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Original article

Sucrose may play an additional role to that of an osmolyte in *Synechocystis* sp. PCC 6803 salt-shocked cells

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Abstract

The role of sucrose in cyanobacteria is still not fully understood. It is generally considered a salt-response molecule, and particularly, in *Synechocystis* sp. strain PCC 6803, it is referred as a secondary osmolyte. We showed that sucrose accumulates transiently in *Synechocystis* cells at early stages of a salt shock, which could be ascribed to salt activation of sucrose-phosphate synthase (SPS, UDP-glucose: D-fructose-6-phosphate 2-α-D-glucosyltransferase; EC 2.4.1.14), the key enzyme in sucrose synthesis pathway, and to an increase of the expression of the SPS encoding gene. Experiments with a mutant strain impaired in sucrose biosynthesis showed that sucrose is essential in stationary phase cells to overcome a later salt stress. Taken together, these results led us to suggest a more intricate function for sucrose than to be an osmoprotectant compound.
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Keywords: Cyanobacterium; Salt stress; Sucrose; Sucrose-phosphate synthase; *Synechocystis* sp. PCC 6803

1. Introduction

Cyanobacteria are oxygenic-photosynthetic prokaryotes worldwide distributed in nature, often abundant in extreme biotopes. This successful spread could be due to their capacity to adapt to adverse environmental conditions (high salinity, extreme temperatures and changes in the surrounding medium (i.e. salt concentrations, temperature, light and nutrient availability) [14,38].

Salinity is an abiotic factor crucial in aquatic ecosystems. An increment in salt concentration in the external medium causes not only a higher osmotic potential, but also a raise of cell ion concentrations as a consequence of the loss of water [14]. In cyanobacteria, strategies developed to tolerate salt stress basically involve two processes: i) the enhancement of active ion export systems, and ii) the accumulation of osmoprotective compounds [32]. After an increase in the medium salinity, a transient increment in Na^+ and Cl^- concentration can be detected inside the cells, followed by the extrusion of Na^+ ions by the action of Na^+/K^+ antiporters [7,29]. The synthesis of organic substances of low molecular mass that do not interfere with the cell metabolism (known as compatible solutes or osmoprotectants) takes place during this period leading to a reduction of the internal osmotic cell potential and preventing denaturation of macromolecules. It has been reported that these molecules are also able to protect the cell from other types of desiccation, as well as from cold and heat stress [11,14,29,32].

Synechocystis sp. PCC 6803 is a unicellular fresh-water cyanobacterium, which is able to tolerate up to 1.2 M sodium chloride. Because of that, it is considered as a moderate salt-tolerant strain that synthesises glucosylglycerol (GG) as the major osmoprotectant compound and sucrose as a secondary osmolyte [14,32]. The biosynthetic pathway leading to GG is a two-step reaction involving the activities of GG-phosphate synthase (GGPS, glucosylglycerol-phosphate synthase; EC 2.4.1.213) and GG-phosphate phosphatase (GGPP, glucosylglycerol-phosphatase; EC 3.1.3.69) [13,17]. In addition to biochemical modulation of enzyme activity, alterations of GGPS

Abbreviations: GG, glucosylglycerol; GGPP, glucosylglycerol-phosphate phosphatase; GGPS, glucosylglycerol-phosphate-synthase; SPP, sucrose-phosphate phosphatase; SPS, sucrose-phosphate synthase.

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gene expression are involved in the regulation of GG production in *Synechocystis* sp. PCC 6803 [18,23]. Cells can also take up GG from the medium via an ABC-type transport system, whose activity is energy-dependent and is enhanced in high-salt adapted cells [14,26,27]. In contrast to the extensive studies related to GG synthesis, much less is known about sucrose. Similarly to the pathway described for plants, the biosynthesis of sucrose in *Synechocystis* sp. PCC 6803 takes place from fructose-6-phosphate and a sugar nucleotide (mainly UDP-Glc) through the sequential action of sucrose-phosphate synthase (SPS, UDP-glucose: D-fructose-6-phosphate 2-α-D-glucosyltransferase; EC 2.4.1.14) and sucrose-phosphate phosphatase (SPP, sucrose-6^F-phosphate-phosphohydrolase; EC 3.1.3.24) [6], previously described in *Anabaena* sp. PCC 7119, a filamentous strain [30]. SPS and SPP encoding genes (*sps*A and *spp*) were identified and characterised in both cyanobacterium strains [4–6,22]. The role of sucrose in cyanobacteria has not been fully elucidated although it is usually associated with responses to increased salt concentrations [15,31,35]. Recently, it was shown in a *Synechocystis* mutant impaired in the synthesis of ADP-Glc (lacking glycogen and GG) that sucrose highly accumulated in salt-stressed cells, allowing the mutant tolerate up to 0.9 M NaCl. These results led to the conclusion that sucrose could act as a similar potent osmoprotectant as GG [25].

The assumption that plant sucrose metabolism has been acquired during the endosymbiotic origin of chloroplast [12] at the time of the cyanobacterial phylogenetic radiation was strongly supported by the fact that plant sucrose enzymes share a common branch with their cyanobacterial homologues in a protein phylogeny [35]. Some responses of living organisms to abiotic stress are well conserved through evolution, such as the accumulation of common intracellular solutes in plants and cyanobacteria to maintain turgor in response to water stress [3]. Therefore, extant cyanobacteria are suitable models for studying the physiology of salt tolerance and the role of response molecules to enlarge our knowledge of similar processes acting in plants.

In the present work we examined the synthesis of sucrose in *Synechocystis* sp. PCC 6803 cells submitted to a salt stress, in an attempt to elucidate the role of this sugar in cyanobacteria. The transient accumulation of sucrose at the onset of the stress and the analysis of a *Synechocystis* mutant strain impaired in sucrose biosynthesis showed that sucrose behaviour differs from that of a typical osmolyte accumulation and that its presence was essential in stationary phase cells to overcome a later salt stress. This study marks the first demonstration of sucrose playing a more complex role than that of a compatible osmolyte for salt tolerance.

2. Results and discussion

2.1. Sucrose accumulation in salt-shocked Synechocystis cells

Salt adapted or salt-shocked cells of *Synechocystis* sp. PCC 6803 accumulate low amounts of sucrose and, therefore, this compound was considered up date as a secondary osmolyte [15,25]. In the present study we were able to show that intracellular sucrose concentration increased immediately after the onset of a NaCl shock, reaching a maximum between 4 and 6 h after salt addition, but decreasing rapidly in the following 20 h up to approximately the initial level (Fig. 1A). For comparison, GG, reported as the dominating osmoprotective compound, was also determined and its concentration was 10-times higher than that of sucrose (Fig. 1B), presenting a similar accumulation pattern as previously reported [10,13,24,25]. Importantly, the kinetics of sucrose accumulation in the PCC 6803 strain (Fig. 1A) contrasts with the typical accumulation pattern of an osmolyte like GG (Fig. 1B), or with the accumulation of sucrose in the *Synechocystis* mutant lacking GG [25].

Upon a sudden increase in salt concentration in the surrounding medium, it was shown that salt might accumulate transiently in cyanobacterial cells to achieve a rapid osmotic balance before sufficient amounts of organic solutes were produced [1]. The initial intracellular salt concentration is likely to cause a direct activation of enzymes related to salt stress responses. Indeed *Synechocystis* GGPS was activated in vitro upon the addition to the assay mixture of a NaCl amount simi-

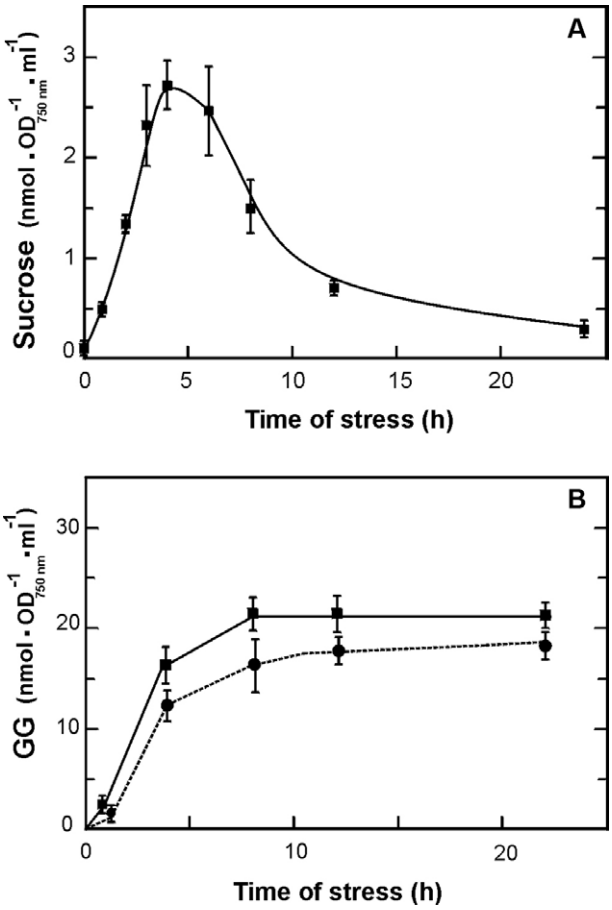

Fig. 1. Sucrose and GG accumulation in *Synechocystis* sp. cells after a salt shock. NaCl was added up to 684 mM (t = 0 h) to cultures; sucrose (A) and GG (B) were quantified in cells of *Synechocystis* sp. PCC 6803 (■) or of the *sps*A$^-$ mutant strain (•).

lar to the apparent intracellular Na^+ level reported for cells adapted to 650 mM NaCl [16,24]. Thus, we tested the effect of different NaCl concentrations on SPS activity in crude extracts of *Synechocystis* cells (Fig. 2). A 2-fold increase of enzyme activity was obtained in the presence of the highest NaCl concentrations, which may be similar to the intracellular salt concentration at the onset of the experiment showed in Fig. 1. In *Synechococcus* sp. PCC 6301, which accumulates high sucrose amounts during salt adaptation, SPS activity also enhanced in the presence of NaCl [15]. We concluded that the activation of SPS by NaCl in *Synechocystis* sp. PCC 6803 might account, at least in part, for sucrose accumulation at the initiation of the salt treatment. Besides we investigated the expression of the gene coding for SPS (*sps*A) at the transcriptional level. The steady-state amount of *sps* transcripts increased very quickly in *Synechocystis* cells after the salt shock, reached a maximum after 30 min and returned during the following hour to initial levels (Fig. 3). In the case of GG production, it has been shown to be primarily activated by a biochemical, salt-dependant mechanism [8,13]. However, when the regulatory events involved in salt stress-related GG accumulation were thoroughly analysed, it was

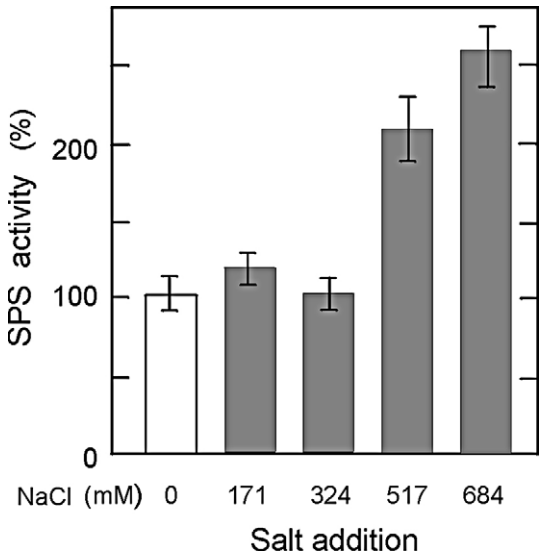

Fig. 2. Effect of NaCl on SPS activity. Crude extracts from *Synechocystis* sp. PCC 6803 cells were assayed for enzyme activity in the presence of different amounts of NaCl. SPS activity (100%) was 6.6 ± 0.6 nkat μl^{-1}. Mean ± S.D., $n = 3$.

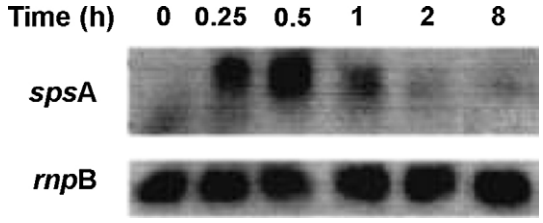

Fig. 3. Effect of NaCl addition on the transcription of *sps*A in *Synechocystis* sp. PCC 6803 cells. Northern blot analysis was performed from total RNA (15 μg) extracted from cells harvested at different times after addition of 684 mM NaCl. Specific probes were used for hybridisation of *sps*A and *rpn*B.

concluded that biochemical regulation might be more important for the immediate reaction to the alarm situation after the salt-shock, including de novo protein synthesis, and that transcriptional control might represent the basis for the fine-tuning of GG synthesis in salt-acclimated cells [23]. In a recent DNA microarray analysis it was reported that the expression of GG synthesis related genes was induced by hyperosmotic stress but SPS encoding gene was not included in the list of strongly transcriptional enhanced genes [20]. This result can be expected in accordance with the transient activation of *sps*A transcription only at the first minutes of the salt treatment (Fig. 3).

Taken together, these results led us to suggest that sucrose may play a role different to that of an osmolyte in *Synechocystis* cells at early stages of salt stress response. The transient accumulation of sucrose tempted us to speculate that the sugar may be acting as a signal molecule. The pivotal role of sugars as signalling molecules is well illustrated by a variety of sugar sensing and signalling mechanisms discovered in free-living microorganisms, in which the process has been dissected in detail [34]. However, the concept of sugars as central molecules, crucial for modulation of gene expression, is relatively novel in plants and still not described in cyanobacteria [21,34,35]. As in higher plants, sucrose might be sensed to transduce a specific signalling pathway in response to salt in *Synechocystis*. Investigation of possible signalling circuitry underlying biological responses in cyanobacteria to changing sugar levels is challenging.

2.2. Effect of NaCl on Synechocystis sp. culture growth

The relevance of sucrose in salt tolerance was investigated in a *Synechocystis* sp. PCC 6803 derivative mutant (*sps*A$^-$), which is unable to synthesise sucrose [6] but that accumulates GG similarly to the wild-type strain (Fig. 1B). Cells of PCC 6803 and *sps*A$^-$ strains at logarithmic or stationary phase were collected and transferred into fresh basal medium with or without 684 mM NaCl, and growth was monitored (Fig. 4). After a short lag period, cells of the wild-type strain initially at logarithmic phase adapted to salt-supplemented medium as they reached similar growth rates in the presence or in the absence of NaCl (Fig. 4A). In contrast, cells of the *sps*A$^-$ mutant resumed growth after a longer lag period, with a rate 20% lower in the presence of NaCl than in basal medium (Fig. 4B). A clear difference in adaptation emerged when cells coming from stationary phase cultures were transferred to fresh medium containing 684 mM NaCl. Wild-type cells became to recover after a 25–50 h lag (Fig. 4C), while *sps*A$^-$ mutant cells could not resume growth (Fig. 4D).

Intracellular sucrose concentration in wild-type cells at exponential phase in basal medium was about 0.27 nmol. Optical density (OD) $_{750\ nm}{}^{-1} \cdot ml^{-1}$ while it was about 10-fold higher (ca. 3 nmol. OD $_{750\ nm}{}^{-1} \cdot ml^{-1}$) in cells of the same strain at stationary phase. Interestingly, sucrose content in cells at the stationary phase in basal medium was similar to the level transiently reached by exponential salt-shocked cells

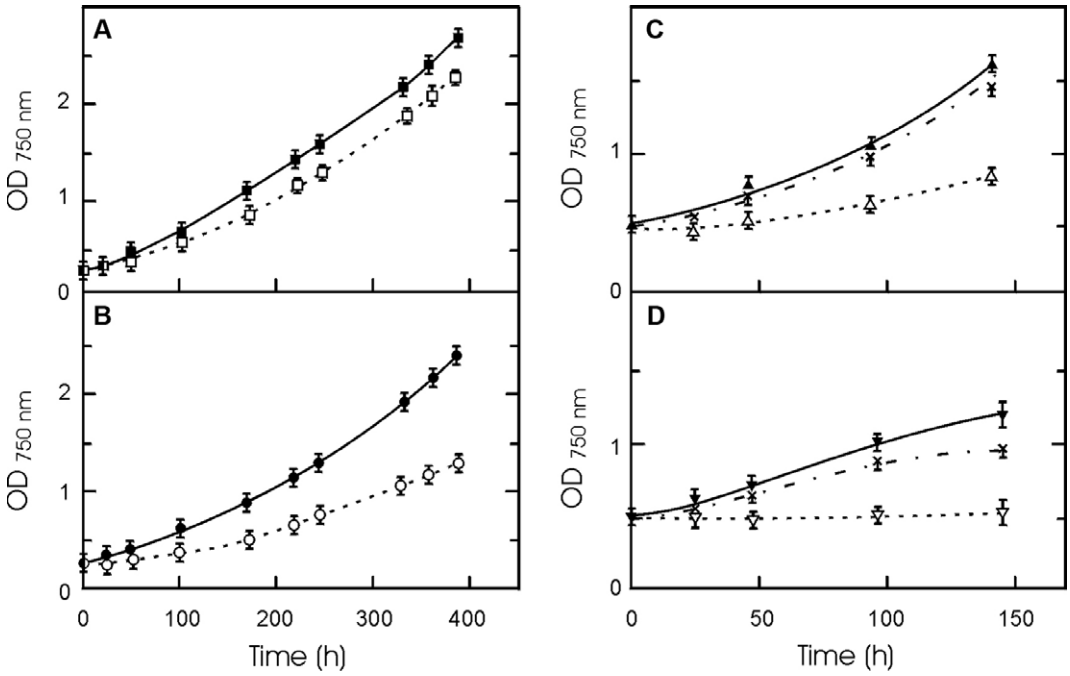

Fig. 4. Effect of NaCl on the growth of *Synechocystis* cultures from exponential (A and B) and stationary phase cells (C and D). (A and C) PCC 6803 strain; (B and D) *sps*A$^-$ mutant strain. Closed symbols (■, ●, ▲, ▼), control BG-11 medium; open symbols (□, ○, Δ, ∇), BG-11 medium supplemented with NaCl 684 mM; crosses (**X**), BG-11 medium supplemented with 684 mM NaCl and 1 mM sucrose.

(Fig. 1). To know if the lack of sucrose in the mutant cells at the stationary phase could be related to its salt-sensitive phenotype, and knowing that external sucrose could enter the cells via specific transporters [26], we followed the growth of PCC 6803 and the *sps*A$^-$ mutant cultures transferred to NaCl-containing medium supplemented with 1 mM sucrose. Under these conditions, both wild-type and *sps*A$^-$ mutant cells resumed growth in the presence of NaCl at rates that were indistinguishable from those in BG-11 medium (Fig. 4C, D). These results suggested that in *Synechocystis* cells submitted to a salt shock, sucrose may play a role at the early stages of the stress, and importantly, its presence should be crucial in stationary phase cells for overcoming a later salt shock. It could be speculated that sucrose function might be related to the regulation of metabolic pathways that are active under the nutritional stress conditions of the stationary phase.

3. Conclusion

Transient sucrose accumulation in *Synechocystis* cells at the onset of a salt stress together with the crucial disaccharide role in stationary phase cells are clear evidence that sucrose may play a role different of being only an osmoprotective compound in salt tolerance. We also showed that sucrose synthesis in salt-treated *Synechocystis* cells may be modulated through both, a salt effect on SPS activity and the transcriptional regulation of SPS encoding gene. The transient accumulation and low levels of sucrose observed in salt stress situations tempted us to speculate that the disaccharide may be involved in triggering signalling cascades, and probably fulfilling a more general function in responses to environmental stresses.

4. Methods

4.1. Bacterial strains and growth

Synechocystis sp. PCC 6803 strain was purchased from the Institut Pasteur. Cells were routinely grown in BG-11 medium (basal medium) [30,33]. The insertional mutant strain (*sps*A$^-$), a derivative from *Synechocystis* sp. PCC 6803 previously generated and formerly named LC20 [6], was cultured in the presence of 20 μg ml^{-1} chloramphenicol. Cell growth was monitored by measuring the OD of the cultures at 750 nm. To study the effect of salt in growth, cells previously cultured at standard conditions up to exponential or stationary phase, were collected and seeded either in basal medium or in basal medium supplemented with NaCl (684-mM final concentration). For sucrose and GG determination, aliquots of cell cultures were taken at different times after the salt shock and cells were harvested by centrifugation.

4.2. Sucrose and GG determination

Packed cells were extracted three times with 80% ethanol at 80 °C. The pooled extracts were evaporated in vacuum; the residue was dissolved in water and aliquots were chromatographed on Whatmann #3 paper using butanol/pyridine/water (6:4:2, v/v/v) as developing solvent. Standard sugars were

chromatographed in parallel strips and visualised by the silver nitrate reagent [37]. Sucrose and GG were determined in eluted fractions located in positions of the chromatograms similar to those of the standards. Sucrose was quantified after hydrolysis with invertase by measuring the formation of NADPH [19]. GG was estimated after acid hydrolysis as described [9].

4.3. Isolation of RNA and Northern hybridisation

RNA was extracted from cells at the exponential phase according to Mohamed and Jansson [28], separated by electrophoresis through agarose gels containing formaldehyde and transferred to nylon membranes (Hybond N, Amersham) [36]. Gene-specific DNA probes were obtained by digestion with *Bam*HI and *Not*I of the plasmid pSySPS harbouring *sps*A [6] and after PCR amplification of the encoding sequence of the subunit of *Synechocystis* RNase P (*Synechocystis* sp. PCC 6803 *rnp*B gene for ribonuclease P RNA, GenBank X65707). DNA probes were labelled with [α-^{32}P]-dCTP using the Random Primer Extension Kit (DuPont). Membranes were hybridised, washed according to standard protocols and exposed on Kodak MS Biomax films [36].

4.4. Protein extraction and enzyme assay

Crude extracts from *Synechocystis* cells were prepared as described [6,30] and desalted before enzyme activity assays. Proteins were quantified by the method of Bradford [2]. SPS activity was determined as previously described [6,30].

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References

[1] E. Blumwald, R.J. Mehlhorn, L. Packer, Ionic osmoregulation during salt adaptation of the cyanobacterium *Synechococcus* 6311, Plant Physiol. 73 (1983) 377–380.

[2] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.

[3] T.J. Close, P.J. Lammers, An osmotic stress protein of cyanobacteria is immunologically related to plant dehydrins, Plant Physiol. 101 (1993) 773–779.

[4] A. Cumino, L. Curatti, L. Giarrocco, G.L. Salerno, Sucrose metabolism: *Anabaena* sucrose-phosphate synthase and sucrose-phosphate phosphatase define minimal functional domains shuffled during evolution, FEBS Lett. 517 (2002) 19–23.

[5] A. Cumino, C. Ekeroth, G.L. Salerno, Sucrose-phosphate phosphatase from *Anabaena* sp. strain PCC 7120: isolation of the protein and gene revealed significant structural differences from the higher-plant enzyme, Planta 214 (2001) 250–256.

[6] L. Curatti, E. Folco, P. Desplats, G. Abratti, V. Limones, L. Herrera-Estrella, et al., Sucrose-phosphate synthase from *Synechocystis* sp. strain PCC 6803: identification of the *sps*A gene and characterization of the enzyme expressed in *Escherichia coli*, J. Bacteriol. 180 (1998) 6776–6779.

[7] I.V. Elanskaya, I.V. Karandashova, M. Hagemann, Functional analysis of the Na^+/H^+ antiporter encoding genes of the cyanobacterium *Synechocystis* PCC 6803, Biochemistry 67 (2002) 432–440.

[8] F. Engelbrecht, K. Marin, M. Hagemann, Expression of the *ggp*s gene, involved in osmolyte synthesis in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002, revealed regulatory differences between this strain and the freshwater strain *Synechocystis* sp. strain PCC 6803, Appl. Environ. Microb. 65 (1999) 4822–4829.

[9] N. Erdmann, S. Fulda, M. Hagemann, Glucosylglycerol accumulation during salt acclimation of two unicellular cyanobacteria, J. Gen. Microbiol. 138 (1992) 363–368.

[10] A. Ferjani, L. Mustardy, R. Sulpice, K. Marin, I. Suzuki, M. Hagemann, et al., Glucosylglycerol, a compatible solute, sustains cell division under salt stress, Plant Physiol. 131 (2003) 1628–1637.

[11] E.A. Galinski, Compatible solutes of halophilic eubacteria: molecular principles, water–solute interactions, stress protection, Experientia 49 (1993) 487–496.

[12] M.W. Gray, W.F. Doolittle, Has the endosymbiont hypothesis been proven? Microbiol. Rev. 46 (1982) 1–42.

[13] M. Hagemann, N. Erdmann, Activation and pathway of glucosylglycerol biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803, Microbiology 140 (1994) 1427–1431.

[14] M. Hagemann, N. Erdmann, Environmental stresses, in: A.K. Rai (Ed.), Cyanobacterial Nitrogen Metabolism and Environmental Biotechnology, Springer Verlag, Narosa Publishing House, New Delhi, 1997, pp. 155–221.

[15] M. Hagemann, K. Marin, Salt-induced sucrose accumulation is mediated by sucrose-phosphate-synthase in cyanobacteria, J. Plant Physiol. 155 (1999) 424–430.

[16] M. Hagemann, A. Schoor, N. Erdmann, NaCl acts as a direct modulator in the salt adaptive response: salt-dependent activation of glucosylglycerol synthesis in vivo and in vitro, J. Plant Physiol. 149 (1996) 746–752.

[17] M. Hagemann, A. Schoor, R. Jeanjean, E. Zuther, F. Joset, The *stp*A gene from *Synechocystis* sp. strain PCC 6803 encodes the glucosylglycerol-phosphate phosphatase involved in cyanobacterial osmotic response to salt shock, J. Bacteriol. 179 (1997) 1727–1733.

[18] M. Hagemann, L. Wöelfel, B. Charger, Alterations of protein synthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 after a salt shock, J. Gen. Microbiol. 136 (1990) 1393–1399.

[19] M.G.K. Jones, W.H. Outlaw Jr., O.H. Lowry, Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues, Plant Physiol. 60 (1977) 379–383.

[20] Y. Kanesaki, I. Suzuki, S.I. Allakhverdiev, K. Mikami, N. Murata, Salt stress and hyperosmotic stress regulate the expression of different sets of genes in *Synechocystis* sp. PCC 6803, Biochem. Biophys. Res. Commun. 290 (2002) 339–348.

[21] K. Koch, Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development, Curr. Opin. Plant Biol. 7 (2004) 235–246.

[22] J.E. Lunn, A.R. Ashton, M.D. Hatch, H.W. Heldt, Purification, molecular cloning and sequence analysis of sucrose-6-phosphate phosphohydrolase from plants, Proc. Natl. Acad. Sci. USA 97 (2000) 12914–12919.

[23] K. Marin, J. Huckauf, S. Fulda, M. Hagemann, Salt-dependent expression of glucosylglycerol-phosphate synthase, involved in osmolyte synthesis in the cyanobacterium *Synechocystis* sp. strain PCC 6803, J. Bacteriol. 184 (2002) 2870–2877.

[24] K. Marin, E. Zuther, T. Kerstan, A. Kunert, M. Hagemann, The *ggp*S gene from *Synechocystis* sp. strain PCC 6803 encoding glucosylglycerol-phosphate synthase is involved in osmolyte synthesis, J. Bacteriol. 180 (1998) 4843–4849.

[25] X. Miao, Q. Wu, G. Wu, N. Zhao, Sucrose accumulation in salt stressed cells of *agp* gene deletion-mutant in cyanobacterium *Synechocystis* sp. PCC 6803, FEMS Microbiol. Lett. 218 (2003) 71–77.

[26] S. Mikkat, U. Effmert, M. Hagemann, Uptake and use of the osmoprotective compounds trehalose, glucosylglycerol, and sucrose by the cyanobacterium *Synechocystis* sp. PCC 6803, Arch. Microbiol. 167 (1997) 112–118.

[27] S. Mikkat, M. Hagemann, A. Schoor, Active transport of glucosylglycerol is involved in salt adaptation of the cyanobacterium *Synechocystis* sp. strain PCC 6803, Microbiology 142 (1996) 1725–1732.

[28] A. Mohamed, C. Jansson, Influence of light on accumulation of photosynthesis-specific transcripts in the cyanobacterium *Synechocystis* 6803, Plant Mol. Biol. 13 (1989) 693–700.

[29] A. Oren, Salts and Brines, in: B.A. Whitton, M. Potts (Eds.), The Ecology of Cyanobacteria, Kluwer Academic Publishers, Dordrecht, 2000, pp. 281–306.

[30] A.C. Porchia, G.L. Salerno, Sucrose biosynthesis in a prokaryotic organism: Presence of two sucrose-phosphate synthases in *Anabaena* with remarkable differences compared with the plant enzymes, Proc. Natl. Acad. Sci. USA 93 (1996) 13600–13604.

[31] R.H. Reed, L.J. Borowitzka, M.A. Mackay, J.A. Chudek, R. Foster, S.R.C. Warr, et al., Organic solute accumulation in osmotically stressed cyanobacteria, FEMS Microbiol. Rev. 39 (1986) 51–56.

[32] R.H. Reed, W.D.P. Stewart, Osmotic adjustment and organic solute accumulation in unicellular cyanobacteria from freshwater and marine habitats, Mar. Biol. 88 (1985) 1–9.

[33] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Genetic assignments, strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol. 111 (1979) 1–61.

[34] F. Rolland, B. Moore, J. Sheen, Sugar sensing and signaling in plants, Plant Cell Suppl. 14 (2002) S185–S205.

[35] G.L. Salerno, L. Curatti, Origin of sucrose metabolism in higher plants: when, how and why? Trends Plant Sci. 8 (2003) 63–69.

[36] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 2001.

[37] I. Smith, Carbohydrates, in: I. Smith (Ed.), Chromatographic and Electrophoretic Techniques, vol. 1, Pitman Press, London, 1960, pp. 246–260.

[38] B.A. Whitton, M. Potts, Introduction to the cyanobacteria, in: B.A. Whitton, M. Potts (Eds.), The Ecology of Cyanobacteria, Kluwer Academic Publishers, Dordrecht, 2000, pp. 1–11.