## Effects of rising $CO_2$ on the harmful cyanobacterium Microcystis

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# Chapter 8

# Potassium sensitivity differs among strains of the harmful cyanobacterium *Microcystis* and correlates with the presence of salt tolerance genes

# Potassium sensitivity differs among strains of the harmful cyanobacterium *Microcystis* and correlates with the presence of salt tolerance genes

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## Abstract

*Microcystis aeruginosa* is a ubiquitous harmful cyanobacterium that causes problems in eutrophic lakes. Potassium ion ($K^+$) addition is one of the suggested methods to combat harmful cyanobacterial blooms (**Figure 8.1**). To investigate the effectiveness of this method, we compared the potassium ion sensitivity of four *Microcystis* strains. *Microcystis* strains PCC 7005 and NIES-843 were very susceptible to potassium ion concentrations of ~12mmol $L^{-1}$, whereas strain PCC 7806 and its non-toxic mutant PCC 7806 Δ*mcyB* were not affected by added potassium ions. The origin of the strain appears to be of importance. Strain PCC 7806 originates from brackish water and possesses genes for the synthesis of the compatible solute sucrose, the water channel protein gene *aqpZ* and the sodium influx gene *nhaS2*, whereas strains PCC 7005 and NIES-843 have a freshwater origin and lack these genes. We conclude that potassium ion addition will not be a successful mitigation strategy in brackish waters, but may temporarily suppress *Microcystis* blooms in freshwater lakes. However, in the long run other *Microcystis* strains or other cyanobacteria with a higher salt tolerance will likely take over. In addition, our results also have implications for the potassium ion concentrations of mineral media used in laboratory studies with cyanobacteria.

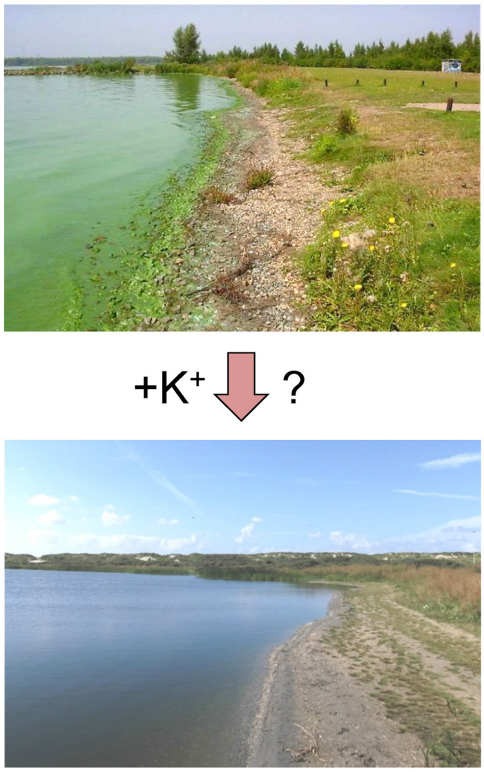


**Figure 8.1. Graphical abstract. Is potassium ion addition an effective method to combat harmful cyanobacterial blooms?**


## Introduction

*Microcystis* is a ubiquitous harmful cyanobacterium that can be found in both fresh and brackish water (Chorus and Bartram, 1999; Tonk *et al.*, 2007; Moisander *et al.*, 2009). The cells can produce gas vesicles that provide buoyancy and during summertime, when conditions are most favourable, the genus can form dense surface blooms and scum layers (Huisman *et al.*, 2005). Many *Microcystis* strains produce the hepatotoxinmicrocystin (Carmichael, 2001;

Dittmann *et al.*, 2013), and bloom development of *Microcystis* in numerous eutrophic lakes, reservoirs and estuaries raises worldwide concerns (Chen *et al.*, 2003; Robson and Hamilton, 2003; Michalak *et al.*, 2013). These blooms have led to the closure of lakes for recreational use, drinking and irrigation water, and aquaculture, with substantial economic damage as a result of this (Verspagen *et al.*, 2006; Dodds *et al.*, 2008; Qin *et al.*, 2010). Moreover, climate change is foreseen to increase the problems with harmful cyanobacterial blooms in eutrophic waters (Paerl and Huisman, 2008; Davis *et al.*, 2009; O'Neil *et al.*, 2012; Verspagen *et al.*, 2014b; Sandrini *et al.*, 2015a).

The addition of low amounts of potassium ions ($K^+$) has been suggested as a method to combat harmful cyanobacterial blooms (Parker *et al.*, 1997; Kolmakov, 2006; Shukla and Rai, 2007). *Microcystis* appears to bemore sensitive to low concentrations of potassium ions (1-5 mmol $L^{-1}$) than to other alkali metal cations such as sodium (Parker *et al.*, 1997). Yet, currently it is not known if all *Microcystis* strains are susceptible to potassium ions, and what the underlying genetic cause is for the observed potassium ion sensitivity.

Potassium ions are involved in a variety of cellular functions such as membrane energetics, pH regulation, enzyme activities and gene expression (Wood, 1999; Gralla and Vargas, 2005). Moreover, potassium ions play a vital role in salt and turgor acclimation (Wood, 1999). Hence, we hypothesize that there might be a relation between the potassium ion sensitivity of cyanobacteria and their salt tolerance. For adjustment to various external salt concentrations and to warrant a balanced water potential, cyanobacteria use a large array of ion transporters, water and mechanosensitive channels, andmay synthesize compatible solutes. These compatible solutes include sucrose and trehalose in freshwater strains, glucosylglycerol and glucosylglycerate in moderately halotolerant strains, and glycine betaine and glutamate betaine in halophilic strains (Hagemann, 2011).

In this brief study, we investigate the effect of potassium ion addition on the specific growth rate of *Microcystis* strain PCC 7806 isolated frombrackishwater and two *Microcystis* strains obtained from freshwater lakes. Furthermore, we investigate whether microcystin synthesis affects potassium ion sensitivity by comparison of the growth rate of the microcystin-producing *Microcystis* PCC 7806 and its non-toxic mutant PCC 7806 Δ*mcyB*. Finally, we compare the genomes of these strains to assess whether differences in potassium ion sensitivity are associated with the presence of specific salt tolerance genes. Our study has implications for the mitigation of harmful cyanobacterial blooms, and for the composition of mineral media used in a wide range of ecological and physiological studies of cyanobacteria.

## Materials and Methods

We investigated four *Microcystis* strains: the non-microcystinproducing strain PCC 7005, the microcystin-producing strains NIES-843 and PCC 7806, and the non-toxic mutant PCC 7806 Δ*mcyB* (Dittmann *et al.*, 1997). The axenic strains were grown in 24-well microplates (Corning Incorporated, New York, NY, USA) using the same experimental set-up as in Sandrini *et al.* (2014). In short, the 24-well microplates were placed in sterilized 1.7 L glass incubation chambers, which in turn were placed in a large Orbital incubator (Gallenkamp, Leicester, UK) that was maintained at 25°C and provided 120 rpm shaking and continuous light from TL-D 30W/33–640 white fluorescent tubes (Philips, Eindhoven, the Netherlands). The photon flux density at the wells was ~15 µmol photons $m^{-2}$ $s^{-1}$. The glass incubation chambers were provided with $CO_2$-enriched air at a flow rate of 25 L $h^{-1}$ containing 400 ppm $pCO_2$.

Nutrients were provided by a modified BG11 medium (Rippka *et al.*, 1979), with 0.2 mmol $L^{-1}$ $K_2HPO_4$, 5 mmol $L^{-1}$ sodium nitrate and 5 mmol $L^{-1}$ sodium chloride, but without addition of sodium bicarbonate or sodium carbonate ('BG11*'). To test the potassium ion sensitivity of the strains, we compared their growth rates with and without an extra addition of 11.5mmol $L^{-1}$ potassium chloride. Hence, the potassium chloride treatment contained ~12mmol $L^{-1}$ of potassium ions, whereas the control contained only 0.4mmol $L^{-1}$. We also measured the growth rates after addition addition of 11.5 mmol $L^{-1}$ sodium chloride and lithium chloride, to compare the potassium ion sensitivity against the sensitivity to two other alkali metal cations.

At the start of the experiments, exponentially growing precultures were diluted with mineral medium to a similar absorbance at 750 nm of $A_{750}$ ~0.015. The strains were randomized over the microplates to minimize position effects, with six replicates per strain and per treatment. The total start volume per well was 2,200 µL. Samples of 100 µL were taken from the wells on an almost daily basis for at least 1 week and transferred in a sterile hood to a 96-well microplate (Corning Incorporated) to measure the $A_{750}$ with a tunable Versamax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Some wells close to the gas inlet showed evaporation and were excluded from the data analyses. Specific growth rates were calculated from six time points during the linear growth phase, using the slope obtained by linear regression of the natural logarithm of $A_{750}$ versus time. The growth data were analysed with two-way analysis of variance to test whether the specific growth rates were affected by the strain type and medium composition. Type III sum of squareswas used to account for unequal sample sizes and *post-hoc* comparisons of the means were based on Tukey's unequal N HSD test, using a significance level $\alpha$ of 0.01.

We screened the genomes of *Microcystis* PCC 7806 (Frangeul *et al.*, 2008), *Microcystis* NIES-843 (Kaneko *et al.*, 2007), *Microcystis* PCC 7005 (Sandrini *et al.*, 2014) and 13 other sequenced *Microcystis* strains (Fiore *et al.*, 2013; Humbert *et al.*, 2013; Yang *et al.*, 2013; Okano *et al.*, 2015) to assess whether differences in potassium ion sensitivity are associated with the presence of specific salt tolerance genes. First, CyanoBase (Nakamura *et al.*, 1998), KEGG (Kanehisa and Goto, 2000) and Genbank (Benson *et al.*, 2013) were used to search and retrieve sequences of proteins involved in salt acclimation of cyanobacteria. Subsequently, BLAST (Altschul *et al.*, 1990) was used to find homologues of cyanobacterial salt stress genes in Microcystis strains, using protein sequences of the cyanobacteria *Synechocystis* PCC 6803, *Nostoc* PCC 7120, *Synechococcus* PCC 7002 and *Synechococcus* WH8102 as reference.

## Results

The results show a strain-specific response to the different salt treatments (**Figure 8.2; Table S8.1, Supplementary Information**). *Microcystis* strains PCC 7806 and its non-toxic mutant PCC 7806 Δ*mcyB* maintained a high growth rate irrespective of the salt supplements. Furthermore, addition of sodium and lithium ions did not have major effects on the growth rates of the strains, although lithium chloride slightly lowered the growth of NIES-843. In contrast, the growth of strains PCC 7005 and NIES-843 was significantly reduced in the treatment with potassium chloride (**Figure 8.2**).

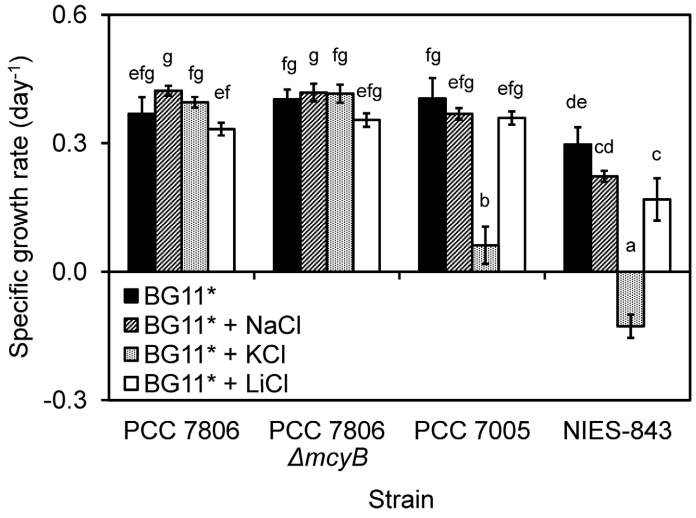


**Figure 8.2. Specific growth rates of selected *Microcystis* strains after addition of 11.5mmol $L^{-1}$ of sodium, potassium and lithium salts to the mineral medium.** Error bars indicate the standard deviations ($n$ = 5-6). Bars with different letters indicate significant differences in growth rate, as tested by a two-way analysis of variance (Table S8.1) followed by *post-hoc* comparison of the means.

To explain differences in potassium ion sensitivity between *Microcystis* strains, the genes involved in salt acclimation were compared between the sequenced *Microcystis* strains PCC 7806, PCC 7005 and NIES-843, and the model cyanobacterium *Synechocystis* PCC 6803 (**Table 8.1**). It appeared that the three *Microcystis* strains have a similar set of ion transporting proteins, with only few differences.

Yet, a notable difference is the presence of the gene *nhaS2* encoding for a sodium/proton antiporter and the gene *trkA* encoding for a homologue of a potassium ion transporter in *Microcystis* strain PCC 7806, whereas both genes are absent in strains PCC 7005 and NIES-843 (**Table 8.1**). Moreover, both genes were also detected in *Microcystis* strains PCC 9432, PCC 9443, PCC 9807, PCC 9809, T1-4 and TAIHU98, but were absent in seven other sequenced *Microcystis* strains (NIES-44, PCC 7941, PCC 9701, PCC 9717, PCC 9806, PCC 9808 and SPC77).

Another notable difference is the presence of *aqpZ*, encoding for a water channel protein, in *Microcystis* PCC 7806 (*IPF_3590*), and absence of this gene in *Microcystis* PCC 7005 and NIES-843 (**Table 8.1**). Besides Microcystis PCC 7806, *aqpZ* was detected in only one other *Microcystis* strain (PCC 9443).

Genes for the synthesis of the compatible solute glucosylglycerol that are present in the genome of the moderately halotolerant cyanobacterium *Synechocystis* PCC 6803 were not detected in the three *Microcystis* strains. However, genes for the synthesis of the compatible solute sucrosewere present in strain PCC 7806, but not in strains PCC 7005 and NIES-843 (**Table 8.1**). We did not detect any other compatible solute gene clusters in these three *Microcystis* strains. Moreover, extended genome analysis demonstrated that compatible solute synthetase genes appeared completely absent in all other *Microcystis* strains that we investigated (PCC 7005, NIES-843, NIES-44, PCC 7941, PCC 9432, PCC 9443, PCC 9701, PCC 9717, PCC 9806, PCC 9807, PCC 9808, PCC 9809, SPC77, T1-4 and TAIHU98).

**Table 8.1. Salt-dependent and salt acclimation genes in *Microcystis* strains.**

| Gene name | Function of gene product | *Synechocystis* PCC 6803 locus tags[a] | Homologue present in *Microcystis* PCC 7806[b] | Homologue present in *Microcystis* PCC 7005[b] | Homologue present in *Microcystis* NIES-843[b] | Locus tags of *Microcystis* homologues[c,e] | Protein BLAST bit score (*Microcystis* strain)[d,e] |
|---|---|---|---|---|---|---|---|
| Sodium transport | | | | | | | |
| *bicA* | Sodium-dependent bicarbonate transporter | *sll0834* | + | + | - | *IPF_4911* | 755 (PCC 7806) |
| *sbtA* | Sodium-dependent bicarbonate transporter | *slr1512* | - | + | + | *MAE_62090* | 614 (NIES-843) |
| *nhaS1* | Sodium/proton antiporter | *slr1727* | + | + | + | *IPF_4370* | 622 (PCC 7806) |
| *nhaS2* | Sodium/proton antiporter | *sll0273* | + | - | - | *IPF_5459* | 521 (PCC 7806) |
| *nhaS3* | Sodium/proton antiporter | *sll0689* | + | + | + | *IPF_4912* | 513 (PCC 7806) |
| *nhaS4* | Sodium/proton antiporter | *slr1595* | - | - | - | n.a. | n.a. |
| *nhaS5* | Sodium/proton antiporter | *slr0415* | - | - | - | n.a. | n.a. |
| *nhaS6* | Sodium/proton antiporter | *sll0556* | + | + | + | *MAE_60970* | 696 (NIES-843) |
| n.a. | Solute/sodium symporter | *sll1087* | + | + | + | *IPF_871* | 89.4 (PCC 7806) |
| *mrp* operon | Multicomponent sodium/proton antiporter | *slr2006-slr2012; ssr3409-ssr3410* | + | + | + | *IPF_634-IPF_641* | n.a. |
| *gltS* | Sodium-dependent glutamate transport | *slr1145* | + | + | + | *IPF_5465* | 561 (PCC 7806) |
| n.a. | Probable sodium-dependent transporter | *sll1428* | + | + | + | *IPF_5556* | 244 (PCC 7806) |
| n.a. | Sodium-dependent glutamate transport | *slr0625* | - | - | - | n.a. | n.a. |
| *gtrABC* | Sodium-dependent glutamate transport | *sll1102-sll1104* | - | - | - | n.a. | n.a. |
| n.a. | Probable sodium/calcium exchanger | *slr0681* | - | - | - | n.a. | n.a. |
| *sac1* | Probable sodium/sulfate symporter | *sll0640* | + | + | + | *MAE_12960* | 784 (NIES-843) |
| Potassium transport | | | | | | | |
| *kchX* | Potassium channel | *sll0993* | + | + | + | *IPF_4613* | 468 (PCC 7806) |
| *kdpA* | Potassium-transporting ATPase subunit A | *slr1728* | + | + | + | *IPF_1236* | 727 (PCC 7806) |
| *kdpB* | Potassium-transporting ATPase subunit B | *slr1729* | + | + | + | *IPF_1235* | 1036 (PCC 7806) |
| *kdpC* | Potassium-transporting ATPase subunit C | *slr1730* | + | + | + | *IPF_1229* | 221 (PCC 7806) |
| *kdpD* | Potassium-transporting ATPase subunit D | *slr1731* | + | + | + | *IPF_5006* | 475 (PCC 7806) |
| *ktrB/ntpJ* | Membrane subunit of a Ktr-like ion transport system | *slr1509* | + | + | + | *IPF_4143* | 526 (PCC 7806) |

Table 8.1 continued.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| n.a. | Potassium channel | *sll0261* | + | + | + | *IPF_2272* | 758 (PCC 7806) |
| *trkA* | Potassium uptake protein TrkA | *slr0773* | + | - | - | *IPF_5457* | 292 (PCC 7806) |
| *krtA* | Potassium uptake protein TrkA | *sll0493* | + | + | + | *IPF_4144* | 283 (PCC 7806) |
| n.a. | Inward rectifier potassium channel | *slr5078* | - | - | - | n.a. | n.a. |
| n.a. | Putative flavoprotein involved in $K^+$ transport | *slr0801* | - | - | - | n.a. | n.a. |
| *kchX* | Probable potassium channel | *sll0536* | + | + | + | *IPF_1361* | 375 (PCC 7806) |
| n.a. | Probable potassium channel | n.a. | + | + | + | *IPF_4452* | n.a. |
| Chloride transport | | | | | | | |
| n.a. | Chloride channel protein | *sll1864* | + | + | + | *IPF_3699* | 865 (PCC 7806) |
| n.a. | Chloride channel protein | *sll0855* | - | - | - | n.a. | n.a. |
| n.a. | Calcium-activated chloride channel homolog | *sll0103* | + | + | + | *IPF_3014* | 516 (PCC 7806) |
| n.a. | Calcium-activated chloride channel homolog | *slr7060* | - | - | - | n.a. | n.a. |
| Sucrose synthesis | | | | | | | |
| *spsA* | Sucrose phosphate synthase | *sll0045* | + | - | - | *IPF_1564* | 201 (PCC 7806) |
| *susA* | Sucrose cleavage glucosyltransferase | n.a. | + | - | - | *IPF_1565* | n.a. |
| *sppA* | Sucrose phosphate phosphatase | *slr0953* | + | - | - | *IPF_1566* | 254 (PCC 7806) |
| Glucosylglycerol synthesis | | | | | | | |
| *ggpS* | Glucosylglycerol-phosphate synthase | *sll1566* | - | - | - | n.a. | n.a. |
| *ggpP* | Glucosylglycerol-phosphate synthase | *slr0746* | - | - | - | n.a. | n.a. |
| *ggtA* | Glucosylglycerol transport system substrate-binding protein | *slr0747* | - | - | - | n.a. | n.a. |
| *ggtB* | Glucosylglycerol transport system substrate-binding protein | *slr0529* | - | - | - | n.a. | n.a. |
| *ggtC* | Glucosylglycerol transport system permease protein | *slr0530* | - | - | - | n.a. | n.a. |
| *ggtD* | Glucosylglycerol transport system permease protein | *slr0531* | - | - | - | n.a. | n.a. |

Table 8.1 continued.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Other genes | | | | | | | |
| *mscS* | Mechanosensitive channel | *slr0765; slr0639* | + (5) | + (3) | + (5) | *IPF_417*; *IPF_829*; *IPF_1474*; *IPF_1762*; *IPF_2625* | n.a. |
| *mscL* | Mechanosensitive channel | *slr0875* | - | - | - | n.a. | n.a. |
| *aqpZ* | Water channel protein | *slr2057* | + | - | - | *IPF_3590* | 119 (PCC 7806) |
| n.a. | Glycine/betaine transport system homolog | n.a. | + | + | + | *IPF_64* | n.a. |

[a]*Synechocystis* PCC 6803 was used as reference strain for comparison with *Microcystis* PCC 7806, PCC 7005 and NIES-843.

[b]Homologues present in the *Microcystis* strains are indicated with a plus sign ('+'), whereas absence is indicated with a dash sign ('–').

[c]The locus tags are from *Microcystis* PCC 7806 (IPF) or NIES-843 (MAE).

[d]The bit scores of the protein sequence alignments were calculated with BLAST (Altschul *et al.*, 1990).

[e]n.a.: not applicable.

## Discussion

Parker *et al.* (1997) previously reported that *Microcystis* is only dominant in lakes with low potassium ion concentrations ≤2.5 mmol $L^{-1}$ and high sodium/potassium ion ratios (>4). They found that growth of field-collected *Microcystis* was strongly reduced after addition of 5 mmol $L^{-1}$ potassium chloride (**Table 8.2**). A study by Shukla and Rai (2007) also showed that *Microcystis* growthwas strongly inhibited by addition of 4-8mmol $L^{-1}$ potassium chloride (**Table 8.2**), and that potassium ion addition inhibited phosphorus and nitrogen metabolism. In our study, exposure to 12 mmol $L^{-1}$ potassium chloride at a sodium/potassium ion ratio of ~0.8 strongly reduced the growth of the microcystin-producing strain NIES-843 and the non-microcystin-producing strain PCC 7005, but did not affect the microcystin-producing strain PCC 7806 and its non-toxic mutant. Hence, our results demonstrate that the effect of potassium ions on *Microcystis* is strain specific. Furthermore, our results show that potassium ion sensitivity does not depend on the capability of strains to produce microcystins.

Strain PCC 7806 was previously shown to withstand relatively high concentrations of sodium ions (~170 mmol $L^{-1}$; Tonk *et al.*, 2007). It was originally isolated from brackish water (the Braakman, the Netherlands), which may explain the high salt tolerance of this strain and its possession of genes against salt stress. The sodium/proton-antiporter gene *nhaS2* was resent in *Microcystis* PCC 7806, but was absent in the potassium ion sensitive strains PCC 7005 and NIES-843 and several other *Microcystis* strains. In *Synechocystis* PCC 6803, *nhaS2* (*sll0273*) plays an important role in the sodium ion influx of the cells. Moreover, Mikkat *et al.* (2000) showed that Δ*nhaS2* mutants of *Synechocystis* PCC 6803 were sensitive to potassium ions. They

also revealed that the potassium ion sensitivity of the Δ*nhaS2* mutants depends not only on the absolute potassium ion concentration but also on the sodium/potassium ion ratio. For instance, the Δ*nhaS2* mutants did not show growth at sodium/potassium ion ratios <2, unless the absolute potassium ion concentration was below 1 mmol $L^{-1}$. Therefore, Mikkat *et al.* (2000) concluded that the Δ*nhaS2* mutants of *Synechocystis* PCC 6803 behaved like potassium-sensitive cyanobacteria. Based on these results, it is likely that the presence of *nhaS2* also allows *Microcystis* PCC 7806 to better cope with low sodium/potassium ion ratios and elevated potassium ion concentrations than *Microcystis* strains lacking *nhaS2*.

Moreover, in contrast to *Microcystis* PCC 7005 and NIES-843, strain PCC 7806 contains the genes *trkA* and *aqpZ*. TrkA is a homologue to a low-affinity potassium ion transporter (Berry *et al.*, 2003), while AqpZ is a water channel protein (Shapiguzov *et al.*, 2005). Although *trkA* has not been studied in detail in cyanobacteria, an *aqpZ* insertion mutant of *Synechocystis* PCC 6803 lacked osmotic shrinkage (Shapiguzov *et al.*, 2005). Therefore, *aqpZ* likely plays an important role in the osmotic stress response of *Microcystis* PCC 7806.

**Table 8.2. Potassium ion sensitivity of various *Microcystis* strains.**

| *Microcystis* strain | Origin | Tested potassium ion concentration (mmol $L^{-1}$) | Growth inhibited by potassium ions | Reference |
|---|---|---|---|---|
| *Microcystis* PCC 7806 | The Netherlands | 12 | No | This study |
| *Microcystis* PCC 7005 | United States | 12 | Yes | This study |
| *Microcystis* NIES-843 | Japan | 12 | Yes | This study |
| *Microcystis* from Laxmi, Varanasi | India | 4.8 | Yes | Parker *et al.* 1997 |
| *Microcystis* UWOCC C3-9 | United States | 5-30 | Yes | Parker *et al.* 1997 |
| *Microcystis* from Luxmikund, Varanasi | India | 4-8 | Yes | Shukla and Rai 2007 |

Depending on the osmotic strength that cyanobacteria have to tolerate because of salts in their environment, a range of compatible solutes has been discovered in cyanobacteria, including sucrose, trehalose, glucosylglycerol, glucosylglycerate, glycine betaine and glutamate betaine (Hagemann, 2011). The involvement of sucrose synthesis in salt stress tolerance of *Microcystis* PCC 7806 was reported previously (Kolman *et al.*, 2012). In our analysis, *Microcystis* PCC 7806 was the only strain with sucrose synthesis genes, which confirms that sucrose synthesis is not common among Microcystis strains (Humbert *et al.*, 2013; Kolman *et al.*, 2015). Genes for the synthesis of other compatible solutes were absent in all sequenced

*Microcystis* strains that we investigated. However, one non-sequenced *Microcystis* strain, Gromov 398, was previously reported to synthesize glycosylglycerol (Erdmann *et al.*, 1992). In contrast to *Microcystis*, many *Anabaena* strains are able to produce sucrose (Reed *et al.*, 1984). Therefore, *Anabaena* could be more resistant to salt stress than most *Microcystis* strains. Yet, whether these compatible solutes play a role only in the protection against salt stress or also in potassium ion tolerance is not entirely clear. Although the synthesis of compatible solutes provides protection against salt stress by counteracting the outflow of water, addition of low potassium ion concentrations (<12 mmol $L^{-1}$) to combat harmful cyanobacterial blooms is likely not enough to be of osmotic significance.

The combined presence of sucrose synthesis genes, *nhaS2* and *aqpZ* in Microcystis PCC 7806 most likely gives this strain increased tolerance to potassium ions and salt stress compared to other *Microcystis* strains. Laboratory studies have shown that *Microcystis* PCC 7806 maintains a high growth rate at salt concentrations up to 10 g $L^{-1}$, and can temporarily endure salinities as high as 17.5 g $L^{-1}$ (Tonk *et al.*, 2007). These lab results are consistent with field observations, reporting dense *Microcystis* blooms in brackish waters across the globe at salinities up to 5-10 g $L^{-1}$, *e.g.*, in upper San Francisco Bay, USA (Lehman *et al.*, 2005; Moisander *et al.*, 2009), the Golden Horn estuary, Turkey (Taş *et al.*, 2006) and in the Swan River, Australia (Orr *et al.*, 2004). Furthermore, lower Microcystis concentrations have been found at salinities up to 18 g $L^{-1}$ (Lehman *et al.*, 2005; Taş *et al.*, 2006). Similar to freshwater ecosystems (Kardinaal *et al.*, 2007), *Microcystis* blooms of brackish waters appear to consist of mixtures of different *Microcystis* strains (Orr *et al.*, 2004; Moisander *et al.*, 2009). Hence, in addition to strain PCC 7806, other *Microcystis* strains tolerating elevated potassium ion concentrations and withstanding salt stress are expected to exist aswell. Potassium ion addition is therefore unlikely to be very successful against *Microcystis* blooms in brackish waters.

In contrast, most *Microcystis* strains isolated from freshwater ecosystems appear to be sensitive to potassium (**Table 8.2**). Hence, addition of 1-5 mmol $L^{-1}$ potassium chloride at a sodium/potassium ion ratio <2 could be very effective to temporarily suppress *Microcystis* blooms in freshwater lakes. Addition of such low concentrations of potassium chloride does not reduce the growth of macrophytes (Parker *et al.*, 1997), and may actually stimulate the growth of water fleas (Civitello *et al.*, 2014). However, *Microcystis* strains that are less sensitive to potassium ions might take over on the long run. Also other harmful cyanobacteria may take over the place of the suppressed *Microcystis* strains. Hence, lake treatments with potassium ionsmay not resolve the problems over a longer time span.

Our results also have implications for laboratory studies of *Microcystis* and other freshwater cyanobacteria. Potassium hydroxide, potassium phosphate and other potassium salts are often used in the mineral media of laboratory experiments with cyanobacteria. For example,

mineral medium with 10 mmol $L^{-1}$ TES buffer may already contain ~6 mmol $L^{-1}$ of potassium ions, if potassium hydroxide is used to set the pH of the buffer at 8.0. This concentration would exceed the tolerance limit of most (but not all) *Microcystis* strains. Although potassium is an essential element for cyanobacterial growth, potassium ion concentrations >1 mmol $L^{-1}$ should be avoided. Because *Microcystis* is much less sensitive to sodium ions, replacement of potassium salts by sodium salts should be the default choice.

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# Supplementary Information

**Table S8.1. Results of the two-way ANOVA, with the specific growth rate as the dependent variable and the *Microcystis* strain type and medium composition as independent variables.**

| Effect | $df_1$, $df_2$ | $F$ | $P$ |
|---|---|---|---|
| *Growth rate* | | | |
| Strain type | 3, 73 | 186.58 | <0.001 |
| Medium composition | 3, 73 | 369.81 | <0.001 |
| Strain type × medium composition | 9, 73 | 76.03 | <0.001 |

Columns indicate the variables of interest, the main effects and interaction effects, the degrees of freedom ($df_1$ and $df_2$), the value of the F-statistic ($F_{df1,df2}$) and the corresponding probability ($P$).

We tested if the specific growth rates were affected by the type of *Microcystis* strain and the medium composition. A two-way ANOVA was used, with the specific growth rate as dependent variable and the *Microcystis* strain type and medium composition as independent variables. All treatments were replicated 5-6 times. Type III Sum of Squares was used to account for unequal samples sizes. *Post-hoc* comparisons of the means were based on Tukey's unequal N HSD test, using a significance level $\alpha$ of 0.001.

The results of the two-way ANOVA show significant main effects of the *Microcystis* strain type and of the medium composition (**Table S8.1, Supplementary Information**). In contrast, the specific growth rate of strain PCC 7005, which contains both *bicA* and *sbtA*, was not significantly affected by the treatments. The results of the *post-hoc* comparison of the means are presented in **Figure 8.2** of the main text.