Supplementary Information for "Engineering the methylerythritol phosphate pathway in cyanobacteria for photosynthetic isoprene production from CO_2"

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Table S1. Isoprene production from 20 engineered *S. elongatus* strains expressing various plant *ispS* genes.

Strain	Promoter	Source organism for *ispS*	Codon-optimized*	Isoprene ($\mu g\ L^{-1}$)†
SE01	P_{psbA2}	*Pueraria montana*	+	59 ± 2
SE02	P_{psbA2}	*Populus trichocarpa*	–	30 ± 2
SE03	P_{psbA2}	*Populus deltoides*	–	26 ± 1
SE04	P_{psbA2}	*Eucalyptus globulus*	–	263 ± 13
SE05	P_{psbA2}	*Populus alba*	–	294 ± 7
SE06	P_{psbA2}	*Populus canescens*	–	273 ± 7
SE16	P_{cpc}	*Pueraria montana*	+	243 ± 17
SE17	P_{cpc}	*Populus alba*	–	1084 ± 52
SE10	P_{cpc}	*Populus alba*	+	1176 ± 34
SE18	P_{cpc}	*Populus canescens*	–	935 ± 58
SE20	P_{cpc}	*Populus canescens*	+	1174 ± 45
SE19	P_{cpc}	*Eucalyptus globulus*	–	3462 ± 378
SE11	P_{cpc}	*Eucalyptus globulus*	+	3063 ± 195
SE09	P_{trc}	*Pueraria montana*	+	283 ± 15
SE07	P_{trc}	*Populus alba*	–	988 ± 21
SE12	P_{trc}	*Populus alba*	+	1365 ± 279
SE14	P_{trc}	*Populus canescens*	–	1797 ± 545
SE15	P_{trc}	*Populus canescens*	+	4357 ± 381
SE08	P_{trc}	*Eucalyptus globulus*	–	5914 ± 291
SE13	P_{trc}	*Eucalyptus globulus*	+	7733 ± 28

*Codon-optimized sequences are indicated by (+) and native sequences are indicated by (–).
†Cells were grown in shake flask cultures for 72 h. IPTG (1 mM) was added to cultures at OD_{730} of about 0.5 if needed. Errors indicate s.d. (*n* = 3).

Table S2. Intracellular concentrations of MEP pathway intermediates quantified by LC-MS.

Strain	Intracellular concentration (µM)*				
	DXP	MEP	CDP-ME	MEcPP	HMBPP
WT	23.5 ± 2.8	3.7 ± 0.2	0.2 ± 0.1	30.1 ± 4.6	1.9 ± 0.1
SE13	20.7 ± 0.5	3.6 ± 0.1	0.3 ± 0.1	1878 ± 227	185.6 ± 5.3
SE23	55.9 ± 1.4	39.5 ± 3.6	0.9 ± 0.2	12127 ± 606	1677 ± 180

*Errors indicate s.d. (*n* = 3).

Table S3. Kinetics of decreases in the unlabeled fraction of MEP pathway intermediates after switching to media with [3-^{13}C]pyruvate.

Time (min)	DXP		MEP		CDP-ME		MEcPP		HMBPP	
	SE32	SE52	SE32	SE52	SE32	SE52	SE32	SE52	SE32	SE52
0	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1	0.9635	0.9414	0.9992	0.9927	0.9917	0.9872	0.9998	0.9691	0.9913	0.9385
2	0.9364	0.9142	0.9836	0.9537	0.9831	0.9630	0.9977	0.9156	0.9890	0.9107
4	0.9168	0.9042	0.9891	0.9627	0.9756	0.9677	0.9913	0.9201	0.9652	0.9234
8	0.9052	0.8886	0.9747	0.9462	0.9756	0.9604	0.9850	0.9150	0.9478	0.9314

Table S4. Photosynthetic carbon fluxes towards biomass synthesis and isoprene production at different growth stages of long-term cultivation.

Strain	Time (d)	Biomass-C (μmol g^{-1} h^{-1})	Isoprene-C (μmol g^{-1} h^{-1})	Isoprene-C Percentage (%)
SE52	0–3	889.3	188.5	17.5
	3–21	24.2	45.9	65.6
SE54	0–3	847.4	147.3	14.8
	3–21	72.5	26.2	26.5

Table S5. Comparison of productivity of various biochemicals from CO_2 in cyanobacteria.

Chemical	Host	Productivity (μg L^{-1} h^{-1})	Inducer	Ref.*
Terpenoids				
β-Caryophyllene	*Synechocystis sp.* 6803	0.3	–	1
β-Phellandrene	*Synechocystis sp.* 6803	1.0	–	2
Farnesene	*Anabaena sp.* 7120	1.29	–	3
α-bisabolene	*Synechococcus sp.* 7002	6.25	–	4
Limonene	*Synechococcus sp.* 7002	41	–	4
Isoprene	*Synechocystis sp.* 6803	4	–	5
Isoprene	***S. elongatus* 7942**	**2489**	**–**	**This work**
Isoprene	***S. elongatus* 7942**	**4260**	**+**	**This work**
Others				
Sucrose	*S. elongatus* 7942	36100	+	6
2,3-Butanediol	*S. elongatus* 7942	9847	+	7
Ethanol	*Synechocystis sp.* 6803	8833	–	8
Isobutyraldehyde	*S. elongatus* 7942	6230	+	9
Fatty acids	*Synechocystis sp.* 6803	4104	–	10
Ethylene	*Synechocystis sp.* 6803	3800	–	11
Mannitol	*Synechococcus sp.* 7002	3819	–	12

Isobutanol	*S. elongatus* 7942	3125	+	9
Lactic acid	*Synechocystis sp.* 6803	2488	+	13
1-Butanol	*S. elongatus* 7942	1403	+	14
3-Hydroxybutyrate	*Synechocystis sp.* 6803	1058	+	15
2-Methyl-1-butanol	*S. elongatus* 7942	694	+	16
1,2-Propanediol	*S. elongatus* 7942	625	+	17
Acetone	*Synechocystis sp.* 6803	375	–	18
Isopropanol	*S. elongatus* 7942	123	+	19
Alka(e)nes	*Synechocystis sp.* 6803	108	+	20
Fatty alcohols	*Synechocystis sp.* 6803	0.5	–	21

*See the references in the last part of the Supplementary materials.

Table S6. Strains and plasmids.

Strains	Genotype	Source
S. elongatus strains		
PCC 7942	Wild type	ATCC
SE01	P_{psbA2} $ispS_{P.m.*}$ integrated at NSII	This work
SE02	P_{psbA2} $ispS_{P.t.}$ integrated at NSII	This work
SE03	P_{psbA2} $ispS_{P.d.}$ integrated at NSII	This work
SE04	P_{psbA2} $ispS_{E.g.}$ integrated at NSII	This work
SE05	P_{psbA2} $ispS_{P.a.}$ integrated at NSII	This work
SE06	P_{psbA2} $ispS_{P.c.}$ integrated at NSII	This work
SE07	P_{trc} $ispS_{P.a.}$ integrated at NSII	This work
SE08	P_{trc} $ispS_{E.g.}$ integrated at NSII	This work
SE09	P_{trc} $ispS_{P.m.*}$ integrated at NSII	This work
SE10	P_{cpc} $ispS_{P.a.*}$ integrated at NSII	This work
SE11	P_{cpc} $ispS_{E.g.*}$ integrated at NSII	This work
SE12	P_{trc} $ispS_{P.a.*}$ integrated at NSII	This work
SE13	P_{trc} $ispS_{E.g.*}$ integrated at NSII	This work
SE14	P_{trc} $ispS_{P.c.}$ integrated at NSII	This work
SE15	P_{trc} $ispS_{P.c.*}$ integrated at NSII	This work
SE16	P_{cpc} $ispS_{P.m.*}$ integrated at NSII	This work
SE17	P_{cpc} $ispS_{P.a.}$ integrated at NSII	This work
SE18	P_{cpc} $ispS_{P.c.}$ integrated at NSII	This work
SE19	P_{cpc} $ispS_{E.g.}$ integrated at NSII	This work
SE20	P_{cpc} $ispS_{P.c.*}$ integrated at NSII	This work
SE07-His	P_{trc} His-tagged $ispS_{P.a.}$ integrated at NSII	This work
SE08-His	P_{trc} His-tagged $ispS_{E.g.}$ integrated at NSII	This work
SE09-His	P_{trc} His-tagged $ispS_{P.m.*}$ integrated at NSII	This work
SE10-His	P_{cpc} His-tagged $ispS_{P.a.*}$ integrated at NSII	This work
SE11-His	P_{cpc} His-tagged $ispS_{E.g.*}$ integrated at NSII	This work
SE12-His	P_{trc} His-tagged $ispS_{P.a.*}$ integrated at NSII	This work
SE13-His	P_{trc} His- tagged $ispS_{E.g.*}$ integrated at NSII	This work

SE23	P_{trc} $ispS_{E.g.*}$ *dxs* integrated at NSII	This work
SE24	P_{trc} $ispS_{E.g.*}$ $idi_{S.e.}$ integrated at NSII	This work
SE25	P_{trc} $ispS_{E.g.*}$ $idi_{H.p.}$ integrated at NSII	This work
SE26	P_{trc} $ispS_{E.g.*}$ $idi_{B.s.}$ integrated at NSII	This work
SE27	P_{trc} $ispS_{E.g.*}$ $idi_{S.c.}$ integrated at NSII	This work
SE28	P_{trc} $ispS_{E.g.*}$ $idi_{S.c.}$ *dxs* integrated at NSII	This work
SE29	P_{cpc} $ispS_{P.a.*}$ *dxs* integrated at NSII	This work
SE30	P_{cpc} $ispS_{P.a.*}$ $idi_{S.c.}$ integrated at NSII	This work
SE31	P_{cpc} $ispS_{P.a.*}$ $idi_{S.c.}$, *dxs* integrated at NSII	This work
SE32	P_{trc} *idi*-GGGS-$ispS_{E.g.*}$ *dxs* integrated at NSII	This work
SE33	P_{trc} $ispS_{E.g.*}$-GGGS-*idi* *dxs* integrated at NSII	This work
SE34	P_{cpc} *idi* -GGGS-$ispS_{P.a.*}$ *dxs* integrated at NSII	This work
SE35	P_{cpc} $ispS_{P.\ a.*}$-GGGS-*idi* *dxs* integrated at NSII	This work
SE36	P_{cpc}*idi* -GSGGGGS-$ispS_{P.a.*}$ *dxs* integrated at NSII	This work
SE37	P_{cpc}*idi* -GSGEAAAK-$ispS_{P.a.*}$ *dxs* integrated at NSII	This work
SE38	P_{cpc}*idi* -GSG(EAAAK)$_2$-$ispS_{P.a.*}$ *dxs* integrated at NSII	This work
SE40	P_{trc} $ispS_{E.g.*}$ *ispD* integrated at NSII	This work
SE41	P_{trc} $ispS_{E.g.*}$ *ispF* integrated at NSII	This work
SE42	P_{trc} $ispS_{E.g.*}$ *ispD* *ispF* integrated at NSII	This work
SE43	P_{trc} $ispS_{E.g.*}$ *idi* *dxs* integrated at NSII P_{tac}*ispD* *ispF* integrated at NSI	This work
SE51	P_{trc} *idi*-GGGS-$ispS_{E.g.*}$ *dxs* integrated at NSII $P_{tac}$$ispG_{S.e.}$ integrated at NSIII	This work
SE52	P_{trc} *idi*-GGGS-$ispS_{E.g.*}$ *dxs* integrated at NSII P_{tac} $ispG_{T.e.}$ integrated at NSIII	This work
SE53	P_{cpc} *idi*-GGGS-$ispS_{P.a.*}$ *dxs* integrated at NSII P_{cpc} $ispG_{S.e.}$ integrated at NSIII	This work
SE54	P_{cpc} *idi*-GGGS-$ispS_{P.a.*}$ *dxs* integrated at NSII P_{cpc} $ispG_{T.e.}$ integrated at NSIII	
Plasmids		
pBluescript KS II (+)	Amp^r; ColE1 ori; P_{T7} MCS	Agilent Technologies
pCL1920	$Spec^r$; SC101 ori;	Ref. [22]
pMMB66EH	Amp^r; P_{tac}	ATCC
pSE01	$Spec^r$; NSII targeting vector; ColE1 ori;	This work
pSE02	$Spec^r$; NSII targeting ; P_{psbA2}	This work
pSE03	$Spec^r$; NSII targeting ; P_{cpc}	This work
pSE04	$Spec^r$; NSII targeting ; P_{trc}	This work
pSE05	$Spec^r$; NSII targeting ; P_{cpc}; containing poly-His tag	This work
pSE06	$Spec^r$; NSII targeting ; P_{trc}; containing poly-His tag	This work
pSE07	$Spec^r$; NSII targeting ; P_{psbA2} $ispS_{P.m.*}$	This work
pSE08	$Spec^r$; NSII targeting ; P_{psbA2} $ispS_{P.t.}$	This work
pSE09	$Spec^r$; NSII targeting ; P_{psbA2} $ispS_{P.d.}$	This work
pSE10	$Spec^r$; NSII targeting ; P_{psbA2} $ispS_{E.g.}$	This work

pSE11	Spec^{r}; NSII targeting ; P_{psbA2} $ispS_{P.a.}$	This work
pSE12	Spec^{r}; NSII targeting ; P_{psbA2} $ispS_{P.c.}$	This work
pSE13	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.m.*}$	This work
pSE14	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.a.}$	This work
pSE15	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.c.}$	This work
pSE16	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{E.g.}$	This work
pSE17	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.c.*}$	This work
pSE18	Spec^{r}; NSII targeting ; P_{trc} $ispS_{P.a.}$	This work
pSE19	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.}$	This work
pSE20	Spec^{r}; NSII targeting ; P_{trc} $ispS_{P.c.}$	This work
pSE21	Spec^{r}; NSII targeting ; P_{trc} $ispS_{p.c.*}$	This work
pSE22	Spec^{r}; NSII targeting ; P_{trc} $ispS_{p.m.*}$	This work
pSE23	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.a.*}$	This work
pSE24	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{E.g.*}$	This work
pSE25	Spec^{r}; NSII targeting ; P_{trc} $ispS_{P.a.*}$	This work
pSE26	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$	This work
pSE27	Spec^{r}; NSII targeting ; P_{cpc} His-tagged-$ispS_{P.a.*}$	This work
pSE28	Spec^{r}; NSII targeting ; P_{cpc} His-tagged-$ispS_{E.g.*}$	This work
pSE29	Spec^{r}; NSII targeting ; P_{trc} His-tagged-$ispS_{P.m.*}$	This work
pSE30	Spec^{r}; NSII targeting ; P_{trc} His-tagged-$ispS_{P.a.}$	This work
pSE31	Spec^{r}; NSII targeting ; P_{trc} His-tagged-$ispS_{E.g.}$	This work
pSE32	Spec^{r}; NSII targeting ; P_{trc} His-tagged-$ispS_{P.a.*}$	This work
pSE33	Spec^{r}; NSII targeting ; P_{trc} His-tagged-$ispS_{E.g.*}$	This work
pSE34	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$ *dxs*	This work
pSE35	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$ $idi_{S.e.}$	This work
pSE36	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$ $idi_{H.p.}$	This work
pSE37	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$ $idi_{B.a.}$	This work
pSE38	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$ $idi_{S.c.}$	This work
pSE39	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$ $idi_{S.c.}$ *dxs*	This work
pSE40	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.a.*}$ *dxs*	This work
pSE41	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.a.*}$ $idi_{S.c.}$	This work
pSE42	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.a.*}$ $idi_{S.c.}$ *dxs*	This work
pSE43	Spec^{r}; NSII targeting ; P_{trc} *idi*-GGGS-$ispS_{E.g.*}$ $dxs_{S.e.}$	This work
pSE44	Spec^{r}; NSII targeting ; P_{trc} $ispS_{E.g.*}$-GGGS-*idi* $dxs_{S.e.}$	This work
pSE45	Spec^{r}; NSII targeting ; P_{cpc} *idi*-GGGS-$ispS_{P.a.*}$ $dxs_{S.e.}$	This work
pSE46	Spec^{r}; NSII targeting ; P_{cpc} $ispS_{P.a.*}$-GGGS-*idi* $dxs_{S.e.}$	This work
pSE47	Spec^{r}; NSII targeting ; P_{cpc} *idi*-GSGGGGS-$ispS_{P.a.*}$ $dxs_{S.e.}$	This work
pSE48	Spec^{r}; NSII targeting ; P_{cpc} *idi*-GSGEAAAK-$ispS_{P.a.*}$ $dxs_{S.e.}$	This work
pSE49	Spec^{r}; NSII targeting ;P_{cpc} *idi*-GSG(EAAAK)$_{2}$-$ispS_{P.a.*}$ $dxs_{S.e.}$	This work
pSE53	Kan^{r}; NSIII targeting vector; SC101 ori;	This work
pSE54	Kan^{r}; NSIII targeting ; P_{tac};	This work
pSE55	Kan^{r}; NSIII targeting ; P_{cpc};	This work
pSE56	Kan^{r}; NSIII targeting ; P_{tac} $ispG_{T.e.}$	This work
pSE57	Kan^{r}; NSIII targeting ; P_{tac} $ispG_{S.e.}$	This work

pSE58	Kan^r; NSIII targeting ; P_{cpc} *ispG*$_{S.e.}$	This work
pSE59	Kan^r; NSIII targeting ; P_{cpc} *ispG*$_{T.e.}$	This work
pET28a-*dxr*$_{E.c.}$	Kan^r; ColE1 ori; P_{T7} *dxr*$_{E.c.}$	This work
pET28a-*idi*$_{S.c.}$	Kan^r; ColE1 ori; P_{T7} *idi*$_{S.c.}$	This work
pET28a- *ispS*$_{P.a.*}$	Kan^r; ColE1 ori; P_{T7} *ispS*$_{P.a.*}$	This work
pET28a-*idi*-GGGS-*ispS*$_{P.a.*}$	Kan^r; ColE1 ori; P_{T7} *idi*-GGGS-*ispS*$_{P.a.*}$	This work
pET28a-*ispS*$_{P.a.*}$-GGGS-*idi*	Kan^r; ColE1 ori; P_{T7} *ispS*$_{P.a.*}$-GGGS-*idi*	This work
pET28a-*ispS*$^{*}_{E.g.}$	Kan^r; ColE1 ori; P_{T7} *ispS*$_{E.g.*}$	This work

Table S7. Oligonucleotides used in this study.

Name	Sequence 5′ → 3′	Used for Plasmid
GX1	AGAGAGCTCGAGAACCGTTCCTGCGCGATCGCTCTTA	pSE01
GX2	AGAGAGGGTACCTAAGCGGGCCACGGCAGCGAAAGGG	pSE01
GX3	AGAGAGGAATTCCCAGCTTGGCTGTTTTGGCGG	pSE01
GX4	AGAGAGAAGCTTAAGGCCCAGTCTTTCGACTGA	pSE01
GX5	AGAGAGAAGCTTAAAAAAATTGAAAAAAGTGTTTC	pSE01
GX6	AGAGAGCTCGAGATCGATTTTCGTTCGTGAATAC	pSE01
GX7	AGAGAGGAGCTCCGATCGCTTTGGGACTTGGAACGGT	pSE01
GX8	AGAGAGGGATCCAAATCACCAGCTGAAACGGTGAAGT	pSE01
GX9	AGAGAGGGATCCATCAGAATCCTTGCCCAGATGC	pSE02
GX10	AGAGAGGAATTCAGAGAGCATATGGTTATAATTCCTTATGTATTTG	pSE02
GX11	AGAGAGGGATCCGTTATAAAATAAACTTAACAAATC	pSE03, pSE05
GX12	AGAGAGGAATTCAGAGAGCATATGATTAATCTCCTACTTGACTTTATG	pSE03
GX13	AGAGAGGGATCCGGTTTTCACCGTCATCACCGAAAC	pSE04, pSE06
GX14	AGAGAGGAATTCAGAGAGCATATGCTGTTTCCTGTGTGAAATTG	pSE04
GX15	AGAGGAATTCAGAGCATATGGCTGTGGTGATGATGGTGATGGCTGCTGCCCATTGAATTA ATCTCCTACTTGACTTTATG	pSE05
GX16	AGAGGAATTCAGAGCATATGGCTGTGGTGATGATGGTGATGGCTGCTGCCCATGGTCTGT TTCCTGTGTGAAATTG	pSE06
GX17	AGAGAGTGTACAAAGAAGGAGATATACCATGCATCTCAGCGAAATTACC	pSE34, pSE40
GX18	AGAGAGGAATTCAGAGAGACTAGTTTAAGCCGAAGCAGCACCAATC	pSE34, pSE40
GX19	AGAGAGTGTACAAAGAAGGAGATATACCATGAACTTCCCGATCGCAGCTG	pSE35
GX20	AGAGAGGAATTCAGAGAGACTAGTTTAGCAGGGTCGCAAGACCCCCGG	pSE35
GX21	AGAGAGTGTACAAAGAAGGAGATATACCATGACTCGAGCAGAACGAAAAAG	pSE37
GX22	AGAGAGGAATTCAGAGAGACTAGTTTATCGCACACTATAGCTTGATG	pSE37

GX23	AGAGAGTGTACAAAGAAGGAGATATACCATGACTGCCGACAACAATAGTATGC	pSE38
GX24	AGAGAGGAATTCAGAGAGACTAGTTTATAGCATTCTATGAATTTGCC	pSE38
GX25	AGAGAGACTAGTAAGAAGGAGATATACCATGCATCTCAGCGAAATTACC	pSE39, pSE42, pSE43, pSE44, pSE45, pSE46
GX26	AGAGAGGAATTCTTAAGCCGAAGCAGCACCAATC	pSE39, pSE42, pSE43, pSE44, pSE45, pSE46
GX27	AGAGCATATGACTGCCGACAACAATAGTATGC	pSE43, pSE45
GX28	TAGCATTCTATGAATTTGCCTGTC	pSE43, pSE45
GX29	GACAGGCAAATTCATAGAATGCTAGGCGGTGGCTCCATGGAAGGACGACGGAGCGCC	pSE43
GX30	AGAGAGTGTACATTACGCGGCTGGACTAATCGG	pSE43
GX31	AGAGCATATGGAAGGACGACGGAGCGCC	pSE44
GX32	GCATACTATTGTTGTCGGCAGTCATGGAGCCACCGCCCGCGGCTGGACTAATCGGTTTAATG	pSE44
GX33	ATGACTGCCGACAACAATAGTATGC	pSE44, pSE46
GX34	AGAGAGTGTACATTATAGCATTCTATGAATTTGCC	pSE44, pSE46
GX35	GACAGGCAAATTCATAGAATGCTAGGCGGTGGCTCCATGCGATGTTCTGTTAGTACC	pSE45
GX36	AGAGAGTGTACATTAACGCTCAAAGGGTAAAATGG	pSE45
GX37	AGAGAGCATATGCGATGTTCTGTTAGTACC	pSE46
GX38	GCATACTATTGTTGTCGGCAGTCATGGAGCCACCGCCACGCTCAAAGGGTAAAATGGGTTC	pSE46
GX39	GACAGGCAAATTCATAGAATGCTAGGCTCCGGTGGCGGTGGCAGTATGCGATGTTCTGTTAGTACC	pSE47
GX40	GACAGGCAAATTCATAGAATGCTAGGCTCCGGTGAAGCCGCTGCCAAAATGCGATGTTCTGTTAGTACC	pSE48
GX41	GACAGGCAAATTCATAGAATGCTAGGCTCCGGTGAAGCCGCTGCCAAAGAAGCCGCTGCCAAAATGCGATGTTCTGTTAGTACC	pSE49

GX50	AGAGAGGTCGACGGCGTTTTCTGCTACATGGGCCG	pSE53
GX51	AGAGAGGGATCCAGAGAGGGTACCTTAGCCGTTGATTGCAGGTGCAGTC	pSE53
GX56	AGAGAGAGATCTTTAAGTCTCTATCTCTGCAGGAG	pSE53
GX57	AGAGAGGAGCTCGGAAGTCCAGCGCAATCAGCGG	pSE53
GX58	AGAGAGGCGGCCGCTTCGTGTCGCTCAAGGCGCAC	pSE54
GX59	AGAGAGGGATCCTGTTTCCTGTGTGAAATTGTTATC	pSE54
GX60	AGAGAGGCGGCCGCGTTATAAAATAAACTTAACAAATC	pSE55
GX61	AGAGAGGGATCCAATCTCCTACTTGACTTTATG	pSE55
GX62	AGAGAGGGATCCATGCAGACCCTCTCCACCCCCAGC	pSE57, pSE58
GX63	AGAGAGCCGCGGTTAGGCAATCGGTTCCGGTTC	pSE57, pSE58
GX89	AGAGAGCATATGAAGCAACTCACCATTCTG	pET28a-$dxr_{E.c.}$
GX90	AGAGAGGTCGACTTAGCTTGCGAGACGCATCACC	pET28a-$dxr_{E.c.}$
GX91	AGAGCATATGACTGCCGACAACAATAGTATGC	pET28a-$idi_{S.c.}$
GX92	AGAGAGGGATCCTTATAGCATTCTATGAATTTGC	pET28a-$idi_{S.c.}$
GX93	AGAGAGCATATGCGATGTTCTGTTAGTACC	pET28a- $ispS_{P.a.*}$
GX94	AGAGAGGGATCCTTAACGCTCAAAGGGTAAAATGG	pET28a- $ispS_{P.a.*}$
GX95	AGAGCATATGGAAGGACGACGGAGCGCC	pET28a-$ispS^{*}_{E.g.}$
GX96	AGAGGGATCCTTACGCGGCTGGACTAATC	pET28a-$ispS^{*}_{E.g.}$
GX100	GTGATGGTGTGCTTGTTGATAGC	NSII validate
GX101	ATGCTGTGGAGTTATCTTTTGGC	NSII validate
GX104	CGGCTGATGCGGAACAGCTAG	NSIII validate
GX105	GATGAGAAATTCGAGGTTCTTGC	NSIII validate

Table S8. Extracellular concentrations of metabolites quantified by GC-MS*

Metabolites	Extracellular concentration (μM)			Limit of detection (μM)
	WT	SE52	SE54	
Pyruvate	NA[b]	NA	NA	1.5
Lactate	NA	NA	NA	3.3
Fumarate	NA	NA	NA	2.0
Succinate	6.1 ± 0.1	5.7 ± 0.2	6.0 ± 0.1	1.5
Malate	NA	NA	NA	2.0
α-Ketoglutarate	NA	NA	NA	2.0
Citrate	13.7 ± 0.3	14.2 ± 0.4	14.8 ± 0.2	1.1
Isocitrate	NA	NA	NA	1.7
Glycolate	NA	8.8 ± 0.1	9.4 ± 0.3	5.3

*For analysis of extracellular metabolites, culture samples (1.5 mL) were harvested by centrifugation. The supernatant was dried in a vacuum centrifuge at room temperature. After redissolved with 50 μL of 20 mg mL^{-1} methoxyamine hydrochloride in pyridine, the sample was incubated at 30 °C for 60 min and then derivatized at 70 °C for 30 min in 50 μL pyridine and 50 μL *N*-methyl-*N*-[tert-butyldimethylsilyl] trifluoroacetamide (Sigma). The GC-MS analysis was performed as described previously.[23] NA, not detectable.

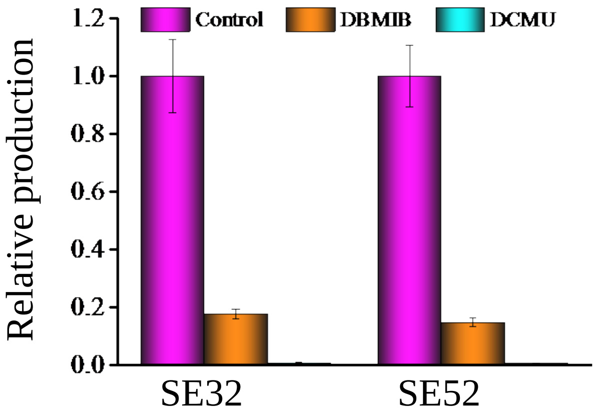

Fig. S1. Effect of treatment with DBMIB and DCMU on isoprene production of strains SE32 and SE52. DCMU (10 μM) or DBMIB (10 μM) was added to the shake flask cultures when OD_{730} reaches about 0.8, and then the cells were cultivated at 30 °C for 24 h. Data are normalized to the isoprene production in the absence of DBMIB or DCMU. Error bars indicate s.d. (*n* = 3).

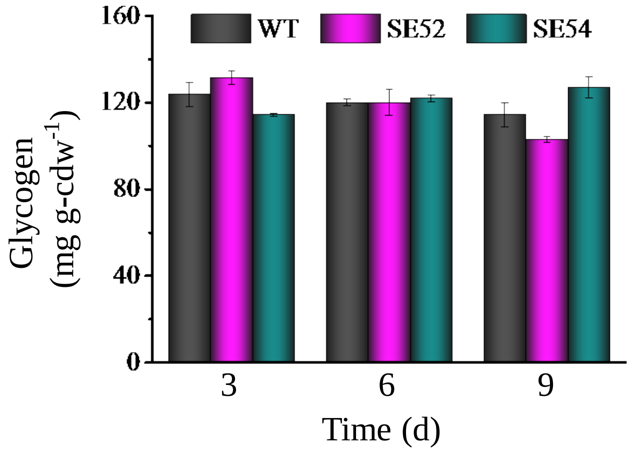

Fig. S2. Intracellular glycogen contents in *S. elongatus* wild type, SE52, and SE53 strains during long-term continuous production of isoprene. Samples were taken at 3, 6, and 9 d. Glycogen content was measured as described previously.[24] Error bars indicate s.d. (n = 3).

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