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Fluorescence in situ hybridization (FISH) of *Microcystis* strains producing microcystin using specific mRNA probes

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Supporting information

Figure S1. Alignment (Clustal Omega; <http://www.ebi.ac.uk/Tools/msa/clustalo/>) of the rRNA 16S sequences of the cyanobacterial strains used in this study. The complementary sequences of the EUB338 and MICR3 probes are highlighted in purple and green, respectively. A star indicates identity. Accessions: *Microcystis aeruginosa* PCC 7806, EMBL: AM778843–AM778958; *Microcystis aeruginosa* PCC 7005: GenBank: AQPY00000000 ; *Chlorogloeopsis fritschii* PCC 6912 GenBank: AJLN01000001–AJLN01000161.

Percent identity matrix for 1474 bases

	PCC 6912	PCC 7806	PCC 7005	
PCC 6912	100.00	89.27	89.27	
PCC 7806	89.27	100.00	99.80	
PCC 7005	89.27	99.80	100.00	
PCC_6912_rRNA16S	AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGTATGCTTAACACATGCAAGTCGAAC			60
PCC_7806_rRNA16S	AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAAC			60
PCC_7005_rRNA16S	AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAAC			60
***** **				
PCC_6912_rRNA16S	GGTCTTTTTCGGAAGATAGTGGCGGACGGGTGAGTAACGCGTGAGAATCTGGCTCTAGGT			120
PCC_7806_rRNA16S	GGGAATCTTCGGATTCTAGTGGCGGACGGGTGAGTAACGCGTAAGAATCTAACTTCAGGA			120
PCC_7005_rRNA16S	GGGAATCTTCGGATTCTAGTGGCGGACGGGTGAGTAACGCGTAAGAATCTAACTTCAGGA			120
** * *****				
PCC_6912_rRNA16S	TCGGGACAACCCTGGAAACGGTGGCTAATACCGGATGTGCCGAAGAGGTGAAAGGCTAG			180
PCC_7806_rRNA16S	CGGGGACAACAGTTGGAAACGACTGCTAATACCGGATGTGCCGCAAG-GTGAAACCTAAT			179
PCC_7005_rRNA16S	CGGGGACAACAGTTGGAAACGACTGCTAATACCGGATGTGCCGCAAG-GTGAAACCTAAT			179
***** ***** ***** * ***** *				
PCC_6912_rRNA16S	CTGCCCTAGAGATGAGCTCGCTCTGATTAGCTAGATGGTGGGGTAAGAGCCTACCATGGC			240
PCC_7806_rRNA16S	TGGCCTGGAGAAGAGCTTTCGCTCTGATTAGCTAGTGGTGGGGTAAGAGCCTACCAAGGC			239
PCC_7005_rRNA16S	TGGCCTGAAGAAGAGCTTTCGCTCTGATTAGCTAGTGGTGGGGTAAGAGCCTACCAAGGC			239
**** * ** *****				
PCC_6912_rRNA16S	GACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG			300
PCC_7806_rRNA16S	GACGATCAGTAGCTGGTCTGAGAGGATGAGCAGCCACACTGGGACTGAGACACGGCCAG			299
PCC_7005_rRNA16S	GACGATCAGTAGCTGGTCTGAGAGGATGAGCAGCCACACTGGGACTGAGACACGGCCAG			299

PCC_6912_rRNA16S	ACTCCTACGGGAGGCAGCAGTGGGGAATTTCCGCAATGGGCGAAAGCCTGACGGAGCAA			360
PCC_7806_rRNA16S	ACTCCTACGGGAGGCAGCAGTGGGGAATTTCCGCAATGGGCGAAAGCCTGACGGAGCAA			359
PCC_7005_rRNA16S	ACTCCTACGGGAGGCAGCAGTGGGGAATTTCCGCAATGGGCGAAAGCCTGACGGAGCAA			359

PCC_6912_rRNA16S	TACCGCGTGAGGGAGGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAGGGAAGAAGAT			420
PCC_7806_rRNA16S	CGCCGCGTGAGGGAGGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAAGGAAGAAGTTCT			419
PCC_7005_rRNA16S	CGCCGCGTGAGGGAGGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAAGGAAGAAGTTCT			419
***** *****				
PCC_6912_rRNA16S	GACGGTACCTGAGGAAGAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAG			480
PCC_7806_rRNA16S	GACGGTACCTGAGGAATCAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGGG			479
PCC_7005_rRNA16S	GACGGTACCTGAGGAATCAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGGG			479
***** ***** **				
PCC_6912_rRNA16S	GATGCAAGCGTTATCCGGAATGATGGGCGTAAAGCGTCCGTAGGTGGTGTGCAAGTCT			540
PCC_7806_rRNA16S	GAGGCAAGCGTTATCCGGAATGATGGGCGTAAAGCGTCCGTAGGTGGTGTGCAAGTCT			539
PCC_7005_rRNA16S	GAGGCAAGCGTTATCCGGAATGATGGGCGTAAAGCGTCCGTAGGTGGTGTGCAAGTCT			539
** *****				
PCC_6912_rRNA16S	ATTGTCAAAGCGTGCGGCTCAACCGCATAAAGGCAGTGAAACTGTGTAACCTAGAGTGCA			600
PCC_7806_rRNA16S	GCCGTCAAATCAGGTTGCTTAACGACCTAAAGGCGGTGGAACTGGCAGACTAGAGAGCA			599
PCC_7005_rRNA16S	GCCGTCAAATCAGGTTGCTTAACGACCTAAAGGCGGTGGAACTGGCAGACTAGAGAGCA			599
***** * * ** ** * *****				
PCC_6912_rRNA16S	TTCGGGGCAGGGGGAATTCCTGGTGTAGCGGTGAAATGCGTAGAGATCAGGAAGAACACC			660
PCC_7806_rRNA16S	GTAGGGGTAGCAGGAATTCAGTGTAGCGGTGAAATGCGTAGAGATTGGGAAGAACATC			659
PCC_7005_rRNA16S	GTAGGGGTAGCAGGAATTCAGTGTAGCGGTGAAATGCGTAGAGATTGGGAAGAACATC			659
* **** * *****				

Figure S2. Alignment (Clustal Omega) of the *rpoB* gene sequences of the *M. aeruginosa* strains used in this study. The complementary sequence of the RPOB probe is highlighted in red. The sequences show 97.10% identity (indicated by a star) for 3312 bases. Accessions: *Microcystis aeruginosa* PCC 7806, EMBL: AM778843–AM778958; *Microcystis aeruginosa* PCC 7005; GenBank: AQPY00000000.

PCC7806rpoB	ATGAACAATCTTACCTTTAATCTCTTACCCGACCTGATCGAAATCCAACACTCCAGTTTT	60
PCC7005RpoB	ATGAACAATCTTACCTTTAATCTCTTACCCGACCTCATCGAAATCCAACACTCCAGTTTT	60

PCC7806rpoB	CGGTGGTTTTTTGGAAGAAGGACTCATCGAGGAACGAATAGCTTTTCCCCCATCAGCGAC	120
PCC7005RpoB	CGGTGGTTTTTTGGAAGAAGGACTAATCGAGGAACGAACAGCTTTTCCCCCATCAGCGAC	120

PCC7806rpoB	TATACAGGTAAATTAGAATACATTTCCCTCGGCCAGGACTACAGACTCAAAGAACCGAAA	180
PCC7005RpoB	TATACAGGTAAATTAGAATACATTTCCCTCGGCCAGGACTACAGACTCAAAGAACCGAAA	180

PCC7806rpoB	TACAACGTCGATGAAGCCAACGACGCGATAGCAGCTATTCTGTTTCAGATGTACGTTCCC	240
PCC7005RpoB	TACAACGTCGATGAAGCCAACGACGCTGATAGCACCTATTCCGTTTCAGATGTACGTTCC	240

PCC7806rpoB	ACCCGTCTAATCAACAAAGAAACGGGAGAAAACATCGAACAGGAAGTTTTATCGGCGAT	300
PCC7005RpoB	ACCCGTCTAATCAACAAAGAAACGGGAGAAAACATCGAACAGGAAGTTTTATCGGCGAT	300

PCC7806rpoB	TTACCCCTGATGACCGAACGGGAACTTTATCATTAACGGGGCTGAACGAGTTCATCGTC	360
PCC7005RpoB	TTACCCCTGATGACCGAACGGGAACTTTATCATTAACGGGGCTGAACGAGTTCATCGTC	360

PCC7806rpoB	AATCAGATCGTCCGTTCCCGGTTTACTACAAAGCCGAAATCGACAAAAACGGTCGC	420
PCC7005RpoB	AATCAGATCGTCCGTTCCCGGTTTACTACAAAGCCGAAATCGACAAAAACGGTCGC	420

PCC7806rpoB	CGCACCTACTCCGTTCCCTGATTTCCCAACCGAGGAGCTTGGTTAAAATTTGAAACCGAC	480
PCC7005RpoB	CGTACCTACTCCGTTCCCTGATTTCCCAACCGAGGAGCTTGGTTAAAATTTGAAACCGAC	480
	** *****	
PCC7806rpoB	AAAAACGGCTTAGTTTGGGTCAGAATCGACAAAACCCGTAATATCCGCCAAGTTCTC	540
PCC7005RpoB	AAAAACGGCTTAGTTTGGGTCAGAATCGACAAAACCCGTAATATCCGCCAAGTTCTC	540

PCC7806rpoB	CTGAAAGCGATCGGATTGAGCGATAACGAAATCTTTGACTCCCTACGCCATCCCGAATTT	600
PCC7005RpoB	CTGAAAGCGATCGGATTGAGCGATAACGAAATCTTTGACTCCCTACGCCATCCCGAATTT	600

PCC7806rpoB	TACCAAAAAACCCTCGACAAAGAAGGCAATCCACGGAAGAAGAGGCCCTGCTCGACCTG	660
PCC7005RpoB	TACCAAAAAACCCTCGACAAAGAAGGCAATCCACGGAAGAAGAGGCACCTCTCGACCTG	660

PCC7806rpoB	TACCGCAAACCTACGCCCGGGAACTCCACCGTCACTGGCGGACAACAACCTACTCGAA	720
PCC7005RpoB	TACCGCAAACCTACGCCCGGGAAACCCCCACCGTCACTGGCGGACAACAACCTACTCGAA	720

PCC7806rpoB	TCGCGCTTTTTTGACAATAAACGCTACGATCTCGGGCGCGTTGGTTCGTTATAAACTCAAC	780
PCC7005RpoB	TCGCGCTTTTTTGACAATAAACGCTACGATCTCGGGCGCGTTGGTTCGTTATAAACTCAAC	780

PCC7806rpoB	AAAAAACTACGCCCTCAACGTCCCCGATAACAGCGCGTTCTCACCACCACCGACATTTTA	840
PCC7005RpoB	AAAAAACTACGCCCTCAACGTCCCCGATAACAGCGCGTTCTCACCACCACCGACATCTTA	840

PCC7806rpoB	TCGGCGATCGACTACCTAATTAACCTCGAATTCGACATCGGTAACACCGACGACATCGAC	900
PCC7005RpoB	TCGGCGATCGACTACCTAATTAACCTCGAATTTGACATCGGCAACACCGACGACATCGAC	900

PCC7806rpoB	CACCTCGGCAACCGTCGCGTCCGTTCCGTTGGGTGAAGTGTCCAAAACCAAGTGCAGTA	960
PCC7005RpoB	CACCTCGGCAACCGTCGCGTCCGTTCCGTTGGGTGAAGTGTCCAAAACCAAGTGCAGTA	960

PCC7806rpoB	GGTTTAAACCGCTTAGAAAGAATCATTCGGGAACGGATGACCGTCAGCGAATCCCAAAAC	1020
PCC7005RpoB	GGTTTAAACCGCTTAGAAAGAATCATTCGGGAACGGATGACCGTCAGCGAATCCCAAAAC	1020

PCC7806rpoB	CTCACCCCGCTTCCCTAGTCAACCCGAAACCCCTAGTAGCGGCGATTAAAGAATCTTTT	1080
PCC7005RpoB	CTCACCCCGCTTCCCTAGTCAACCCGAAACCCCTAGTAGCGGCGATTAAAGAATCTTTT *****	1080
PCC7806rpoB	GGTTCCTCGCAACTCTCCAGTTTATGGATCAAACCAACCCCTAGCGGAATTGACCCAT	1140
PCC7005RpoB	GGTTCCTCGCAACTCTCCAGTTTATGGATCAAACCAACCCCTAGCGGAATTGACCCAT *****	1140
PCC7806rpoB	AAACGCCGATTTCCGCCCTTGGACCCGGCGGTCTCACCCGGGAACGGGCCGTTTGGCC	1200
PCC7005RpoB	AAACGCCGATTTCCGCCCTTGGACTGGCGGTCTCACCCGGGAACGGGCCGTTTGGCC *****	1200
PCC7806rpoB	GTGCGGACATTCACCCCTCCACACCGTTCGATTTGTCCGGTGGAAACCCAGAAAGGT	1260
PCC7005RpoB	GTGCGGACATTCACCCCTCCACACCGTTCGATTTGTCCGGTGGAAACCCAGAAAGGT *****	1260
PCC7806rpoB	CCCAACGCTGGTTTAAATCGGTTCCCTAGCCACCTACGCCCGGTCAACGACTACGGTTTT	1320
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PCC7005RpoB	ATTGAAACCCCTGTTATGGGGTGGAAAAATGGCCGAGTTCGCTACGATCTCCCGTCAA *****	1380
PCC7806rpoB	TACCTCACCGCCGACGAAGAAGACGATCTCAGAGTCGCCCGGGAGACGTGGCCACCGAT	1440
PCC7005RpoB	TACCTCACCGCCGACGAAGAAGACGATCTCAGAGTCGCCCGGGAGACGTGGCCACCGAT *****	1440
PCC7806rpoB	GAAATGGTTATATCCTCGGGCAAATATCCCGTGCCTATCGGCAGGAATTCTCCACC	1500
PCC7005RpoB	GAAATGGTTATATCCTCGGGCAAATATCCCGTGCCTATCGGCAGGAATTCTCCACC *****	1500
PCC7806rpoB	ACCTCCCCGAACAAGTGGACTACGTTTGTGTTTCCCCGTGCAAATGTCTCGGTGGCC	1560
PCC7005RpoB	ACTTCCCCGAACAAGTGGACTACGTTTGTGTTTCCCCGTGCAAATGTCTCGGTGGCC ** *****	1560
PCC7806rpoB	ACTTCCCTGATTCCCTTCCCTCGAACACGACGACGCTAACCGCGCCCTCATGGGTCCAAT	1620
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PCC7806rpoB	ATGCAGCGACAAGCGGTTCCCTGCTGCGTCCCGAACGTCCCTTGTGGGAACGGGATTG	1680
PCC7005RpoB	ATGCAGCGACAAGCGGTTCCCTGCTGCGTCCCGAACGTCCCTTGTGGGACTGGATTG *****	1680
PCC7806rpoB	GAAGCACAGGCCGCTCGCGACTCCGGCATGGTTCATCGTTTCCCGTACCAATGGAGTAGTT	1740
PCC7005RpoB	GAAGCACAGGCCGCTCGCGACTCCGGCATGGTTCATCGTTTCCCGTACCAATGGGTCGTT *****	1740
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PCC7806rpoB	GGCAAGAGCGAGATCGAGTACGAAATTCAGAAATATCAACGCTCTAACCAAGATACCTGC	1860
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PCC7005RpoB	ATGCCCTGGGAAGGCTATAACTACGAAGATGCGATTTTAATCAGTGAAAGACTGGTGGCA *****	2040
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PCC7005RpoB	CAAGACACCTACACAGTATCCACATTGAAAAATACGAAATCGAAGCCCGTCAAACATAA *****	2100
PCC7806rpoB	CTCGGACCAGAGGAAATCACCAGGAAATTCCCAACGTCGGTGAAGATGCCCTGCGAAAC	2160
PCC7005RpoB	CTAGGACCAGAGGAAATCACCAGGAAATTCCCAACGTCGGTGAAGATGCCCTGCGAAAC ** *****	2160
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PCC7806rpoB	CACGCCCGTCTACCGGTCCCTACTCCTTGGTTACTCAGCAACCCCTGGGCGGTAAAGCT	2940
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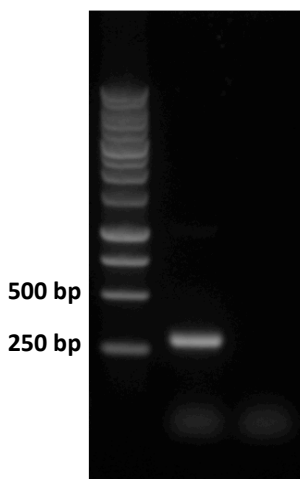
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PCC7806rpoB	AATGCCCCGATGGTACTTCCCGCATGTGGAGGTGGATCTGATGGTCGATGTCGGTCTG	3240
PCC7005RpoB	AATGCCCCGATGGTACTTCCCGCATGTGGAGGTGGATCTGATGGTCGATGTCGGTCTG	3240

PCC7806rpoB	CGCGCCCCCTCTCGCCCCACTACGAATCCTTAACCACAGAAGACCTTGAGGAAGAAGAA	3300
PCC7005RpoB	CGCGCCCCCTCTCGCCCCACTACGAATCCTTAACCACAGAAGACCTTGAGGAAGAAGAA	3300

PCC7806rpoB	TCGGAAGTGTA 3312	
PCC7005RpoB	TCGGAAGTGTA 3312	

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 CCTCGATTTTCCAGTAGCGCACTTGAATCAACAGGAATTAGACGATATTTCTGGCAGAAATTGACTAA 8388



Ladder
 PCC 7806
 PCC 7005

Figure S4. Epifluorescence micrographs (FITC filter set; $\times 40$ magnification; 100 ms exposure; scale bar 30 μm) of *Microcystis aeruginosa* PCC 7806 (A), of *Microcystis aeruginosa* PCC 7005 (B) and of *Chlorogloeopsis fritschii* PCC 6912 (C) with the EUB338-HRP probe.

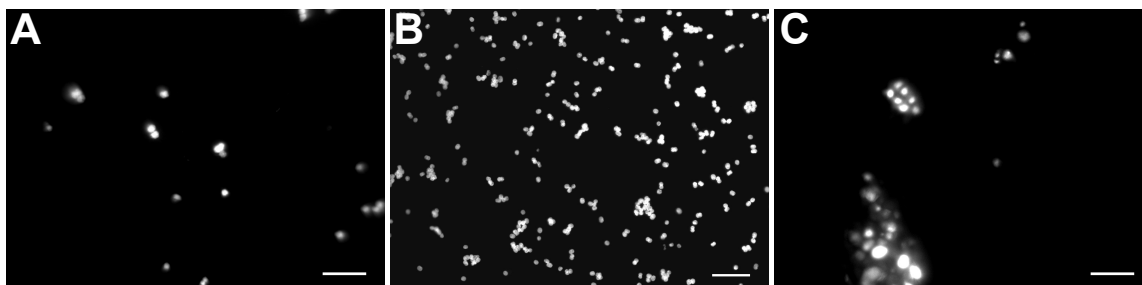


Figure S5. Epifluorescence micrographs (superposition of two images, one obtained with the Cy3 filter set, in red, and the other one obtained with the FITC filter set, in green; $\times 63$ magnification; 500 ms exposure; scale bar 30 μm) of *Microcystis aeruginosa* PCC 7806 (A) unlabeled, (B) labeled with probe MCYA-HRP and (C) labeled with probe RPOB-HRP, and of *Microcystis aeruginosa* PCC 7005 (D) unlabeled, (E) labeled with probe MCYA-HRP and (F) labeled with probe RPOB-HRP.

