CHAPTER TWO

Genomics of Salt Acclimation: Synthesis of Compatible Solutes among Cyanobacteria

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Contents

Abstract

During their long evolution, cyanobacteria were able to inhabit all light-exposed ecosystems. One of the main environmental factors determining cyanobacterial distribution is the salinity of the surrounding medium. Among cyanobacterial strains, three main salt-tolerance groups can be distinguished: low- and moderate-halotolerant cyanobacteria as well as hypersaline strains. Regardless of the final salt resistance, all cyanobacteria apply two basic strategies for a successful acclimation to enhanced salt concentrations: accumulation of compatible solutes combined with active export of toxic ions, particularly Na^+ and Cl^-. During the years 1991–2010, the molecular basis of these mechanisms has been elucidated. Today, many complete genome sequences appear in databases of cyanobacterial strains, which are often difficult to cultivate in the laboratory. These data were used here to screen the genomes of more than 60 cyanobacteria regarding their compatible solute accumulation capacities. Hence, the existing knowledge about cyanobacterial salt acclimation was used to annotate basic salt-resistance mechanisms on the basis of genome information. Understanding the basic salt acclimation among cyanobacteria will also be useful for their future biotechnological application, which will be performed preferentially in saline waters.

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1. INTRODUCTION

Cyanobacteria can be found in every light-exposed habitat on Earth. The majority of cyanobacterial strains in culture collections (e.g. Pasteur culture collection of cyanobacteria, PCC, Paris, France) were isolated from aquatic habitats, but many cyanobacteria are also found in diverse terrestrial habitats such as on soils or surfaces of rocks. Beside nutrients, light and temperature, the availability of water and the amount of dissolved ions (total salinity) are important environmental factors determining the occurrence of strains in specific environments. Because total salinity and water amount are closely linked, e.g. during desiccation of soil, the amount of water is decreasing and, in parallel, the total salt concentration is increasing, it is not surprising that acclimation towards drought and high salinity employs overlapping mechanisms. In both cases, the maintenance of water and turgor pressure inside the cell is one of the central issues during the acclimation (experimentally verified for cyanobacteria by Ladas and Papageorgiou (2000)). Because water uptake is a passive process following the water potential gradient, growing microbial cells need to establish a lower water potential inside the cell relative to the surrounding medium, which is achieved by regulating the cellular osmotic potential via varying amounts of low molecular compounds.

The main difference between pure water or osmotic stress and salt stress is the additional direct ion effect on metabolic activities in the latter case. In the nature exists large variations regarding the amount and composition of inorganic salts, which clearly affects cyanobacterial distribution (Oren, 2000). In addition to the problem that high total ion content generally makes it difficult to maintain water and turgor inside the cell, many ions are toxic for living cells. This direct toxicity is true not only for heavy metals but also for any ion at nonphysiological high cellular concentrations.

This chapter deals with cyanobacterial salt acclimation in the genomic era. Because the annotation of genome information relies on the knowledge of well-studied model organisms, the author will use here the term high salt equivalent for an enhanced Na^+ and Cl^- contents, the main inorganic ions in the marine environment, which are usually used in laboratory experiments to mimic salt stress.

2. BASIC SALT ACCLIMATION STRATEGY

To acclimate to a quick increase in the external salinity or to live permanently in the high salt environment, cyanobacteria as most other cells apply the so-called 'salt-out' strategy (Galinski, 1995). The other strategy is called 'salt-in' strategy, which is used by some halophilic Archaea and Bacteria. The latter prokaryotes accumulate high internal amounts of inorganic salts (especially KCl) exceeding the external salt concentration. The accumulation of inorganic ions is metabolically cheaper to increase internal osmotic concentrations and to ensure water uptake, turgor pressure and growth. However, the presence of high salt concentrations in the metabolically active compartment needed an adaptation of all organic macromolecules to this new environment, which seemed to be difficult to achieve during evolution. This assumption explains why the energetic favourable 'salt-in' strategy is restricted to a few prokaryotes.

In contrast, organisms using the 'salt-out' strategy can keep the normal set of low-salt-resistant proteins for metabolic activity but needed to find an energetically more expensive strategy to balance the osmotic potential difference. These organisms are characterized by almost unchanged internal ion concentration after acclimation to high NaCl concentrations. This observation is especially true for amounts of Na^+ and Cl^- (e.g. shown for *Synechocystis* sp. PCC 6714 by Reed, Warr, Richardson, Moore, and Stewart (1985)). To keep the low internal NaCl concentration in the presence of high external salinities, Na^+ and Cl^- are actively pumped out from the cells. Multiple transporters are used for Na^+ export by cyanobacteria. Predominantly, specific members of the Na^+/H^+ antiporter family seem to be involved (Elanskaya, Karandashova, Bogachev, & Hagemann, 2002; Inaba, Sakamoto, & Murata, 2001; Waditee et al., 2001, 2002; Wang, Postier, & Burnap, 2002). Additionally, the Mrp system is used for Na^+ export (Blanco-Rivero, Leganés, Fernández-Valiente, Calle, & Fernández-Piñas, 2005; Fukaya et al., 2009). These transporters receive the energy for ion export from the proton gradient at the membrane. For many years, it was discussed if cyanobacteria and eukaryotic algae also employ primary active Na^+-ATPases for ion export during salt acclimation (Gimmler, 2000). Only recently, direct experimental evidence for the presence and activity of Na^+-ATPases of the F_1F_0-type was presented for *Aphanothece halophytica* (Soontharapirakkul et al., 2011) and from genome information for some more

strains like *Acaryochloris marina* (Dibrova, Galperin, & Mulkidjanian, 2010). Compared to Na^+, the export of Cl^- is much less well understood among cyanobacteria (for a review, see Hagemann (2011)).

Additional to the energy demand for ion export, cells using the 'salt-out' strategy need energy and organic matter for the accumulation of high amounts of compatible solutes, which are used instead of inorganic ions to balance the osmotic potential and to maintain turgor. These compounds are low-molecular-mass organic molecules that are highly water soluble and usually do not carry net charge at physiological pH. Compatible solutes can be accumulated in high (molar) amounts without negative interference (i.e. being compatible) towards the metabolisms (Brown, 1976). In addition to the osmotic equilibrium, the compatible solutes can also exhibit direct protective effects towards sensitive macromolecules. The protective effect explains why often the accumulation of rather low amounts of compatible solutes, i.e. at concentrations not making big contribution to the intracellular osmotic potential, results in significant increase of salt or drought stress tolerance (for a review, see Chen and Murata (2011)).

3. COMPATIBLE SOLUTES

After the first description of glucosylglycerol (GG) as compatible solute in a marine *Synechococcus* strain (Borowitzka, Demmerle, Mackay, & Norton, 1980), about 200 cyanobacteria were screened for such compounds and their salt-resistance range (see Hagemann (2011) for a comprehensive table). This data set revealed that a rather small spectrum of compatible solutes is found in salt-loaded cyanobacteria, and, that a correlation between the chemical nature of the compatible solute and the salt resistance limits exists (Reed, Borowitzka et al., 1986). Accordingly, group 1 of low salt tolerance accumulates sucrose and/or trehalose (150 examples), group 2 of moderate halotolerance prefers GG (71 examples), and group 3 of halophilic strains have an absolute requirement for a minimal salt concentration and synthesize glycine betaine (22 examples) as characteristic compatible solute. Unfortunately, cyanobacterial taxonomy is problematic. Species names as well as strain numbers have been changed or mixed. Therefore, it is difficult to make a direct comparison of the strain list with compatible solutes (see Hagemann, 2011) and the list of compatible solute genes derived from genome sequences of cyanobacteria (Table 2.1).

In this chapter, the author will often refer to two big groups of cyanobacteria, which were initially distinguished according to their RubisCO

(one carrying RubisCO form I-A or RubisCO form I-B) and carboxysome types (Hess et al., 2001). Later they were named alpha-cyanobacteria (mostly picoplanktonic oceanic *Prochlorococcus* and *Synechococcus* strains) and beta-cyanobacteria (Badger, Hanson, & Price, 2002). To analyse the molecular basis of compatible solute synthesis, mostly beta-cyanobacterial strains have been investigated: *Nostoc* (*Anabaena*) sp. PCC 7120 (hereafter *Nostoc* 7120) for group 1, *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803) for group 2, and *A. halophytica* (hereafter *Aphanothece*) for group 3.

3.1. Sucrose

Sucrose (α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside) accumulation was often found in salt-stressed cyanobacteria. Still it is possible to conclude that all heterocystous, N_2-fixing cyanobacteria use sucrose as major compatible solute at elevated salinity, while generally the preference for a specific compatible solute is not correlated with any other specific cyanobacterial clade (Fig. 2.1). The widespread occurrence of sucrose in salt-stressed cyanobacteria is not surprising because sucrose plays a central role in the carbon metabolism in photoautotrophic organisms (Kolman, Torres, Martin, & Salerno, 2011; Lunn, 2002).

It has been shown that four enzymes are crucial for sucrose accumulation inside photoautotrophic cells. Sucrose-phosphate synthase (Sps) and sucrose-phosphate phosphatase (Spp) are the main sucrose biosynthesis enzymes:

(Sps) UDP-glucose + fructose 6-phosphate → sucrose-phosphate + UDP

(Spp) Sucrose-phosphate → sucrose + Pi

The sucrose synthase (Sus) can catalyse the following reversible reaction; however, it is believed to predominantly degrade sucrose:

(Sus) Sucrose + UDP ↔ UDP-glucose + fructose

Additionally, invertase (sucrase) is known to irreversibly hydrolyse sucrose:

Sucrose → fructose + glucose.

Thus, the activity of sucrose synthesis enzymes relative to the two sucrose degrading enzymes should determine the sucrose steady-state level in cyanobacterial cells (Kolman et al., 2011).

Table 1 Sixty seven cyanobacterial genomes were searched in GenBank for genes coding enzymes for compatible solute biosynthesis by the BLAST algorithm (Altschul et al., 1997)

Strain	SpsA (Sll0045)	SpsA* (Alr3370)	SpsA* (All4376)	Spp (Slr0953)	OtsAB	TreZ
Beta-cyanobacteria						
Acaryochloris marina MBIC11017		YP_001517507.1	YP_001517507.1	YP_001517199.1		
Acaryochloris sp. CCMEE 5410		ZP_09247255.1	ZP_09247255.1	ZP_09249474.1		
Acaryochloris sp. CCMEE 5410		ZP_09251279.1	ZP_09251279.1			
Acaryochloris sp. CCMEE 5410		ZP_09251468.1	ZP_09251468.1			
Anabaena variabilis ATCC 29413		YP_323804.1	YP_323804.1	YP_323329.1		YP_321946.1
Anabaena variabilis ATCC 29413		YP_323913.1	YP_323913.1			
Arthrospira maxima CS-328						ZP_03275188.1
Arthrospira platensis NIES-39						
Arthrospira platensis str. Paraca						ZP_06384104.1
Arthrospira sp. PCC 8005						
Cyanobacterium UCYN-A						
Crocosphaera watsonii WH 8501					ZP_00516247.1	
Cyanothece sp. ATCC 51142				YP_001802623.1		
Cyanothece sp. CCY0110				ZP_01727894.1		ZP_01730695.1
Cyanothece sp. PCC 7424		YP_002378525.1	YP_002377234.1	YP_002379708.1		YP_002379851.1
Cyanothece sp. PCC 7424		YP_002377234.1	YP_002378525.1			
Cyanothece sp. PCC 7425			YP_002484407.1	YP_002485261.1		
Cyanothece sp. PCC 7425		YP_002482481.1	YP_002482481.1			
Cyanothece sp. PCC 7425		YP_002483625.1	YP_002483625.1			
Cyanothece sp. PCC 7822		YP_003885909.1	YP_003885909.1	YP_003887642.1		YP_003889479.1
Cyanothece sp. PCC 7822		YP_003886096.1	YP_003886096.1			
Cyanothece sp. PCC 8801						YP_002371610.1
Cyanothece sp. PCC 8802						YP_003137180.1
Cylindrospermopsis raciborskii CS-505		ZP_06309461.1	ZP_06309461.1	ZP_06306557.1		
Fischerella sp. JSC-11		ZP_08987552.1	ZP_08987552.1	ZP_08986780.1		ZP_08984802.1
Gleobacter violaceus PCC 7421		NP_926786.1	NP_926786.1	NP_926785.1		
Lyngbya sp. PCC 8106						ZP_01620214.1
Microcoleus chthonoplastes PCC 7420		ZP_05024229.1	ZP_05024229.1	ZP_05024293.1		ZP_05027447.1
Microcoleus chthonoplastes PCC 7420				ZP_05029649.1		
Microcoleus vaginatus FGP-2		ZP_08493316.1	ZP_08493316.1	ZP_08495240.1		ZP_08493057.1
Microcystis aeruginosa NIES-843						

TreY	TreA	GgpS	GgpP	GpgS	GgpgP	GsmT	Dmt
		YP_001516020.1	YP_001516026.1	YP_001517954.1	YP_001517956.1		
		ZP_09250227.1	ZP_09250231.1	ZP_09250357.1	ZP_09250359.1		
YP_321945.1	YP_321944.1						
ZP_03275187.1		ZP_03273530.1	ZP_03275431.1	ZP_03275227.1	ZP_03274421.1		
BAI87980.1		BAI93049.1		BAI91147.1	BAI90985.1		
ZP_06384103.1		ZP_06380107.1	ZP_06380589.1	ZP_06384311.1	ZP_06380864.1		
ZP_09784258.1		ZP_09780739.1	ZP_09781502.1		ZP_09782039.1		
		YP_003421551.1					
		YP_001803806.1	YP_001804705.1				
	ZP_01732618.1	ZP_01726261.1	ZP_01726605.1				
YP_002379850.1							
YP_003889480.1							
YP_002371611.1	YP_002371612.1						
YP_003137181.1							
ZP_08984801.1							
ZP_01620213.1				ZP_01620950.1	ZP_01623921.1	ZP_01618784.1	ZP_01618784.1
ZP_05027675.1		ZP_05025446.1	ZP_05024720.1	ZP_05025802.1	ZP_05030789.1		
ZP_08493056.1							

Continued

Table 1 Sixty seven cyanobacterial genomes were searched in GenBank for genes coding enzymes for compatible solute biosynthesis by the BLAST algorithm (Altschul et al., 1997)—cont'd

Strain	SpsA (Sll0045)	SpsA* (Alr3370)	SpsA* (All4376)	Spp (Slr0953)	OtsAB	TreZ
Microcystis aeruginosa PCC 7806		CAO88729.1	CAO88729.1	CAO88727.1		
Moorea product 3L		ZP_08427749.1	ZP_08427749.1	ZP_08432285.1		
Moorea product 3L						
Nodularia spumigena CCY9414		ZP_01631316.1	ZP_01631316.1	ZP_01628463.1		ZP_01632239.1
Nodularia spumigena CCY9414	ZP_01629520.1			ZP_01629520.1		
Nostoc azollae 0708		YP_003720706.1	YP_003720706.1	YP_003721505.1		
Nostoc azollae 0708		YP_003722939.1	YP_003722939.1			
Nostoc punctiforme PCC 73102		YP_001865190.1	YP_001865190.1	YP_001866500.1		YP_001868949.1
Nostoc punctiforme PCC 73102		YP_001866499.1	YP_001866499.1	YP_001866908.1		
Nostoc punctiforme PCC 73102		YP_001867882.1	YP_001867882.1			
Nostoc sp. PCC 7120		NP_488416.1	NP_488416.1	CAC43285.1		NP_484212.1
Nostoc sp. PCC 7120		NP_487410.1	NP_487410.1	NP_484420.1		
Oscillatoria sp. PCC 6506						ZP_07112724.1
Synechococcus elongatus PCC 6301	YP_171440.1			YP_171440.1		
Synechococcus elongatus PCC 7942	YP_399827.1			YP_399827.1		
Synechococcus sp. JA-2-3B'a(2-13)				YP_478310.1		YP_478726.1
Synechococcus sp. JA-3-3Ab				YP_473831.1		YP_473674.1
Synechococcus sp. PCC 7002	AAR31179.1			AAR31179.1		
Synechococcus sp. PCC 7002				YP_001734147.1		
Synechococcus sp. PCC 7335						ZP_05036594.1
Synechocystis sp. PCC 6803	NP_442711.1			NP_441739.1		
Synechocystis sp. PCC 6803				NP_442711.1		
Thermosynechococcus elongatus BP-1	NP_681372.1			NP_681372.1		
Thermosynechococcus elongatus BP-1		NP_682380.1	NP_682380.1			
Trichodesmium erythraeum IMS101						
Alpha-cyanobacteria						
Cyanobium sp. PCC 7001	ZP_05045051.1			ZP_05045051.1		
Cyanobium sp. PCC 7001				ZP_05045012.1		
Prochlorococcus marinus str. AS9601	YP_001010309.1					
Prochlorococcus marinus str. CCMP1375	NP_876271.1					
Prochlorococcus marinus str. CCMP1986	NP_893828.1					
Prochlorococcus marinus str. MIT 9211	YP_001551734.1					
Prochlorococcus marinus str. MIT 9215	YP_001485182.1					

TreY	TreA	GgpS	GgpP	GpgS	GgpgP	GsmT	Dmt
							ZP_08431884.1 ZP_08430526.1
ZP_01632238.1							
YP_001868950.1	YP_ 001868951.1						
NP_484211.1	NP_ 484210.1						
ZP_07112723.1							
YP_478367.1					YP_478588.1		
YP_473881.1					YP_475220.1		
		YP_ 001736074.1	YP_001736065.1	YP_001735263.1	YP_001735265.1		
ZP_05038218.1	ZP_ 05036580.1	ZP_05035448.1	ZP_05036760.1				
		NP_441672.1	NP_442928.1				
							YP_722132.1
		ZP_05043859.1	ZP_05046321.1				
			YP_001009088.1	YP_001009287.1	YP_001009289.1		
			NP_875188.1	NP_875119.1	NP_875121.1		
			NP_892757.1	NP_893079.1	NP_893077.1		
			YP_001550632.1	YP_001550559.1	YP_001550561.1		
			YP_001483923.1	YP_001484127.1	YP_001484129.1		

Continued

Table 1 Sixty seven cyanobacterial genomes were searched in GenBank for genes coding enzymes for compatible solute biosynthesis by the BLAST algorithm (Altschul et al., 1997)—cont'd

Strain	SpsA (Sll0045)	SpsA* (Alr3370)	SpsA* (All4376)	Spp (Slr0953)	OtsAB	TreZ
Prochlorococcus marinus str. MIT 9301	YP_001092125.1					
Prochlorococcus marinus str. MIT 9303	YP_001019012.1			YP_001019012.1		
Prochlorococcus marinus str. MIT 9312	YP_398301.1					
Prochlorococcus marinus str. MIT 9313	NP_896092.1			NP_896092.1		
Prochlorococcus marinus str. MIT 9515	YP_001012216.1					
Prochlorococcus marinus str. NATL1A	YP_001016015.1			YP_001016015.1		
Prochlorococcus marinus str. NATL2A	YP_292514.1			YP_292514.1		
Synechococcus sp. BL107	ZP_01469083.1			ZP_01469083.1		
Synechococcus sp. CB0101	ZP_07974999.1			ZP_07972524.1		
Synechococcus sp. CB0101				ZP_07974999.1		
Synechococcus sp. CB0205	ZP_07969740.1			ZP_07969422.1		
Synechococcus sp. CB0205				ZP_07969740.1		
Synechococcus sp. CC9311	YP_732123.1			YP_732123.1		
Synechococcus sp. CC9605	YP_382969.1			YP_382969.1		
Synechococcus sp. CC9902	YP_378316.1			YP_378316.1		
Synechococcus sp. RCC307	YP_001228785.1			YP_001226627.1		
Synechococcus sp. RS9916	ZP_01471531.1			ZP_01471531.1		
Synechococcus sp. RS9917	ZP_01079206.1			ZP_01079206.1		
Synechococcus sp. WH 5701	ZP_01083582.1			ZP_01083584.1		
Synechococcus sp. WH 5701	ZP_01083584.1			ZP_01083585.1		
Synechococcus sp. WH 5701				ZP_01084261.1		
Synechococcus sp. WH 5701				ZP_01086245.1		
Synechococcus sp. WH 7803	YP_001226250.1			YP_001226250.1		
Synechococcus sp. WH 7805	ZP_01124878.1			ZP_01124878.1		
Synechococcus sp. WH 8016	ZP_08957469.1			ZP_08957469.1		
Synechococcus sp. WH 8102	NP_898609.1			NP_898609.1		
Synechococcus sp. WH 8109	ZP_05789248.1			ZP_05789248.1		

Sucrose-phosphate synthases (SpsA) involved in sucrose biosynthesis were searched with SpsA (Sll0045) from *Synechocystis* 6803 (proteins are similar with $2e^{-104}$ or better). Another group of sucrose-phosphate synthases (SpsA⋆) involved in sucrose biosynthesis were searched with SpsA⋆ (Alr3370 or All4376) from *Nostoc* 7120 (proteins are similar with $3e^{-72}$ or better). Sucrose-phosphate phosphatases (Spp) involved in sucrose biosynthesis were searched with Spp (Slr0953) from *Synechocystis* 6803 (proteins are similar with $9e^{-9}$ or better). Maltooligosyltrehalose synthases (TreY) involved in trehalose biosynthesis were searched with TreY (All0167) from *Nostoc* 7120 (proteins are similar with $7e^{-46}$ or better). Maltooligosyltrehalose trehalohydrolases (TreZ) involved in trehalose biosynthesis were searched with TreZ (All0168) from *Nostoc* 7120 (proteins are similar with $6e^{-113}$ or better). Glucosylglycerol-phosphate synthases (GgpS) involved in glucosylglycerol biosynthesis were searched with GgpS (Sll1566) from *Synechocystis* 6803 (proteins are similar with $3e^{-98}$ or better). Glucosylglycerol-phosphate phosphatases (GgpP) involved in glucosylglycerol biosynthesis were searched with GgpP (Slr0746) from *Synechocystis* 6803 (proteins are

TreY	TreA	GgpS	GgpP	GpgS	GgpgP	GsmT	Dmt
			YP_001090890.1	YP_001091118.1	YP_001091120.1	YP_001017710.1	YP_001017711.1
			YP_001017345.1				
			YP_397136.1	YP_397333.1	YP_397335.1		
			NP_894695.1			NP_894385.1	NP_894384.1
			YP_001011021.1	YP_001011359.1	YP_001011357.1		
			YP_001014527.1	YP_001014736.1	YP_001014738.1		
			YP_291274.1	YP_291440.1	YP_291442.1		
		ZP_01467787.1	ZP_01468168.1	ZP_01469150.1			
		ZP_07973425.1	ZP_07973431.1				
		ZP_07971307.1	ZP_07971313.1	ZP_07970707.1	ZP_07970705.1		
		YP_730607.1	YP_730381.1	YP_729592.1	YP_729594.1		
		YP_381716.1	YP_382080.1	YP_382889.1	YP_382887.1		
		YP_377087.1	YP_376875.1	YP_378245.1	YP_378243.1		
		YP_001227629.1	YP_001227635.1	YP_001228257.1	YP_001228259.1		
		ZP_01472077.1	ZP_01470228.1	ZP_01471834.1	ZP_01471831.1	ZP_01471810.1	ZP_01471811.1
		ZP_01080283.1	ZP_01080399.1	ZP_01080889.1	ZP_01080887.1	ZP_01080893.1	ZP_01080894.1
		ZP_01083670.1	ZP_01083664.1		ZP_01086608.1		
		YP_001224958.1	YP_001225121.1	YP_001226198.1	YP_001226196.1	YP_001224084.1	YP_001224083.1
			ZP_01124278.1	ZP_01124959.1	ZP_01124961.1	ZP_01123473.1	ZP_01123474.1
		ZP_08955132.1	ZP_08954908.1	ZP_08956138.1	ZP_08956136.1	ZP_08956143.1	ZP_08956144.1
		NP_897374.1	NP_896953.1	NP_898525.1	NP_898523.1	NP_898005.1	NP_898004.1
		ZP_05788791.1	ZP_05789870.1	ZP_05789780.1	ZP_05788408.1		

similar with $8e^{-93}$ or better). Glucosylglycerate-phosphate synthases (GpgS) involved in glucosylglycerate biosynthesis were searched with GggS (SYNPCC7002_A2021) from *Synechococcus* 7002 (proteins are similar with $1e^{-96}$ or better). Glucosylglycerate-phosphate phosphatases (GpgP) involved in glucosylglycerate biosynthesis were searched with GpgP (SS120_Pro0729) from *Prochlorococcus* SS120 (proteins are similar with $1e^{-11}$ or better). Glycine/sarcosine-*N*-methyltransferases (GSMT) involved in glycine betaine biosynthesis were searched with GSMT (Q33WC4.1) from *Aphanothece* (proteins are similar with $2e^{-116}$ or better). Dimethylglycine-*N*-methyltransferases (DMT) involved in glycine betaine biosynthesis were searched with DMT (Q83WC3.1) from *Aphanothece* (proteins are similar with $7e^{-76}$ or better).

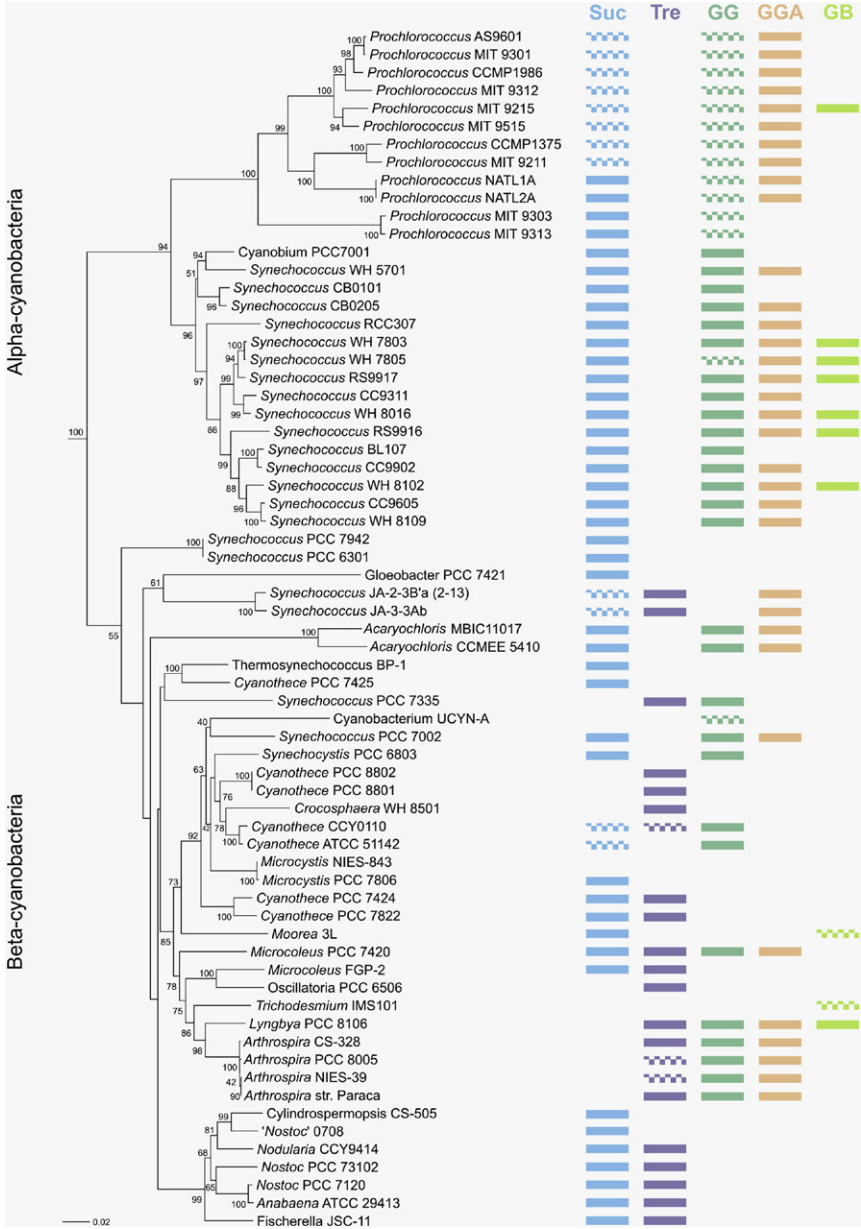

Figure 2.1 Phylogenetic tree (neighbour joining algorithm) of 67 cyanobacterial strains with known genome sequence. The tree is divided into the large groups of alpha- and beta-cyanobacteria. The colour bars represent whether or not genes for compatible solute biosynthesis (Suc – sucrose; Tre – trehalose; GG – glucosylglycerol; GGA – glucosylglycerate; GB – glycine betaine) were found in the genome sequences (see Table 2.1). Dotted bars indicate that the pathway is only incomplete. See the colour plate.

The involvement of Sps in salt-induced sucrose synthesis has been verified for the cyanobacterial strains *Anabaena* sp. PCC 7119 (Porchia & Salerno, 1996), *Nostoc* 7120 (Cumino, Curatti, Giarrocco, & Salerno, 2002), *Synechocystis* 6803 (Curatti et al., 1998; Hagemann & Marin, 1999), and *Synechococcus* sp. PCC 7002 (Cumino, Perez-Cenci, Giarrocco, & Salerno, 2010). These studies revealed that Sps is the sucrose synthesis enzyme among cyanobacteria because purified native or recombinant enzymes showed sucrose synthesis activities, the corresponding transcripts and proteins are accumulated under salt-stress conditions, and *spsA* mutants lost the ability to accumulate sucrose. The cyanobacterial Sps seems to use UDP-glucose rather than ADP-glucose as glucosyl donor, because mutation of the ADP-glucose synthesis enzyme abolished glycogen and GG synthesis but not sucrose accumulation in *Synechocystis* 6803 (Miao, Wu, Wu, & Zhao, 2003).

Searching the presently available cyanobacterial genomes (April 2012) revealed that *spsA* gene can be found in many but not in all cyanobacterial strains (Table 2.1). A closer look shows that at least two different subclasses of Sps proteins exist. First, the Sps protein from *Synechocystis* 6803, which represents a biochemical characterized protein with a biochemically active Sps domain and a biochemically inactive Spp domain (Lunn et al., 1999), was used in BLAST searches (Altschul et al., 1997). These searches indicated that beside *Synechocystis* 6803, only a few other beta-cyanobacteria harbour proteins of high similarity, while all alpha-cyanobacteria encode those Sps proteins in their genomes (Table 2.1). During the analysis of the genetic and biochemical basis for salt-induced sucrose accumulation of heterocystous strains, two genes were identified coding for Sps proteins, which are shorter than the Sps from *Synechocystis* 6803 because the Spp domain is missing (Cumino et al., 2002). Moreover, these proteins showed also lower similarities to SpsA from *Synechocystis* 6803 than sucrose synthases from filamentous strains. This second type of Sps is marked by ⋆ in Table 2.1. Additional to the *Anabaena*/*Nostoc* strains, these Sps⋆ proteins are frequently found in genomes of other beta-cyanobacteria, mostly in two copies. There are only a few strains, e.g. *Nodularia spumigena* CCY9414, harbouring genes for the two Sps types in one genome. Sps⋆ protein-coding genes seem not to exist in alpha-cyanobacterial genomes.

Interestingly, there are a few cyanobacterial strains with completely known genomes, e.g. *Microcystis aeruginosa* NIES-843 or *Crocosphaera watsonii* WH 8501, which are virtually free from any Sps-coding genes. For the latter strains, we showed recently that sucrose is indeed not accumulated under saline conditions (Pade, Compaoré, Klähn, Stal, & Hagemann, 2012). However, whether or not those strains are completely unable to make

sucrose is not known. Alternatively, one can assume that sucrose synthases (Sus), or another member of an uncharacterized glucosyltransferase group, could also be used for sucrose biosynthesis, despite the usual preference of the sucrose hydrolysis reaction. However, at least for the strains *Anabaena* sp. PCC 7119 and *Nostoc* 7120, it has been shown that sucrose synthase plays rather an important role for N_2-fixation, probably producing precursors for cell wall synthesis or for nitrogen fixation from sucrose (Cumino, Marcozzi, Barreiro, & Salerno, 2007). Similar to *spsA*, *sus* genes are also not universal among cyanobacteria (data not shown here). For example, the genome of *M. aeruginosa* NIES-843 has neither *spsA* nor *sus*.

The second step in sucrose biosynthesis is catalysed by SPP. For this enzyme, separate genes are found in almost all cyanobacterial genomes (Table 2.1). Generally *spsA* containing genomes contain at least one Spp-coding sequence. In most cases, *spsA* and *spp* genes are not linked, however, in *Synechococcus* sp. PCC 7002, they form a salt-regulated operon (Cumino et al., 2010). Interestingly, among *Prochlorococcus* spp., many genomes contain an *spsA* but no *spp* gene (Table 2.1; Scanlan et al., 2009). Whether, in these cases, the C-terminal Spp domain of the SpsA protein is biochemical active or another sugar phosphatase performs the second step in sucrose biosynthesis is not known. It has been experimentally proven that *Prochlorococcus* strains accumulate sucrose as main compatible solute (Klähn, Steglich, Hess, & Hagemann, 2010), therefore, they are obviously able for *de novo* sucrose synthesis and should perform Spp activity.

3.2. Trehalose

Trehalose (α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside) is a widespread compatible solute, which not only is protecting against salt but also helps to tolerate heat, dehydration and many other stresses (Furuki, Oku, & Sakurai, 2009). Among cyanobacteria, trehalose is made by two different biosynthetic pathways. The OtsAB pathway was initially elucidated in *Escherichia coli* and employs two enzymes (Strøm & Kaasen, 1993), trehalose-phosphate synthase (OtsA) and subsequently the trehalose-phosphate phosphatase (OtsB) catalysing the following two reactions:

(OtsA) UDP-glucose + glucose 6-phosphate → trehalose-phosphate + UDP

(OtsB) Trehalose-phosphate → trehalose + Pi

This pathway is used by most bacteria and eukaryotic organisms for stress-induced trehalose biosynthesis. However, in the past years, alternative

trehalose synthesis pathways were found (e.g. Wolf, Krämer, & Morbach, 2003), such as TreYZ. In this pathway, a polysaccharide (i.e. glycogen) precursor is first changed at the terminal end to α 1,1 sugar bound by maltooligosyltrehalose synthase (TreY), and then the trehalose is cleaved off by maltooligosyltrehalose trehalohydrolase (TreZ):

(TreY) α-1,4-polyglucose → α-1,1-maltooligosyltrehalose

(TreZ) α-1,1-maltooligosyltrehalose → trehalose + α-1,4 polyglucose (n-2)

Molecular analyses of cyanobacterial trehalose synthesis revealed that the TreYZ pathway seems to be mostly used. The occurrence of the TreYZ pathway among cyanobacteria was first verified in *Nostoc* 7120 during the study of a gene cluster, which was previously found to be upregulated under desiccation (Higo, Katoh, Ohmori, Ikeuchi, & Ohmori, 2006). In addition to genes for TreY (*all*0167), TreZ (*all*0168), this operon codes for a trehalase (TreA, *all*0166), which is able to hydrolyse trehalose. It should be mentioned that *Nostoc* 7120 accumulates only sucrose under salt stress, while trehalose seems to be made in response to drought stress. Subsequently, the TreYZ pathway has also been found in other trehalose-accumulating cyanobacteria, such as *Nostoc punctiforme* IAM M-15 (Yoshida & Sakamoto, 2009), *Arthrospira platensis* NIES-39 (Ohmori, Ehira, Kimura, & Ohmori, 2009), and *Nostoc flagelliforme* (Wu, He, Shen, Zhang, & Wang, 2010). Using the TreYZA proteins from *Nostoc* 7120 in Blast searches, genes showing high similarities were found in 19 cyanobacterial genomes (Table 2.1). These strains all belong to the group of beta-cyanobacteria; many of them are filamentous and/or N_2-fixing cyanobacteria, which display often a high-desiccation tolerance (e.g. *Microcoleus vaginatus* FGP-2; Starkenburg et al., 2011). However, genomes of a few unicellular, non-N_2-fixing strains contain also these genes. Interestingly, the genomes of the two thermophilic *Synechococcus* strains (JA-3-3Aba) from Yellowstone National Park, USA contain TreYZ-coding genes. Most probably, these strains synthesize trehalose as thermoprotectant in their hot environment because this disaccharide exhibits excellent stabilizing activity for macromolecules at high temperatures (Furuki et al., 2009).

With the only exception *C. watsonii* strain WH 8501, genes coding the *E. coli*-like OtsAB pathway for trehalose synthesis are absent from cyanobacteria (Table 2.1). Only the genome of this unicellular, marine N_2-fixing cyanobacterium codes for a large fusion protein comprising OtsA and OtsB

domains (Pade et al., 2012). Corresponding to the occurrence of the *otsAB* gene, *Crocosphaera* accumulates trehalose as only compatible solute. Overexpression of this gene in *E. coli* verified that the gene codes for a functional trehalose biosynthesis enzyme. Interestingly, *C. watsonii* WH 8501 acquired this gene by a lateral gene transfer event (Pade et al., 2012). Cyanobacteria closely related to *Crocosphaera* usually accumulate GG, however, these genes are missing (lost?) in this cyanobacterium.

3.3. Glucosylglycerol

Glucosylglycerol (GG; α-D-glucopyranosyl-(1 → 2)-glycerol) was the first compatible solute, whose accumulation was found in a cyanobacterium (Borowitzka et al., 1980). Later on, it was detected in many cyanobacterial strains and was regarded to be characteristic for the group of moderate halotolerance (Hagemann, 2011; Reed, Borowitzka et al., 1986). While GG-accumulating beta-cyanobacteria are mostly euryhaline, i.e. they can be cultivated in freshwater and salt-containing media (e.g. *Synechocystis* 6803 or *Synechococcus* sp. PCC 7002; Engelbrecht, Marin, & Hagemann, 1999; Marin, Zuther, Kerstan, Kunert, & Hagemann, 1998), the GG-accumulating picoplanktonic *Synechococcus* strains, which belong to the alpha-cyanobacteria, show only a small range of halotolerance, i.e. they can be cultivated only in media near the normal seawater salinity level (Klähn, Steglich et al., 2010). Beside its osmotic function, GG has good direct membrane and protein stabilizing properties, which are also of biotechnological interest (Borges, Ramos, Raven, Sharp, & Santos, 2002; Hincha & Hagemann, 2004; Sawangwan, Goedl, & Nidetzky, 2010).

Cyanobacteria use a two-step biosynthesis for GG (Hagemann & Erdmann, 1994) with an initial GG-phosphate synthase (GgpS) and a subsequent GG-phosphate phosphatase (GgpP):

(GgpS) ADP-glucose + glycerol 3-phosphate → glucosylglycerol-phosphate + ADP

(GgpP) Glucosylglycerol-phosphate → glucosylglycerol + Pi

This biosynthetic pathway is similar to that of sucrose, trehalose and glucosylglycerate (Klähn & Hagemann, 2011). However, sucrose and trehalose biosyntheses prefer UDP-glucose as glucosyl donor, while cyanobacterial GG synthesis is strictly dependent on ADP-glucose (Hagemann & Erdmann, 1994; Miao et al., 2003). Many heterotrophic bacteria also accumulate GG

under saline conditions. These organisms use ADP- and also UDP-glucose for the GgpS reaction. Moreover, in many heterotrophic bacteria, the two enzyme activities, GgpS and GgpP, are found in one continuous protein (Hagemann et al., 2008). Such fused GG synthesis proteins have not yet been found in any cyanobacterial genome.

Genome searches using the GgpS protein (Sll1566) from *Synechocystis* 6803 (Marin et al., 1998) identified 28 genes coding for highly similar proteins in other cyanobacteria (Table 2.1). The closest homologue is the GgpS from *Synechococcus* sp. PCC 7002, which has been functionally verified (Engelbrecht et al., 1999). Among beta-cyanobacteria, it is also found in many euryhaline and marine strains such as *Arthrospira* (Yoshikawa et al., 2011), *Acaryochloris*, and some but not all *Cyanothece* strains (Table 2.1). However, there are also many marine strains, which do not harbour *ggpS* genes in their genomes and are accordingly not using GG as main compatible solute. Interestingly, the marine mat-forming *Microcoleus chtonoplastes* contains genes for GG synthesis, whereas the terrestrial *M. vaginatus* strain FGP-2 is only able to synthesize trehalose (Table 2.1). Probably, in the terrestrial habitat, desiccation is the main stress, which is usually tolerated by trehalose accumulation, while the marine *Microcoleus* is faced by salt stress, therefore preferring GG.

As previously reported (Scanlan et al., 2009), practically all of the marine picoplanktonic *Synechococcus* strains (only exception is *Synechococcus* sp. WH 7805) contain *ggpS* genes, whereas the related *Prochlorococcus* strains miss it all (Table 2.1). The accumulation of GG in marine *Synechococcus* and its absence in *Prochlorococcus* strains have been recently verified (Klähn, Steglich et al., 2010).

The GgpP (StpA was used synonymously) protein is a specific GG-phosphate phosphatase. Homologues of this protein are restricted to cyanobacteria and are characterized by a specific protein domain called Salt_tol_Pase superfamily. The only functional characterized GgpP enzyme is encoded by *slr*0746 in *Synechocystis* 6803 (Hagemann, Schoor, Jeanjean, Zuther, & Joset, 1997). Highly similar proteins are found in all cyanobacterial genomes harbouring a *ggpS* gene. Among beta-cyanobacteria *ggpS* and *ggpP* never form an operon. However, in the oceanic picoplanktonic *Synechococcus* strains, these genes coding for functional linked proteins are often found adjacent to each other indicative for operon structures.

In one case, *Synechococcus* sp. RCC307, not only *ggpS* and *ggpP* are linked but also the four subunits for the putative ABC-type GG-transport system

ggtABCD are situated downstream in the genome (Scanlan et al., 2009). Because a clear functional assignment of ABC transporters by sequence similarity searches is difficult, the occurrence of GG-transporter genes as well as genes for other compatible uptake systems was not analysed here. Generally, it can be assumed that at least transporters for their own main compatible solute are present among cyanobacteria, which prevent leakage of compatible solutes in the medium as has been shown for *ggtA* mutant of *Synechocystis* 6803 (Hagemann, Richter, & Mikkat, 1997; Mikkat, Effmert, & Hagemann, 1997).

Surprisingly, *ggpP* genes are also present in the *Prochlorococcus* genomes, which do not contain *ggpS* genes. Since the only known biochemical function of GgpP is dephosphorylation of the intermediate GG-phosphate, one can speculate that the common ancestor of alpha-cyanobacteria harboured *ggpS* and *ggpP* genes (Scanlan et al., 2009). Early in the evolution of the *Prochlorococcus* clade, the *ggpS* gene was lost and sucrose replaced GG as major compatible solute. Why the *ggpP* gene was kept is uncertain, possibly the GgpP is able to dephosphorylate also other sugar phosphates. An interesting possibility would be that in *Prochlorococcus* strains, the GgpP may act as sucrose-phosphate phosphatase, because separate *spp* genes are missing from all *Prochlorococcus* genomes (Table 2.1).

3.4. Glucosylglycerate

Glucosylglycerate (GGA) is an uncommon compatible solute because it carries a net charge at physiological pH. GGA has been early detected in extracts of the cyanobacterium *Synechococcus* sp. PCC 7002 (*Agmenellum quadruplicatum*) (Kollman, Hanners, London, Adame, & Walker, 1979). GGA and its structural relative mannosylglycerate have been extensively analysed in thermophilic, heterotrophic bacteria (Empadinhas & da Costa, 2008). The identification of the structural genes for GGA synthesis revealed that genes for similar proteins occur also in cyanobacterial genomes (Costa et al., 2006). The biosynthetic pathway resembles that of GG, sucrose and trehalose, a GGA-phosphate synthase (GpgS) cooperates with a phosphatase (GpgP):

(GpgS) NDP-glucose + glycerate 3-phosphate → GGA-phosphate + NDP

(GpgP) GGA-phosphate → GGA + Pi

Recently, we could confirm that GGA is used as compatible solute among cyanobacteria (Klähn, Steglich et al., 2010). GGA accumulation was found

in many alpha-cyanobacteria, i.e. *Prochlorococcus* and *Synechococcus* spp., and in the beta-cyanobacterium *Synechococcus* sp. PCC 7002. An interesting observation was that the GGA amount was not only dependent from the salinity level; its accumulation became clearly stimulated or even induced when salt addition was combined with nitrogen limitation. Especially, the *Synechococcus* strains were virtually free of GGA under N-excess but contained high internal amounts at N-limited growth (Klähn, Steglich et al., 2010). This finding gave rise to the hypothesis that the charged compatible solute GGA is replacing glutamate under N-limiting conditions and serve as organic counterion for cations, especially K^+, inside the salt-loaded cells of marine cyanobacteria, which are usually faced by N-limiting conditions.

Overexpression of the *gpgS* gene from *Synechocystis* sp. PCC 7002 allowed the purification of recombinant GpgS protein, which showed the expected biochemical activity. Similar proteins are coded in the genomes of most alpha-cyanobacteria (only two *Prochlorococcus* and two *Synechococcus* strains miss *gpgS* genes, Table 2.1), while only a few beta-cyanobacterial genomes code for this enzyme. As in heterotrophic bacteria, in alpha-cyanobacteria, the *gpgS* genes are usually found in an operon with two other genes coding further proteins for GGA metabolism, i.e. GGA hydrolase and GpgP. Genes for GpgP (mostly wrongly annotated as mannosyl-3-phosphoglycerate phosphatase in cyanobacterial genomes) are found in almost all cyanobacterial genomes with *gpgS* genes (only exception is *Synechococcus* sp. BL107). There are few genomes (e.g. the two thermophilic *Synechococcus* strains from Yellowstone National Park) carrying the *gpgP* but no *gpgS* gene, which could be taken as an indication that the GGA biosynthesis became stepwise lost in these strains.

3.5. Glycine Betaine

Glycine betaine (*N,N,N*-trimethylglycine) is a widespread compatible solute, which has been reported from many salt-stressed organisms (for a review, see Chen and Murata (2011)). In most organisms, glycine betaine is synthesized by a two-step oxidative pathway using choline as precursor, in which choline dehydrogenase (CDH) encoded by *betA* and betaine aldehyde dehydrogenases (BADH) encoded by *betB* in *E. coli* (Andresen, Kaasen, Styrvold, Boulnois, & Strøm, 1988) are cooperating.

$$\text{(CDH) Choline} + NAD^+ \leftrightarrow \text{betaine aldehyde} + NADH$$

$$\text{(BADH) Betaine aldehyde} + NAD^+ + H_2O \leftrightarrow \text{glycine betaine} + NADH + 2H^+$$

Later on, genes and proteins for alternative glycine betaine synthesis were identified in heterotrophic bacteria (Nyyssola, Kerovuo, Kaukinen, von Weymarn, & Reinikainen, 2000). This pathway is performed by two enzymes, glycine/sarcosine-*N*-methyltransferase (GSMT) and then dimethylglycine-*N*-methyltransferase (DMT), which catalyse the three-step methylation of glycine with the methyl-donor S-adenosyl-methionine (SAM):

(GSMT) Glycine + SAM → *N*-methylglycine (sarcosine) + SAH

(GSMT) Sarcosine + SAM → *N,N*-dimethylglycine + SAH

(DMT) *N,N*-dimethylglycine + SAM → *N,N,N*-trimethylglycine (glycine betaine) + SAH

The direct methylation of glycine seems to be used by all glycine-betaine-accumulating cyanobacteria. Its activity has been verified in the hypersaline model strain *Aphanothece* and in the picoplanktonic *Synechococcus* sp. WH 8102 (Lu, Chi, & Su, 2006; Waditee et al., 2003, 2005). Screening the database with the GsmT and Dmt sequences from *Aphanothece*, which itself is not completely sequenced, showed only a small number of highly similar proteins in cyanobacterial genomes. Among beta-cyanobacteria, only *Lyngbya* sp. PCC 8106 and a related newly described species *Moorea product* 3L (Engene et al., 2012), which was isolated from seagrasses, contain these genes for glycine betaine synthesis. The filamentous N_2-fixing *Trichodesmium erythraeum* IMS101 harbours only a Dmt-coding gene in its chromosome, while the GsmT sequence is missing. In the moment, it is unknown which compatible solute is accumulated in this marine strains. Our own screenings of salt-loaded cells of *Trichodesmium* did not detect any glycine betaine but another, yet unknown compound in osmotic significant concentrations (Hagemann et al., unpublished results). Among the alpha-cyanobacteria, genes coding for GsmT and Dmt are found in eight *Synechococcus* or *Prochlorococcus* genomes. These strains contain also genes for many other compatible solute biosynthesis enzymes and seem to be rather euryhaline and able to live in dynamic ecosystems (Mao et al., 2010; Scanlan et al., 2009).

Compared to the number of strains accumulating other compatible solutes, a rather low number of glycine betaine accumulators can be predicted from the genome information. One explanation could be that cyanobacteria can also use the oxidative *E. coli*-like BetAB pathway for glycine betaine synthesis. Using the BetAB sequences in genome searches is not very

informative since many noncharacterized aldehyde oxidase/dehydrogenases display a certain degree of similarity. However, those proteins are also frequently found in genomes of strains, which definitely do not accumulate glycine betaine. In the moment, it seems to be more likely that cyanobacteria use exclusively the methylation pathway for glycine betaine synthesis and the oxidative pathway is not active. This view is indirectly supported by the results of two studies, which introduced the oxidative glycine betaine pathway into the freshwater strain *Synechococcus* sp. PCC 6301 (Deshnium, Los, Hayashi, Mustardy, & Murata, 1995; Nomura, Ishitani, Takabe, Rai, & Takabe, 1995). Significant glycine betaine accumulation was only detected when the medium was supplemented with the BetAB pathway precursor choline, which seems to be limiting in cyanobacteria, whereas glycine as precursor for the methylation pathway is available in high amounts.

4. REGULATION

Compatible solute synthesis needs to be regulated according to the external salt conditions, i.e. the cellular concentration of compatible solutes varies according to the amount of external total salts. There are many examples showing that transcripts for compatible solute biosynthesis genes increase after salt-shock treatments (e.g. *ggpS* for GG synthesis, Marin, Huckauf, Fulda, & Hagemann, 2002; *sps* for sucrose biosynthesis, Cumino et al., 2010). In most cases, the transcription is highly stimulated after the salt-shock treatment, in the case of *ggpS* up to 50-fold, whereas the final steady-state contents are only slightly elevated and depend on the external salt concentration (Fig. 2.2). Interestingly, the transcript amounts of glucosylglycerate biosynthesis genes showed a rather small increase after salt addition, which became much more pronounced and extended when salt-stress treatments were done under N-limiting conditions (Klähn, Steglich et al., 2010).

The sensing of the salt-stress signal and its transduction to the gene expression is less good understood. Many potential signals are discussed to inform the cell about the external salinity (Wood, 1999). Intracellular inorganic ions or turgor changes are among those possible signals. In the case of *E. coli*, a transient rise in K^+ amounts, which has been also observed in salt-shocked cells of *Synechocystis* sp. PCC 6714 (Reed et al., 1985), changes the promoter-binding specificity of the RNA polymerase towards promoters for genes activated after salt shocks (Gralla & Vargas, 2005). In cyanobacteria, a GAF-domain-containing adenylate cyclase has been characterized as sodium sensor (Cann, 2007). Moreover, knocking out the water channel

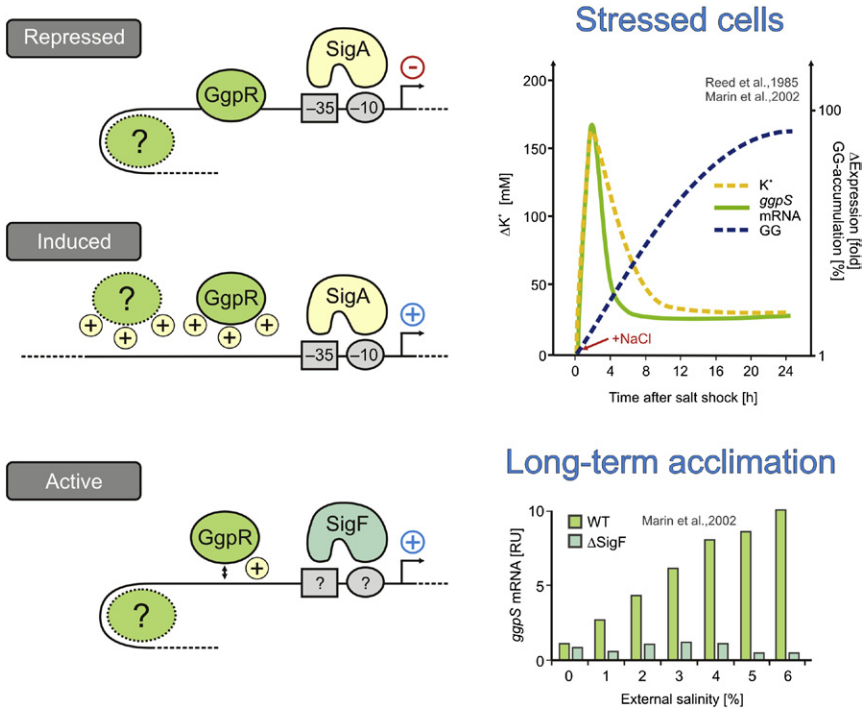

Figure 2.2 Model of transcriptional regulation of the *ggpS* gene for glucosylglycerol (GG) synthesis (left panels) in *Synechocystis* 6803 in comparison to experimental data (right panels). In low-salt grown cells, the *ggpS* gene is repressed leading to GG-free cells. Salt-shock treatments induce the highest *ggpS* expression and quick GG accumulation because the influx of inorganic ions releases the repressor GgpR from the *ggpS* promoter in salt-stressed cells. In long-term salt-acclimated cells, the *ggpS* expression remains active but depends on the external salinity. Moreover, the *ggpS* expression in salt-acclimated cells seems to be driven by SigF instead of SigA because a SigF deletion abolishes *ggpS* expression to a large extent. Possibly, additional regulatory factors (marked by ?) are also involved. See the colour plate.

AqpZ affected not only water flow and turgor, but also the expression of many salt-regulated genes was changed as well in *Synechocystis* 6803 (Shapiguzov et al., 2005).

Analyses of the impact of mutations of two component systems in *Synechocystis* 6803 on salt-regulated gene expression revealed that defined groups of these genes are controlled by pairs of histidine kinases and their cognate response regulators (Marin et al., 2003; Shoumskaya et al., 2005). However, none of the genes involved in GG synthesis or transport showed changes in the salt regulation in any of these mutants. Other candidates for regulating

salt-stress-stimulated gene expression are alternative sigma factors. A mutation of the group 3 sigma factor SigF resulted in the decreased expression of many salt-stress proteins including GgpS (Huckauf, Nomura, Forchhammer, & Hagemann, 2000; Marin et al., 2002) (Fig. 2.2). Moreover, knocking out genes for group 2 sigma factors (especially SigB) also lead to decreased salt tolerance in *Synechocystis* 6803 (Nikkinen et al., 2012). The bioinformatic analysis of the genome sequence and the analysis of salt-regulated transcriptional changes resulted in the prediction that the alternative sigma factor σ38 seems to control the salt-stress-related gene expression pattern in the alpha-cyanobacterium *Synechococcus* sp. WH 8102 (Mao et al., 2010).

A transcriptional factor specifically regulating compatible solute biosynthesis genes or other groups of salt-induced genes is not known among cyanobacteria. The promoter of the salt-regulated *ggpS* gene was mapped in *Synechocystis* 6803. This study revealed that the *ggpS* expression is negatively regulated. An ORF for a small protein was discovered in the *ggpS* promoter region, which codes for GgpR repressing *ggpS* under low salt conditions (Klähn, Höhne, Simon, & Hagemann, 2010). The binding of GgpR to the *ggpS* promoter region is influenced by the concentration of inorganic ions (Klähn et al., unpublished results). This finding lead to the model that the transient accumulation of K^+ in salt-shocked *Synechocystis* 6803 cells releases the GgpR protein from the *ggpS* promoter resulting in its maximal activity. Under steady-state conditions, when the K^+ content decreased, GgpR is loosely associated to the *ggpS* promoter and alternative sigma factors (e.g. SigF) guarantee a stress-proportional *ggpS* expression (Fig. 2.2). Because *ggpR* mutations did not completely abolish the salt regulation of *ggpS*, the involvement of additional regulatory molecules is possible (marked by ? in Fig. 2.2). Interestingly, a small ORF, similar to *ggpR*, also exists upstream of the *ggpS* gene of *Synechococcus* sp. PCC 7002 (Klähn, Höhne et al., 2010).

Additional to transcriptional regulation, compatible solute biosynthesis is tightly regulated on biochemical level. A reversible activation and inactivation of the GgpS activity by addition of high salt and removal of inorganic ions, respectively, has been early described (Hagemann & Erdmann, 1994). It took almost 20 years to elucidate the molecular mechanism. Novak, Stirnberg, Roenneke, and Marin (2011) discovered a new biochemical switch to activate or inactivate GgpS activity. It was found that GgpS can bind DNA or RNA in a sequence-unspecific manner, which switches off the enzyme activity. In the presence of increasing concentrations of inorganic ions, particularly K^+, the GgpS is released and becomes increasingly active. The model explains why GgpS is maximally active in salt-shocked cells

when the internal ion content is high, whereas its steady-state activity corresponds to the external salinity, because the steady-state amount of internal K^+ increases gradually in cells acclimated to increasing salinities (Novak et al., 2011; Reed et al., 1985).

5. CYANOBACTERIAL BIOTECHNOLOGY AND SALT ACCLIMATION

Due to environmental concerns and the future decline in oil production, cyanobacteria are increasingly investigated as alternative sources for green energy and chemical feedstock (e.g. Ducat, Way, & Silver, 2011). To minimize competition with agricultural food production, the mass cultivation of cyanobacteria should be done on land not suited for agriculture and by using salt water instead of freshwater. For this purpose, it is important to gain more knowledge on salt acclimation of a broader set of cyanobacteria. Future production strains will be certainly optimized regarding salt-tolerance mechanisms, i.e. by engineering the energetic cheapest strategy to minimize negative impacts on product biosynthesis.

Compatible solutes will be not only important for enabling high growth rates of production strains in salty media, these chemicals itself are of relatively high value, because they can be used in cosmetics and pharmaceuticals as moisturizers and stabilizers. Presently, GG (Sawangwan et al., 2010) but particularly ectoine, a compatible solute from halobacteria, is produced by the BITOP GmbH (http://www.bitop.de/cms/website.php?id=/en/index.htm). The compatible solute ectoine is harvested by a so-called bacterial milking process. It has been previously shown that the alternative incubation in salt medium (production phase) and subsequent incubation in distilled water (harvesting phase) could also be used to elute compatible solutes from cyanobacteria (Fulda, Hagemann, & Libbert, 1990; Reed, Warr, Kerby, & Stewart, 1986). Recently, *Synechococcus* sp. PCC 7942 strains have been engineered, which express a sucrose export system. These strains did not only continuously excrete sucrose into the culture supernatant when grown in saline medium, the overall sucrose biosynthesis and photosynthesis rate were also increased (Ducat, Avelar-Rivas, Way, & Silver, 2012).

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