Electronic Supplementary Information for:

Production of C3 platform chemicals from CO_2 by genetically engineered cyanobacteria

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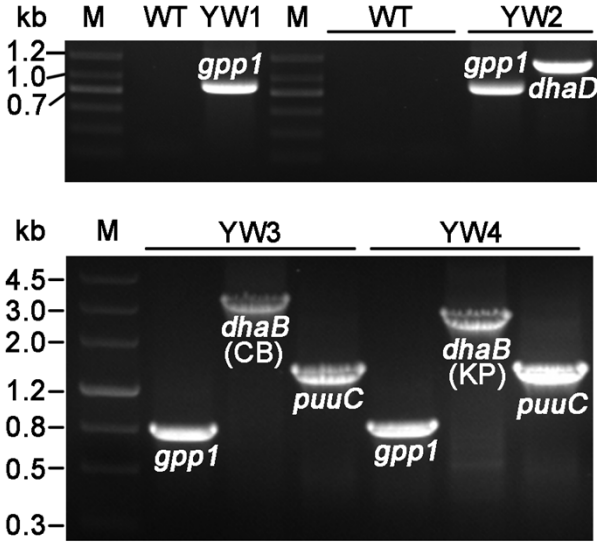

Fig. S1 Colony PCR results confirming the integration of foreign genes in engineered cyanobacteria.

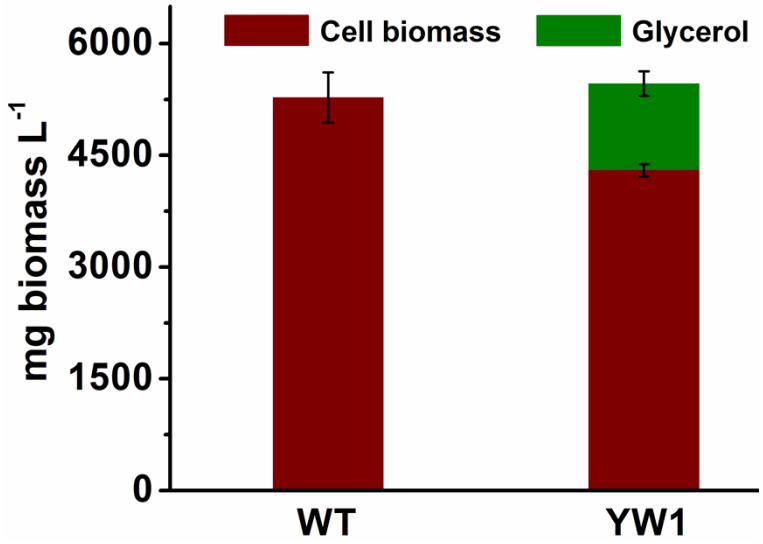

Fig. S2 Total biomass production of *Synechococcus elongatus* wild-type (WT) and YW1 strains incubated with bubbling ordinary air for 20 days. Cell biomass was determined as cell dry weight. Total biomass was cell biomass plus glycerol produced. Error bars indicate SD (n = 3).

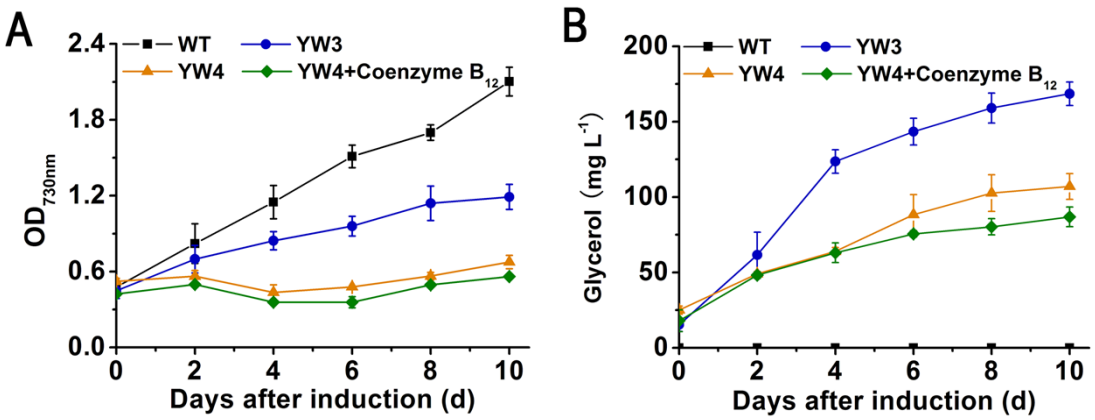

Fig. S3 Cell density and glycerol production of *Synechococcus elongatus* wild-type (WT), YW3 and YW4 strains. (A) Cell density. (B) Glycerol production. Strains were incubated under standard oxygenic incubation with constant light exposure. Coenzyme B_{12} of 2.5 μM was added periodically in the cultures of YW4 at day 0, 4, and 8 following induction. Error bars indicate SD (n = 3).

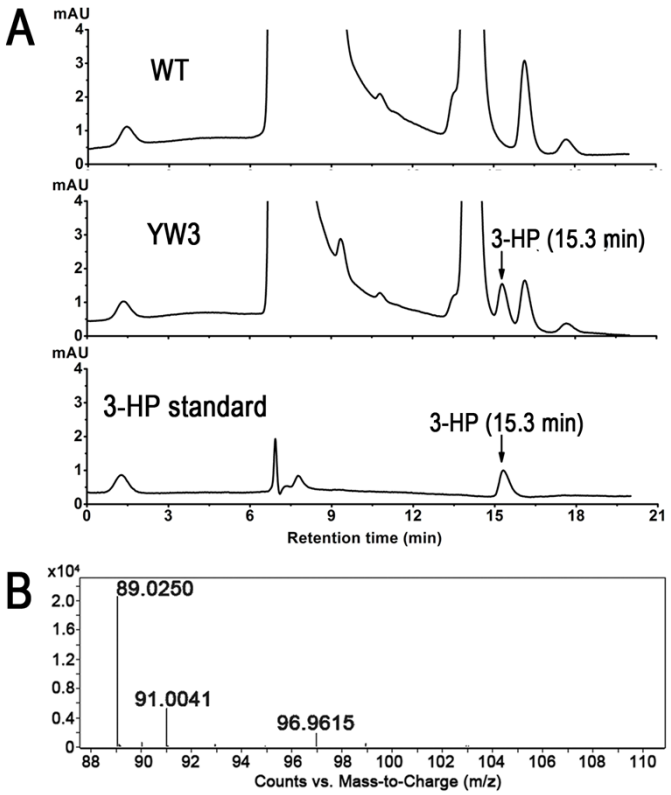

Fig. S4 Identification of 3-HP produced by engineered cyanobacteria. (A) HPLC profile of cultures of *Synechococcus elongatus* wild-type (WT) and YW3 strains and the 3-HP standard. (B) The mass spectrum of the peak at 15.3 min (3-HP) in the culture of strain YW3. Selected-ion monitoring (SIM) parameter: m/z = 89.0250 (3-HP).

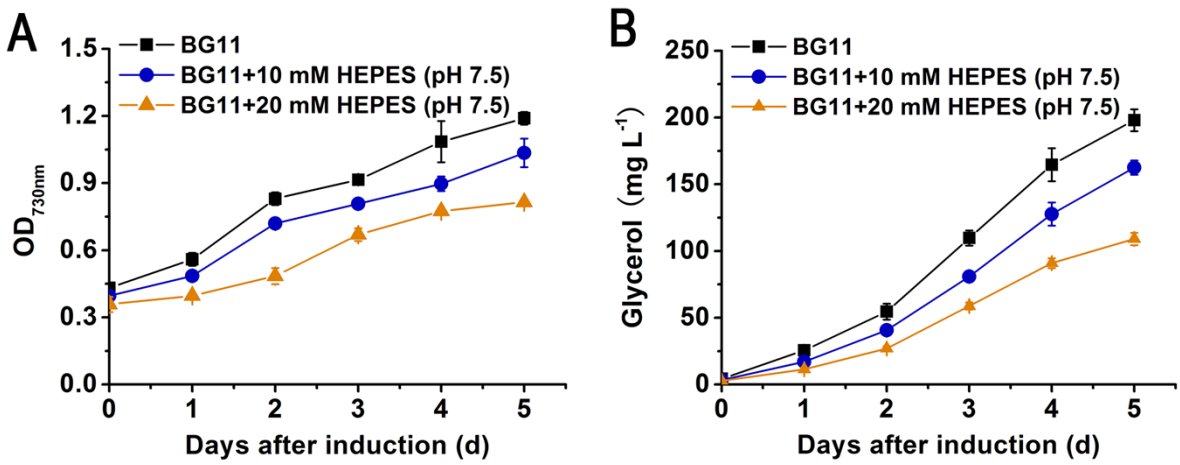

Fig. S5 Effects of HEPES buffer (pH 7.5) on cell growth and glycerol production of strain YW1. (A) Cell growth. (B) Glycerol production. YW1 strain was cultured under standard oxygenic incubation with constant light exposure.

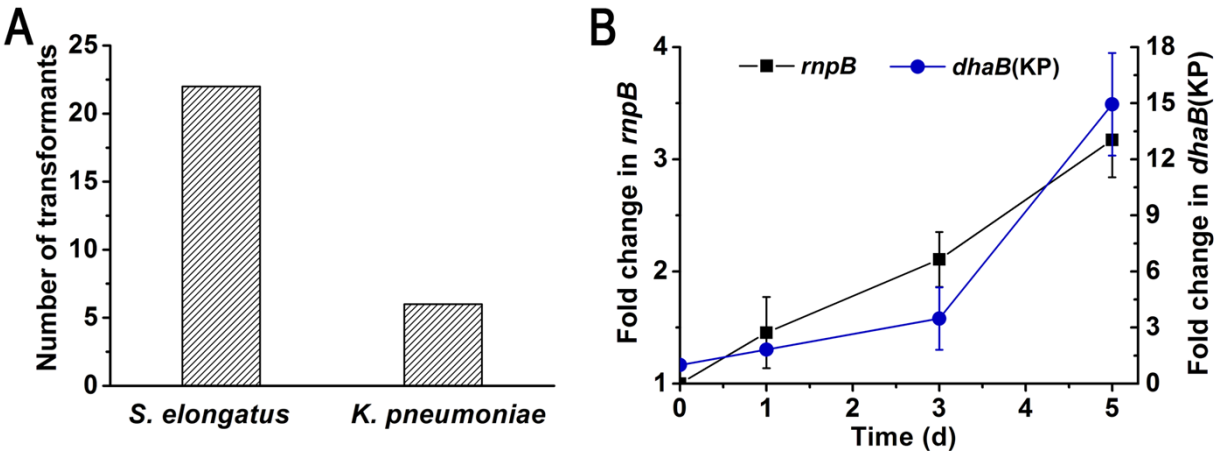

Fig. S6 Characterization of the species formed in the co-cultivation processes. (A) Number of transformants whose plasmids contain the 16S rRNA gene of *Synechococcus elongatus* or *Klebsiella pneumoniae*. The total DNA of the co-culture on day 5 was extracted. 16S rRNA genes were amplified by PCR. The PCR products were subcloned into the clone vector and the products were transformed into *Escherichia coli*. Total 32 individual transformants were picked, and the plasmids were extracted and sequenced. Among them, 28 transformants harbored plasmids which contained the 16S rRNA genes, and the rest 4 transformants harbored empty plasmids. (B) Quantitative PCR (qPCR) analysis of *rnpB* gene of *Synechococcus elongatus* and *dhaB* gene of *Klebsiella pneumoniae* (*dhaB*(KP)) in the co-culture. Samples (1 mL) collected from the co-culture on day 0, 1, 3, and 5 were used for extraction of the total DNA, which was then used as the template of qPCR. Primers for *rnpB* and *dhaB* genes were QrnpB-F/QrnpB-R and QdhaB(KP)-F/QdhaB(KP)-R, respectively (Table S3).

As shown in Fig. S6A, only the 16S rRNA genes of *Synechococcus elongatus* (22 transformants) and *Klebsiella pneumoniae* (6 transformants) were detected, suggesting the existence of both species in the co-culture and no bacterial contamination. The house keeping gene *rnpB* of *Synechococcus elongatus* and glycerol dehydratase encoding gene *dhaB* of *Klebsiella pneumoniae* were quantified by using qPCR to demonstrate the growth of the two species. The increased amount of *rnpB* and *dhaB* genes suggests the cell growth of both *Synechococcus elongatus* and *Klebsiella pneumoniae* in the co-culture (Fig. S6B).

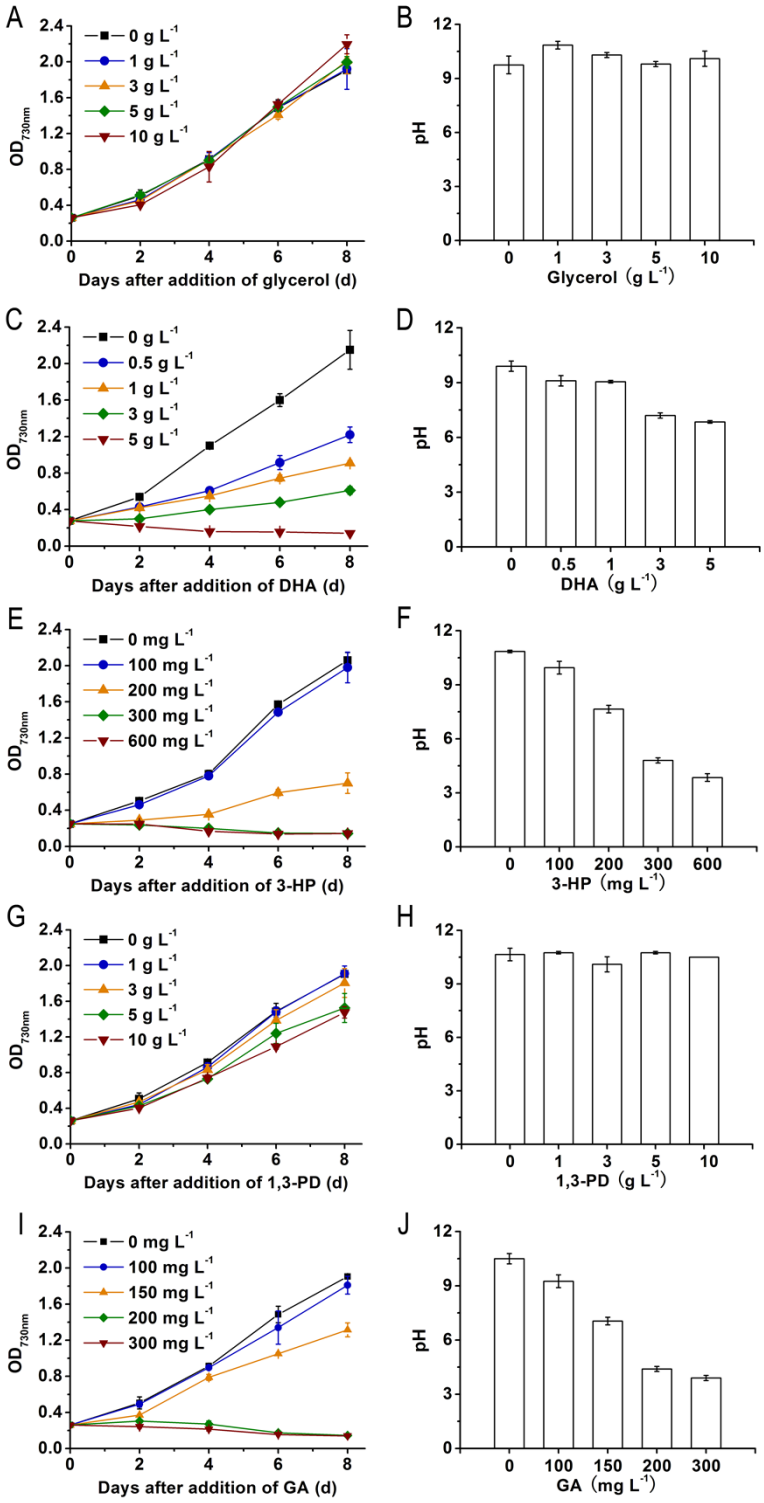

Fig. S7 Effects of potential products on cell growth of *Synechococcus elongatus* wild-type strain and pH of the medium. (A, B) Glycerol. (C, D) DHA. (E, F) 3-HP. (G, H) 1,3-PD. (I, J) GA. Various concentrations of chemicals were added into the culture medium when the cell density of the cultures reached an OD_{730nm} of 0.25, and the growth was monitored for the next few days. Error bars indicate SD (n = 3).

Table S1 Glycerol production by strain YW1 under different culture conditions.

	Glycerol concentration (mg L^{-1})		
Time (d)	**Stationary culture**	**Air bubbling**	**5% CO_2 bubbling**
0	4.45 ± 0.29	4.74 ± 0.67	6.09 ±1.35
2	60.80 ± 6.65	147.18 ± 46.28	164.56 ± 14.42
4	168.90 ± 5.03	518.39 ± 31.79	605.76 ± 23.83
6	252.47 ± 11.69	777.21 ± 58.13	866.96 ± 75.86
8	295.36 ± 28.05	919.30 ± 66.49	931.45 ± 51.21
10	348.23 ± 29.59	1044.55 ± 97.10	1046.78 ± 117.13
12	375.71 ± 12.36	1086.78 ± 118.63	1102.34 ± 38.56
14	412.42 ± 19.59	1125.89 ± 78.12	1166.16 ± 65.29
16	437.18 ± 27.03	1135.54 ± 81.46	1201.70 ± 32.00
18	479.44 ± 16.12	1166.16 ± 82.70	1179.09 ± 117.92
20	500.83 ± 2.37	1168.51 ± 83.72	1237.56 ± 122.08

Table S2 Strains and plasmids used in this study

Strain	**Genotype**	**Reference**
Escherichia coli DH5α	*supE44ΔlacU169* (Φ*80 lacZΔM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Novagen
Escherichia coli BL21 (DE3)	Source of *puuC* gene	Novagen
Saccharomyces cerevisiae	Source of *gpp1* gene	Lab collection
Klebsiella pneumoniae	Source of *dhaD* and *dhaB*(KP) genes	ATCC 25955
Clostridium butyricum	Source of *dhaB*(CB) gene	DSM 10702
Synechococcus elongatus PCC7942	Wild-type	ATCC 33912
YW1	P_{trc}::*gpp1*, spec^r integrated into NSI	This study
YW2	P_{trc}::*gpp1*, P_{trc}:: *dhaD*, spec^r integrated into NSI	This study
YW3	P_{trc}::*gpp1*, P_{trc}:: *dhaB*(CB) *puuC*, spec^r integrated into NSI	This study
YW4	P_{trc}:: *dhaB*(KP) *puuC*, P_{trc}::*gpp1*, spec^r integrated into NSI	This study
Plasmid	**Genotype**	**Reference**
pEASY-Blunt	Ap^r, cloning vector	Transgene
pAM2991	P_{trc}:: MCS, ColE1, *lacI^q*, NSI targeting, spec^r	Ref. 1
pAM-MCS12	pAM2991 derivative, MCS was replaced with MCS1&2 of pETDuet	Lab collection
pAM-YW1	P_{trc}::*gpp1*, *lacI^q*, NSI targeting, spec^r	This study
pAM-P2	pAM-MCS12 derivative harbouring two P_{trc}	This study
pAM-YW2	P_{trc}::*gpp1*, P_{trc}:: *dhaD*, *lacI^q*, NSI targeting, spec^r	This study
pAM-YW3	P_{trc}::*gpp1*, P_{trc}:: *dhaB*(CB) *puuC*, *lacI^q*, NSI targeting, spec^r	This study
pAM-YW4	P_{trc}:: *dhaB*(KP) *puuC*, P_{trc}::*gpp1*, *lacI^q*, NSI targeting, spec^r	This study

1. S. A. Bustos and S. S. Golden, *J. Bacteriol.*, 1991, **173**, 7525–7533.

Table S3 Synthetic oligonucleotides used in this study

Name	Squence 5' to 3'	Plasmid
Ptrc-F	*AGATCT*GACAGCTTATCATCGACT	pAM-P2
Ptrc-R	*CTCGAG*TTCCATGGTCTGTTTCCT	pAM-P2
gpp1-F1	*GAATTC*ATGCCTTTGACCACAAAACC	pAM-YW1, pAM-YW2 & pAM-YW3
gpp1-R1	*AGATCT*TTACCATTTCAACAAGTCAT	pAM-YW1, pAM-YW2 & pAM-YW3
dhaD-F	*CTCGAG*ATGCTAAAAGTTATTCAAT	pAM-YW2
dhaD-R	*GGATCC*TTAACGCGCCAGCCACTGC	pAM-YW2
dhaB-F1	*CTCGAG*ATGATAAGTAAAGGATTTAG	pAM-YW3
dhaB-R1	*GCGGCCGC*TTACTCAGCTCCAATTGTGC	pAM-YW3
puuC-F1	*GCGGCCGC*ACAGGAGTCATAATGAATTT	pAM-YW3
puucC-R1	*GGATCC*TCAGGCCTCCAGGCTTAT	pAM-YW3
gpp1-F2	*CTCGAG*ATGCCTTTGACCACAAAACC	pAM-YW4
gpp1-R2	*GGATCC*TTACCATTTCAACAAGTCAT	pAM-YW4
dhaB-F2	*GAATTC*ATGAAAAGATCAAAACGA	pAM-YW4
dhaB-R2	*GGCGCGCC*TTAGCTTCCTTTACGCAGCTT	pAM-YW4
puuC-F2	*GGCGCGCC*ACAGGAGTCATAATGAATTTTC	pAM-YW4
puuC-R2	*AGATCT*TCAGGCCTCCAGGCTTAT	pAM-YW4
Qgpp1-F	CAAGAGACCAGAATACTTCATCAC	Quantitative PCR
Qgpp1-R	AGCCTTACCAGCAGCAATAC	Quantitative PCR
QdhaB(CB)-F	GCTTAACAGGTGCTACTCCAGATG	Quantitative PCR
QdhaB(CB)-R	GTCCAGATACATCACAGCCTCTTG	Quantitative PCR
QdhaB(KP)-F	CAACGACATCAATGACTATC	Quantitative PCR
QdhaB(KP)-R	ATACCGCCTTATTCAATGG	Quantitative PCR
QpuuC-F	CCGTCTTCGTCAATAACTAC	Quantitative PCR
QpuuC-R	CTCCAGGCTTATCCAGATG	Quantitative PCR
QrnpB-F	AGCAAGGTGGAGGGACAAC	Quantitative PCR
QrnpB-R	CGAAGACAGAGGGCAGTTATC	Quantitative PCR