

Supporting information

Engineering an obligate photoautotrophic cyanobacterium to utilize glycerol for growth and chemical production

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Table S1 Oligonucleotides used in this study

Name	Sequence (5'-3')
MK139	CGACTGCACGGTGCACCAAT
MK140	CTGTTTCCTGTGTGAAATTGTTAT
MK141	ATTGGTGCACCGTGCAGTCGCTCGAGTCTCATCCCTAAC
MK142	CAATTTACACAGGAAACAGGAATTCATTAAAGAGGAGAAAGG
MK372	TTGCACCAGGATCCCGCTCGCGACTGCACGGTGCACCAAT
MK375	GACTGGAAAGCGGGCAGTGAATTAATGCAGCTTAAGGTTG
MK376	CAACCTTAAGCTGCATTAATTCAGTCCCGCTTTCCAGTC
MK377	TGCACCGTGCAGTCGCGAGCGGGATCCTGGTGCAA
MK662	TTTGAATGTATTTAGAAAAAGGCTGCCAGCCCGAAACAGC
MK663	AGTAACAACCTATATCGTATCCCTGCTCGTCACGCTTTCA
MK664	TGAAAGCGTGACGAGCAGGGATACGATATAAGTTGTTACT
MK665	CATCTTCCTGCTCCAGAAGCCTCGCCCAATGGACAAGGGA
MK666	TCCCTTGTCATTGGGCGAGGCTTCTGGAGCAGGAAGATG
MK667	ACTCAGGAGAGCGTTCACCGATGGATCTGACCAACATGAT
MK668	ATCATGTTGGTCAGATCCATCGGTGAACGCTCTCCTGAGT
MK669	GCTGTTTCGGGCTGGCAGCCTTTTTCTAAATACATTCAA
MK671	ATTGTGAGCGGATAACAATTAACATTAACCTTTCAGGAT
MK672	CCTCATTCACTTTTCGTAAATTATTCGTCTGTTCTTCCC
MK673	GGGAAGAACACGACGAATAATTTAACGAAAGTGAATGAGG
MK674	GCAGTTCCTACTCTCGCTTTTACGACGCCAGCGATAACC
MK675	GGTTATCGCTGGCGTCGTAAAAGCGAGAGTAGGGAAGTGC
MK676	TGAAGAGTTAATGTTTAATTGTTATCCGCTCACAATTC
MK1204	ATTGTGAGCGGATAACAATTAAGCGTGGAGAATTAAATG
MK1205	GCAGTTCCTACTCTCGCTTTTAAGCCTGTTTAGCCGCTTC
MK1206	AAGCGGCTAAACAGGCTTAAAGCGAGAGTAGGGAAGTGC
MK1207	CATTTTAATTCTCCACGCTTAATTGTTATCCGCTCACAATTC
MK1212	ATTGTGAGCGGATAACAATTTATGACTACGGGACAATTAA
MK1213	TTAATTGTCCCGTAGTCATAAATTGTTATCCGCTCACAATTC
MK1233	AACAAAAAGCTTCGCTGTAATGCTGGAGCAAAATAATGAA
MK1234	TGCGGTCCATAATTGCTCCTTTAACCCCTGACGGTTGAAAC
MK1235	GTTTCAACCGTCAGGGTTAAAGGAGCAATTATGGACCGCA
MK1236	GCAGTTCCTACTCTCGCTTTTATTCCCACTCTTGCAGGA
MK1237	TCCTGCAAGAGTGGGAATAAAAGCGAGAGTAGGGAAGTGC

MK1238	TTCATTATTTTGCTCCAGCATTACAGCGAAGCTTTTTGTT
MK1311	GCAGTTCCTACTCTCGCTTTTACAGCGAAGCTTTTTGTT
MK1312	AACAAAAAGCTTCGCTGTAAAAGCGAGAGTAGGGAACTGC

Table S2 Plasmid construction by SLIC

Plasmid	PCR					
	Primers	Template	Insert(s)	Primers	Template	
pAL1136	MK139/MK140	pAL40 ^a	<i>P</i> trc	MK141/MK142	pAL1040 ^b	backbone
pAL1533	MK376/MK377	pAL1040 ^b	<i>lac</i> ^R	MK1312/MK375	pAL974 ^c	backbone
	MK671/MK1311	<i>E. coli</i> gDNA	<i>glpF</i>			
	MK372/MK676	pAL40 ^a	<i>P</i> trc			
pAL1310	MK671/MK672	<i>E. coli</i> gDNA	<i>glpF-glpK</i>	MK675/MK676	pAL1533	backbone
	MK673/MK674	<i>E. coli</i> gDNA	<i>glpD</i>			
pAL1498	MK674/MK1212	pAL1310	<i>glpK-glpD</i>	MK675/MK1213	pAL1310	backbone
pAL1506	MK1233/MK1234	<i>E. coli</i> gDNA	<i>dhaKLM</i>	MK1237/MK1238	pAL1310	backbone
	MK1235/MK1236	<i>E. coli</i> gDNA	<i>gldA</i>			
pAL1314	MK662/MK663	<i>S. elongatus</i> gDNA	5'NSI	MK668/MK669	pZE12-luc	backbone
	MK666/MK667	<i>S. elongatus</i> gDNA	3'NSI			
	MK664/MK665	pAL40 ^a	<i>spec</i> ^R - <i>lac</i> ^R - <i>P</i> trc: <i>galP</i>			
pAL1495	MK1204/MK1205	<i>E. coli</i> gDNA	<i>tpiA</i>	MK1206/MK1207	pAL1314	backbone

^a McEwen *et al.*, 2013,¹ ^b Nozzi *et al.*, 2015,² ^c Oliver *et al.*, 2015.³

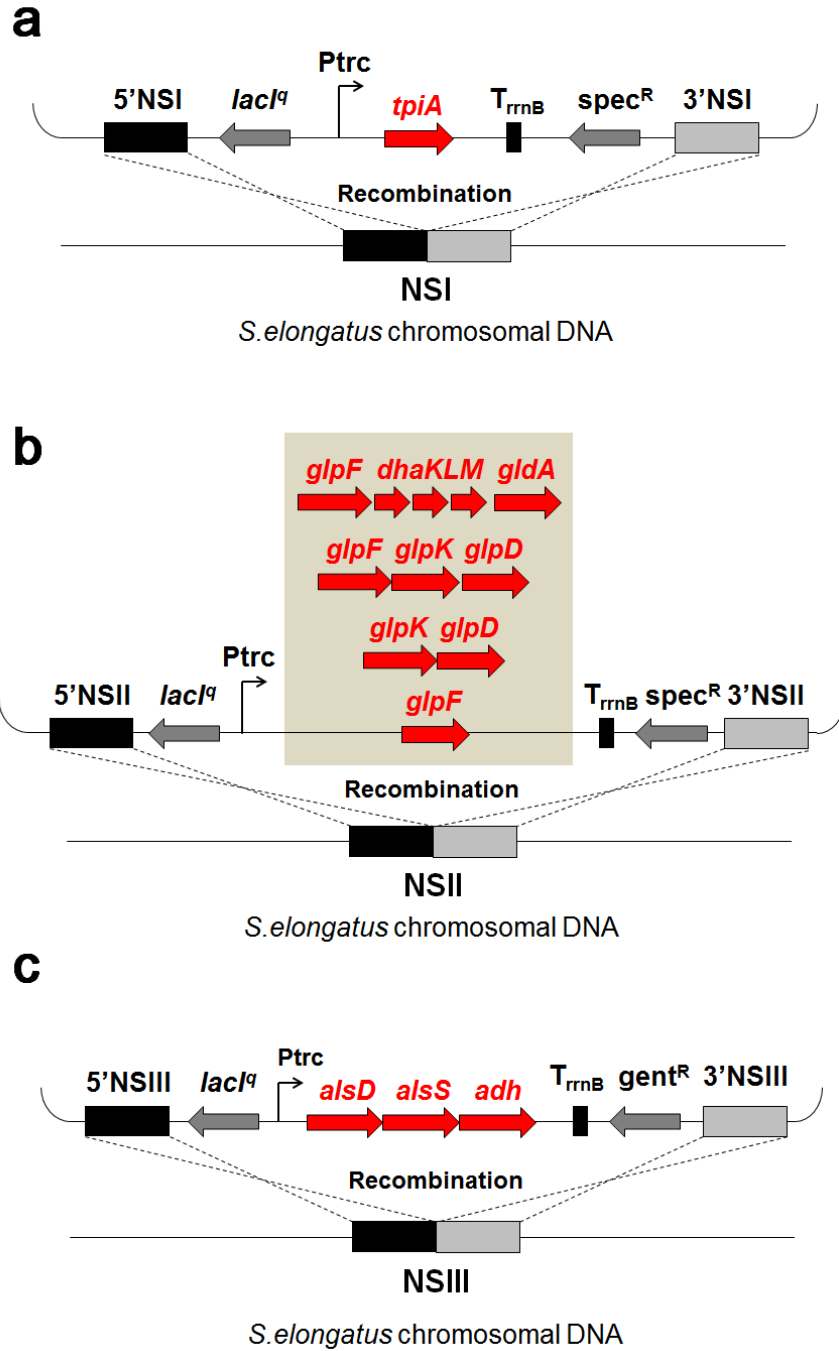


Fig. S1 Schematic of genome engineering in *S. elongatus*.

Schematic of gene integration into NSI⁴ (a), NSII⁵ (b) and NSIII¹ (c). pAL1495 (*tpiA*), pAL1533 (*glpF*), pAL1498 (*glpK-glpD*), pAL1310 (*glpF-glpK-glpD*), pAL1506 (*glpF-dhaKLM-gldA*), pAL1136 (*alsD-alsS-adh*) (Table 1) were utilized for transformation.

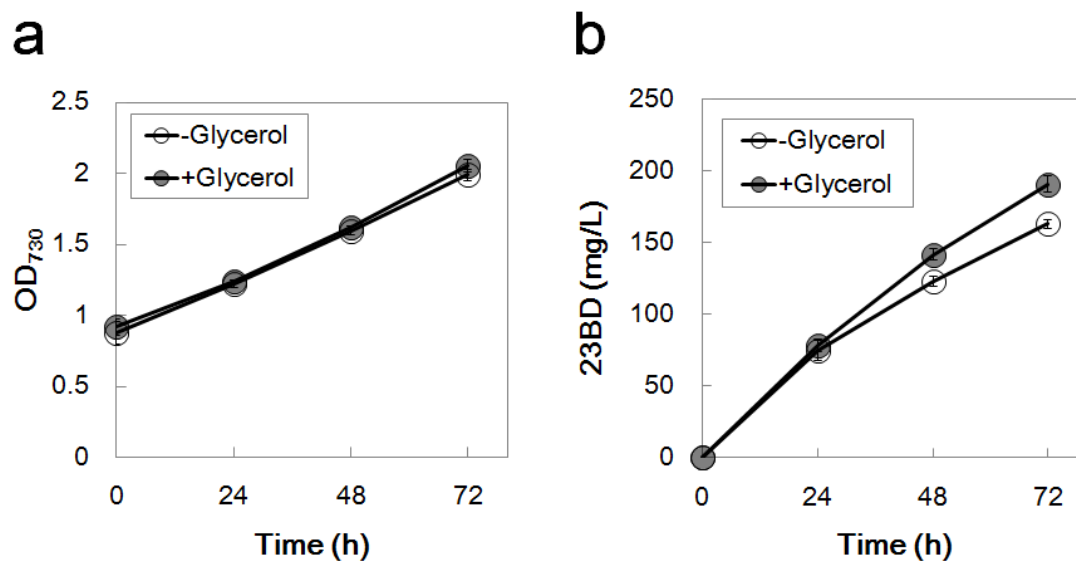


Fig. S2 Growth and 23BD production profiles of Strain 1 with glycerol.

Strain 1 harboring the 23BD biosynthesis pathway was cultured in 10 mL of BG11 media containing 3 g/L glycerol (closed circle) or no glycerol (open circle) and 20 mM NaHCO₃ under continuous light conditions. IPTG (0.1 mM) was added at 0 h. Growth (a) and 23BD production (b) of **Strain 1** over 72 hours where n = 3 biological replicates, and error bars represent standard deviation.

Supporting References.

- [1] McEwen, J. T., Machado, I. M., Connor, M. R., and Atsumi, S. (2013) Engineering *Synechococcus elongatus* PCC 7942 for continuous growth under diurnal conditions, *Appl Environ Microbiol* 79, 1668-1675.
- [2] Nozzi, N. E., and Atsumi, S. (2015) Genome engineering of the 2,3-butanediol biosynthetic pathway for tight regulation in cyanobacteria, *ACS Synth Biol* 4, 1197-1204.
- [3] Oliver, J. W., and Atsumi, S. (2015) A carbon sink pathway increases carbon productivity in cyanobacteria, *Metab Eng* 29, 106-112.
- [4] Bustos, S. A., and Golden, S. S. (1992) Light-regulated expression of the *psbD* gene family in *Synechococcus* sp. strain PCC 7942: evidence for the role of duplicated *psbD* genes in cyanobacteria, *Mol Gen Genet* 232, 221-230.
- [5] Anderson, S. L., and McIntosh, L. (1991) Light-activated heterotrophic growth of the cyanobacterium *Synechocystis* sp. strain PCC 6803: a blue-light-requiring process, *J Bacteriol* 173, 2761-2767.