Biochemical and Biophysical Research Communications 433 (2013) 84–89

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Salinity impacts photosynthetic pigmentation and cellular morphology changes by distinct mechanisms in *Fremyella diplosiphon*

Shailendra P. Singh [a], Beronda L. Montgomery [a,b,*]

[a] Department of Energy—Plant Research Laboratory, Michigan State University, Plant Biology Laboratories, 612 Wilson Road, Room 106, East Lansing, MI 48824-1312, United States
[b] Department of Biochemistry and Molecular Biology, Michigan State University, 603 Wilson Road, Room 212, East Lansing, MI 48824, United States

ARTICLE INFO

Article history:
Received 31 January 2013
Available online 27 February 2013

Keywords:
Cellular morphology
Cyanobacteria
Oxidative stress
Osmotic stress
Photosynthetic pigments
Salinity

ABSTRACT

Fremyella diplosiphon is a freshwater cyanobacterium that exhibits complementary chromatic adaptation (CCA), which allows the organism to alter its pigmentation and cellular morphology to maximally harvest available green light (GL) and red light (RL) at different depth levels in its aquatic ecosystem. We tested the effect of salinity on CCA-associated pigment and morphological changes in *F. diplosiphon*. Sodium chloride (NaCl) salt at a concentration of 200 mM was found to maximally inhibit growth, chlorophyll levels, and accumulation of phycoerythrin (PE) and phycocyanin (PC) under GL and RL, respectively. NaCl also affected cellular morphology resulting in a larger cell size under both light conditions. Cell length decreased while width increased under GL in the presence of salt, and both cell length and width were increased under RL with salt. The addition of osmoprotectant glycine betaine (GB) to the growth medium in the presence of salt resulted in a reversion of the morphology to that of cells growing in the absence of salt, whereas GB treatment in the presence of salt did not have a major effect on growth or on PE and PC biosynthesis or accumulation. Thus, salt affects cellular morphology due to osmotic stress, while pigmentation is likely affected by ionic toxicity. Understanding the distinct mechanisms of salt-mediated changes on pigmentation and morphology may increase the suitability of strains such as *F. diplosiphon*, which harbor pigments that allow growth in low light and shaded environments, for adaptation as energy strains.

1. Introduction

Cyanobacteria are gram-negative prokaryotes performing plant-like photosynthesis, which are found in all ecological niches of terrestrial and aquatic ecosystems. Cyanobacteria have an inherent ability to utilize the visible region of the solar spectrum (photosynthetically active radiation or PAR, 400–700 nm) and water molecules to perform photosynthesis to produce carbohydrate and the byproduct oxygen [1]. Biomass produced by cyanobacteria supports the food web in both aquatic and terrestrial ecosystems. In aquatic environments, the quality and quantity of visible light changes significantly at different depth levels of the water column, which can limit the ecologically important process of photosynthesis [2]. However, many cyanobacteria have evolved an ability to sense and adapt to changes in light quality and quantity [3–5]. Cyanobacteria biosynthesize accessory photosynthetic pigments in addition to chlorophyll and carotenoids that can harvest available light wavelengths at different depth levels to sustain photosynthesis in an aquatic ecosystem [6]. These additional ecologically important photosynthetic pigments, called phycobiliproteins (PBPs), are assembled in a macromolecular structure called the phycobilisome (PBS), which transfers absorbed light energy to the chlorophyll-containing photosynthetic reaction center within thylakoid membranes to fuel photosynthesis [7]. PBPs are water-soluble proteins which are stacked together by mostly non-pigmented linker proteins to give the three dimensional structure of PBSs [8,9]. There are three major classes of PBPs: red-light (RL) absorbing allophycocyanin (AP; $\lambda_{max} \sim 650$ nm), RL-absorbing phycocyanin (PC; $\lambda_{max} \sim 620$ nm), and green-light (GL) absorbing phycoerythrin (PE; $\lambda_{max} \sim 565$ nm). AP makes up the core of PBSs, whereas PC and PE make up the outward-radiating rods of PBSs [10]. The ratio of PE and PC and the length of rods in PBSs depend on the ambient light quality and quantity, respectively [10].

Several cyanobacteria have the ability to alter the composition of the outer rods of the PBSs in response to available light quality, i.e., under green-enriched environments, the outer rod is composed of PE; however, PC constitutes the outer part of the rods under red-enriched environments [10,11]. This ecologically important

Abbreviations: AP, allophycocyanin; CCA, complementary chromatic adaptation; GB, glycine betaine; GL, green light; PBP, phycobiliprotein; PBS, phycobilisome; PC, phycocyanin; PE, phycoerythrin; RL, red light.

* Corresponding author at: Michigan State University, MSU-DOE Plant Research Laboratory, Plant Biology Laboratories, 612 Wilson Road, Room 106, East Lansing, MI 48824-1312, USA. Fax: +1 517 353 9168.
E-mail address: montg133@msu.edu (B.L. Montgomery).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.060

phenomenon which allows cyanobacteria to fine tune photosynthesis by altering pigment composition of the rods of PBSs in response to ambient light quality is called complementary chromatic adaptation (CCA) [10,12]. CCA has been studied in detail in the freshwater cyanobacterium *Fremyella diplosiphon* (also called *Tolypothrix or Calothrix* sp. PCC 7601), which can alter both PE and PC under GL and RL [3]. In addition to pigment changes, cellular and filament morphologies of *F. diplosiphon* also change during CCA. Filaments are long, and cells are rectangular in shape under GL, whereas short filaments having spherical cells are characteristic of RL-grown cultures [13,14].

Nutrient availability and light quality have been shown to affect CCA by regulating the composition and abundance of PBS [15]. There is no information available regarding the effect of salinity on CCA, although salinization and its impacts on freshwater and other environments is increasing due to environmental changes and human impact due to practices such as agriculture and road salting [16,17]. Furthermore, there is a great interest in using cyanobacteria or algae for biofuels production due to the ability of these organisms to grown in non-arable or brackish environments [18]. Cyanobacteria such as *F. diplosiphon*, which produce PE that allows growth in low light and shaded environments, have great potential for development as production-scale biofuel strains [19]. Salt has been shown to have detrimental effects on growth in cyanobacterial systems [20–24]. Thus, understanding the specific impacts of salt on PE-producing strains such as *F. diplosiphon* may increase their suitability for adaptation as biofuel strains. In this study, we investigated the effect of salt stress on CCA-associated pigment accumulation and morphological alterations in *F. diplosiphon*, and report that salinity alters cellular morphology and lowers photosynthetic pigment levels, including a downregulation of chlorophyll accumulation and of PC and PE in the presence of salt.

2. Materials and methods

2.1. Experimental organism and growth condition

F. diplosiphon wild-type pigmentation strain SF33 [25] was used in this study. *F. diplosiphon* was grown in autoclaved BG-11 medium (Fluka, Buchs, Switzerland) containing 10 mM HEPES at pH 8.0 under continuous white fluorescent light at an intensity of ~15 μmol m^{-2} s^{-1}. The exponentially growing cultures, which were diluted to an initial optical density at 750 nm (OD750) of 0.2, were transferred to either GL or RL growth chambers with continuous shaking at 175 rpm at 28 °C. GL and RL sources were those reported earlier [13]. The intensity of GL and RL in growth chamber was on average ~15 μmol m^{-2} s^{-1} during all experiments.

2.2. Salt, ascorbic acid and glycine betaine treatment

An autoclaved stock solution of 5 M sodium chloride salt (NaCl) was prepared, and added to cultures at a final concentration of 50, 100 or 200 mM (equivalent to an osmotic concentration of about 100, 200 or 400 mOsmol L^{-1}). Both GL- and RL-grown cultures without added salt served as a control. Similarly, a filter sterilized 1 M stock solution of glycine betaine (GB) in water was added to the cultures at 0.5, 1 or 2 mM final concentration, and filter sterilized 1 M ascorbic acid (AA) was added at 3 mM final concentration when indicated.

2.3. Growth measurements

The growth of the cyanobacterium under RL and GL was estimated by scattering of light or optical density at 750 nm (OD750) measured using a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA).

2.4. Pigment extraction and quantification

The concentration of chlorophyll *a* (chl*a*) was estimated from 1 ml of culture using the method of Tandeau de Marsac and Houmard [26] as previously detailed [27]. PBPs were extracted from 1 ml of cell culture using the method detailed by Kahn et al. [28] with modifications previously detailed [27], except that the extraction was done for 1 h. PBP levels were calculated using equations from Tandeau de Marsac and Houmard [26] and reported relative to chl*a* as described [13].

2.5. Cellular morphology analysis

The cellular morphology of *F. diplosiphon* was analyzed using images acquired with an inverted Axiovert 200 Zeiss LSM 510 Meta confocal laser scanning microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA) using differential interference contrast (DIC) optics and fluorescence excitation and emission filters as described previously [13,29]. Slides of live, immobilized *F. diplosiphon* cells were prepared according to the method of Bordowitz and Montgomery (2010). The length and width of the cells were measured using LSM FCS Zeiss 510 Meta AIM imaging software provided by the manufacturer.

2.6. Statistical analysis

All experiments were performed in three independent biological replicates and repeated twice. The length and width measurements of cells were conducted on 50–100 cells. All data were analysed by one-way analysis of variance using OpenStat statistical software (version 10.01.08; W.G. Miller [http://www.Statprograms4U.com/OpenStatMain.html]). Once a significant difference was detected post hoc multiple comparisons were made by using the Tukey test. The level of significance was set at 0.05 for all tests.

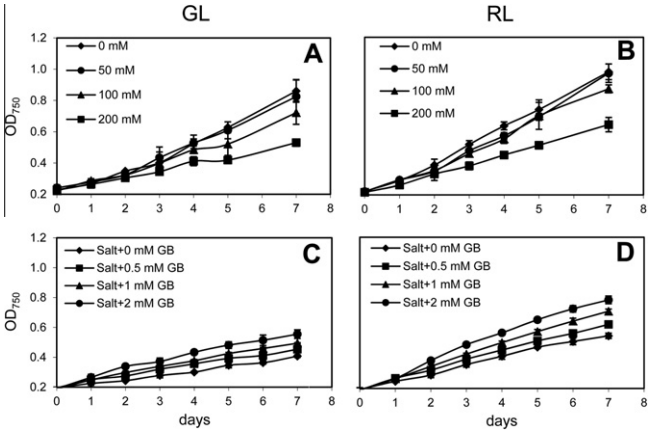

Fig. 1. Measurement of the impact of salt on growth in *Fremyella diplosiphon*. Growth of *F. diplosiphon* over time under (A, C) green light (GL) and (B, D) red light (RL). (A, B) Cells grown in the presence of 0, 50, 100 or 200 mM final concentration of sodium chloride salt or (C, D) cells grown in the presence of 200 mM sodium chloride salt and different concentrations of glycine betaine (GB; 0, 0.5, 1 or 2 mM final concentration). Cell growth was measured as scattering of light at 750 nm (optical density or OD) every 24 h or daily. Average OD_{750} (±SD, $n = 3$) is shown for each time point.

3. Results and discussion

3.1. Salt impairs growth and photosynthetic pigment accumulation in *F. diplosiphon*

CCA is an ecologically important phenomenon that allows cyanobacteria to adjust cellular photosynthetic machinery in accordance to prevailing light conditions in their habitats [6]. The process of CCA has been shown to be susceptible to nitrogen, sulphur or phosphorus deprivation [15] or iron limitation [30]. Furthermore, correlations between CCA-associated regulation of pigmentation and morphology and other environmental parameters, including light intensity [31] and temperature [32], have been noted. Here, we studied the effect of salinity on pigmentation and morphology associated with CCA in *F. diplosiphon*. Salt at 100 mM or 200 mM under GL or 200 mM under RL inhibited *F. diplosiphon* growth (Fig. 1A and B).

To assess the impact of NaCl on photosynthetic pigmentation, we measured chlorophyll and PBP concentrations of cells grown in the presence and absence of salt over time. The chl*a* concentration was significantly lower in the presence of 200 mM salt under both light conditions (Fig. 2A and D). Extraction and quantification of PBPs from different salt-treated samples indicated that the presence of 200 mM NaCl in the growth medium has a maximum inhibitory effect on pigments under both GL and RL (Fig. 2B, C, E and F). Thus, growth, chl*a* and PBP levels were severely reduced in the presence of 200 mM salt under both GL and RL conditions, which indicates that salt impacts pigment accumulation but not the process of CCA specifically. Reductions in growth, chl*a* levels and PBP accumulation in the presence of salt in this study are in

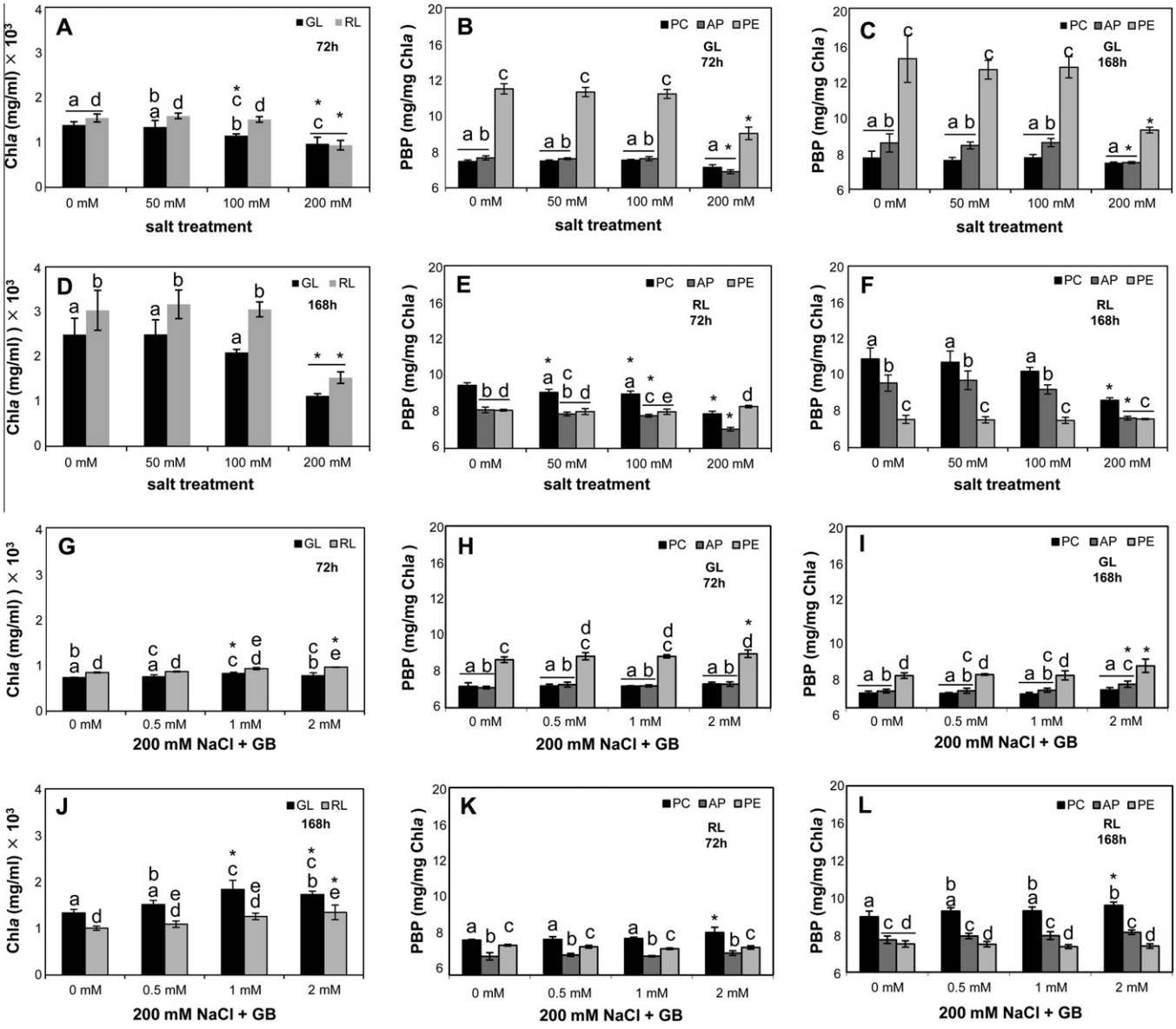

Fig. 2. Impact of salinity in the absence or presence of glycine betaine on photosynthetic pigment levels in response to sodium chloride salt over time. (A–F) Effect of sodium chloride (NaCl) salt (at 0, 50, 100 or 200 mM final concentration) or (G–L) 200 mM NaCl and glycine betaine (GB; at 0, 0.5, 1, or 2 mM) on chlorophyll *a* (chl*a*) and phycobiliprotein (PBP), i.e., phycoerythrin (PE), allophycocyanin (AP), and phycocyanin (PC), content in *Fremyella diplosiphon* after 72 and 168 h of growth under green light (GL) or red light (RL). Chl*a* (A, D, G, J) and PBP (B, C, E, F, H, I, K, L) levels under GL or RL as indicated. Asterisks indicate a significant difference ($p < 0.05$) from 0 mM salt-treated sample (A–F) or 0 mM GB treated sample (G–L). Identical letters over bars represent homogenous mean groups ($p > 0.05$), whereas lines over bars indicate no significant difference ($p > 0.05$) between GL and RL (in case of chl*a*) and PBPs at each salt concentration (A–F) or at each GB concentration (G–L). Average pigment levels (±SD, $n = 3$) are shown.

accordance with earlier studies with cyanobacteria [20,23,24]. However, here we also report that salinity inhibits PE in addition to PC, the latter of which was reported earlier for PC-predominant cyanobacterial strains. As 200 mM salt concentration had a maximum impact on all of the physiological parameters we tested, this concentration was used in further experiments.

3.2. Oxidative stress is not involved in the salt-mediated alteration of cellular morphology in F. diplosiphon

The cellular morphology of *F. diplosiphon* differs between GL and RL growth conditions, in addition to pigment changes [13,14]. The presence of 200 mM salt in BG-11 growth medium was found to alter cellular morphology under both GL and RL (Fig. 3). Cells were bigger than cells of the control sample where salt was omitted from growth medium (Fig. 3A). Salt treatment under RL also resulted in vacuolation, indicating a higher level of stress in the presence of salt under RL (Fig. 3A).

Recently, we showed that light-dependent alterations in cellular morphology during CCA in *F. diplosiphon* are associated with an increased level of oxidative stress under RL compared to GL [33]. As salt stress also causes oxidative stress [21,24], we tested the effect of adding the antioxidant ascorbic acid (AA) in the presence of salt on cellular morphology. AA was not able to revert the cellular morphology in the presence of salt to that of cells grown in the absence of salt under either light condition (Fig. 3), suggesting that oxidative stress is not a major factor involved in the salt-mediated alteration of cell shape.

3.3. Osmotic stress mediates salt-associated morphology changes, but not photosynthetic pigment defects

High salt concentration in the growth medium is known to lower the water potential in addition to the cellular toxicity caused by high ion concentrations [34]. Thus, salt stress also induces osmotic

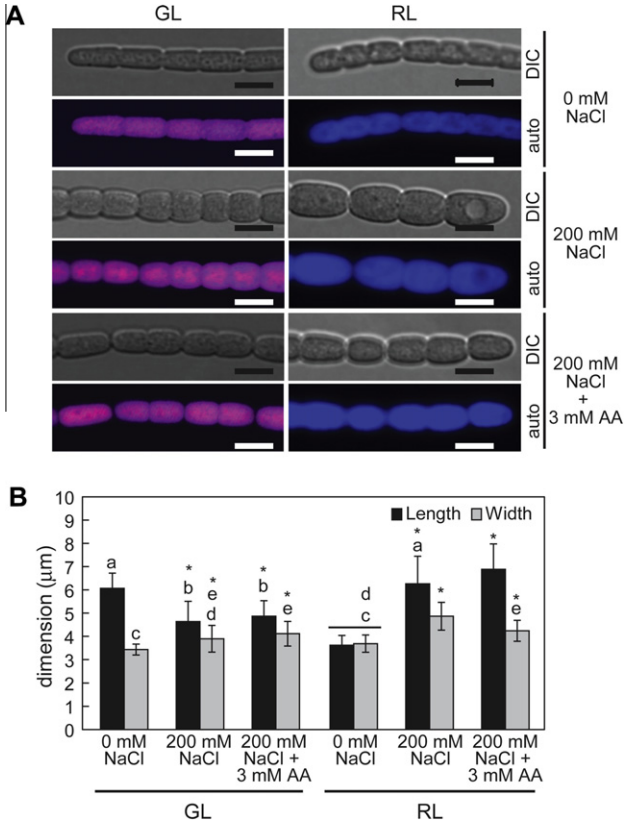

Fig. 3. Confocal laser scanning microscopy analyses of the cellular morphology of *Fremyella diplosiphon* grown with or without sodium chloride salt and/or ascorbic acid under GL or RL. (A) Representative optical slices from a Z-series of differential interference contrast (DIC) images and corresponding maximum intensity projection phycobiliprotein autofluorescence (auto) images indicating the effect of sodium chloride salt (NaCl; 0 or 200 mM final concentration) or ascorbic acid (AA; 3 mM final concentration) in the presence of 200 mM NaCl on cellular morphology in *F. diplosiphon* grown under GL or RL for 72 h. Images were obtained using a 40× oil-immersion objective with 2× zoom setting. Bars, 5 µm. (B) Cell length and width measurements of *F. diplosiphon* cells grown under GL or RL with or without 200 mM NaCl or 200 mM NaCl + 3 mM AA. Asterisks indicate a significant difference ($p < 0.05$) from 0 mM NaCl treatment under GL and RL, while identical letters over bars represent a homogeneous mean group ($p > 0.05$). Line over bars indicates no significant difference ($p > 0.05$) between length and width of cells. Average cell length or width (±SD) are shown ($n = 50–100$ cells).

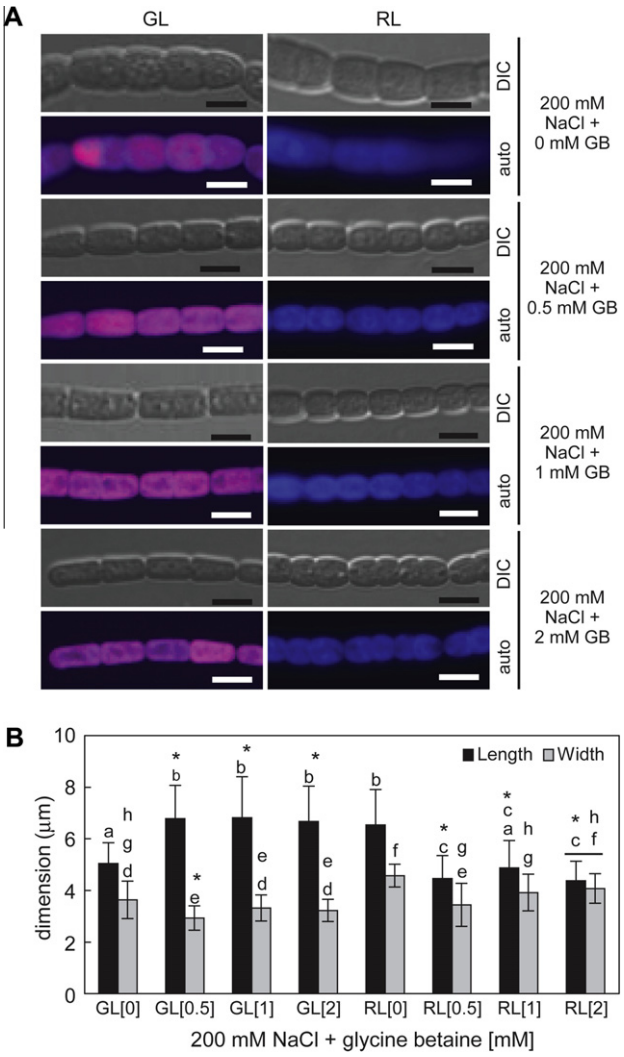

Fig. 4. Confocal laser scanning microscopy analyses of the cellular morphology of *Fremyella diplosiphon* grown with sodium chloride salt and/or glycine betaine under GL or RL. (A) Representative optical slices from a Z-series of differential interference contrast (DIC) images and corresponding maximum intensity projection phycobiliprotein autofluorescence (auto) images indicating the effect of glycine betaine (GB; 0, 0.5, 1 or 2 mM) in the presence of 200 mM sodium chloride (NaCl) salt on cellular morphology in *F. diplosiphon* grown under GL or RL for 72 h. Images were obtained using a 40× oil-immersion objective with 2× zoom setting. Bars, 5 µm. (B) Cell length and width measurements of *F. diplosiphon* cells grown as described in panel (A). Asterisks indicate a significant difference ($p < 0.05$) from 0 mM GB treated sample under GL and RL, while identical letters over bars represent a homogeneous mean group ($p > 0.05$). Line over bars indicates no significant difference ($p > 0.05$) between length and width of cells. Average cell length or width (±SD) is shown ($n = 50–100$ cells).

stress, and several organisms including cyanobacteria accumulate or biosynthesize compatible solutes or osmoprotectants to maintain lower water potential inside the cell under these conditions [35,36]. Sucrose, trehalose, glucosylglycerol and GB are commonly accumulated osmoprotectants; however, GB has been shown to be the major osmoprotectant accumulated by bacterial systems in response to external salinity [34,37–40]. Notably, a transcript encoding a protein with similarity to a component of a glycine-betaine transport system was identified in RNA-seq analyses of *F. diplosiphon* (data not shown), indicating an ability for the strain to take up GB. We added different concentrations of GB (0, 0.5, 1 or 2 mM) to the growth medium to determine whether it impacts normal growth, pigmentation and cellular morphology of *F. diplosiphon* in the presence of 200 mM salt. The addition of different concentrations of GB improved the growth of *F. diplosiphon* under both light conditions in the presence of NaCl (Fig. 1C and D). However, none of the GB concentrations used was able to revert the growth of *F. diplosiphon* similar to the condition where salt was absent (Fig. 1). The addition of GB, mainly at 2 mM, in the presence of 200 mM salt significantly increases chl*a* and some PBPs under GL and RL (Fig. 2G–L); however, pigment concentrations never reached the values comparable to those obtained in absence of salt.

The effect of adding different concentrations of GB on cellular morphology in the presence of salt under GL and RL was also tested. All concentrations of GB added to the growth medium successfully reverted cells to GL- and RL-specific cellular morphology in *F. diplosiphon* with a maximum effect seen in the presence of 0.5 mM GB under GL and 2 mM GB under RL (Fig. 4).

The addition of GB to the growth medium in our study resulted in the reversion of a salt-induced larger cell morphology to a normal sized GL- and RL-specific rectangular and spherical cell morphology, respectively. However, GB addition did not show a major effect on growth and pigmentation defects induced by salt in *F. diplosiphon*. Taken together, these results suggest that morphological abnormalities induced by salt are most likely caused by salt-induced osmotic stress as the addition of osmoprotectant GB to growth medium could prevent salt-induced cellular morphology defects, whereas salt effects on photosynthetic pigments are likely caused by an irreversible damaging effect of high intracellular ions, the latter of which was previously postulated [41,42].

Acknowledgments

This research was supported by the U.S. Department of Energy (Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, Office of Science, Grant No. DE–FG02–91ER20021 to B.L.M.). Support for Shailendra P. Singh was from a CAREER award from the National Science Foundation (Grant No. MCB–0643516 to B.L.M.). We thank Britney Robinson, for whom funding was from the National Science Foundation (Grant no. MCB–0919100 to B.L.M.) and Haley Miller, for whom funding was provided by the Professorial Assistantship Program of the Honors College at Michigan State University, for technical assistance.

References

[1] R.Y. Stanier, G. Cohen-Bazire, Phototrophic prokaryotes: the cyanobacteria, Annu. Rev. Microbiol. 31 (1977) 225–274.
[2] C. Postius, O. Neuschaefer-Rube, V. Haid, P. Böger, N2-fixation and complementary chromatic adaptation in non-heterocystous cyanobacteria from Lake Constance, FEMS Microbiol. Ecol. 37 (2001) 117–125.
[3] A. Gutu, D.M. Kehoe, Emerging perspectives on the mechanisms, regulation, and distribution of light color acclimation in cyanobacteria, Mol. Plant 5 (2012) 1–13.
[4] B. Pattanaik, M.J. Whitaker, B.L. Montgomery, Light quantity affects the regulation of cell shape in *Fremyella diplosiphon*, Front. Microbiol. 3 (2012) 170.
[5] B.L. Montgomery, Shedding new light on the regulation of complementary chromatic adaptation, Cent. Eur. J. Biol. 3 (2008) 351–358.
[6] D. Campbell, Complementary chromatic adaptation alters photosynthetic strategies in the cyanobacterium *Calothrix*, Microbiology 142 (1996) 1255–1263.
[7] L. Bogorad, Phycobiliproteins and complementary chromatic adaptation, Annu. Rev. Plant Physiol. 26 (1975) 369–401.
[8] E. Gantt, Phycobilisomes, Annu. Rev. Plant Physiol. 32 (1981) 327–347.
[9] A.N. Glazer, Phycobilisomes: structure and dynamics, Annu. Rev. Microbiol. 36 (1982) 173–198.
[10] D.M. Kehoe, A. Gutu, Responding to color: the regulation of complementary chromatic adaptation, Annu. Rev. Plant Biol. 57 (2006) 127–150.
[11] D.M. Kehoe, Chromatic adaptation and the evolution of light color sensing in cyanobacteria, Proc. Natl. Acad. Sci. USA 107 (2010) 9029–9030.
[12] N. Tandeau de Marsac, Occurrence and nature of chromatic adaptation in cyanobacteria, J. Bacteriol. 130 (1977) 82–91.
[13] J.R. Bordowitz, B.L. Montgomery, Photoregulation of cellular morphology during complementary chromatic adaptation requires sensor-kinase-class protein RcaE in *Fremyella diplosiphon*, J. Bacteriol. 190 (2008) 4069–4074.
[14] A. Bennett, L. Bogorad, Complementary chromatic adaptation in a filamentous blue-green alga, J. Cell. Biol. 58 (1973) 419–435.
[15] J.L. Collier, A.R. Grossman, A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria, EMBO J. 13 (1994) 1039–1047.
[16] S.S. Kaushal, P.M. Groffman, G.E. Likens, K.T. Belt, W.P. Stack, V.R. Kelly, L.E. Band, G.T. Fisher, Increased salinization of fresh water in the northeastern United States, Proc. Natl. Acad. Sci. USA 102 (2005) 13517–13520.
[17] W.D. Williams, Salinization of rivers and streams: an important environmental hazard, Ambio 16 (1987) 180–185.
[18] A. Parmar, N.K. Singh, A. Pandey, E. Gnansounou, D. Madamwar, Cyanobacteria and microalgae: a positive prospect for biofuels, Bioresour. Technol. 102 (2011) 10163–10172.
[19] Z. Dubinsky, N. Stambler, Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications, Aquat. Microb. Ecol. 56 (2009) 163–176.
[20] P. Bhadauriya, R. Gupta, S. Singh, P.S. Bisen, Physiological and biochemical alterations in a diazotrophic cyanobacterium *Anabaena cylindrica* under NaCl stress, Curr. Microbiol. 55 (2007) 334–338.
[21] A.K. Srivastava, P. Bhargava, R. Thapar, L.C. Rai, Salinity-induced physiological and proteomic changes in *Anabaena doliolum*, Environ. Exp. Bot. 64 (2008) 49–57.
[22] I.M. Rafiqul, A. Hassan, G. Sulebele, C.A. Orosco, P. Roustaian, K.C.A. Jalal, Salt stress culture of blue green algae *Spirulina fusiformis*, Pak. J. Biol. Sci. 6 (2003) 648–650.
[23] K. Marin, Y. Kanesaki, D.A. Los, N. Murata, I. Suzuki, M. Hagemann, Gene expression profiling reflects physiological processes in salt acclimation of *Synechocystis* sp. strain PCC 6803, Plant Physiol. 136 (2004) 3290–3300.
[24] A.K. Srivastava, Assessment of salinity-induced antioxidative defense system of diazotrophic cyanobacterium *Nostoc muscorum*, J. Microbiol. Biotechnol. 20 (2010) 1506–1512.
[25] J.G. Cobley, E. Zerweck, R. Reyes, A. Mody, J.R. Seludo-Unson, H. Jaeger, S. Weerasuriya, S. Navankasattusas, Construction of shuttle plasmids which can be efficiently mobilized from *Escherichia coli* into the chromatically adapting cyanobacterium, *Fremyella diplosiphon*, Plasmid 30 (1993) 90–105.
[26] N. Tandeau de Marsac, J. Houmard, Complementary chromatic adaptation: physiological conditions and action spectra, Methods Enzymol. 167 (1988) 318–328.
[27] S.P. Singh, B.L. Montgomery, Temporal responses of wild-type pigmentation and RcaE-deficient strains of *Fremyella diplosiphon* during light transitions, Commun. Integr. Biol. 4 (2011) 503–510.
[28] K. Kahn, D. Mazel, J. Houmard, N. Tandeau de Marsac, M.R. Schaefer, A role for *cpeYZ* in cyanobacterial phycoerythrin biosynthesis, J. Bacteriol. 179 (1997) 998–1006.
[29] J.R. Bordowitz, B.L. Montgomery, Exploiting the autofluorescent properties of photosynthetic pigments for analysis of pigmentation and morphology in live *Fremyella diplosiphon* cells, Sensors 10 (2010) 6969–6979.
[30] B. Pattanaik, B.L. Montgomery, FdTonB is involved in the photoregulation of cellular morphology during complementary chromatic adaptation *in Fremyella diplosiphon*, Microbiology 156 (2012) 731–741.
[31] B. Pattanaik, M.J. Whitaker, B.L. Montgomery, Light quantity affects the regulation of cell shape in *Fremyella diplosiphon*, Front. Microbiol. 3 (2012) 170.
[32] N.K. Singh, A. Parmar, R.R. Sonani, D. Madamwar, Isolation, identification and characterization of novel thermotolerant *Oscillatoria* sp. N9DM: change in pigmentation profile in response to temperature, Process Biochem. 47 (2012) 2472–2479.
[33] S.P. Singh, B.L. Montgomery, Reactive oxygen species are involved in the morphology-determining mechanism of *Fremyella diplosiphon* cells during complementary chromatic adaptation, Microbiology 158 (2012) 2235–2245.
[34] M. Hagemann, Molecular biology of cyanobacterial salt acclimation, FEMS Microbiol. Rev. 35 (2011) 87–123.
[35] E. Bremer, R. Krämer, Coping with Osmotic Challenges: Osmoregulation Through Accumulation and Release of Compatible Solutes in Bacteria, in: R. Hengge-Aronis (Ed.), Bacterial Stress Responses, ASM Press, Washington, DC, 2000, pp. 79–96.
[36] A.D. Brown, Microbial water stress, Bacteriol. Rev. 40 (1976) 803–846.
[37] F.A.A. Mohammad, R.H. Reed, W.D.P. Stewart, The halophilic cyanobacterium *Synechocystis* DUN52 and its osmotic responses, FEMS Microbiol. Lett. 16 (1983) 287–290.

[38] R.H. Reed, J.A. Chudek, R. Foster, W.D.P. Stewart, Osmotic adjustment in cyanobacteria from hypersaline environments, Arch. Microbiol. 138 (1984) 333–337.
[39] U. Vijaranakul, M.J. Nadakavukaren, B.L. de Jonge, B.J. Wilkinson, R.K. Jayaswal, Increased cell size and shortened peptidoglycan interpeptide bridge of NaCl-stressed *Staphylococcus aureus* and their reversal by glycine betaine, J. Bacteriol. 177 (1995) 5116–5121.
[40] S. Klähn, M. Hagemann, Compatible solute biosynthesis in cyanobacteria, Environ. Microbiol. 13 (2011) 551–562.
[41] A.A. Zorina, K.S. Mironov, N.S. Stepanchenko, M.A. Sinetova, N.V. Koroban, V.V. Zinchenko, E.V. Kupriyanova, S.I. Allakhverdiev, D.A. Los, Regulation systems for stress responses in cyanobacteria, Russ. J. Plant Physiol. 58 (2011) 749–767.
[42] S.I. Allakhverdiev, Y. Nishiyama, S. Miyairi, H. Yamamoto, N. Inagaki, Y. Kanesaki, N. Murata, Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in *Synechocystis*, Plant Physiol. 130 (2002) 1443–1453.