphate uptake rate was analyzed as a function of the external phosphate concentration. In the short-term experiments, the phosphate uptake by *Microcystis* was affected by the external

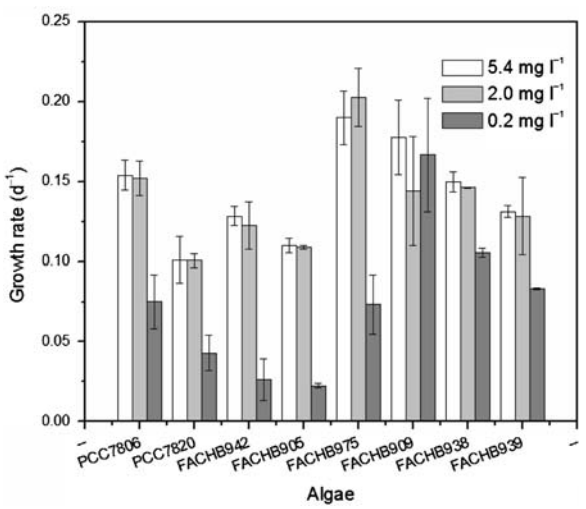

Fig. 1 The specific growth rates of 4 unicellular strains (PCC7806, PCC7820, FACHB905 and FACHB942), 1 small colonial strain (FACHB975) and 3 large colonial strains (FACHB909, FACHB938 and FACHB939) of *Microcystis* in the logarithmic growth phase at varying P concentrations

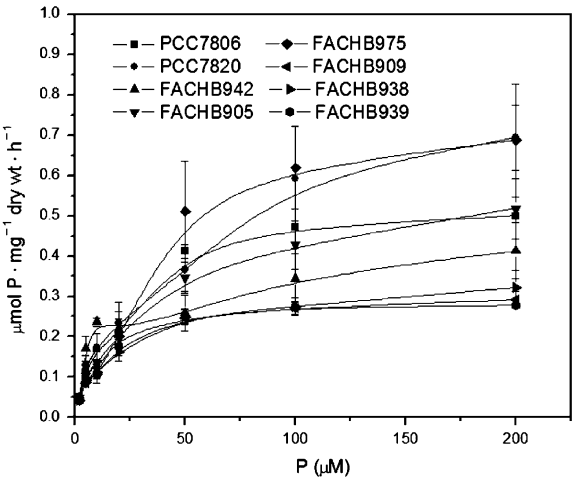

Fig. 2 Phosphate uptake rates of 4 unicellular strains (PCC7806, PCC7802, FACHB942, and FACHB905) and 4 colonial strains (FACHB975, FACHB909, FACHB938, and FACHB939) of *Microcystis* as a function of the external phosphate concentration (25°C)

phosphate concentrations, which can be described by the Michaelis-Menten model ($V = V_{max}\cdot[C]/(K_m + [C])$) (Fig. 2). The unicellular *Microcystis* strains exhibited higher K_m and V_{max} ($P < 0.05$) values than those exhibited by the colonial *Microcystis* strains (Table 2). In the long-term experiment, a higher P concentration, the residual P concentration in the unicellular *Microcystis* cultures was lower than that in the colonial cultures ($P < 0.05$), whereas no significant differences were observed between the two phenotypes at a lower P concentration ($P > 0.05$) (Fig. 3). The P cell quota of each strain increased considerably after 36 h of incubation; however, it ceased to change at each initial P concentration. The P cell quota in the unicellular strains decreased remarkably during the time period from 36 to 120 h (Table 3), indicating that they consumed more P than the colonial strains.

Effects of P_i on the APA in unicellular and colonial Microcystis strains

Extracellular APAs were affected differently by varying P_i concentration in the unicellular and colonial *Microcystis* strains (Fig. 4). APA was greater in the unicellular and small colonial

Table 2 Kinetic constants for P_i uptake in 4 unicellular *Microcystis* strains (PCC7806, PCC7802, FACHB 942, and FACHB 905) and 4 colonial *Microcystis* strains (FACHB 975, FACHB 909, FACHB 938, and FACHB 939) (25°C)

Sharp	Strains	K_m (μM P)	V_{max} (μM P mg dry $wt^{-1} \cdot h^{-1}$)
Unicell	PCC 7806	17.045 ± 1.572	0.479 ± 0.031
Unicell	PCC 7820	19.475 ± 2.503	0.571 ± 0.082
Unicell	FACHB 942	16.617 ± 1.596	0.488 ± 0.044
Unicell	FACHB 905	14.921 ± 1.749	0.407 ± 0.028
Small colony	FACHB 975	14.720 ± 2.138	0.386 ± 0.043
Large colony	FACHB 909	9.523 ± 0.486	0.273 ± 0.015
Large colony	FACHB 938	11.836 ± 2.477	0.275 ± 0.022
Large colony	FACHB 939	12.138 ± 1.379	0.288 ± 0.024

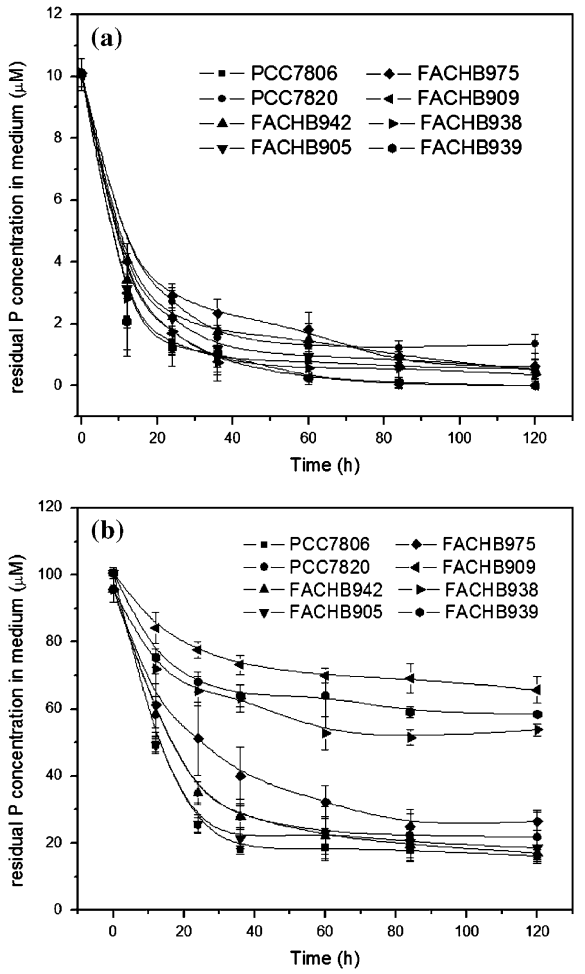

Fig. 3 Variation in residual P concentration in the medium as a function of time in 4 unicellular strains (PCC7806, PCC7820, FACHB942, and FACHB905) and 4 colonial strains (FACHB975, FACHB909, FACHB938, and FACHB939) of *Microcystis*. (**a**) 10 μM, (**b**) 100 μM

strains than in the colonial strains. APA significantly increased by 2.4–10.45 times in all the unicellular and small colonial strains at lower P_i concentrations ($P < 0.05$) as compared to that at higher P_i concentrations. However, APA was not markedly induced by low P_i concentrations in large colonial strains. At the beginning of the experiment, extracellular APA was not detected in all the large colonial strains (data not shown); however, after 10 d, low amounts of APA could be detected in FACHB909 ($P > 0.05$), and it increased in FACHB938 and FACHB939 grown in initial P_i concentration of 0.2 mg l^{-1} ($P < 0.05$).

Effects of P_i on the photosynthetic parameters of the 8 strains

After incubation in the different initial P concentrations for 5 d, the photosynthetic oxygen evolution activity in the unicellular and small colonial *Microcystis* strains decreased slightly at an P_i initial concentration of 0.2 mg l^{-1} (6.93%, PCC7806; 13.07%, PCC7820; 7.69%, FACHB942; 9.72%, FACHB905; and 10.97%, FACHB975) (Fig. 5); however, in the colonial strains, no significant differences were observed at the varying initials concentrations ($P > 0.05$). In contrast, the photosynthetic oxygen evolution activity was substantially higher in the large colonial strains (215.12–322.80 μM $O_2 \cdot mg^{-1}$ Chla$\cdot h^{-1}$) than in the unicellular and small colonial strains (131.58–160.23 μM $O_2 \cdot mg^{-1}$ Chla$\cdot h^{-1}$).

The maximum effective quantum yield of PSII (F_v/F_m) was measured under various P levels at initial concentrations of 5.4, 2, and 0.2 mg l^{-1}. The effects of P_i on the PSII activities of the *Microcystis* species were related to its colony size (Fig. 6). The *Fv*/*Fm* values in the colonial *Microcystis* strains did not significantly change with varying initial P concentrations ($P > 0.05$) during

Table 3 Changes in cellular P quota under different initial P_i concentrations in 4 cellular *Microcystis* strains (PCC7806, PCC7820, FACHB942, and FACHB 905) and 4 colonial *Microcystis* strains (FACHB942, FACHB909, FACHB938, and FACHB939)

$C_{initial\ Pi}$ (μM)	Time (h)	Q_P (μg P mg dry wt^{-1})							
		PCC 7806	PCC 7820	FACHB 942	FACHB 905	FACHB 975	FACHB 909	FACHB 938	FACHB 939
10	0	3.06 ± 0.33	2.05 ± 0.04	2.91 ± 0.02	4.26 ± 0.24	1.19 ± 0.16	6.30 ± 0.64	3.11 ± 0.15	3.65 ± 0.21
	36	14.17 ± 1.24	13.78 ± 1.07	15.15 ± 1.05	16.88 ± 0.60	11.76 ± 1.21	21.29 ± 1.78	20.76 ± 1.67	19.28 ± 1.52
	120	11.84 ± 1.81	10.29 ± 1.15	9.93 ± 1.47	11.76 ± 0.56	8.11 ± 1.17	17.63 ± 0.60	18.13 ± 1.50	17.16 ± 2.30
100	36	42.25 ± 1.49	40.60 ± 3.44	39.61 ± 4.27	45.33 ± 0.84	35.81 ± 5.59	29.04 ± 1.43	31.02 ± 2.92	25.81 ± 1.46
	120	27.04 ± 1.30	24.40 ± 2.52	21.08 ± 1.52	23.05 ± 1.46	19.79 ± 2.84	22.10 ± 1.43	20.33 ± 0.96	17.61 ± 0.41

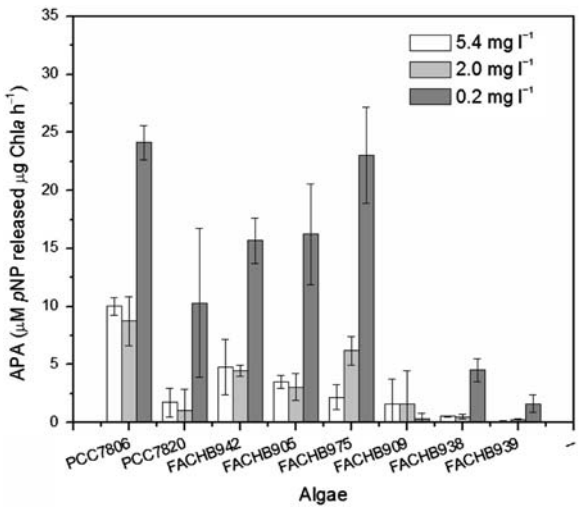

Fig. 4 Alkaline phosphatase activity of 4 unicellular strains (PCC7806, PCC7820, FACHB905, and FACHB942), 1 small colonial strain (FACHB975), and 3 large colonial strains (FACHB909, FACHB938, and FACHB939) of *Microcystis* grown for 10 d at varying P concentrations

the experiments, and those of unicellular and small colonial strains did not changed at initial P concentrations of 5.4 and 2.0 mg l^{-1} (the data not showed). However, the F_v/F_m values in the unicellular and small colonial strains decreased dramatically after 12 d of incubation in 0.2 mg l^{-1} P medium ($P < 0.05$) (Fig. 6).

The *ETR* of the 2 phenotypes demonstrated different trends at the initial P concentration of 0.2 mg l^{-1} (Fig. 7). The initial slope of the light response curve (α) in the unicellular strains decreased with incubation time (Fig. 7a), and

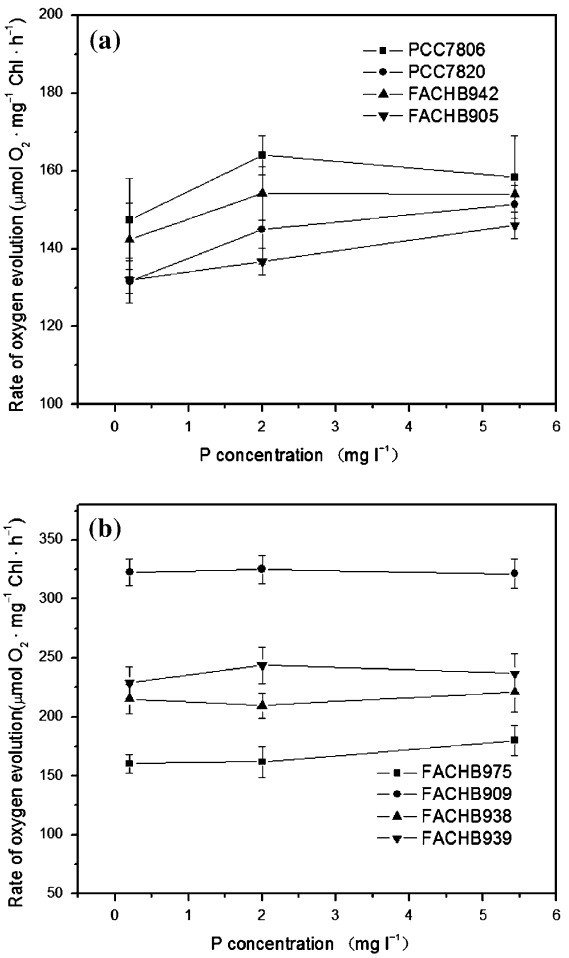

Fig. 5 Oxygen evolution in 8 *Microcystis* strains grown for 5 d at varying P concentrations. (**a**) unicellular *Microcystis* strains (PCC7806, PCC7820, FACHB905, and FACHB942); (**b**) colonial *Microcystis* strains (FACHB975, FACHB909, FACHB938, and FACHB939)

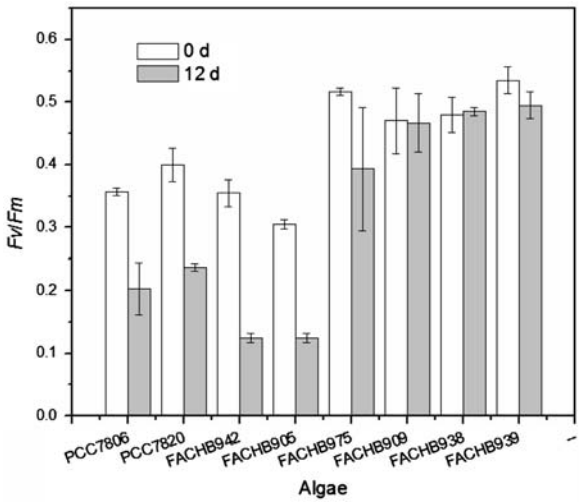

Fig. 6 Chlorophyll fluorescence ratio (*Fv*/*F*m) of 4 unicellular strains (PCC7806, PCC7820, FACHB905, and FACHB942) and 4 colonial strains (FACHB975, FACHB909, FACHB938, and FACHB939) of *Microcystis* grown for 12 d at low P concentrations (0.2 mg l^{-1})

that in the colonial strains increased with incubation time (Fig. 7b). An identical phenomenon was also observed for ETR_{max} (data not shown).

Discussion

To date, no study has addressed the response of the two *Microcystis* phenotypes to P. The availability of both unicellular and colonial *Microcystis* strains in our laboratory facilitated comparative studies on the physiological response of these phenotypes to P. Our results indicated that the 2 strains differed substantially in their response to P, as indicated by the growth rate, P uptake and storage, APA, and photosynthetic activity.

Our study revealed that the response of the two phenotype strains varied under P-limited conditions. Several studies have reported that P-limited conditions inhibits of algal growth (Lee et al., 2005; Litchman et al. 2003). Ou et al. (2005) discovered that *M. aeruginosa* biomasses decreased with a decrease in P_i concentration. Nalewajko and Murphy (2001) suggested that the *Microcystis* species would not be able to grow well in P-limited waters. In the present study, the growth patterns of all the unicellular strains but not those of the colonial strains were quite similar

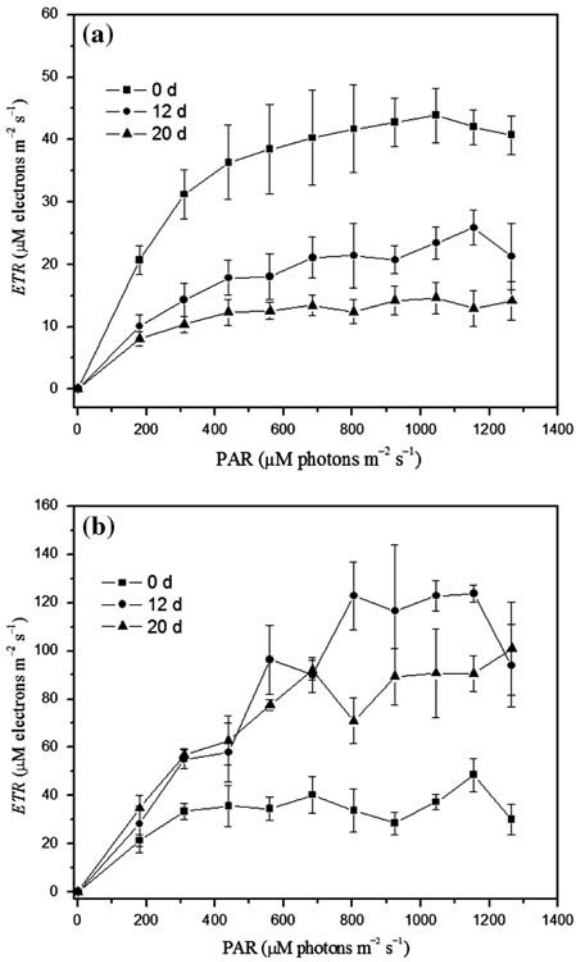

Fig. 7 Electron transport rate (*ETR*) curves for different *Microcystis* strains grown for 0, 12, and 20 d at low P concentrations (0.2 mg l^{-1}). (**a**) unicellular *Microcystis* strain (FACHB7806), (**b**) colonial *Microcystis* strain (FACHB909)

to those in the abovementioned studies. The colonial strains were more resistant to low P concentrations than the unicellular and small colonial strains were. The reason for the difference in growth patterns under P-limited conditions may be attributed to the difference in the extracellular structure between the two phenotype strains. The colonial *Microcystis* cells are embedded in a mucilaginous sheath comprising several polysaccharide compounds (Rohrlack et al., 1999), thereby imparting resistance to unfavorable environmental conditions (Kolmakov, 2006), it is thus beneficial for the colonial strains. As also suggested by Reynolds (2007),

metabolic self-regulation of mucilage may help the colonial strains survive in nutrient-poor water. Organisms that produce conspicuously variable amounts of mucilage produce more mucilage when nutrient (especially P) concentrations are depleted.

Difference in properties of P uptake and storage in the two phenotype strains could further explain the difference in the growth of these strains under P-limited conditions. The constant K_s reflects the relative ability of phytoplankton to use nutrients at low levels, and V_m is considered the maximum specific growth rate of the organism based on the external conditions (Eppley et al., 1969). The K_s values for the colonial strains were lower than those for the unicellular strains, which indicates that the former had a higher affinity for P_i at low concentrations. Stolte & Riegman (1995) discovered that the initial specific nitrate uptake rates in small and large cells of the diatom *Ditylum brightwellii* did not significantly differ, but the larger cells maintained a high uptake rate for a longer period. Our results contradicted theirs. Similar to that observed in the high P_i culture, the uptake rate in all the strains was higher than that in the low P_i culture. With regard to the unicellular *Microcystis* strains, the increase in the uptake rate was even more pronounced. This result demonstrated that luxury uptake of P_i occurs in *Microcystis*, and this characteristic assists them in outcompeting other strains under P_i-limited conditions. While the P cell quota was generally higher in colonial strains than in unicellular strains, the latter consumed more P than the former, indicating that the unicellular strains require more P for growth. Low K_s values and a high P cell quota may be the key factors that determine the success of the colonial strains in P-limited conditions.

The better performance of the colonial strains at low P_i concentrations might be due to the role of mucilage in nutrient sequestration and processing. However, all phytoplankton cells can effectively take up nutrients both actively and endothermically but only from the boundary layer adjacent to the cell. It is thus possible that the provision of a mucilaginous coat offers an inexpensive way of increasing the size of the algal ''target'' and its prospect of encountering nutrient molecules within the water mass without raising the demand for nutrient. Simultaneously, a distinct microenvironment is maintained wherein the rapid uptake of nutrients across the cell wall leaves the immediate environment more dilute than the exterior environment, hence facilitating a beneficial inward diffusion gradient from the medium to the mucilage (Reynolds, 2007). On the other hand, the difference between the two phenotypes with regard to their response to P may also be partly attributed to the presence of heterotrophic bacteria associated with the *Microcystis* strains, especially in the colonial strains. Jiang et al. (2007) discovered that P transfer occurred between *M. aeruginosa* and the attached *Pseudomonas* species, and that the mucilage microenvironment contained relatively high amounts of P. It was proposed that *M. aeruginosa*- attached bacteria might act as a temporary P bank for the growth of *M. aeruginosa* in the microenvironment.

At very low external P_i concentrations in the water column during summer algal blooms, the degree to which phytoplankton may benefit from organic P compounds may be decisive to the competition for P (Spijkerman & Coesel, 1998). Alkaline phosphatases (APases) play an important role in supplying P during P depletion. APases are inducible enzymes, and their expression in algae is generally regulated by the external P_i concentration (Jansson & Petterson, 1988; Hoppe, 2003). Several algae display high APA levels when cultured in P_i-deficient conditions (Fitzgerald & Nelson, 1966; Hernández et al., 1993; Dignum et al., 2004). Further, high APA levels have been used as an indicator of P_i deficiency in productivity (Jansson & Petterson, 1988; Weich & Granéli, 1989). In the present study, the unicellular *Microcystis* strains demonstrated higher APA levels after treatment with 0.2 mg l^{-1} P, implying that P at this concentration was not sufficient for adequate growth. On the other hand, the higher P cell quota observed in the colonial *Microcystis* strains leads to low extracellular APA. Some strains display undetectable or very low maximum APA levels even at low external P concentrations, indicating that they satisfy most of their P demand directly from P_i (Hernández et al. 1999; Lee 2000). This phenomenon was also explained by the observation

of high P cell quotas during assays (Hernández et al., 2002). The higher APA in the unicellular strains than that in the colonial strains under lower P concentration suggests that the latter experience lower P limitation. This result agrees with that regarding the growth rate of the two phenotypes under lower P concentrations. Further investigation is required to evaluate the correlation between APA and P_i affinity for *Microcystis* and the trophic conditions of water columns.

The F_v/F_m value indicates PSII function and oxygen evolution is also considered one of the most sensitive photosynthetic parameters. Photosynthetic efficiency (F_v/F_m) was reduced in *Microcystis* with a decrease in the P concentration of the medium (Ou et al., 2005). Kromkamp & Peene (1999) discovered that low F_v/F_m values coincided with low phosphate and silicate concentrations. Thus, they concluded that the PSII quantum efficiency could be used to detect nutrient limitation. The reduced F_v/F_m values in the unicellular *Microcystis* strains at a P concentration of 0.2 mg l^{-1} suggest that F_v/F_m may be used as an indicator of P limitation deficiency for the unicellular strains. Finkel & Irwin (2000) thought that microalgal photosynthesis could be predicted based on cell size. In our experiments, the *Microcystis* photosynthetic parameters depended on the colony size. The F_v/F_m, oxygen evolution, and ETR_{max} values in the colonial strains were obviously higher than those in the unicellular strains; this might facilitate improved resistance to nutrient stress.

Our previous studies illustrated that the colonial *Microcystis* strains were identified by their strong resistance to Cu stress and higher affinity for inorganic carbon (Wu et al., 2007; Xu & Song 2007). Based on these results, we may conclude that the colonial strains endure stress better than the unicellular strains. In other words, the ability of *Microcystis* species to resist stress correlates positively with colony size. Some studies have demonstrated that bloom-forming algae exhibit characteristics of K-strategy (Kruskopf & Plessis 2004; Krivtsov et al., 2005). They had a relatively low growth rate under optimal conditions but a high growth rate under nutrient-limited conditions. Based on the above results, it appears that K-strategy traits are more pronounced in the colonial strains, allowing them to maintain a prolonged competitive advantage.

Conclusion

The two different phenotypes of bloom-forming *Microcystis* display the same physiological response to P_i-repleted conditions. However, the growth rate, APA, F_v/F_m, and ETR_{max} of the unicellular *Microcystis* strains were more sensitive to P_i deficiency. In contrast, at low P concentrations, the colonial strains grew better than the unicellular stains because they required less P for growth. Moreover, the colonial strains possess a higher affinity for low levels of P_i, high P cell quota, and high photosynthetic efficiency, which facilitates the maintenance of normal growth for long periods under conditions of fluctuating P concentrations. Mucilage might influence the physiological characteristic of the *Microcystis* species conferring a competitive advantage on the colonial strains in natural water columns with regard to nutrient acquisition.

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