Assessment of salinity-induced photorespiratory glycolate metabolism in *Anabaena* sp. PCC 7120

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This paper reports an investigation of salinity-induced glycolate metabolism in the cyanobacterium *Anabaena* sp. PCC 7120 (hereafter *Anabaena* PCC 7120). Quantitative analysis of transcripts for the photosynthesis-associated genes encoding ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), phosphoribulokinase and transketolase, as well as those involved in glycolate metabolism (phosphoglycolate phosphatase, glycolate oxidase, alanine-glyoxylate aminotransferase and serine hydroxymethyltransferase) was performed. The expression of all investigated photosynthesis-associated genes except Rubisco was downregulated after 24 h NaCl treatment. However, under the same conditions, the transcripts encoding enzymes involved in glycolate metabolism were overexpressed. This was further confirmed by the quantitative analysis of the intermediates involved in glycolate metabolism. The intracellular levels of organic acids (glyceric, glycolic and glyoxylic acids) and amino acids (glycine and serine) were elevated in salt-treated cells as compared to those in the control cells. Transcriptional inhibition of photosynthesis-associated genes, and upregulation of genes and enhanced synthesis of intermediates associated with glycolate metabolism, indicate the occurrence of this photorespiratory metabolic pathway in *Anabaena* PCC 7120 under salt stress.

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INTRODUCTION

The evolution of cyanobacteria over 2.7 billion years significantly contributed to a major transition in the history of life on Earth (Buick, 1992). Cyanobacterial photosynthesis resulted in a tremendous increase in the oxygen concentration of the atmosphere and the emergence of oxygen-dependent life. Due to their ability to adapt frequently to changing environments, cyanobacteria currently occupy most ecological niches on our planet.

An important habitat for cyanobacteria is rice fields, where they significantly contribute to the availability of nitrogen for the crop (Singh, 1961). The rice agro-ecosystem, including cyanobacteria, is subjected to several abiotic stresses, including salt stress caused by anthropogenic activities (Srivastava *et al.*, 2009). It has been reported that high salinity not only inhibits photosynthesis and hence the carbon pool of freshwater cyanobacteria (Srivastava *et al.*, 2008) but also decreases the amount of fixed carbon available for the synthesis of compatible solutes (Ferjani

et al., 2003). Responses of freshwater cyanobacteria to salt stress have been studied by several research groups at the genomic, transcriptomic and proteomic levels (Fulda *et al.*, 2006; Huang *et al.*, 2006; Kanesaki *et al.*, 2002; Marin *et al.*, 2004; Srivastava *et al.*, 2008). These studies reported salt stress responses of several cyanobacterial species in relation to specific genes encoding stress proteins, photosynthetic proteins and proteins involved in the synthesis of compatible solutes. However, salinity-induced changes in metabolism related to glycolate metabolism (photorespiration) have been little studied in cyanobacteria.

Photorespiration evolved as an essential disadvantage of oxygenic life, as this pathway utilizes about a quarter of the carbon fixed from photosynthesis (Eckardt, 2005). It widely depends on the activity of type I ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), commonly found in cyanobacteria, algae and higher plants, which has both carboxylase and oxygenase activity. However, cyanobacterial type I Rubisco has a low affinity for carbon dioxide and therefore cyanobacteria have evolved a carbon-concentrating mechanism. By placing this enzyme in carboxysomes, an increase in CO₂ concentration (about 1000-fold) around the active site of the protein occurs (Badger & Price, 2003).

Abbreviations: DEPC, diethylpyrocarbonate; Rubisco, ribulose-1,5-bisphosphate carboxylase oxygenase.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are GU560731–GU560737.

The existence of photorespiration in cyanobacteria has been questioned (Colman & Norman, 1997). Several reports have dealt with discrete biochemical reactions related to photorespiration (Bergman *et al.*, 1984; Eisenhut *et al.*, 2006, 2008; Norman & Colman, 1988; Renström & Bergman, 1989); most of these studies suggested the presence of an incomplete cyanobacterial photorespiratory glycolate metabolism. However, Bauwe *et al.* (2010) and Eisenhut *et al.* (2006) reported that *Synechocystis* PCC 6803 exhibits glycolate metabolism as well as possessing a glycerate pathway. More recently, Srivastava *et al.* (2008) proposed glycolate metabolism in *Anabaena doliolum* exposed to salt stress. However, the physiological and proteomic characterization of salinity-induced glycolate metabolism in *A. doliolum* using two-dimensional protein gel electrophoresis was interpreted against several methodological variables, including sample preparation, isoelectric focusing strip length, voltage during first dimension and staining procedure (Bhargava *et al.*, 2008; Görg *et al.*, 1999; López, 2007). Thus the present study was designed to validate and build on earlier reports of glycolate metabolism by using transcription analysis of the various enzymes proposed to be involved. Evidence was sought for changes in the expression of these different enzymes and the accumulation of intermediates of the glycolate pathway. *Anabaena* PCC 7120 was selected as the test organism due to the availability of its published and characterized genome.

To achieve the above-mentioned objectives, quantitative reverse transcription PCR (qRT-PCR) was performed for four genes involved in photorespiration, namely those encoding phosphoglycolate phosphatase (*alr4944*), glycolate oxidase (*all0170*), alanine-glyoxylate aminotransferase (*alr1004*) and serine hydroxymethyltransferase (*alr4806*), as well as three genes that play a role in carbon fixation: those encoding the Rubisco small subunit (*alr1526*), phosphoribulokinase (*alr2350*) and transketolase (*alr3344*). In addition, intracellular concentrations of metabolic intermediates (glycine, serine, glyceric, glycolic and glyoxylic acids) were determined.

METHODS

Organism and growth condition. Cultures of *Anabaena* PCC 7120 were maintained in Jaworski's medium (Thompson *et al.*, 1988) buffered with Tris/HCl (pH 7.5). The culture was grown at 24 ± 2 °C under fluorescent tubes emitting $72 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR (photosynthetically active radiation) with a photoperiod of 14:10 h (light:dark). The cultures were continuously shaken. The experiments were conducted in three sets: (i) cells grown in normal culture conditions, (ii) cells exposed to 150 mM NaCl for 1 h and (iii) cells exposed to 150 mM NaCl for 24 h. This concentration of NaCl was selected since it is the LC_{50} dose obtained by the colony count method (Rai & Raizada, 1985). The selected dose and duration of salt treatment has been found to alter photosynthesis, antioxidant defence and protein profiles in cyanobacteria (Srivastava *et al.*, 2005, 2008). All experiments were performed as independent biological triplicates and repeated twice to confirm the findings.

Gene expression studies of glycolate metabolism and bioinformatic analysis. The gene sequences encoding relevant proteins involved in glycolate metabolism were obtained from the genome of *Anabaena* PCC 7120 (<http://genome.kazusa.or.jp/cyanobase/Anabaena>) and used for primer design. Percentage similarity and identity of those genes to other translated sequences were determined using BLAST in conjunction with the National Center for Biotechnology Information (NCBI, NIH). Primers for photosynthesis-associated genes encoding the Rubisco small subunit, phosphoribulokinase and transketolase, and genes encoding enzymes involved in glycolate metabolism (phosphoglycolate phosphatase, glycolate oxidase, alanine-glyoxylate aminotransferase and serine hydroxymethyltransferase) were designed using Primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Details of the primers used in this study are given in Table 1.

DNA isolation, gene amplification and sequencing. Genomic DNA was isolated using the method described by Srivastava *et al.* (2007). PCR was performed in a 20 μl reaction mixture containing 100 ng DNA, PCR buffer, 4.5 mM MgCl_2 , 200 μM dNTPs, 10 pmol of each primer and 0.2 U *Taq* DNA polymerase. Thermal cycling was performed using the following programme: initial denaturation for 5 min at 94 °C followed by 35 cycles each consisting of 30 s denaturation at 94 °C, 30 s annealing at specific temperature depending on primers, 45 s elongation at 72 °C and a final 7 min elongation at 72 °C. PCR products were purified and subjected to automated DNA sequencing using a PRISM BigDye cycle sequencing system with the DNA sequencing reaction containing BigDye, sequencing buffer and 3.2 pmol of either of the primers. Thermal cycling for the sequencing reaction was 96 °C for 3 min, 30 cycles of 96 °C for 10 s and 50 °C for 5 s, followed by 60 °C for 4 min. Sequencing was performed to confirm the amplified gene identity. The sequences were submitted to GenBank under accession numbers GU560731 to GU560737.

RNA isolation and DNase treatment. Total RNA was isolated using the method of Schmidt-Goff & Federspiel (1993). Briefly, 5 ml cyanobacterial culture (OD_{730} 0.5) was centrifuged at 5000 *g* and frozen in liquid nitrogen for 5 min. This was followed by the addition of 0.5 mm zirconium silicate beads, 20 % SDS, 3 % celite and Tris-buffered phenol. The aqueous layer was collected after centrifugation and 1 ml chloroform was added. After centrifugation, freshly prepared precipitation buffer (4 M LiCl, 200 mM Tris, pH 7.5; 10 mM EDTA, pH 8.0) was added followed by incubation overnight at -20 °C. This was followed by centrifugation at 16 000 *g* and addition of 3 M sodium acetate and 95 % ethanol. After precipitation at -80 °C, the pellet was collected by centrifugation and resuspended in DEPC-treated water.

DNase treatment was carried out for 4 h at 37 °C. Twenty nanograms of RNA was treated with 6 U of TURBO DNase (Ambion). This was followed by a TRIzol (Invitrogen) extraction according to the manufacturer's instructions.

Total cDNA synthesis. RNA quantity was estimated using a Nanodrop spectrophotometer (Nanodrop Technologies). To check for DNA contamination in the RNA samples, 16S rDNA primers were used in a PCR (Neilan *et al.*, 1997). An equal amount (250 ng) of RNA isolated from control and treated samples was used for cDNA synthesis. Reverse transcription was performed using the First Strand cDNA synthesis kit (Marligen Biosciences) according to the manufacturer's instructions with slight modifications. Synthesis was done in a 20 μl reaction as follows: one cycle of 22 °C for 5 min, two cycles of 42 °C for 90 min and one cycle of 85 °C for 5 min. This was again followed by cDNA quantification using a Nanodrop spectrophotometer.

qRT-PCR. For quantification of transcripts from selected genes of *Anabaena* PCC 7120 exposed to salt stress, qRT-PCR was performed

Table 1. Primers used for analysis of selected transcripts of *Anabaena* PCC 7120

Enzyme/product	Gene	Primers	Annealing temp. (°C)	Expected product size (bp)	Primer efficiency (%)	Reference
Rubisco small subunit	<i>alr1526</i>	F: ATTTCCAAATAGCAACCAATTATCA R: CAATAAAGTTCGGTAGGTTTCAGAAA	61	140	96.5	Bhargava <i>et al.</i> (2008)
Transketolase	<i>alr3344</i>	F: CGGTTCCCCTAACAAATCTAATACT R: CTTTGTAATCTGCGTAGGTTTGT	61	180	100.0	Bhargava <i>et al.</i> (2008)
Phosphoribulokinase	<i>alr2350</i>	F: TGACTAATGGTAGTTCTGTGTCAGC R: GTAACCTTTGTAGTGTTCGCCAGTT	61	175	95.0	Bhargava <i>et al.</i> (2008)
Glycolate oxidase	<i>all0170</i>	F: ATTAGCCAAATGCAACCCTG R: CAATGGCGTTTCAATGTTTG	61	280	99.5	This study
Serine hydroxymethyltransferase	<i>alr4806</i>	F: TGGATTTGTCTCATGGTGA R: ACTAAACCTGCAATGTGGGC	61	220	100.0	This study
Phosphoglycolate phosphatase	<i>alr4944</i>	F: TGATGGCACGATTGCGGATACC R: CCGAACTCTACCGCCAAGCG	57	70	91.0	This study
Alanine-glyoxylate aminotransferase	<i>alr1004</i>	F: CACAGGCGCACGTCAACCCT R: GGCGAAGCGCCAGGAGAACA	57	150	97.0	This study
RNA polymerase (<i>rpoC1</i>)	<i>alr1595</i>	F: CCTCAGCGAAGATCAATGGT R: CCGTTTTTGCCCTTACTTT	57	160	100.0	Ginn <i>et al.</i> (2010)

using the RotorGene 3000 thermal cycler (Corbett Research). qRT-PCR was performed in a 25 µl volume containing 12.5 µl enzyme-nucleotide-dye (SYBR) mix (Invitrogen), 0.5 µl reference dye (ROX), 10 pmol of each primer and 500 ng cDNA. Negative controls with no cDNA were included. All reactions were done in triplicate. The amplification programme consisted of an initial hold at 60 °C for 5 min and denaturation at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, 61 or 57 °C (Table 1) for 30 s. The *rpoC1* gene was used as a reference for relative quantification of selected transcripts (Ginn *et al.*, 2010). Primer efficiency curves and relative quantification were performed as described by Pfaffl (2001).

Measurement of organic acids. Intracellular glyceric, glycolic and glyoxylic acids were extracted in 80 % ethanol, and the quantities measured by HPLC (Eisenhut *et al.*, 2006) using an Aminex column HPX-87H (300 × 7.8) (Bio-Rad) equipped with a UV detector. The ethanol extract was dried in a Speedvac concentrator (Savant). MilliQ water was added to the dried samples for analysis of organic acids. The organic acids were detected at 210 nm using a UV spectrophotometer. Separations were performed at 50 °C and eluted at 0.6 ml min⁻¹ using 5 mM H₂SO₄. The culture medium was also analysed for possible excretion of organic acids. Standards containing analytical-grade components were used to calibrate the system.

Amino acid analysis. Amino acids were extracted in ethanol using the method described by Eisenhut *et al.* (2006). The ethanol extract was dried in a Speedvac concentrator. MilliQ water was added to the dried samples and sonicated at room temperature. The suspension was centrifuged at 14 000 g and subjected to amino acid analysis using Water AccQTag Ultra chemistry.

Statistical analysis. Results of organic acids and amino acids were statistically analysed using a one-way ANOVA followed by Duncan's new multiple range test (DMRT) using SPSS version 15.0. Three independent variables were considered for each experiment.

RESULTS

Sequence analysis and primer efficiency

In order to understand the regulation of the photorespiration pathway under salt-stress conditions in *Anabaena* PCC 7120, we looked into whole-genome information for this organism and selected candidate genes based on information from previous studies (Eisenhut *et al.*, 2006; Srivastava *et al.*, 2008). The sequences obtained were analysed and compared with the sequences available in GenBank using BLASTN, except for the phosphoglycolate phosphatase gene (*alr4944*), for which BLASTX was performed. Among those genes, that encoding Rubisco (*alr1526*) showed 100 % identity to those of *A. doliolum* LCR1. All other genes showed similarity with the respective genes of *Anabaena* PCC 7120. No significant DNA similarity was observed for the phosphoglycolate phosphatase gene (*alr4944*) due to the short sequence length; however, translated (BLASTX) analysis revealed 86 % similarity with the same enzyme from *Anabaena* PCC 7120. Interestingly, none of these genes

had significant sequence similarity with their counterparts from unicellular cyanobacteria. The efficiency curve for each primer set revealed between 91 and 100 % efficiency (Table 1).

Effect of high salinity on transcription of the selected genes

Data pertaining to salinity-induced alterations in transcripts of selected genes are shown in Table 2. Among the photosynthesis-related genes in *Anabaena* PCC 7120 that were investigated, the Rubisco gene (*alr1526*) from the salt-stressed cells showed 2.6-fold higher expression after 24 h NaCl treatment compared to the control cells. However, the other two genes, encoding phosphoribulokinase (*alr2350*) and transketolase (*alr3344*), showed approximately 10- and 3.3-fold lower expression respectively after 24 h NaCl treatment compared to the control cells. After 1 h NaCl treatment, very similar mRNA expression levels from both salt-treated and control cells were detected.

In contrast to the above results, all genes related to glycolate metabolism, except the gene encoding serine hydroxymethyltransferase (*alr4806*), showed overexpression after 1 h salt treatment. The highest induction (7.5-fold) was observed for the serine hydroxymethyltransferase gene (*alr4806*) transcript after 24 h NaCl treatment relative to the control. The glycolate oxidase (*all0170*) and phosphoglycolate phosphatase (*alr4944*) genes were overexpressed 1.9- and 2.3-fold, respectively, after 24 h NaCl treatment. Alanine-glyoxylate aminotransferase (*alr1004*) was upregulated 1.4- and 6.9-fold after 1 and 24 h NaCl treatment, respectively. Transcription of the *rpoC1* gene (*alr1595*), selected as a housekeeping gene, showed insignificant changes in both control and salt-treated cells at both time points.

Organic acid content

Glyceric, glycolic and glyoxylic acids, which are intermediates involved in glycolate metabolism, were measured to confirm the findings of the transcript analysis described above. Intracellular concentrations of these organic acids increased following NaCl treatment (Fig. 1a). The glyceric acid content of the cells increased by 2.1- and 3.2-fold after 1 and 24 h NaCl treatment, respectively. The glycolic and glyoxylic acid concentrations were 4.2- and 3.9-fold higher, respectively, after 24 h exposure to 150 mM NaCl as compared to control.

Amino acid content

Fig. 1(b) shows the change in concentration of glycine and serine, amino acids which are metabolites of the glycolate pathway. The concentration of both amino acids increased, by 1.9- (glycine) and 2.2-fold (serine), following the 24 h NaCl treatment as compared to the control culture. Interestingly, the free alanine concentration was below detection limits in the control cells but after 1 and 24 h treatment its levels rose to 0.08 and 0.16 $\mu\text{g (mg protein)}^{-1}$, respectively.

DISCUSSION

It is well known that high salinity affects cell growth and inhibits photosynthesis in cyanobacteria (Fulda *et al.*, 1999; Moisaner *et al.*, 2002; Srivastava *et al.*, 2005, 2008). Among the genes involved in photosynthesis that were studied here, only *alr1526*, encoding Rubisco, was overexpressed in response to high concentrations of NaCl. This finding is supported by a previous study by Srivastava *et al.* (2008), who reported salinity-induced hyper-accumulation of Rubisco in *A. doliolum*. Salt stress has been found to induce Rubisco activity and its transcription in *Sesbania*

Table 2. Change in transcript level of selected genes of *Anabaena* PCC 7120 exposed to 150 mM NaCl for 1 and 24 h as revealed by qRT-PCR analysis

All values are mean $\pm$ SD. Values indicate fold change in the transcripts compared to the non-treated control.

Gene encoding	Change in transcript level after NaCl exposure for:	
	1 h	24 h
Rubisco small subunit (<i>alr1526</i>)	2.40 $\pm$ 0.17*	2.57 $\pm$ 0.15*
Transketolase (<i>alr3344</i>)	1.14 $\pm$ 0.22	0.33 $\pm$ 0.01†
Phosphoribulokinase (<i>alr2350</i>)	1.20 $\pm$ 0.12	0.12 $\pm$ 0.02†
Glycolate oxidase (<i>all0170</i>)	1.69 $\pm$ 0.15*	1.93 $\pm$ 0.14*
Serine hydroxymethyltransferase (<i>alr4806</i>)	0.50 $\pm$ 0.11†	7.51 $\pm$ 0.40*
Phosphoglycolate phosphatase (<i>alr4944</i>)	1.57 $\pm$ 0.13*	2.30 $\pm$ 0.16*
Alanine-glyoxylate aminotransferase (<i>alr1004</i>)	1.40 $\pm$ 0.13	6.93 $\pm$ 0.19*
RNA polymerase (<i>rpoC1</i> , <i>alr1595</i>)	0.97 $\pm$ 0.11	0.99 $\pm$ 0.09

*' and '†' indicate significant repression and overexpression, respectively, of transcripts after 150 mM NaCl treatment compared to control cells. All analyses were performed in triplicate using biologically independent repeats.

Fig. 1. Contents of (a) organic acids (glyceric, glycolic and glyoxylic acids) and (b) free amino acids (glycine and serine) of control and 150 mM NaCl-treated *Anabaena* PCC 7120. Error bars show SD. Different analysis was done for each column (Duncan's new multiple range test). Bars with different letters are significantly different ($P < 0.01$).

and alfalfa (Sivakumar *et al.*, 2000; Winicov & Seemann, 1990). In contrast to the Rubisco gene, transcription of the phosphoribulokinase (*alr2350*) and transketolase (*alr3344*) genes was downregulated after 24 h NaCl treatment, suggesting inhibition of carbon assimilation under high-salinity conditions. Similar findings were also reported by Seki *et al.* (2002) and Chaves *et al.* (2009). A decrease in carbon assimilation may also be attributed to the selective inhibition of photosystem II as reflected in NADPH production (Lu & Zhang, 2000; Srivastava *et al.*, 2008; Sudhir *et al.*, 2005). An enhanced oxygenase activity of Rubisco is therefore postulated due to a reduction in photosynthetic carbon assimilation that may decrease the amount of carbon in the carboxysome, exposing Rubisco to a higher oxygen partial pressure.

All genes related to the glycolate metabolic pathway showed upregulation after salt treatment for 24 h. Overexpression of the glycolate oxidase gene (*all0170*) supports the observations of Srivastava *et al.* (2008), who reported increased enzyme activity in *A. doliolum* exposed

to salt stress. Betsche *et al.* (1992) also reported glycolate oxidase activity in the endosymbiotic cyanobacterium *Cyanophora paradoxa*. Likewise, overexpression of phosphoglycolate phosphatase during the salt stress response observed in this study was also shown by Chaves *et al.* (2009). It is worth mentioning that the genome analysis of *Anabaena* PCC 7120 suggested that it has no homologue to the glutamate-glyoxylate aminotransferase gene of *Synechocystis* PCC 6803 (Eisenhut *et al.*, 2006). Instead, *Anabaena* PCC 7120 has an alanine-glyoxylate aminotransferase (*alr1004*) gene, which showed upregulation under salt stress conditions (Table 2). Although the cellular alanine content was below the detection limit of the assay in the untreated cells, elevated levels were found in the salt-treated cultures. Upregulation of alanine-glyoxylate aminotransferase (*alr1004*) together with the hyper-accumulation of alanine suggest the possible involvement of this enzyme in the glycolate metabolism of *Anabaena* PCC 7120. In contrast to this, Renström-Kellner & Bergman (1989) suggested the glutamate-mediated conversion of glyoxylate to glycine by a glutamate-glyoxylate aminotransferase in *Anabaena cylindrica*.

Overexpression of serine hydroxymethyltransferase (*alr4806*) after 24 h NaCl treatment is reflected by the increased accumulation of serine in the cell (Fig. 1b). Serine hydroxymethyltransferase activity was not reported in *Coccochloris penicostis* (Norman & Colman, 1992); however, significant activity was reported in *Synechocystis* PCC 6803 (Eisenhut *et al.*, 2006). The genome of *Anabaena* PCC 7120 also suggests probable absence of genes involved in a bacterial-like glycerate pathway, as in *Synechocystis* PCC 6803 (Eisenhut *et al.*, 2006). The relatively lower upregulation of these genes may be due to the specific carbon-concentrating mechanism of cyanobacteria.

Glycolate metabolism was further confirmed by the assessment of its metabolites. Amino acids (glycine and serine) and organic acids (glyceric, glycolic and glyoxylic acids) showed a time-dependent increase after NaCl treatment. Eisenhut *et al.* (2006) observed that a small change in the CO_2 level, favouring photorespiratory conditions, resulted in a significant change in the glycine to serine ratio. Further, increased levels of glycine and serine induced by high salinity have been reported in *Anabaena oryzae*, *A. doliolum* and the chlorophyte *Cladophora vagabunda* (Alia & Gahiza, 2007; Rani, 2007; Srivastava *et al.*, 2008). Similar observations were also reported for rice when exposed to salt stress (Dubey & Rani, 1989). The increase in serine content is confirmed by the overexpression of transcripts of genes of glycolate metabolism in general and of serine hydroxymethyltransferase (*alr4806*) in particular. Glycine and serine are not only the intermediates of glycolate metabolism but also play a significant role in maintaining the osmotic status of cells exposed to high salt concentrations (Kirst & Bisson, 1979; Measures, 1975).

The increase in organic acid content mirrors the upregulation of various genes involved in glycolate metabolism. Glycolate is a toxic compound and must be metabolized immediately. Thus, glycolate is converted to glyoxylate via the activity of glycolate oxidase, which would result in an increase of the glyoxylic acid content in the cells exposed to salt stress. Glyoxylate can be directly transaminated to glycine (Bergman, 1986); however, this may not be the major pathway for the metabolism of this compound due to increased serine and ammonia production (Srivastava *et al.*, 2008). It is also known that nitrogen-fixing cyanobacteria (cultured in nitrogen-deficient medium) metabolize glycolate via the glycine and serine *de novo* pathway (Renström-Kellner & Bergman, 1990). The increase in glyceric acid content results from enhanced respiration and glycolate metabolism in the salt-stressed cells, as reported previously (Srivastava *et al.*, 2008). Although cyanobacteria have four different routes to metabolize glyoxylate, it is primarily metabolized through the photorespiratory process as in higher plants (Bergman, 1986; Bergman *et al.*, 1985). Hyperaccumulation of glycolic and glyoxylic acid, as well as the observable increase in glycine and serine content in salt-stressed cells, supports the hypothesis of a photorespiratory pathway being present in *Anabaena* PCC 7120. However, the modest increases observed in the expression of particular transcripts and concentrations of metabolites involved in glycolate metabolism indicate that salinity does not completely disrupt the carbon-concentrating mechanism of this cyanobacterium.

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