

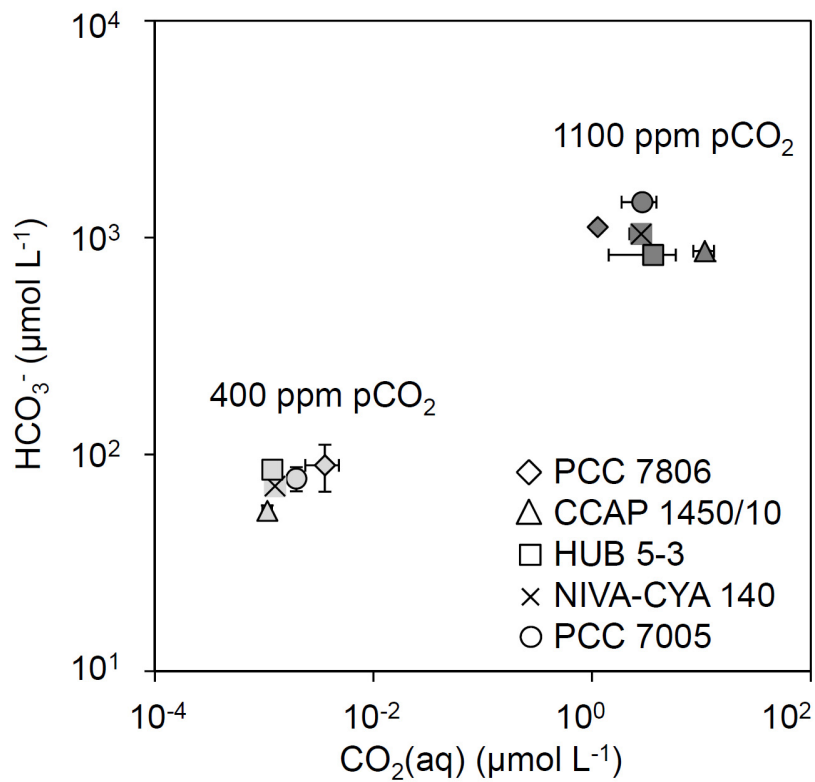
Supplemental Material

**Strains of the harmful cyanobacterium *Microcystis* differ in gene expression and activity of inorganic carbon uptake systems at elevated CO<sub>2</sub>**

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**FIG S1** Dissolved CO<sub>2</sub> (CO<sub>2</sub>(aq)) and bicarbonate concentrations in batch cultures of *Microcystis* strains exposed to ambient pCO<sub>2</sub> (400 ppm; light grey symbols) or elevated pCO<sub>2</sub> (1100 ppm; dark grey symbols) in the gas flow. CO<sub>2</sub>(aq) and bicarbonate concentrations were determined after four days of culturing. Error bars indicate standard deviation ( $n = 4$ ).

**TABLE S1** *Microcystis* primers used in this study for RT-qPCR gene expression analyses. The locus tags are from *Microcystis* PCC 7806 (IPF) and *Microcystis* NIES-843 (MAE). The amplification efficiency E for the individual primers sets was based on 32-48 amplification curves, except for *ccaA2* that was based on only 8 curves.

Primer name	Sequence 5'→3' (length)	Gene symbol	Function of complete protein/complex	Locus tag	Accession no. (Genbank)	Expected product size (bp)	Amplification efficiency E	Reference
16SrRNA-F	GTCGAACGGGAAT CTTCGGAT (21)	<i>16S rRNA</i>	Used here as reference gene for quantification of gene expression	IPF_5548	AM778951.1	157	1.91x±0.07	Sandrini <i>et al.</i> , 2015
16SrRNA-R	GCTAATCAGACGC AAGCTCTTC (22)							Sandrini <i>et al.</i> , 2015
cmpA-F	GTAAACACCCAG GGTAACGGA (22)	<i>cmpA</i>	High-affinity ATP- dependent bicarbonate uptake system	IPF_2181	AM778958.1	180	1.86±0.02	Sandrini <i>et al.</i> , 2015
cmpA-R	GCTAACAGTAAC GAATCCAGAAGT (25)							Sandrini <i>et al.</i> , 2015
sbtA-F	CTGGCCTTTTGAT TGGTGG (20)	<i>sbtA</i>	High-affinity bicarbonate/sodium symporter	MAE_62090	AP009552.1	143	1.87±0.02	This study
sbtA-R	AGGTTGGAATTGC GGATGG (19)							This study
bicA-F	CAAGCTAACGGTC GCATCAT (20)	<i>bicA</i>	Low-affinity bicarbonate/sodium symporter	IPF_4911	AM778949.1	132	1.88±0.02	Sandrini <i>et al.</i> , 2015
bicA-R	AGGCACATCACTC AAGTCCA (20)							Sandrini <i>et al.</i> , 2015
chpX-F	CCTGTCAAGTCTC CTCTCAT (21)	<i>chpX</i>	Low-affinity CO <sub>2</sub> uptake system	IPF_1842	AM778957.1	113	1.88±0.02	Sandrini <i>et al.</i> , 2015
chpX-R	TTCAGGATACCCA CTACCTCG (21)							Sandrini <i>et al.</i> , 2015
chpY-F	ATATCGCCAAAAT GCCGACC (20)	<i>chpY</i>	High-affinity CO <sub>2</sub> uptake system	IPF_1545	AM778958.1	114	1.80±0.04	Sandrini <i>et al.</i> , 2014
chpY-R	GACATCATCCGCA CCTGTTC (20)							Sandrini <i>et al.</i> , 2014
ccmR-F	CCTACCGTCTCAAC CCAAGT (20)	<i>ccmR</i>	Transcriptional regulator of CCM genes	IPF_1549	AM778958.1	109	1.88±0.02	Sandrini <i>et al.</i> , 2014
ccmR-R	ACAGTAATTCCTG ACCCGCTT (21)							Sandrini <i>et al.</i> , 2014
ccmR2-F	TCCTGGGATAAA CCACATACCA (23)	<i>ccmR2</i>	Transcriptional regulator of CCM genes	IPF_2166	AM778949.1	204	1.86±0.02	Sandrini <i>et al.</i> , 2014
ccmR2-R	TTTTCTCGACCATG GCATCAC (21)							Sandrini <i>et al.</i> , 2014
rbcX-F	CGGATCATGACGG TAAGAGAACA (23)	<i>rbcX</i>	RuBisCO chaperone, in same operon as genes of small and large subunit of RuBisCO	IPF_2531	AM778933.1	157	1.86±0.01	Sandrini <i>et al.</i> , 2015
rbcX-R	ATCCGATGTCTCT GGTTGACT (22)							Sandrini <i>et al.</i> , 2015

ccmM-F	AAGTCCACACCTTC TCTAACCTC (23)	<i>ccmM</i>	Carboxysomal protein; in same operon as other carboxysomal genes	IPF_5695	AM778933.1	118	1.87±0.02	Sandrini <i>et al.</i> , 2014
ccmM-R	CTGTGTCGCCAAT GTGAA (19)							Sandrini <i>et al.</i> , 2014
ccaA1-F	ACTCCTGCGGTAA TACTGTGG (22)	<i>ccaA</i>	Carboxysomal carbonic anhydrase	IPF_5538	AM778919.1	97	1.88±0.02	Sandrini <i>et al.</i> , 2014
ccaA1-R	GATAAATGCGATC AGCTTGGGAG (23)							Sandrini <i>et al.</i> , 2014
ccaA2-F	ATTCGTTCCCGATT ACATCAAGG (23)	<i>ccaA2</i>	Carboxysomal carbonic anhydrase	MAE_36560	AP009552.1	137	1.88±0.02	Sandrini <i>et al.</i> , 2014
ccaA2-R	AAGTTGCTCAACG GGATCG (19)							Sandrini <i>et al.</i> , 2014
ecaA-F	CCCAAGAACCTTCT CCTGAAATG (23)	<i>ecaA</i>	$\alpha$ -type carbonic anhydrase; presumably mainly present in the periplasmic space	IPF_4566	AM778932.1	187	1.86±0.02	Sandrini <i>et al.</i> , 2014
ecaA-R	GCCAATTGTTGCA GTTGTTGG (21)							Sandrini <i>et al.</i> , 2014
mcyB-F	ATCCCATGCTCAG AGACGTT (20)	<i>mcyB</i>	Microcystin synthesis	IPF_375	AM778952.1	163	1.86±0.03	Sandrini <i>et al.</i> , 2014
mcyB-R	AGATGTCCGCAGG GATTCAT (20)							Sandrini <i>et al.</i> , 2014

**TABLE S2** Changes in gene expression at elevated CO<sub>2</sub> for each of the six *Microcystis* strains. Gene expression changes were obtained by RT-qPCR applied to samples taken before and 20 h after increasing the pCO<sub>2</sub> level from 400 to 1100 ppm. Bold values in the ‘Log2 relative gene expression’ column indicate expression changes < -0.8 or > 0.8. Bold values in the ‘FDR adjusted *p*-values’ column indicate significant changes (*p* < 0.05).

<i>Microcystis</i> strain	Gene	Log2 relative gene expression change	Standard deviation	FDR adjusted <i>p</i> -values
PCC 7806	<i>cmpA</i>	<b>-6.998</b>	1.456	<b>0.011</b>
	<i>bicA</i>	<b>-3.093</b>	0.407	<b>0.007</b>
	<i>chpX</i>	0.132	0.097	0.130
	<i>chpY</i>	-0.101	0.205	0.458
	<i>ccmR</i>	<b>-3.778</b>	0.548	<b>0.007</b>
	<i>ccmR2</i>	-0.436	0.079	<b>0.010</b>
	<i>rbcX</i>	0.062	0.509	0.848
	<i>ccmM</i>	-0.244	0.204	0.168
	<i>ccaA1</i>	0.144	0.091	0.104
	<i>ecaA</i>	-0.367	0.046	<b>0.007</b>
<i>mcyB</i>	-0.276	0.107	<b>0.040</b>	
NIES-843	<i>cmpA</i>	<b>-1.713</b>	0.486	<b>0.022</b>
	<i>sbtA</i>	<b>-1.818</b>	0.273	<b>0.007</b>
	<i>chpX</i>	0.154	0.261	0.412
	<i>chpY</i>	0.575	0.607	0.254
	<i>ccmR</i>	<b>-3.683</b>	0.377	<b>0.007</b>
	<i>ccmR2</i>	-0.393	0.256	0.109
	<i>rbcX</i>	<b>0.839</b>	0.369	0.052
	<i>ccmM</i>	0.642	0.387	0.096
	<i>ccaA1</i>	0.246	0.178	0.462
	<i>ccaA2</i>	-0.169	0.284	0.412
<i>mcyB</i>	0.758	0.539	0.127	
CCAP 1450/10	<i>cmpA</i>	<b>-3.830</b>	0.171	<b>0.007</b>
	<i>sbtA</i>	<b>-2.870</b>	0.590	<b>0.011</b>
	<i>chpX</i>	-0.036	0.483	0.906
	<i>chpY</i>	0.026	0.541	0.930
	<i>ccmR</i>	<b>-2.165</b>	0.428	<b>0.011</b>
	<i>ccmR2</i>	-0.695	0.369	0.072
	<i>rbcX</i>	0.399	0.051	<b>0.007</b>
	<i>ccmM</i>	0.041	0.304	0.843
	<i>ccaA1</i>	-0.279	0.485	0.414
	<i>ecaA</i>	-0.253	0.402	0.399
<i>mcyB</i>	-0.252	0.415	0.411	

NIVA-CYA 140	<i>cmpA</i>	<b>-7.225</b>	0.348	<b>0.002</b>
	<i>sbtA</i>	-0.432	0.766	0.417
	<i>bicA</i>	0.274	0.198	0.127
	<i>chpX</i>	-0.366	0.373	0.246
	<i>chpY</i>	0.111	0.229	0.459
	<i>ccmR</i>	<b>-1.837</b>	0.291	<b>0.007</b>
	<i>ccmR2</i>	-0.370	0.704	0.444
	<i>rbcX</i>	-0.712	0.318	0.053
	<i>ccaA1</i>	-0.482	0.733	0.399
	<i>ecaA</i>	0.094	0.144	0.399
	<i>mcyB</i>	<b>0.966</b>	0.290	<b>0.023</b>
HUB 5-3	<i>cmpA</i>	<b>-1.169</b>	0.433	<b>0.037</b>
	<i>sbtA</i>	<b>-2.703</b>	0.619	<b>0.013</b>
	<i>bicA</i>	<b>-3.235</b>	0.775	<b>0.014</b>
	<i>chpX</i>	-0.162	0.414	0.530
	<i>chpY</i>	-0.390	0.446	0.271
	<i>ccmR</i>	<b>-1.425</b>	0.723	0.068
	<i>ccmR2</i>	<b>-0.820</b>	0.552	0.114
	<i>rbcX</i>	0.221	0.111	0.068
	<i>ccmM</i>	0.092	0.247	0.542
	<i>ccaA1</i>	-0.310	0.356	0.271
	<i>ecaA</i>	-0.458	0.511	0.269
PCC 7005	<i>cmpA</i>	<b>-4.328</b>	0.600	<b>0.007</b>
	<i>sbtA</i>	0.518	0.489	0.124
	<i>bicA</i>	0.591	0.202	<b>0.031</b>
	<i>chpX</i>	0.346	0.177	0.068
	<i>chpY</i>	0.123	0.301	0.521
	<i>ccmR</i>	<b>-1.635</b>	0.349	<b>0.011</b>
	<i>ccmR2</i>	0.369	0.395	0.254
	<i>rbcX</i>	-0.612	0.956	0.399
	<i>ccmM</i>	-0.513	0.768	0.399
	<i>ccaA1</i>	0.306	0.123	<b>0.043</b>
<i>ecaA</i>	0.519	0.155	<b>0.023</b>	

***References***

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