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Carbon limitation enhances CO_2 concentrating mechanism but reduces trichome size in *Arthrospira platensis* (cyanobacterium)

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Abstract *Arthrospira* species grow well under highly enriched inorganic carbon concentrations, but little is known on the effects of inorganic carbon (Ci) limitation on its physiological performance. When *Arthrospira platensis* D-0083 was grown in a modified medium without $NaHCO_3$ under ambient air of 380 ppm CO_2, its trichomes became disassembled while the growth and photosynthetic rates were severely reduced. Phycocyanin and allophycocyanin contents decreased but the carotenoid content increased under the Ci limitation. Compared with the cells grown in Zarrouk medium, the trichomes grown under the Ci limitation increased their photosynthetic apparent affinity for Ci by about 14 times but photochemical quenching capacity was reduced. It appeared that *A. platensis* increased its CO_2 concentrating mechanism by inducing HCO_3^- transporters and reducing the trichome size which increased filamentous surface to volume ratio.

Keywords *Arthrospira platensis* · Inorganic carbon (Ci) · CO_2 concentration mechanism (CCM) · Morphology · Photosynthesis

Introduction

Cyanobacteria are known to be able to take up both CO_2 or HCO_3^- as inorganic carbon (Ci) source for photosynthesis (Raven et al. 2011). Assimilation of CO_2 in cyanobacteria cannot usually be optimized because of a rather low affinity of Rubisco for CO_2 and constant fluctuations in Ci levels within cells during photosynthesis. When the cellular CO_2 concentration ranges between 10 and 12 μmol CO_2 L^{-1} (equivalent level when CO_2 equilibrium between the air and the water is reached), photosynthetic CO_2 fixation could hardly take place (Badger and Price 2003; Badger et al. 2006). To overcome these challenges, cyanobacteria have developed an effective CO_2 concentration mechanism (CCM) to increase the intracellular CO_2 level around Rubisco, up to 1,000 times higher than that in the external medium (Kaplan and Reinhold 1999; Badger and Price 2003; Ogawa and Kaplan 2003; Giordano et al. 2005; Badger et al. 2006; Raven et al. 2011).

Morphology and photosynthetic efficiency in cyanobacteria can be altered by availability of Ci or changes in the carbonate system (Beardall et al. 2009; Singh and Montgomery 2011; Gao et al. 2012). Low carbon availability produces a rigid cell wall and induces akinete formation (Kaplan-Levy et al. 2010; Singh and Montgomery 2011), which was thought to be a strategy to survive under carbon-starved conditions for some cyanobacteria. Conversely, HCO_3^- transport could be stimulated under low CO_2 concentration with simultaneous intensive irradiance (Benschop et al. 2003; McGinn et al. 2003; Eisenhut et al. 2007). In *Arthrospira* species, elevated PAR levels decrease the helix pitch of *Arthrospira* (*Spirulina*) *platensis* (Ma and Gao 2009), while UV radiation has led to more compressed spirals (Wu et al. 2005). Oxidative pressure induced by light or UV stresses leads to broken trichomes in *A. platensis* and *Anabaena variabilis* PCC 7937 (Ma and Gao 2010; Rastogi et al. 2010).

Arthrospira platensis is an economically important cyanobacterium and commercially cultured around the world to supply biomass and a rich source of protein for the health food industry (Torzillo and Vonshak 2003; Sili et al. 2012). Natural habitats of most *Arthrospira* species are alkaline waters

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with adequate Ci supply. However, some species are occasionally found in puddles, flowing and stagnant fresh water, springs, stagnant, and sulfur-containing water, ponds, filter beds, reservoirs, and tanks with inadequate Ci (Sili et al. 2012). To date, little is known on the effects of limited Ci concentrations on the physiological performance and morphological regulation in *Arthrospira* species.

Material and methods

Arthrospira platensis D-0083 was obtained from Hainan Dainippon Ink and Chemicals (DIC) Microalgae Co. Ltd., Hainan province, China. A single healthy spiral was isolated and used for all trichome propagations. The cells were pre-cultured at 25 °C and 60 μmol photons m^{-2} s^{-1} PAR (12 L/12 D) in Zarrouk medium (Zarrouk 1966) containing 200 mmol L^{-1} $NaHCO_3$ and 0.22 mmol L^{-1} EDTA(Na). The culture was aerated with ambient air at a flow rate of 100 mL min^{-1}. Cells in the exponential growth phase were used in subsequent experiments on growth of *A. platensis* at various levels of Ci.

Low and high dissolved inorganic carbon (DIC) treatments

Strains of *A. platensis* D-0083 cultured in normal Zarrouk medium were designated as high-Ci (high-DIC) treatment while those grown in modified medium where $NaHCO_3$ and EDTA(Na) were eliminated were referred to as low-Ci (low-DIC) treatment. The pH of the low-DIC medium was adjusted to the same as that of the high-DIC one by adding NaOH solution (1 M) before the cells were inoculated. Therefore, the dissolved inorganic carbon (DIC) and CO_2 concentrations were very different although the pH was identical in low and high-DIC media. Cultures (400 mL) with the same cell density (OD_{560nm}= 0.04) in the high and low-Ci media were irradiated with 60 μmol photons m^{-2} s^{-1} PAR (12 L/12 D) at 25 °C. Both high and low-Ci cultures were continuously aerated (100 mL min^{-1}) with ambient air containing about 380 ppmv CO_2. Three replicates each were done for each treatment, and the data are reported as means and standard deviation for each Ci level.

Preparation of Ci-free reaction medium

For measurements of photosynthetic O_2 evolution at different levels of Ci, Ci-free reaction solution was prepared by removing $NaHCO_3$ and EDTA(Na) from Zarrouk medium, adjusting the pH to about 2.0 with 1 mol L^{-1} HCl, aerating with pure N_2 gas for 30 min and then re-adjusting the pH back to 8.0 in the presence of N_2 with freshly prepared supersaturated NaOH solution. The pH of the medium was measured by using a pH meter (Mettler Toledo DL15 Titrator, Sweden), which was calibrated with standard NBS buffer solution (Hanna).

Determination of the carbonate system in low and high DIC treatments

The cell densities and pH of cultures in both Ci treatments were monitored everyday at the beginning of the light period. The optical cell densities (OD_{560nm}) were determined with a spectrophotometer (Shimadzu, UV 2501-PC) and the pH was measured. Concentrations of Ci in the culture media were measured with a total organic carbon analyzer (TOC-5000A, Shimadzu, Japan), which measures the CO_2 released from the acidified aliquot of the media and automatically determines the concentration of Ci. HCO_3^-, CO_3^{2-}, CO_2, and total alkalinity (TA) of the carbonate system were computed with CO_2SYS software (Lewis and Wallace 1998) based on the known values of DIC, pH, salinity, and nutrients, K_1 and K_2 for carbonic acid dissociation (Roy et al. 1993) and K_B for boric acid (Dickson 1990).

Determination of pigments content

For photosynthetic pigments analysis, 20 mL cultures that had been grown under high and low Ci treatments for 2 weeks were filtered (GF/F, Whatman) and extracted overnight in the dark with absolute methanol at 4 °C. Absorption spectra of the supernatant (centrifuged at 5,000×*g* for 5 min) were measured with a spectrophotometer (Shimadzu, UV 2501-PC). Chlorophyll *a* (Chl *a*) concentration was calculated according to Porra (2002) and carotenoids (Car) were determined using the formulae of Parsons and Strickland (1963).

Extraction of phycocyanin (PC) and allophycocyanin (APC) were carried out by re-suspending the harvested cells (from 20 mL cultures) in sodium phosphate buffered at pH 6.7 containing 0.2 mmol L^{-1} NaCl, homogenized with a ultrasonic homogenizer (CPX600, Cole-Parmer, USA) in an ice bath. Concentrations of PC and APC were determined from absorbance values at 615 and 652 nm of the supernatant (Bennet and Bogard 1973).

Morphological examination

The morphological change of *A. platenssis* D-0083 was examined with a microscope (Zeiss Axioplan 2; Carl Zeiss, Germany) everyday. Digital images were recorded weekly with a Zeiss Axicam HRC color camera (Carl Zeiss, Jena, Germany) ,and analyzed with a Vision Analysis system (Axio Vision 3.0).

Measurements of photosynthetic oxygen evolution under saturated irradiance

Photosynthetic oxygen evolution was measured with a Clark type oxygen electrode (Chlorolab 3, Hansatech, UK) at 400 μmol photons m^{-2} s^{-1} and 25 °C. The oxygen electrode

was calibrated with air-equilibrated distilled water for the full scale and nitrogen-bubbled distilled water for zero point. Temperature was controlled by a refrigerated circulator. PAR levels were obtained by variable distances between the halogen lamp and the reaction chamber. Irradiance was measured with a quantum sensor (QRT1; Hansatech Instrument Ltd).

Filaments of low- and high-Ci-acclimated *A. platensis* D-0083 were re-suspended in the Ci-free reaction medium (pH 8.0) in equal chlorophyll *a* concentration (100 μg L^{-1}), and the complete depletion of intracellular Ci was confirmed before a known amount of $NaHCO_3$ was added to achieve a desired Ci level. Photosynthetic O_2 evolution was measured with a Clark type oxygen electrode (Chlorolab 3, Hansatech, UK) and parameters for the photosynthetic response to concentrations of Ci were obtained by fitting the net photosynthetic rates to the levels of Ci following the Michaelis-Menten formula. The theoretical production rates of CO_2 derived from uncatalysed dehydration of HCO_3^- at pH 8.0 were estimated according to formulae of Matsuda et al. (2001).

Determination of chlorophyll fluorescence under stressful irradiance

To investigate the effects of Ci deficiency on resistance of *A. platensis* D-0083 to stressful irradiance, filaments that had been pre-cultured under high and low-Ci treatments for two weeks were harvested. Subsequently, the cells were inoculated in a 24-well microtiter plate (2 mL/well) after the intracellular Ci was exhausted under PAR of 400 μmol photons m^{-2} s^{-1}. Then $NaHCO_3$ solution was added to every well to attain the Ci levels of 0, 100, 200, 500, 1,000, 2,000, 5,000, and 10, 000 μmol L^{-1}, with each Ci level in three wells (i.e., triplicates). PSII quantum yield was determined with an Imaging-PAM (Heinz Walz GmbH, Germany) after the cells in different Ci levels were exposed to stressful PAR of 1, 500 μmol photons m^{-2} s^{-1} for 20 min: The minimum fluorescence (F_0) was determined by illuminating the sample with the low intensity light (600 Hz, 665 nm, 0.3 μmol photons m^{-2} s^{-1}) after the samples were kept in the dark for 1 h. Subsequently, the maximal fluorescence (F_m) was determined with a 0.8-s pulse of saturating red light of 5, 600 μmol photons m^{-2} s^{-1}. The variable florescence (F_v) was defined as $F_v=F_m-F_0$, and the optimal quantum yield was determined as F_v/F_m. After 30 s darkness, the actinic light (60 μmol photons m^{-2} s^{-1}) was turned on until the state fluorescence level (F_t) was reached. Subsequently, the measuring procedure was repeated and F_0' and F_m' were determined in the presence of actinic light and the fluorescence parameters were used for calculating the quenching coefficients: the photochemical quenching of Chl fluorescence (qP) was determined as $qP=(F_m'-F_t)/(F_m'-F_0')$ and the non-photochemical quenching of chlorophyll fluorescence was determined as $NPQ=(F_m-F_m')/F_m'$. According to Campbell et al. (1998), the NPQ is independent of F_0 and not subject to distortion by underlying phycobiliprotein fluorescence.

Statistical analysis

One-way ANOVA, non-parametric analysis (Kruskal–Wallis analysis) and Kendall tests were used to establish differences among treatments, with a significant level set at 5 % ($p=0.05$).

Results

Changes in the carbonate system

Carbonate chemistry parameters for high and low-Ci culture media (normal and modified Zarrouk media) changed continuously (Table 1). At the beginning of culturing, the contents of DIC, HCO_3^-, and CO_2 were 203.3 (±1.8), 175.5 (±2.1), and 0.8 (±0.0) mmol kg^{-1}, respectively, in high-Ci cultures, and 3.3 (±.02), 2.8 (±0.0), and 0.0 (±0.0) mmol kg^{-1}, respectively, in low-Ci cultures. After 2 weeks culture, the DIC, HCO_3^-, and CO_2 in high-Ci cultures decreased to 145.8 (±2.18), 15.6 (±0.2), and 0.0 (±0.0)mmol kg^{-1}; however, in the low-Ci cultures, the DIC and HCO_3^- increased to 4.1 (±0.0) and 3.3 (±0.1) mmol kg^{-1} but CO_2 was unchanged (Table 1). After 2 weeks, TA increased to 281.6 (±4.4) and 8.2 (±0.2) mmol kg^{-1} from 232.8 (±1.5) and 6.9 (±0.0) mmol kg^{-1} at the beginning of inoculation in high and low-Ci cultures, respectively.

Effects of Ci limitation on growth and pigments

The pH of high-Ci (Zarrouk medium) and low-Ci (modified Zarrouk medium) cultures increased in the 7 days and slowed down and even leveled off after day 10 or so (Fig. 1a). pH values increased to 10.14 (±0.01) and 8.58 (±0.05) from 8.41 (±0.01) and 8.43 (±0.02) in high-Ci and low-Ci cultures, respectively. The optical cell density linearly increased in both high and low-Ci cultures during the whole culture period, with significantly higher ($p<0.05$) increase rate in the former compared with the latter (Fig. 1b). It increased to 0.59 (±0.01) in high-Ci culture and 0.40 (±0.01) in low-Ci culture from the same inoculated optical cell density (0.04±0.00).

Chl *a* content of cells at high-Ci medium was 21.11 (±1.33) mg g^{-1} DW and was not significantly ($p>0.05$) different to those cultured under the low-Ci treatment (21.68±0.25 mg g^{-1} DW). The PC and APC contents were significantly higher ($p<0.01$) at 199.13 (±0.31) and 149.08 (±1.83)mg g^{-1} DW of cells in Ci-sufficient cultures than in Ci-deficient medium at 178.67 (±10.08) and 130.42 (±7.09)mg g^{-1} DW cells (Table 2). However, the carotenoid content of cells grown in sufficient

Table 1 Carbonate system parameters in the cultures of *Arthrospira platensis* D-0083 grown at low and high concentrations of dissolved inorganic carbon (DIC)

Time (day)	Treatments	pH_{NBS}	DIC (mmol kg^{-1})	HCO_3^- (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	CO_2 (mmol kg^{-1})	TA (mmol kg^{-1})
0	High-Ci	8.41±0.01	203.3±1.8	175.5±2.1	27.1±0.30	0.8±0.0	232.8±1.5
	Low-Ci	8.43±0.02	3.3±0.0	2.8±0.0	0.5±0.0	0.0±0.0	6.9±0.0
14	High-Ci	10.14±0.01	145.8±2.1	15.6±0.2	130.2±2.2	0.0±0.0	281.6±4.4
	Low-Ci	8.58±0.05	4.1±0.1	3.3±0.1	0.8±0.1	0.0±0.0	8.2±0.2

Parameters of the carbonate system were computed with the CO_2SYS software (Lewis and Wallace 1998) based on the known values of DIC, pH, salinity (22.13), and nutrients (phosphate, 2,604 μmol L^{-1}), the equilibrium constants K_1 and K_2 for carbonic acid dissociation after Roy et al. (1993), and K_B for boric acid after Dickson (1990) were used. The values for Ci species shown here were derived by calculation assuming complete chemical equilibrium among the inorganic carbon species. The means and standard errors are based on triplicate incubations

NBS National Bureau of Standards

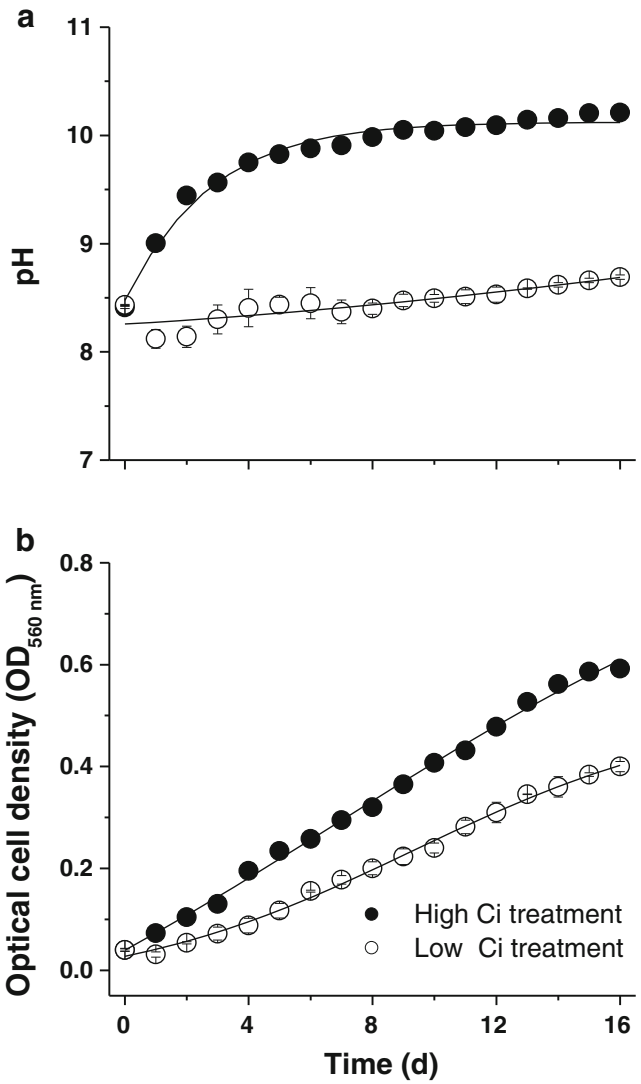

Fig. 1 Changes in pH of high- (control) and low-Ci culture media (**a**) and the growth of *A. platensis* D-0083 (**b**) during the culture period. The cultures were aerated with ambient air (100 mL min^{-1}) and irradiated with PAR (60 μmol photons m^{-2} s^{-1}) at 25 °C. The means and standard errors are based on triplicate incubations

Ci cultures was significantly lower ($p<0.01$) at 4.33 (±0.15) mg g^{-1} cell compared with those grown in deficient Ci cultures.

Effects of Ci limitation on filamentous morphology

Besides the impact of Ci limitation on the growth and pigments contents of *Arthrospira* cells, the morphology of trichomes was affected. Compared with the uniform and regular spirals in high-Ci medium throughout the culture period (Fig. 2a), the morphology of the trichomes in low-Ci cultures changed with prolonged culture period. At the beginning (one week) of low-Ci cultures, the filaments were fragmented or disassembled, and resulted in smaller-sized filaments (Fig. 2b). In addition to being much smaller trichomes with one to two spirals, the largest filaments in low-Ci cultures were about half the length of large fragments in high-Ci cultures (Fig. 2a, b).

Effects of Ci limitation on photochemical performance

The maximal photosynthetic rate (P_{max}) and affinity to Ci ($K_{0.5}$ DIC, the Ci concentration at which the photosynthetic rate was 1/2 of P_{max}) significantly changed after the cells of *A. platensis* D-0083 were exposed to low-Ci treatment for 2 weeks. P_{max} of low-Ci-acclimated cells decreased by 25 % compared with those cultured under high-Ci condition, but its photosynthetic rates were much higher than their counterparts when the Ci concentrations were lower than 500 μmol L^{-1} (Fig. 3). The Ci levels which supported the P_{max} for the low and high-Ci-acclimated cells were 200 and 2,000 μmol L^{-1}, respectively. Respectively, P_{max} values for low and high-Ci-acclimated cells were 15.51 (±0.15) and 21.41(±0.65) μmol O_2 L^{-1} min^{-1} (amounting to 180.77 (±1.75) and 249.54 (±7.58) μmol O_2 mg^{-1} Chl *a* h^{-1}) (Table 3). The $K_{0.5}$ values for low and high-Ci cultured ones was 14.78 (±0.85) and 219.89 (±35.28) μmol L^{-1} respectively, meaning that the affinity to Ci of the low-Ci-acclimated cells was about 15-fold of that of high-Ci-acclimated ones (Table 3). The CO_2 supplying rates

Table 2 Photosynthetic pigment contents of *Arthrospira platensis* D-0083 that had been cultured for 2 weeks under low and high-Ci treatments

Photosynthetic pigments	High-Ci (control)	Low-Ci
Chlorophyll *a* (mg g^{-1})	21.11±1.33	21.68±0.25
Carotenoids (mg g^{-1})	4.33±0.15	4.87±0.06**
Phycocyanin (mg g^{-1})	199.13±2.31	178.67±10.08**
Allophycocyanin (mg g^{-1})	149.08±1.83	130.42±7.09**

The cultures were aerated with air (100 mL min^{-1}) and irradiated with PAR (60 μmol photons m^{-2} s^{-1}) at 25 °C. The means and standard errors are based on triplicate incubations

**p =0.01—significant difference

of CO_2 converted from uncatalyzed HCO_3^- at $K_{0.5}$ were 0.62 and 9.17 μmol L^{-1} min^{-1}. Therefore, the photosynthetic O_2 evolution rates for low and high-Ci-acclimated cells were 12.58- and 1.17-fold of CO_2 supplying rate at $K_{0.5}$ (0.5 V_{max} O_2/0.5 V_{max} CO_2).

Effects of Ci limitation on photosynthetic response to stress irradiance

The photosynthetic capabilities of low and high-Ci-acclimated cells were inhibited by intensive PAR (1, 500 μmol photons m^{-2} s^{-1}, 20 min). The optical quantum yield (F_v/F_m) of low-Ci cultured cells were much lower than the high-Ci-acclimated cells although it increased with Ci levels lower than 2,000 μmol L^{-1} and the trend leveled off after that critical Ci-level (Fig. 4a). The photochemical quenching of chlorophyll fluorescence (qP) in low-Ci-acclimated cells was much lower (p <0.01) than that in cells cultured at high-Ci treatment (Fig. 4b). The values of qP increased with enhanced Ci levels lower than 2,000 μmol L^{-1} in the low-Ci-acclimated cells but with very minimal change for the high-Ci-acclimated cells. The non-photochemical quenching of chlorophyll fluorescence (NPQ) changed in a similar pattern with increased Ci level in both high and low-Ci-acclimated cells and with higher (p <0.05) values in the former (Fig. 4c).

Discussion

The carbonate system or pH was not stable in the present experimental setup, and so it was obvious that the cells in the low and high-DIC cultures were exposed to significantly different levels of DIC through out the comparative growth experiment. While the medium pCO_2 might not be identical to that in the aerated air, pCO_2 was should be higher in the high-DIC cultures. Obviously, growth of *A. platensis* D-0083 in Ci-deficient medium regulated its growth rate and maximal net photosynthetic rate, changed its pigments contents, reduced its trichome size, enhanced its photosynthetic affinity to DIC but aggravated its photoinhibition to stressful irradiance.

Culture of *A. platensis* D-0083 under Ci deficient condition decreased its growth (Fig. 1b), lysed the filaments in initial stage and ultimately induced much smaller-sized trichomes (Fig. 2a). Reactive oxygen species (ROS) induced by stressful irradiance had been proven to provide the cue for the filament break and cell damage in *A. platensis* and *A. variabilis* (Ma and Gao 2010; Rastogi et al. 2010). Furthermore, enhanced production of H_2O_2 had been observed when CO_2 fixation was interrupted (Allahverdiyeva et al. 2005; Takahashi and Murata 2008). The filaments of *Arthrospira* spp. multiplied by breaking off at necridia (dead cells with no inclusion) (Tomaselli et al. 1981). Therefore, the smaller sized filaments may be caused by the easier formation of necridia due to enhanced intracellular ROS production under Ci limited conditions.

The contents of light-harvesting pigments PC and APC in *A. platensis* D-0083 decreased but the contents of photoprotective and antioxidative carotenoid pigments increased under Ci limitation condition (Table 2). Decreases in growth, photosynthetic pigments content and photosynthetic performance are typical responses of algae grown in nutrient limited environments including Ci deficiency (Gordillo et al. 1999; Markou and Georgakahis 2011). Carotenoids are antioxidants, and the enhanced content could alleviate the oxidative damage to cells (Davison et al. 2002; Sharma et al. 2012). Therefore, the increased carotenoid amount may be an inducible protective strategy to alleviate the damage of cell membranes by increased ROS under Ci limitation although the filaments were still damaged.

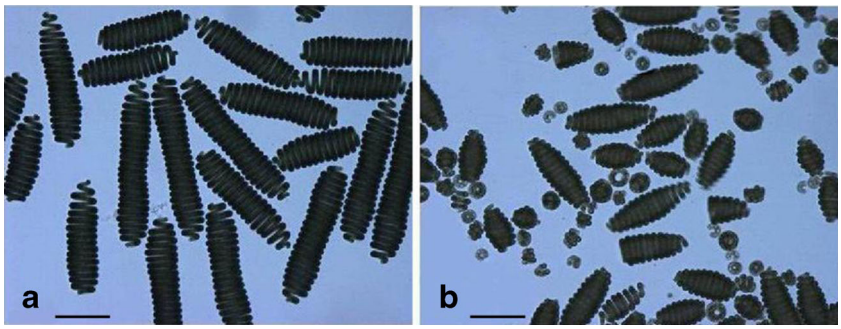

Fig. 2 Morphological characters of *A. platensis* D-0083 filaments cultured under high- (**a**) and low-Ci treatments (**b**) for 2 weeks, which was aerated with ambient air (100 mL min^{-1}) and irradiated with PAR (60 μmol photons m^{-2} s^{-1}) at 25 °C. *Scale bars*, 100 μm

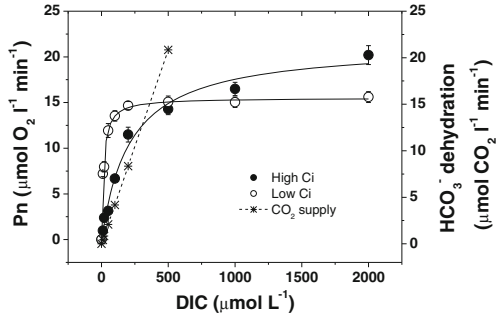

Fig. 3 Photosynthetic O_2 evolution as a function of DIC ($NaHCO_3$) for *A. platensis* D-0083 that had been cultured for 2 weeks under high- (control) and low-Ci treatments. Photosynthetic rates were measured in cultures in equal chlorophyll *a* concentration at pH 8.0, 25 °C and 300 μmol photons m^{-2} s^{-1}. The *dotted line* is the theoretical photosynthetic rate that maximally could be supported by the uncatalyzed conversion of HCO_3^- to CO_2. The means and standard errors are based on triplicates

Our observations that Ci limitation increased its CCM but decreased their maximal photosynthetic capability (Fig. 3; Table 3) are consistent with the results from other cyanobacteria species (Ogawa and Kaplan 2003). Cyanobacteria usually possess CCM that enables efficient CO_2 fixation despite the low affinity of their Rubisco for CO_2 (Kaplan and Reinhold 1999; Ogawa and Kaplan 2003; Badger et al. 2006). The cells of model cyanobacteria species such as *Synechococcus* PCC7942 and *Synechosystis* PCC6803 grown at low-Ci (<200 μmol L^{-1}, pH 8.0) have a high photosynthetic affinity for Ci ($K_{0.5}$ DIC, <20 μmol L^{-1}), while those grown at high-Ci (>2 mmol L^{-1}, pH 8.0) have a reduced affinity ($K_{0.5}$ DIC, >200 μmol L^{-1}) (McGinn et al. 2003; Woodger et al. 2003). Recent studies have shown that the variation in affinity was solely due to the induction of various high and medium affinity HCO_3^- transporters (i.e., BCT1, SbtA, and BicA) and the high affinity CO_2 uptake system (NDH-1_3) (McGinn et al. 2003;

Table 3 Photosynthetic responses of *Arthrospira platensis* D-0083 cultured under low- and high-Ci treatments for 2 weeks

Parameters	High-Ci (control)	Low-Ci
P_{max} (μmol O_2 mg^{-1} Chl *a* h^{-1})	249.54±7.58	180.77±1.75
P_{max} (μmol O_2 L^{-1} min^{-1})	21.41±0.65	15.51±0.15**
$K_{0.5}$ (μmol L^{-1})	219.89±35.28	14.78±0.85**
0.5 V_{max} CO_2 (μmol L^{-1} min^{-1})	9.17	0.62
0.5 P_{max} O_2/0.5 V_{max} CO_2	1.17	12.51

Photosynthetic rates were measured in the buffered media at pH 8.0 at 300 μmol photons m^{-2} s^{-1}. Parameters were estimated from Fig. 3. The means and standard errors are based on triplicate incubations

P_{max} the maximal photosynthetic rate, $K_{0.5}$ the inorganic carbon concentration at which the photosynthetic rate was 1/2 of P_{max}, *0.5* V_{max} CO_2 the maximal CO_2 production rate at $K_{0.5}$, *0.5* P_{max} O_2/*0.5* V_{max} CO_2 the ratio of photosynthetic O_2 evolution rate to CO_2 supply rate at $K_{0.5}$

**p=0.01, significant difference

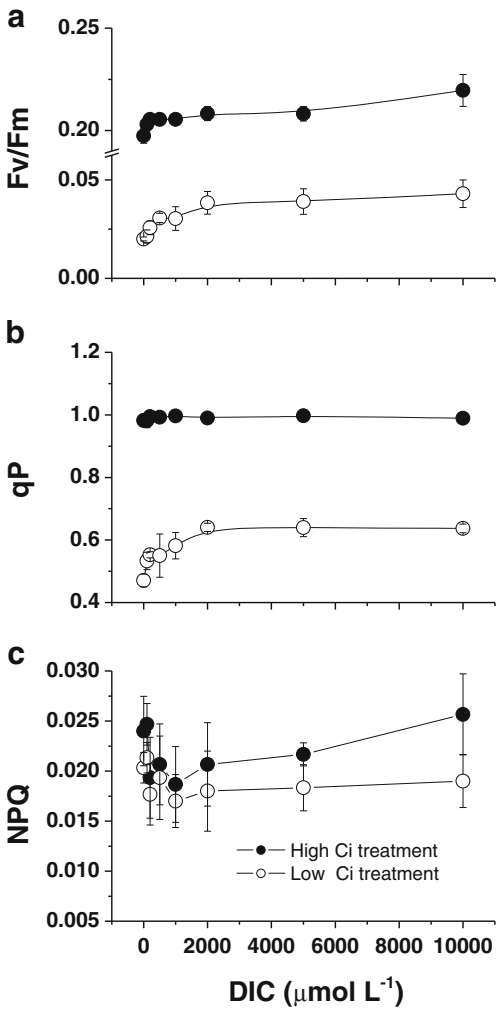

Fig. 4 Effect of Ci level on the optimal quantum yields (**a**), photochemical quenching (**b**), and non-photochemical quenching (**c**) of *A. platensis* D-0083 which had been cultured for 2 weeks under high (control) and low-Ci treatments. The pelleted cells were resuspended with Ci-free reaction medium, known amounts of $NaHCO_3$ were added after intracellular Ci depletion at 300 μmol photons m^{-2} s^{-1}, then intensive PAR (1,500 μmol photons m^{-2} s^{-1}) was provided for 20 min. All samples were dark adapted for 1 h before the chlorophyll fluorescence parameters were measured. The means and standard errors are based on triplicates

Woodger et al. 2003; Price et al. 2008). Compared with the high-Ci-acclimated cells of *A. platensis* D-0083 (219.89±35.28 μM), the photosynthetic affinity of low-Ci-acclimated ones to Ci (14.78±0.85 μM) increased about 14 times. Furthermore, the O_2 evolution rate of low Ci-acclimated cells was 12.51 times of CO_2 supply rate at the Ci concentration of $K_{0.5}$DIC, much higher than that (1.17 times) of high Ci-acclimated ones (Table 3). These observations indicate that some high affinity HCO_3^- transporters must be induced in low-Ci-acclimated cells. On the other hand, for *A. platensis* D-0083 cultured under high-Ci treatment, the 17 % higher O_2 evolution rate than the uncatalyzed CO_2 supply rate at Ci level of $K_{0.5}$ DIC can be ascribed to the constitutive HCO_3^- transporters situated in cellular membrane because *Arthrospira* spp. has been shown to have HCO_3^-

utilization capability (Kaplan 1981; Binaghi et al. 2003). Furthermore, the smaller sized filaments induced under Ci-deficient media could increase its contacting area to ambient Ci by increasing the surface area to volume ratio (Fig. 2b), which may be partly responsible for the increased CCM (Table 3).

Strong irradiance would inevitably induce damage of photosystem II (PSII) and cause decreased photochemical efficiency (Long et al. 1994; Mattoo et al. 1999). Ci transport and accumulation is suggested as an active process, with evidence that adenosine triphosphate derived from cyclic photophosphorylation driven by electron transport around photosystem I (PSI) was involved in the process (Ogawa et al. 1985; Palmqvist et al. 1990). CO_2 fixation coupled to operation of a CCM is thought to be energetically costly (Tchernov et al. 1997; Beardall et al. 1998), supported by the observation that rates of Ci transport and CCM activity are greatest under high-photon flux intensities (Beardall 1991) and operation of the CCM diminishes photodynamic damage by dissipating excess light energy (Qiu and Liu 2004). In this study, the F_v/F_m of *A. platensis* D-0083 decreased with the increased Ci level which ranged from 0 to 2,000 μmol L^{-1} (Fig. 4a). Furthermore, F_v/F_m, qP, and NPQ of the low-Ci-acclimated cells were much lower than those of high-Ci-acclimated ones (Fig. 4a–c). Therefore, we have reason to believe that the down regulation of photosynthetic performance (decrease in both the maximal oxygen evolution rate and chlorophyll fluoresces parameters under stressful irradiance) of the low-Ci-acclimated *A. platensis* D-0083 (Figs. 3 and 4), at least partially, is due to the fact that dissipated energy during CCM operation was negligible relative to the received stress irradiance (1,500 μmol photons m^{-2} s^{-1}). The damage to PSII and the blocking of its repair induced by ROS produced under Ci limiting condition and stressful irradiance (Nishiyama et al. 2005; Murata et al. 2007; Ma and Gao 2010; Rastogi et al. 2010), may be the principal cause of decreased photosynthetic performance.

In conclusion, both the smaller-sized filaments and decreased photosynthetic performance of *A. platensis* D-0083 induced by Ci limitation revealed the reason why *Arthrospira* species have been isolated mainly from alkaline, brackish, and saline waters in tropical and semitropical regions with an unlimited supply of CO_2 (Sili et al. 2012). Furthermore, the results also indicated that *Arthrospira* species could acclimate to Ci-deficient habitats by enhancing its CCM and decreasing its filament size. This has an ecological significance in that the geographical distribution of *Arthrospira* species will expand in the future due to global warming caused by accumulation of anthropogenic CO_2 in the atmosphere.

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