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Minireview

Compatible solute biosynthesis in cyanobacteria

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Summary

Compatible solutes are a functional group of small, highly soluble organic molecules that demonstrate compatibility in high amounts with cellular metabolism. The accumulation of compatible solutes is often observed during the acclimation of organisms to adverse environmental conditions, particularly to salt and drought stress. Among cyanobacteria, sucrose, trehalose, glucosylglycerol and glycine betaine are used as major compatible solutes. Interestingly, a close correlation has been discovered between the final salt tolerance limit and the primary compatible solute in these organisms. In addition to the dominant compatible solutes, many strains accumulate mixtures of these compounds, including minor compounds such as glucosylglycerate or proline as secondary or tertiary solutes. In particular, the accumulation of sucrose and trehalose results in an increase in tolerance to general stresses such as desiccation and high temperatures. During recent years, the biochemical and molecular basis of compatible solute accumulation has been characterized using cyanobacterial model strains that comprise different salt tolerance groups. Based on these data, the distribution of genes involved in compatible solute synthesis among sequenced cyanobacterial genomes is reviewed, and thereby, the major compatible solutes and potential salt tolerance of these strains can be predicted. Knowledge regarding cyanobacterial salt tolerance is not only useful to characterize strain-specific adaptations to ecological niches, but it can also be used to generate cells with increased tolerance to adverse environmental conditions for biotechnological purposes.

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Introduction

Cyanobacteria have colonized all light-exposed habitats on Earth and can be used as excellent models to study the environmental adaptation of photoautotrophic organisms. The majority of cyanobacterial strains are found in waters of different or changing salinities. They have clearly developed mechanisms to adapt to a wide range of and fluctuations in external salinity. High salt concentrations generate two major problems for living systems. First, a low water potential results in a loss of water and turgor pressure, which is compensated by the accumulation of osmotically active compounds. Second, the high ionic strength of the surrounding medium results in a continuous influx of inorganic ions (mainly Na^+ and Cl^-, Fig. 1). Halophilic *Archaea* and several halophilic bacteria equilibrate their osmotic potential via the accumulation of inorganic ions and have consequently adapted their cellular environment to tolerate high internal concentrations of inorganic ions, e.g. evolution of macromolecules with a high capacity to bind salts and water ('salt-in' strategy; Eisenberg and Wachtel, 1987; Galinski and Trüper, 1994; Ventosa *et al*., 1998; Müller and Oren, 2003). The majority of organisms (including cyanobacteria) limit the cytoplasmic ionic strength to rather low levels, because most intracellular macromolecules are sensitive to high levels of inorganic ions. To maintain a favourable ion composition, ions, especially Na^+ and Cl^-, are pumped out of the cytoplasmic space ('salt-out' strategy, Fig. 1). Simultaneously, these cells accumulate so-called compatible solutes, which adjust the cellular osmotic potential to levels that allow water uptake via osmosis; this accumulation is the basis for turgor pressure and growth (e.g. Kempf and Bremer, 1998).

The concept of compatible solutes was introduced by Brown (1976) and has led to the definition of a functional group of low molecular mass, organic compounds without net charge that do not disturb cellular metabolism (compatible) at the high (molar) concentrations necessary to equilibrate osmotic conditions. The advantage and great success of the 'salt-out' strategy is based on the ability to acclimate towards low as well as high salt environments, because none of the other cellular constituents (e.g. membranes, proteins) had to be changed during evolution. The structural variety of these compounds is

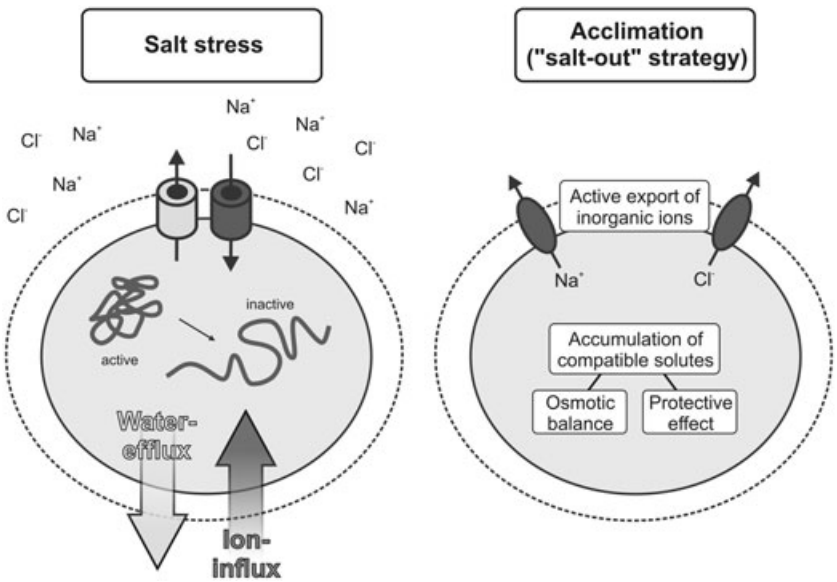

Fig. 1. Schematic view on the principle of salt acclimation strategy of cyanobacteria. Salt-stressed cells have to cope with a low water potential resulting in water efflux through aquaporins. Moreover, intracellular accumulation of inorganic ions causes toxic effects on cellular metabolism by interference with macromolecules (including denaturation of proteins). Cyanobacteria and most other microbes acclimate to those conditions via active ion extrusion and simultaneous accumulation of compatible solutes, which are necessary for osmotic balance and for direct and/or indirect protection of macromolecules.

relatively limited, because only a small number of chemical structures meet the special requirements of compatibility with cell metabolism. Based on their chemical structure, compatible solutes can be divided into various groups: carbohydrates, polyols, heterosides, amino acids and their derivatives. Some solutes are widespread among pro- and eukaryotes, whereas others are restricted to specialized organisms (Santos and Costa, 2002). In addition to their osmotic functions, compatible solutes can also protect macromolecules such as proteins and membranes against denaturation (Luzardo *et al*., 2000; Borges *et al*., 2002; Hincha and Hagemann, 2004), which provides an explanation for their participation in the acclimation of cells exposed to freezing, desiccation or heat (Eleutherio *et al*., 1993; Ko *et al*., 1994; Welsh and Herbert, 1999).

In this review, we describe that among cyanobacteria the accumulation of defined major compatible solutes results in a characteristic final salt tolerance limit. In addition to the compensation of osmotic potential differences during salt acclimation, compatible solutes display direct protective effects towards critical macromolecules, which explains their function to achieve tolerance towards general stresses such as desiccation and high temperatures. During recent years, the biochemical and molecular basis of compatible solute accumulation has been characterized using cyanobacterial model strains that comprise different salt tolerance groups. Based on these data, the distribution of genes involved in compatible solute synthesis among sequenced cyanobacterial genomes is reviewed, and thereby, the major compatible solutes and potential salt tolerance of these strains can be predicted (see Table 1).

Sucrose and trehalose as compatible solutes in strains of low salt tolerance

Organisms from freshwater habitats are adapted to an environment of rather low ionic strength; therefore, under standard conditions, compatible solute accumulation is of low importance or lacking, and these cells require active uptake systems for inorganic ions, e.g. K^+. However, almost all freshwater cyanobacteria display certain flexibility towards increased salt concentrations, in which stenohaline strains tolerate only small changes, and euryhaline strains are able to grow in a larger range of changes. The screening of many cyanobacterial genera has revealed the general trend that freshwater isolates mainly accumulate sucrose [Suc, α-D-glucopyranosyl-(1→2)-β-D-fructofuranose, Fig. 2] as the primary compatible solute under saline conditions (Reed *et al*., 1984a). The importance of Suc synthesis for salt acclimation was first recognized by its salt stress-induced accumulation in *Nostoc muscorum* (Blumwald and Tel-Or, 1982), *Synechococcus* sp. PCC6301 (Blumwald *et al*., 1983) and *Anabaena variabilis* (Erdmann, 1983). In addition to Suc, trehalose (Tre, 1-α-glucopyranosyl-1-α-glycopyranoside, Fig. 2) is also a dominant, salt-inducible osmolyte in cyanobacteria from freshwater, as well as coastal waters and terrestrial habitats. Tre accumulation was first demonstrated in *Rivularia atra*, a coastal strain that lives in the tidal zone (Reed and Stewart, 1983). Interestingly, Tre-accumulating cyanobacteria are mostly filamentous strains, which often form mats or aggregates. It is well documented that extracellular polysaccharide (EPS) sheaths in combination with Tre-accumulation protect cyanobacteria against desiccation, but the role of such

Table 1. Distribution of gene orthologues encoding proteins potentially involved in compatible solute synthesis in cyanobacteria.

Genus/strain	Genome Accession No. (GenBank)	Origin	Suc synthesis			Tre synthesis					GG synthesis		GGA synthesis		GB synthesis[a]		Compatible solute accumulation
			SpsA	Spp	Sus	TPS/OtsA	TPP/OtsB	TreY	TreZ	TreS	GgpS	GgpP	GpgS	GpgP	GSMT	SDMT	
Anabaena variabilis ATCC29413 (N-Fix)	CP000117.1	Limnic	–	+	2	–	–	+	+	–	–	–	–	–	–	–	Tre, Suc (p)
Cyanothece sp. PCC7424 (N-Fix)	CP001291.1	Limnic	–	+	+	–	–	+	+	+	–	–	–	–	–	–	Tre, Suc (p)
Cyanothece sp. PCC7425 (N-Fix)	CP001344.1	Limnic	–	+	2	–	–	–	–	–	–	–	–	–	–	–	Suc (p)
Cyanothece sp. PCC8801 (N-Fix)	CP001287.1	Limnic	–	–	–	–	–	+	+	–	–	–	–	–	–	–	Tre (p)
Microcystis aeruginosa PCC7806	AM778843–AM778958	Limnic	+	+	+	–	–	–	–	–	–	–	–	–	–	–	Suc (p)
Nostoc sp. PCC7120 (N-Fix)	BA000019.2	Limnic	–	+	2	–	–	+	+	–	–	–	–	–	–	–	Tre (e)
Synechococcus elongatus PCC7942	CP000100.1	Limnic	+	+	–	–	–	–	–	–	–	–	–	–	–	–	Suc (p)
Synechococcus elongatus PCC6301	AP008231.1	Limnic	+	+	–	–	–	–	–	–	–	–	–	–	–	–	Suc (p)
Synechocystis sp. PCC6803	BA000022.2	Limnic	+	+	–	–	–	–	–	–	+	+	–	–	–	–	GG, Suc (f)
Acaryochloris marina MBIC11017	CP000828.1	Marine	–	+	+	–	–	–	–	–	+	+	+	+	–	–	GG, GGA (p)
Crocosphaera watsonii WH8501 (N-Fix)	AADV00000000.2	Marine	–	–	–	+[b]	+[b]	–	–	–	–	–	–	–	–	–	Tre (a)
Cyanothece sp. ATCC 51142 (N-Fix)	CP000806.1	Marine	+	+	–	–	–	–	–	–	+	+	–	–	–	–	GG (p)
Microcoleus chthonoplastes PCC7420	ABRS00000000	Marine	–	+	+	–	–	+	+	+	+	+	+	+	–	–	GG, GGA, Tre, Suc (p)
Prochlorococcus sp. NATL2A	CP000095.2	Marine	+	–	–	–	–	–	–	–	–	+	+	+	–	–	Suc, GGA (b)
Prochlorococcus sp. SS120	AE017126	Marine	+	–	–	–	–	–	–	–	–	+	+	+	–	–	Suc, GGA (b)
Prochlorococcus sp. MIT9313	BX548175.1	Marine	+	–	–	–	–	–	–	–	–	+	–	–	+	+	Suc, GB (b)
Synechococcus sp. PCC7002	CP000951.1	Marine	+	–	–	–	–	–	–	–	+	+	+	+	–	–	GG, GGA, Suc (b)
Synechococcus sp. WH8102	BX548020.1	Marine	+	–	–	–	–	–	–	–	+	+	+	+	+	+	GG, GB (b)
Synechococcus sp. WH7803	CT971583.1	Marine	+	–	–	–	–	–	–	–	+	+	+	+	+	+	GG, Suc, GB (b)
Trichodesmium erythraeum IMS101 (N-Fix)	CP000393.1	Marine	–	–	–	–	–	–	–	–	–	–	–	–	–	+	not investigated
Arthrospira maxima CS-328	ABYK00000000	Salt lake	–	–	+	–	–	+	+	+	+	+	+	+	–	–	GG, GGA, Tre, Suc (p)
Arthrospira (*Spirulina*) *platensis* NIES-39	AP011615	Salt lake	–	–	+	–	–	+	+	+	+	+	+	+	–	–	GG (c), Tre (d), GGA (p)
Nodularia spumigena CCY9414 (N-Fix)	AAVW00000000	Brackish water	+	+	2	–	–	+	+	+	–	–	–	–	–	–	Tre, Suc (p)
Lyngbya sp. PCC8106 (N-Fix)	not finished	Coastal/marine	–	–	–	–	–	+	+	+	–	–	+	+	+	+	GGA, Tre, GB (p)
Gloeobacter violaceus PCC7421	BA000045.2	Epilithic	+	+	–	–	–	–	–	–	–	–	–	–	–	–	Suc (p)
Nostoc punctiforme PCC73102 (N-Fix)	CP001037.1	Symbiontic	+	+	2	–	–	+	+	–	–	–	–	–	–	–	Tre, Suc (p)
Synechococcus sp. JA-2-3B'a(2–13)	CP000240.1	Hot spring	–	–	–	–	–	+	+	+	–	–	–	–	–	–	Tre (p)
Synechococcus sp. JA-3-3Ab	CP000239.1	Hot spring	–	–	–	–	–	+	+	+	–	–	–	–	–	–	Tre (p)
Thermosynechococcus elongatus BP-1	BA000039.2	Hot spring	+	+	+	–	–	–	–	–	–	–	–	–	–	–	Suc (p)

a. All presently known cyanobacterial genomes lack further genes for GB synthesis known from other organisms such as choline dehydrogenase (CDH), betaine-aldehyde dehydrogenase (BADH), choline oxidase (CO), choline mono-oxygenase (CMO).

b. Combined protein with putative TpS and TpP activity.

(p) – postulated from gene occurrence; (a) – own preliminary measurements; (b) – Klähn and colleagues (2010); (c) – Warr and colleagues (1985a); (d) – Ohmori and colleagues (2009); (e) – Higo and colleagues (2006); (f) – Reed and Stewart (1985).

Sequences of functionally characterized proteins involved in compatible solute biosyntheses were used for BLASTP searches: SpsA, sucrose-phosphate synthase from *Synechocystis* sp. PCC 6803 (Accession No. BAA10782); SPP, sucrose-phosphate phosphatase from *Synechocystis* sp. PCC 6803 (Accession No. AAG31136); Sus, sucrose synthase from *Anabaena variabilis* ATCC 29413 (Accession No. CAC00631); TPS/OtsA, trehalose 6-phosphate synthase from *E. coli* (Accession No. AP_002515); TPP/OtsB, trehalose 6-phosphate phosphatase from *E. coli* (Accession No. AP_002516); TreY/Mts, maltooligosyltrehalose synthase from *Nostoc* sp. PCC 7120 (Accession No. NP_484211); TreZ/Mth, maltooligosyltrehalose trehalohydrolase from *Nostoc* sp. PCC 7120 (Accession No. NP_484212); TreS, trehalose synthase from *Pimelobacter* sp. (Accession No. BAA11303); GgpS, glucosylglycerol-phosphate synthase from *Synechocystis* sp. PCC 6803 (Accession No. P74258); GgpP, glucosylglycerol-phosphate phosphatase from *Synechocystis* sp. PCC 6803 (Accession No. BAA18740); GpgS, glucosyl 3-phosphoglycerate synthase from *Persephonella marina* (Accession No. ABX75857); GpgP, glucosyl 3-phosphoglycerate phosphatase from *Persephonella marina* (Accession No. ABX75858); GSMT, glycine-sarcosine-*N*-methyltransferase from *Aphanothece halophytica* (Accession No. BAC56939); SDMT, sarcosine-dimethylglycine-*N*-methyltransferase from *Aphanothece halophytica* (Accession No. BAC56940).

–, gene is absent; +, gene is present in one copy; 2, gene is present in two copies; N-Fix, N_2-fixing strains.

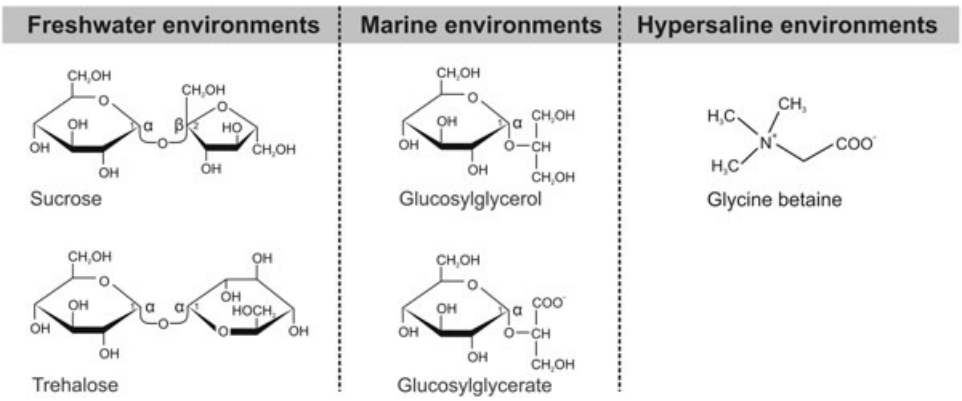

Fig. 2. Structure of major compatible solutes of cyanobacteria in correlation with the preferred habitats.

sheaths for salt acclimation is less investigated (Potts, 1994).

In general, the accumulation of Suc and Tre as main compatible solutes is associated with a rather low salt tolerance (stenohaline behaviour). The upper salt tolerance limit of Suc-accumulating strains comprises salinities equivalent to 50–100% seawater (Reed and Stewart, 1985). The participation of Suc in primary metabolism might be a reason for restricting its accumulation to lower concentrations that are insufficient to mediate high salt resistance. Furthermore, evidence has suggested that Suc and Tre are more important as general stress protectants (e.g. against desiccation) rather than salt stress-specific protectants. This view is supported by findings that their transient accumulation commonly occurs in more halotolerant cyanobacteria during the early acclimation phase. Moreover, a mutant of *Synechocystis* sp. PCC6803 that is unable to accumulate Suc displays a reduced survival rate of stationary phase cells, whereas steady-state growth under saline conditions is not significantly affected (Desplats *et al*., 2005).

Recently, Suc was identified as a main compatible solute in marine *Prochlorococcus* strains (Klähn *et al*., 2010). This unexpected finding indicate that it is likely not the Suc or Tre accumulation that restricts most of the freshwater strains to low salinities, but rather the capacity for inorganic ion export, which is the second prerequisite for successful salt acclimation that is limiting in most freshwater strains.

Glucosylglycerol as compatible solute in strains of moderate salt tolerance

Many cyanobacteria originating from marine environments are characterized by a moderate salt tolerance that allows them to grow from very low to up to threefold increases in seawater concentrations (seawater contains 3.5 g l^{-1} of total salts, about 600 mM, which is mostly represented by sodium and chloride ions comprising about 85% of total ions, i.e. equivalent to 510 mM or 3% of NaCl). In most cases, the accumulation of glucosylglycerol [GG, *O*-α-D-glucopyranosyl-(1→2)-glycerol, Fig. 2] provides a characteristic degree of salt resistance that is needed in marine habitats and is significantly higher than that of Suc- and/or Tre-accumulating strains. Accordingly, GG mainly occurs in strains from marine environments but was also found in freshwater isolates such as *Synechocystis* sp. PCC6803. Suc and/or Tre often occur as secondary solutes in these strains. In general, GG-accumulating strains are euryhaline because they can grow in a wide range of salinities, with an upper limit of 1.8 M NaCl (Reed and Stewart, 1985). However, marine picoplanktonic *Synechococcus* strains that accumulate GG display a rather stenohaline behaviour, which restricts them to salinities associated with normal oceanic conditions (Lu *et al*., 2006). Cells of euryhaline GG-producing strains contain virtually no GG when grown in low-salt mineral media. A sudden increase in external salinity activates GG synthesis immediately, and a stress-proportional steady-state level of GG is achieved after several hours (Reed and Stewart, 1985; Reed *et al*., 1985; Warr *et al*., 1985a; Hagemann *et al*., 1987).

GG was the first compatible solute identified in a cyanobacterial strain (Borowitzka *et al*., 1980). Initially, it was thought that GG synthesis and accumulation is restricted to cyanobacteria. Meanwhile, it has also been shown to function as an osmoprotectant in many heterotrophic bacteria, such as different *Pseudomonas* and *Stenotrophomonas* strains (Pocard *et al*., 1994; Mikkat *et al*., 2000; Roder *et al*., 2005). In addition to prokaryotes, steric isomers of GG have also been found in plants such as *Lilium japonicum* (Kaneda *et al*., 1984) and the resurrection plant *Myrothamnus flabellifolia* (Bianchi *et al*., 1993).

In general, it is assumed that compatible compounds do not carry a net charge at physiological pH, except for

some negatively charged solutes found in *Archaea* (Kempf and Bremer, 1998; Roeßler and Müller, 2001). Recently, in addition to Suc, the negatively charged compound glucosylglycerate [GGA, *O*-α-D-glucopyranosyl-(1→2)-glycerate, Fig. 2], which is structurally very similar to GG, was detected in osmotically relevant concentrations in several *Prochlorococcus* strains. GGA was also found in small amounts in the euryhaline, coastal strain *Synechococcus* sp. PCC7002 (Kollman *et al*., 1979; Klähn *et al*., 2010). Interestingly, GGA accumulation increases when cells are shifted to high-salt conditions under N-limited conditions. A stimulated accumulation of GGA under limiting N-conditions has also been detected in salt-treated cells of *Prochlorococcus* SS120 and of the picoplanktonic *Synechococcus* strains WH7803 and WH8102 (Klähn *et al*., 2010). Moreover, an N-starvation-dependent stimulation of GGA accumulation has been previously reported in salt-treated cells of the heterotrophic bacterium *Erwinia chrysanthemi* (Goude *et al*., 2004). In oceanic cyanobacteria, GGA acts to compensate for the positive charge of inorganic cations and seems to replace glutamate, which is usually used as counterion for K^+ in salt-stressed cells of microorganisms such as *Escherichia coli* (Dinnbier *et al*., 1988). The preference of GGA over glutamate is because cyanobacterial growth is usually limited by the amount of available combined nitrogen in the open ocean (Capone *et al*., 2008). In support of this hypothesis, GGA synthesis genes were not found in the genomes of marine N_2-fixing strains (Klähn *et al*., 2010; see Table 1).

Glycine betaine as compatible solute in strains of highest salt tolerance

Cyanobacteria are also common in hypersaline environments, in which they must cope with a very low osmotic potential and a high ionic strength up to saturated NaCl concentrations. The quaternary ammonium compound glycine betaine (GB, *N*,*N*,*N*-trimethylglycine, Fig. 2) is a typical major compatible solute in hypersaline cyanobacteria (Mackay *et al*., 1984; Reed *et al*., 1984b). Moreover, it is also a characteristic compatible solute in halotolerant heterotrophic bacteria and plants, which suggests that it possesses high protection efficiency. In addition to GB, those strains usually accumulate Suc, Tre, GG and/or other compounds at lower concentrations as additional compatible solutes. In salt-shocked cells of *Aphanothece halophytica* (*Synechococcus* sp. PCC7418) isolated from the Solar Lake (Israel), the synthesis of GB is preceded by a transient accumulation of GG and proline. However, in completely salt-acclimated *Aphanothece* cells, GB is clearly dominant, and GG is present only as a minor osmolyte (Fulda *et al*., 1999). Interestingly, GB accumulation has also been detected in cells of the marine *Synechococcus* strains WH7803 and WH8102 and in *Prochlorococcus* MIT9313 (Lu *et al*., 2006; Klähn *et al*., 2010). However, cells of strain WH8102 do not grow in medium containing more than 1 M NaCl (Lu *et al*., 2006). Clearly, the accumulation of GB is not always sufficient to increase the upper salinity tolerance limit to that of hypersaline strains. In addition to the widespread GB, the quaternary ammonia compound glutamate betaine has been reported in two hypersaline *Calothrix* strains (Mackay *et al*., 1984).

Compatible solutes of cyanobacteria from extreme environments

In extreme environments (e.g. desert crusts, hot springs, meltwater ponds), cyanobacteria are exposed to harsh and challenging conditions such as desiccation or high as well as low temperatures. Desiccated cells have little or no metabolic activity but rapidly resume metabolism upon rehydration. For example, desiccated cells of the terrestrial cyanobacterium *Nostoc commune* retain the capacity for cell growth for over 100 years (Lipman, 1941; Cameron, 1962). In natural habitats, *N. commune* forms visible colonies that consist of structurally complex EPS, which provide the basis for the desiccation tolerance of the embedded cell filaments (Hill *et al*., 1997). In addition to the EPS sheath, the cells of desiccation-tolerant *Nostoc* strains contain Tre and Suc for regulation of the intracellular water potential and/or membrane stabilization (Hill *et al*., 1994; 1997). In *N. commune* and the closely related *N. punctiforme* strain IAM M-15, Tre accumulation occurs in response to water loss during desiccation, whereas fully hydrated cells contain virtually no sugar (Sakamoto *et al*., 2009; Yoshida and Sakamoto, 2009). Similarly, addition of 0.2 M NaCl to *Nostoc* cells induces Tre accumulation to levels observed after desiccation. Albeit to a lesser extent, the Suc content was also increased in response to desiccation and salt treatments (Yoshida and Sakamoto, 2009). Tre and Suc accumulation in response to matric water and/or osmotic stress has been observed in other drought-resistant strains, such as *Scytonema* sp., *Phormidium autumnale* and a *Chroococcidiopsis* strain (Hershkovitz *et al*., 1991; Page-Sharp *et al*., 1999). The hypothesis that Tre and Suc have beneficial effects in tolerance to desiccation was confirmed in experiments with *E. coli*, in which both osmotically induced Tre accumulation and genetically established Suc accumulation also mediated enhanced desiccation tolerance, whereas GB accumulation did not (Welsh and Herbert, 1999; Billi *et al*., 2000).

Thermophilic organisms often accumulate special low-molecular-mass compounds as thermoprotectants. However, for cyanobacteria living in hot environments only a few data are available. Di-myo-inositol-1,1′-phosphate

(DIP) or mannosylglycerate (MG) are characteristic solutes of thermophilic *Archaea* and *Bacteria* (Santos and Costa, 2002). In general, MG seems to have a primary role in salt acclimation, because its accumulation is regulated mainly by salt in thermophilic organisms. In contrast, DIP functions as a true thermoprotectant that accumulates specifically at suboptimal growth temperatures. In addition to thermospecific solutes, compatible solutes such as Tre are also found in cells of heat-tolerant prokaryotes (Santos and Costa, 2002). To date, DIP and MG have not been detected in cyanobacteria. The strains *Fischerella* sp. PCC7414 and *Nostoc* sp. PCC7524, which were isolated from hot springs, accumulate Tre or Suc as compatible solutes (Reed *et al*., 1984a). However, the carbohydrate accumulation was only analysed in response to salt stress and not at different temperatures. Therefore, it is not clear whether real heat-specific protective compounds are present in these strains. As mentioned, the frequent accumulation of Suc or Tre as a secondary or tertiary compound in halotolerant strains may be related to its general protective role (e.g. towards higher temperatures) rather than to a salt-specific response. In this regard, it is interesting to note that the growth temperature also has an impact on the synthesis of both compounds. An increase in the Suc/GG ratio at elevated temperatures has been observed in salt-treated cells of *Synechocystis* sp. PCC6714 (Warr *et al*., 1985b). A crucial role for Tre accumulation in mediating thermotolerance has also been shown for yeast in which mutations of Tre synthesis result in a significant decrease of thermotolerance (De Virgilio *et al*., 1994). Therefore, Tre or Suc accumulation might be beneficial for thermophilic cyanobacteria, and accordingly, such strains harbour genes that encode for proteins potentially involved in Tre or Suc biosyntheses (see Table 1).

Cyanobacteria are also found in cold habitats such as meltwater ponds. Those strains are often psychrophilic and show growth optima below 15°C (Nadeau and Castenholz, 2000). Since compatible solutes play also an important role during the acclimation to low temperatures or freezing one could expect their temperature- or stress-dependent accumulation in psychrophilic cyanobacteria. However, compatible solute accumulation has not been investigated in those strains.

Direct protective effects of compatible solutes on macromolecules

The loss of cellular water through osmosis or dehydration results in profound changes in the physical properties of biomolecules, particularly phospholipids and proteins (Crowe *et al*., 1990). In this context, compatible solutes are important stabilizing agents. During recent years, the protection of cellular macromolecules against denaturation by e.g. heat, freezing or drying has been analysed *in vitro* for various compatible solutes, including those that are commonly detected in cyanobacteria. In general, it is difficult to extrapolate data obtained from an artificial system in a physiological context, because stress acclimation (involving protection of cell components) is not exclusively associated with the accumulation of compatible solutes (e.g. the general stress response also includes stabilization by chaperons or alteration of the membrane composition).

Borges and colleagues (2002) assessed protection of the model enzyme lactate dehydrogenase (LDH, isolated from rabbit muscle) *in vitro* against thermal inactivation by compatible solutes that occur naturally in prokaryotes. Interestingly, GG, which is widespread among marine cyanobacteria, was found to function as a stabilizer in a manner similar to MG, which is mainly found among thermophilic prokaryotes. Interestingly, the components of GG, glucose and glycerol showed no significant thermoprotective effects. For Tre, an intermediate behaviour was observed. Analyses of the thermostabilizing effects on malate dehydrogenase (isolated from pig heart) revealed a similar protection capacity of Tre and GB, but both performed better than glycerol, which is a canonical enzyme stabilizer used in biotechnological applications (Diamant *et al*., 2001). Recently, a stabilizing effect was also demonstrated for GGA, which protects LDH against thermal inactivation to a greater extent than does Tre and even GG (Sawangwan *et al*., 2010). However, the thermal stability of the other two enzymes (starch phosphorylase from *Corynebacterium callunae* and xylose reductase from *Candida tenuis*) was positively affected by GGA in a manner similar to that observed with Tre and GG. These data indicate that even small differences in chemical structure may be crucial for the ability of a certain solute to stabilize a given enzyme, but the degree of the protective effect often differs depending on the selected test enzyme. It is interesting to note that direct protective effects of compatible solutes are usually exhibited at lower concentrations than are necessary for osmotic effects.

The residual activity of enzymes in the presence or absence of various compatible solutes has also been considered following exposure to freeze-thawing cycles or freeze-drying followed by rehydration, which simulate frost or desiccation stresses. The stabilization of proteins during drying requires a direct interaction between the stabilizing molecule and the protein, and it probably involves hydrogen bonding between the stabilizer and polar residues in the protein (Crowe *et al*., 1990; Potts, 1994). Tre and Suc were found to be the best stabilizers for LDH and phosphofructokinase under both conditions: freeze-thawing and freeze-drying (Carpenter and Crowe, 1988; Carpenter *et al*., 1990). In contrast, glycerol, which is often used for cryoprotection of proteins, demonstrated

a lower efficiency in the protection of phosphofructokinase activity against freezing and showed no protective effect when the enzyme was exposed to freeze-drying. GB also demonstrated a significant protection of LDH during freezing but with a lower efficiency than those of Tre and Suc. Moreover, the residual activity of mannitol 2-dehydrogenase from *Pseudomonas fluorescens* after a freeze-thawing cycle in the presence of GG and GGA revealed a protection comparable to that of Tre (Sawangwan *et al*., 2010). It should be mentioned that freezing and desiccation are fundamentally different stresses. Whereas the specificity of solutes in the stabilization of macromolecules during freezing is low, only a few (of which carbohydrates are the most effective) mediate a similar protection during drying (Crowe *et al*., 1990). The efficiency of GG, GGA and GB in mediating protein protection during drying has not been investigated so far.

Because membranes are often the primary targets of cellular damage under stress conditions, the protective properties of compatible solutes have also been examined for liposomes (Crowe *et al*., 1990; Hincha and Hagemann, 2004). These studies revealed that only carbohydrates were effective for this purpose. The stabilization of phospholipid bilayers during drying requires a direct interaction between the sugar and polar head groups of the phospholipids (Crowe *et al*., 1990). Accordingly, the reverse order among the tested compatible solutes was found when their ability to confer salt stress tolerance to cyanobacteria was assessed *in vivo*. Suc and Tre were the best protectants for membrane integrity, whereas GG was less effective, and GB showed only minimal effects (Hincha and Hagemann, 2004). This result implies that membrane stability may not be the limiting factor for salt-stressed cyanobacterial cells. Suc and Tre accumulation mostly results in a rather low degree of salt resistance, but both are used as minor solutes in more halotolerant strains to provide greater general stress tolerance (Hagemann, 2010). As mentioned above, Suc and Tre are often found in desiccation-tolerant strains, which is consistent with the superior protein- and membrane-protective properties of Suc and Tre during drying. Both carbohydrates may also contribute to membrane stability at increased temperatures. Correspondingly, the genome sequences of thermophilic strains frequently reveal the presence of Tre or Suc biosynthesis-related genes (see Table 1).

Biochemical and molecular basis for compatible solute accumulation

In photoautotrophic organisms such as cyanobacteria, compatible solutes are synthesized by *de novo* synthesis. Additionally, uptake systems for these compounds are known, and they mainly serve to reuptake lost compatible solutes (e.g. Mikkat *et al*., 1996; Hagemann *et al*., 1997a; Mikkat and Hagemann, 2000). In contrast, in heterotrophic bacteria, the uptake of compatible solutes is preferred (Bremer and Krämer, 2000). The biosynthesis of most compatible solutes and the corresponding genes have been revealed during recent years. Sugar (Suc, Tre) and heteroside (GG, GGA) pathways share clear similarities. In most organisms, a two-step reaction is used that starts with a glucosyltransferase reaction that leads to a phosphorylated intermediate, which is subsequently hydrolysed to the final compatible solute via a phosphatase reaction (Fig. 3).

Salt-induced Suc biosynthesis in cyanobacteria is achieved by sucrose-phosphate synthase (SPS, EC 2.4.1.14), which produces the intermediate sucrose-phosphate from UDP-glucose and fructose 6-phosphate (Porchia and Salerno, 1996). Sucrose 6-phosphate is subsequently hydrolysed to Suc by sucrose-phosphate phosphatase (SPP, EC 3.1.3.24). The *spsA* gene encodes a large protein of approximately 80 kDa with an N-terminal domain that contains all of the features required for SPS activity and with a C-terminal domain of about 20 kDa that shares similarities with SPP. Knockout mutations of this gene result in a complete absence of Suc synthesis and SPS activity in *Synechocystis* sp. PCC6803 (Hagemann and Marin, 1999). Despite its structural similarity to the combined SPS/SPP proteins from plants, the SPS from *Synechocystis* sp. PCC6803 displays only SPS activity (Lunn *et al*., 1999). The gene encoding the cooperating SPP was identified later (Lunn, 2002). Interestingly, in some strains, the combined SPS/SPP protein is probably also capable of performing the phosphatase reaction, because no separate SPP protein has been discovered (see Table 1). Additionally, a one-step reaction that lacks a phosphorylated intermediate catalysed by sucrose synthase (Sus, EC 2.4.1.13) has also been found (Lunn, 2002); however, this reaction functions mostly to degrade Suc and is not involved in Suc accumulation (Curatti *et al*., 2002). Among cyanobacteria, the ability to synthesize Suc seems to be universal, because at least one Suc-synthesizing enzyme is encoded in almost all of the known genome sequences, including the reduced genomes of the marine picoplanktonic strains (see Table 1; Scanlan *et al*., 2009).

The biosynthetic pathway for Tre was initially investigated in *E. coli*, where the trehalose 6-phosphate synthase (TPS, EC 2.4.1.15) converts UDP-glucose and glucose 6-phosphate to trehalose 6-phosphate, which is then hydrolysed by trehalose 6-phosphate phosphatase (TPP, EC 3.1.3.12; Giæver *et al*., 1988; Kaasen *et al*., 1992; Fig. 3). Homologues of these proteins are widespread among bacteria and eukaryotes. In the genome of *Crocosphaera watsonii* WH8501, a unicellular, oceanic N_2-fixing cyanobacterium, similarity searches have

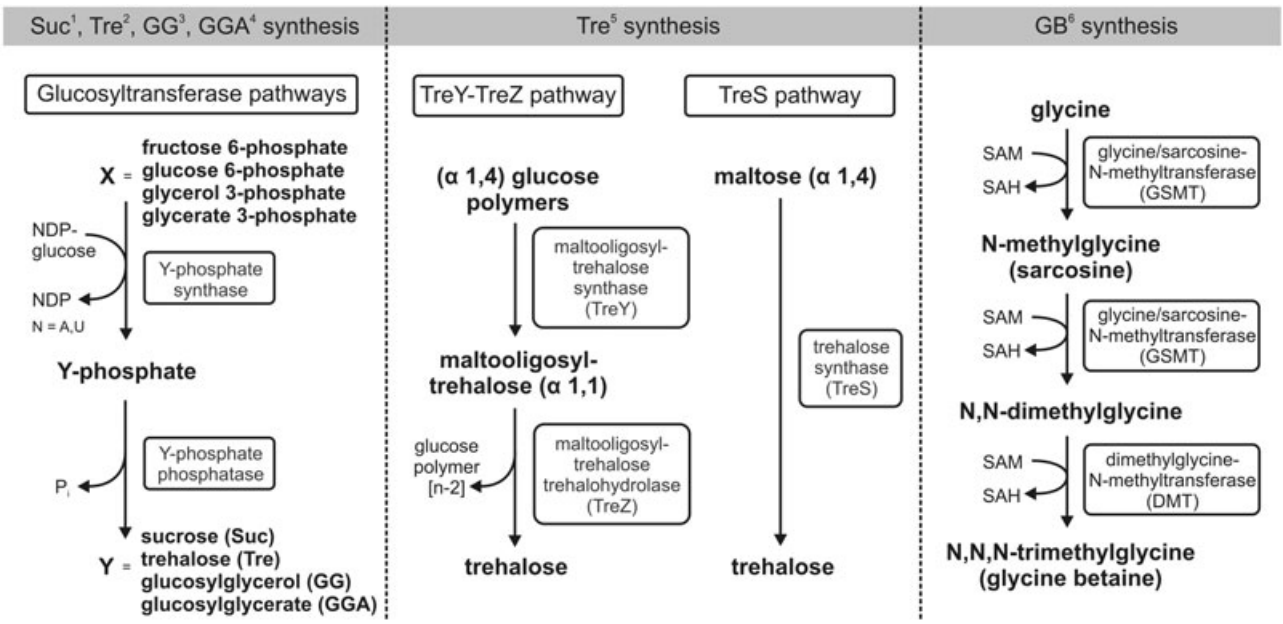

Fig. 3. Synthesis of compatible solutes among cyanobacteria. Superscripted numbers indicate strains in which the biosynthetic pathway was verified or predicted. 1 – *Synechocystis* sp. PCC6803; 2 – *Crocosphaera watsonii* WH8501 (postulated); 3 – *Synechocystis* sp. PCC6803, *Synechococcus* sp. PCC7002; 4 – *Synechococcus* sp. PCC7002, *Prochlorococcus* sp. SS120; 5 – *Nostoc* sp. PCC7120, *Nostoc punctiforme* IAM M-15; 6 – *Aphanothece halophytica*, *Synechococcus* sp. WH8102; NDP-glucose – nucleoside diphospho-glucose (A = Adenosine, U = Uridine); SAM – S-adenosylmethionine; SAH – S-adenosylhomocysteine.

revealed a fusion protein that contains TPS and TPP domains (Table 1). Combined TPS/TPP proteins are known to be involved in Tre synthesis in plants and yeasts. Surprisingly, no other TPS and/or TPP homologues have been discovered among cyanobacteria, although many freshwater and desiccation-tolerant strains are able to produce Tre. In these strains, Tre is synthesized by other pathways that involve the degradation of polysaccharides to the disaccharide Tre (TreY/TreZ pathway) or the inversion of maltose (TreS pathway, Fig. 3). Stress regulation of the alternative TreY/TreZ pathway has been verified in the model strain *Nostoc* (*Anabaena*) sp. PCC7120 (Higo *et al*., 2006). The primary reaction is catalysed by the maltooligosyltrehalose synthase (Mts, EC 5.4.99.15) encoded by *treY*. This enzyme converts the terminal α(1,4)-linked residue of a glucose polymer to an α(1,1) linkage. In the subsequent step, the terminal disaccharide is cleaved by the maltooligosyltrehalose trehalohydrolase (Mth, EC 3.2.1.141) encoded by *treZ*. Similar proteins involved in Tre synthesis have also been found in the related strain *Nostoc punctiforme* IAM M-15 (Yoshida and Sakamoto, 2009) and in *Arthrospira* (*Spirulina*) *platensis* NIES-39 (Ohmori *et al*., 2009) and are present in the genomes of many other cyanobacteria (Table 1). In addition, homologues of a maltose glucosylmutase that converts maltose to Tre [= trehalose synthase (TreS), EC 5.4.99.16, Nishimoto and colleagues 1996] are also present in many cyanobacterial genomes (Table 1). Interestingly, the thermophilic strains *Synechococcus* JA-2-3B'a (2–13) and JA-3-3Ab also harbour these genes, which suggests that Tre is accumulated. In contrast, the thermophilic strain *Thermosynechococcus elongatus* BP-1 appears to accumulate mainly Suc, because no known genes involved in Tre biosynthesis could be detected in its genome. The enzymes for other heat-specific solutes (e.g. MG) were not detected in any of these strains.

Concerning GG, the first reaction is carried out by the GG-phosphate synthase (GgpS, EC 2.4.1.213) with glycerol 3-phosphate and ADP-glucose, and the final product, GG, is generated by the GG-phosphate phosphatase (GgpP, EC 3.1.3.69) (Hagemann and Erdmann, 1994; Fig. 3). The corresponding genes *ggpS* and *ggpP* have been identified in *Synechocystis* sp. PCC6803 (Hagemann *et al*., 1997b; Marin *et al*., 1998). Cyanobacterial mutants with inactivated *ggpS* genes demonstrate a salt-sensitive phenotype despite their increased content of the secondary compatible solute Suc (Marin *et al*., 1998; Engelbrecht *et al*., 1999). Homologous proteins for GG synthesis are present in the genomes of all strains that are known to produce GG, including marine, picoplanktonic isolates of the genus *Synechococcus* (Scanlan *et al*., 2009; Klähn *et al*., 2010; Table 1). Interestingly, *Prochlorococcus* spp. probably lost the ability to synthesize GG after they diverged from the *Synechococcus* clade. A gene very similar to *ggpP* is present in the

genomes of all *Prochlorococcus* strains, but these strains do not carry a *ggpS* gene and accordingly do not accumulate GG (Klähn *et al*., 2010). In many heterotrophic bacteria that accumulate GG after salt stress, biosynthesis is accomplished by a large fusion protein called GgpPS that possesses GgpS activity in its C-terminal region and GgpP activity in its N-terminal region (Hagemann *et al*., 2008).

Biosynthesis of the structurally related but negatively charged GGA also involves a glucosyltransferase, glucosyl-phosphoglycerate synthase (GpgS), which uses an NDP-glucose (preferentially ADP-glucose in cyanobacteria) and glycerate 3-phosphate to produce GGA-phosphate, which is hydrolysed to GGA by the glucosyl-phosphoglycerate phosphatase (GpgP) (Costa *et al*., 2006). Based on GpgS and GpgP sequences from heterotrophic bacteria, genes encoding these proteins were identified in the genomes of many marine cyanobacteria. In *Synechococcus* sp. PCC7002 and *Prochlorococcus* SS120, the biological activity of these gene products was verified (Klähn *et al*., 2010). The capacity to synthesize GGA seems to be common to all oceanic cyanobacteria that are incapable of fixing N_2 (see Table 1; Klähn *et al*., 2010).

In most bacteria and plants, GB is synthesized by a two-step oxidative pathway that begins with choline via the toxic intermediate betaine aldehyde (Bremer and Krämer, 2000; Chen and Murata, 2002), whereas a three-step methylation pathway that starts with glycine followed by GB has been demonstrated in the halophilic cyanobacterium *Aphanothece halophytica* (Waditee *et al*., 2003). The latter reactions are catalysed by glycine-sarcosine-*N*-methyltransferease (GSMT, EC 2.1.1.156), which methylates glycine to sarcosine (*N*-methylglycine), and sarcosine-dimethylglycine-*N*-methyltransferase (SDMT, EC 2.1.1.157), which catalyses the methylation of sarcosine and dimethylglycine to dimethylglycine and betaine respectively (Fig. 3). Recently, it has been demonstrated that this pathway is also used by a few marine picoplanktonic *Synechococcus* and *Prochlorococcus* strains (Lu *et al*., 2006; Klähn *et al*., 2010). In addition to the genes encoding GSMT and SDMT, no genes coding for enzymes that are known to synthesize GB via another pathway are present in the complete genome sequences of these and other cyanobacterial strains (Table 1).

Conclusions and perspectives

The accumulation of compatible solutes is an important key step in stress acclimation not only towards salt but also in response to other stress treatments. Based on the accumulated knowledge of compatible solute synthesis obtained using cyanobacterial model strains, the salt acclimation strategy of less investigated strains (e.g. cyanobacteria that are difficult to cultivate such as picoplanktonic strains) can be predicted based on the increased amount of available genome information (e.g. Scanlan *et al*., 2009), including metagenomics. However, to date, salt acclimation was mostly studied after exposure to defined NaCl concentrations, which does not reflect the natural diversity of saline waters characterized by widely differing ion compositions and pH values. It is also interesting to note that even in the case of the best investigated model strains, many open questions concerning basic salt acclimation strategies remain to be elucidated. For example, transcriptomics using salt-stressed cells of *Synechocystis* sp. PCC6803 revealed a salt-induced upregulation of several hundred genes (Kanesaki *et al*., 2002; Marin *et al*., 2003; 2004); however, among the genes that demonstrated the most robust induction, about 20% encoded hypothetical proteins of a completely unknown function.

The existing knowledge regarding the genetic basis of cyanobacterial compatible solute accumulation could also be used to improve the stress tolerance of sensitive organisms. Several attempts have been undertaken to transfer compatible solute-synthesizing genes from cyanobacteria (and other microorganisms) into plants. In the model plant *Arabidopsis thaliana*, coexpression of the GSMT and SDMT genes from *A. halophytica* leads to significant amounts of GB in plant tissues and consequently to improved growth performance under saline conditions (Waditee *et al*., 2005). Recently, GG synthesis was also established in *Arabidopsis* using the *ggpPS* genes from heterotrophic bacteria, and accordingly, GG accumulation resulted in improved salt tolerance (Klähn *et al*., 2009). Similar results were obtained using salt-sensitive cyanobacterial cells as recipients of such salt resistance genes (e.g. Deshnium *et al*., 1995; Waditee *et al*., 2005). For the future production of bioenergy, the mass cultivation of cyanobacteria and algae will preferentially be performed in saline waters. A detailed knowledge on the natural acclimation of these organisms to adverse environmental conditions will also facilitate the design of advanced cyanobacterial production strains for biotechnological purposes.

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