Supplementary information for

Bioderivatization as a concept for renewable production of chemicals that are toxic or poorly soluble in the liquid phase

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SI Materials and Methods

Construction of *E. coli* strains for 1-octanol and octyl acetate production

To obtain Strain No. 3 to 5 in Table S1, *E. coli* strain C43 (DE3) was transformed with plasmid pET-TPC3 harboring *tes3, sfp*, and *car* genes. Plasmid pACYC-petF-fpr plasmid was used as a template to amplify the *cat* gene. The strawberry alcohol acetyltransferase gene (*saat*) from *Fragaria x ananassa* (UniProtKB: Q9FVF1) and the alcohol O-acetyltransferase gene (*atf1*) from *Saccharomyces cerevisiae* (UniProtKB: P40353) were chemically synthesized from Integrated DNA Technologies (IDT) and codon optimized for *E. coli*. The *saat* and *atf1* genes were individually stored in a blunt-ended pJET1.2 plasmid (Thermo Fisher Scientific), resulting in pJET-SAAT and pJET-ATF1 plasmids, respectively. Next, pCDF-GFP plasmid harboring T7 promoter was constructed as described in Table S7. The *cat, saat,* and *atf1* genes were then sub-cloned into pCDF-GFP plasmid using *Bsa*I restriction site which was introduced by PCR using the oligonucleotide primers listed in Table S4. The resulting plasmid was transformed into the *E. coli* C43 (DE3) strain carrying the pET-TPC3 plasmid, generating Strain No. 3 to 5 in Table S1.

Next, BASIC method was used to construct plasmids in Strain No. 7 to 15 in Table S1. First, plasmid pET-PA1lacO-1-GFP and pCDF-PA1lacO-1-GFP harboring PA1lacO-1 promoter were constructed as shown in Table S6 No. 1 and 2, respectively. Next, the *tes3* and *sfp-car* genes were amplified from plasmid pET-TPC3 using primers listed in Table S4 and cloned into a blunt-ended plasmid pJET1.2 (Thermo Fisher Scientific). Other genes encoding thieosterases: ‘*CpFatB1, ‘CaFatB3,’CpFatB1-4,* and ‘*CaFatB3-5* genes were chemically synthesized as gBlocks from Integrated DNA Technologies (IDT) and cloned into pJET1.2 blunt. The resulting plasmids were used to create plasmids used in Strain No. 7 to 15 in Table S1. The linkers and plasmids used for BASIC method are listed in Table S6 No. 3 to 9. The amino acid sequence of ‘CpFatB1, ‘CaFatB3, ‘CpFatB1-4, and ‘CaFatB3-5 and their wild types are shown in Fig. S1.

Construction of *E. coli* strains for octyl glucoside production

To obtain Strain No. 16-22 in Table S1, *E. coli* strain BW25113 was transformed with pET-‘CaFatB3-5-Sfp-CAR. The glycosyltransferase (GT) from *Actinidia deliciosa* (*AdGT4*), *Vitis vinifera* (*VvGT1*), *Medicago truncatula* (*MtGT1*), *Arabidopsis thaliana* (*AtGT1*) and Medicago truncatula (MtH2) and the gene encoding sucrose synthase from *Arabidopsis thaliana* (*AtSUS1*) were chemically synthesized as gBlocks from Integrated DNA Technologies (IDT) and codon-optimized for *E. coli*. The UniProtKB ID for all the genes used in this study is listed in Table S3. All gBlocks were cloned into blunt-ended pJET1.2 plasmid (Thermo Fisher Scientific). The resulting plasmids are listed in Table S8 and used for Biopart Assembly Standard for Idempotent Cloning (BASIC) method in Table S6. All the linkers (Table S5) and plasmids used to create CloDF13-based plasmids harboring the glycosyltransferase and sucrose synthase under the PA1lacO1 promoter are provided in Table S6. The resulting plasmid was then transformed into *E. coli* BW25113 strain carrying pET-‘CaFatB3-5-Sfp-CAR plasmid.

Construction of cyanobacterial strains for 1-octanol and octyl acetate production

To create strain 6803-Δ*aas*-PnrsB-Sfp-CAR, a suicide plasmid targeting phaA and phaB sites (encoded by *slr1993* and *slr1994*) was first constructed. All the genetic parts used for this plasmid construction are listed in Table S9. Next, the plasmids carrying the upstream (pIY453) and downstream (pIY454) genetic parts were assembled using the linkers shown in Table S10. Next, the suicide plasmid (pIY475) carrying a *gfp* dropout gene were created as shown in Table S11. Next, the *sfp* and *car* genes were then amplified using primers listed in Table S9 and sub-cloned into pJET1.2 blunt plasmid to give plasmid pIY485. Finally, the *sfp* and *car* genes were cloned into plasmid pIY475 using the linkers shown in Table S12. The expression of Sfp and CAR were under controlled of a nickel-inducible promoter PnrsB. Plasmid pIY706 (pMB1-Amp-phaAup-PnrsB-Sfp-CAR-termB15-Sp-phaBdown) was then naturally transformed into 6803-Δ*aas* strain. In brief, 6803-Δ*aas* strain was inoculated in 25 ml of BG11-Co liquid medium at 30°C with continuous illumination at 60 μmol photons/m^2.s and 1% (v/v) CO_2 in the Algaetron AG 230 (Photon Systems Instruments). When the OD_{730} reached 0.3-0.4, the cells were harvested and resuspended in 500 μL fresh BG11-Co medium. One hundred microliters of concentrated liquid culture were mixed with four to seven micrograms of plasmid pIY706 and incubated at at 60 μmol photons/m^2.s and 1% (v/v) CO_2 for 12-16 h prior to plating on BG11-Co agar containing 20 μg/ml spectinomycin. The plate was then incubated at 30°C with continuous illumination at 60 μmol photons/m^2.s for 1-2 weeks or until the colonies appeared. To promote segregations, individual colonies were restreaked on BG11-Co containing 50 μg/ml spectinomycin and 100 μg/ml spectinomycin. A fully segregated mutant was confirmed by PCR using primers IY293 (5'-GGCAAAGCTTTATTTGCCAATGCG-3') and IY292 (5'-CCGATGACACTAATCTCAAGGCGG-3') and used in the subsequent experiments.

Next, to obtain strain 6803-Δ*aas*-PnrsB-Sfp-CAR-Pcoa-'CpFatB1-4 and 6803-Δ*aas*-PnrsB-Sfp-CAR-Pcoa-'CpFatB1-4-SAAT, plasmid pIY849 (Table S6 No. 10, pRSF1010-Ery-Pcoa-'CpFatB1-4) and pIY887 (Table S6 No. 11, pRSF1010-Ery-Pcoa-'CpFatB1-4-SAAT) were individually transformed into 6803-Δ*aas*-PnrsB-Sfp-CAR strain by triparental conjugation. In brief, plasmid pIY849 and pIY887 was first transformed individually into *E. coli* cargo HB101 strain carrying pRL623 plasmid (1). Next, one hundred microliters or the cargo strain carrying both pRL623 and pIY849 or pIY887, conjugal strain carrying plasmid pRL443 (1) and the 6803-Δ*aas*-PnrsB-Sfp-CAR strain were mixed and incubated for 2 h (30°C, 60 μmol photons/m^2.s). Prior to mixing, all strains were washed with LB medium (for *E. coli*) or BG11-Co medium (for cyanobacteria) to remove all the antibiotics. After 2 h of incubation, the mixed culture was then plated on BG11-Co agar plates without antibiotic and incubated for two days (30°C, 60 μmol photons/m^2.s). After 2 days of incubation, biomass was scraped from the agar plates and transferred onto a new agar plate containing 20 μg/ml erythromycin. Individual colonies were restreaked onto a new agar plate containing 20 μg/ml erythromycin, 10 μg/ml spectinomycin, and 10 μg/ml kanamycin and incubated in the Algaetron AG 230 for 4-7 days before it was used for subsequent experiments. Empty plasmid pIY606 was also used as control to obtain strain 6803-Δ*aas*-PnrsB-Sfp-CAR-Pcoa-empty.

All plasmids used in this study are listed in Table S8.

Preparation of BG11-Co liquid medium

The standard 1X BG11-Co liquid medium was prepared by mixing 10 ml 100X BG11-Co, 1 ml 1000X ferric ammonium citrate, 1 ml 1000X Na_2CO_3, and 1 ml 1000X K_2HPO_4 in 1 L of ultrapure water (PURELAB flex 2). The concentrated BG11-Co stock solution (100X BG11-Co) containing 149.6 g $NaNO_3$, 7.49 g $MgSO_4.7H_2O$, 3.6 g $CaCl_2.2H_2O$, 0.89 g Na-citrate.$2H_2O$, 1.12 ml 0.25 M NaEDTA pH 8.0, and 100 ml 1000X trace mineral solution in 1 L ultrapure water was prepared. The 1000X trace mineral solution was made by dissolving 2.86 g H_3BO_3, 1.81 g $MnCl_2.4H_2O$, 0.22 g $ZnSO_4.7H_2O$, 0.39 g $Na_2MoO_4.2H_2O$, and 0.079 g $CuSO_4.5H_2O$ in 1 L ultrapure water and stored at 4°C until being used. The 1000X ferric ammonium citrate, 1000X Na_2CO_3, and 1000X K_2HPO_4 solutions were prepared by dissolving 0.6 g ferric ammonium citrate, 2 g Na_2CO_3, and 3.05 g K_2HPO_4 in 100 ml ultrapure water, respectively. The 1 L standard 1X BG11-Co solution in a 1-L Duran bottle was sterilized by autoclaving.

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'CpFatB1-4             ------------------------------------------------------------ 0
CpFatB1_wild-type      MVAAAASSACFPVPSPGASPKPGKLGNWSSSLSPSLKPKSIPNGGFQVKANASAHPKANG 60
'CpFatB1               ------------------------------------------------------------ 0
CaFatB3_wild-type      MVAAAASSAFFSVPVPGTSPKPGKFRIWPSSLSPSFKPKPIPNGGLQVKANSRAHPKANG 60
'CaFatB3               -------------MHHHHHHKPGKFRIWPSSLSPSFKPKPIPNGGLQVKANSRAHPKANG 47
'CaFatB3-5             ------------------------------------------------------------ 0

'CpFatB1-4             ----------------------------------------------------MFDRKSKR 8
CpFatB1_wild-type      SAVTLKSGSLNTQEDTLSSSPPPRAFFNQLPDWSMLLTAITTVFVAPEKRWTMFDRKSKR 120
'CpFatB1               ----------------------------------------------------------MR 2
CaFatB3_wild-type      SAVSLKSGSLNTQEDT-SSSPPPRTFLHQLPDWSRLLTAITTVFVKS-KRPDMHDRKSKR 118
'CaFatB3               SAVSLKSGSLNTQEDT-SSSPPPRTFLHQLPDWSRLLTAITTVFVKS-KRPDMHDRKSKR 105
'CaFatB3-5             ----------------------------------------------------MHDRKSKR 8
                                                                          *

'CpFatB1-4             PSMLMDSFGLERVVQDGLVFRQSFSIRSYEICADRTASMETVMNHVQETSLNQCKSIGLL 68
CpFatB1_wild-type      PNMLMDSFGLERVVQDGLVFRQSFSIRSYEICADRTASIETVMNHVQETSLNQCKSIGLL 180
'CpFatB1               PNMLMDSFGLERVVQDGLVFRQSFSIRSYEICADRTASIETVMNHVQETSLNQCKSIGLL 62
CaFatB3_wild-type      PDMLMDSFGLESIVQEGLEFRQSFSIRSYEIGTDRTASIETLMNYLQETSLNHCKSTGIL 178
'CaFatB3               PDMLMDSFGLESIVQEGLEFRQSFSIRSYEIGTDRTASIETLMNYLQETSLNHCKSTGIL 165
'CaFatB3-5             PSMLMDSFGLESIVQEGLEFRQSFSIRSYEIGTDRTASMETLMNYLQETSLNHCKSTGIL 68
                       *.********* :**:** ************ :*****:**:**::******:*** *:*

'CpFatB1-4             DDGFGRSPEMCKRDLIWVVTRMKIMVNRYPTWGDTIEVSTWLSQSGKIGMGRDWLISDCN 128
CpFatB1_wild-type      DDGFGRSPEMCKRDLIWVVTRMKIMVNRYPTWGDTIEVSTWLSQSGKIGMGRDWLISDCN 240
'CpFatB1               DDGFGRSPEMCKRDLIWVVTRMKIMVNRYPTWGDTIEVSTWLSQSGKIGMGRDWLISDCN 122
CaFatB3_wild-type      LDGFGRTPEMCKRDLIWVVTKMKIKVNRYPAWGDTVEINTWFSRLGKIGKGRDWLISDCN 238
'CaFatB3               LDGFGRTPEMCKRDLIWVVTKMKIKVNRYPAWGDTVEINTWFSRLGKIGKGRDWLISDCN 225
'CaFatB3-5             LDGFGRTPEMCKRDLIWVVTKMKIKVNRYPAWGDTVEINTWFSRLGKIGKGRDWLISDCN 128
                                   *****:*************:*** *****:****:*:.**:*: ****
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'CpFatB1-4             TGEILVRATSVYAMMNQKTRRFSKLPHEVRQEFAPHFLDSPPAIEDNDGKLQKFDVKTGD 188
CpFatB1_wild-type      TGEILVRATSVYAMMNQKTRRFSKLPHEVRQEFAPHFLDSPPAIEDNDGKLQKFDVKTGD 300
'CpFatB1               TGEILVRATSVYAMMNQKTRRFSKLPHEVRQEFAPHFLDSPPAIEDNDGKLQKFDVKTGD 182
CaFatB3_wild-type      TGEILIRATSAYATMNQKTRRLCKLPYEVHQEIAPLFVDSPPVIEDNDLKLHKFEVKTGD 298
'CaFatB3               TGEILIRATSAYATMNQKTRRLCKLPYEVHQEIAPLFVDSPPVIEDNDLKLHKFEVKTGD 285
'CaFatB3-5             TGEILIRATSAYATMNQKTRRLSKLPYEVHQEIAPLFVDSPPVIEDNDLKLHKFEVKTGD 188
                                   *****:****.** *******:.***:**:**:** *:****.*****
**:**:*****

'CpFatB1-4             SIRKGLTPGWYDLDVNQHVSNVKYIGWILESMPTEVLETQELCSLTLEYRRECGRDSVLE 248
CpFatB1_wild-type      SIRKGLTPGWYDLDVNQHVSNVKYIGWILESMPTEVLETQELCSLTLEYRRECGRDSVLE 360
'CpFatB1               SIRKGLTPGWYDLDVNQHVSNVKYIGWILESMPTEVLETQELCSLTLEYRRECGRDSVLE 242
CaFatB3_wild-type      SIHKGLTPGWNDLDVNQHVSNVKYIGWILESMPTEVLETQELCSLALEYRRECGRDSVLE 358
'CaFatB3               SIHKGLTPGWNDLDVNQHVSNVKYIGWILESMPTEVLETQELCSLALEYRRECGRDSVLE 345
'CaFatB3-5             SIHKGLTPGWNDLDVNQHVSNVKYIGWILESMPTEVLETQELCSLALEYRRECGRDSVLE 248
                       **:******* **********************************:**************

'CpFatB1-4             SVTSMDPSKVGDRFQYRHLLRLEDGADIMKGRTEWRPKNAGTNGAISTGKT*------- 299
CpFatB1_wild-type      SVTSMDPSKVGDRFQYRHLLRLEDGADIMKGRTEWRPKNAGTNGAISTGKT-------- 411
'CpFatB1               SVTSMDPSKVGDRFQYRHLLRLEDGADIMKGRTEWRPKNAGTNGAISTGKT*------- 293
CaFatB3_wild-type      SVTAMDPTKVGGRSQYQHLLRLEDGTDIVKCRTEWRPKNPGANGAISTGKTSNGNSVS- 416
'CaFatB3               SVTAMDPTKVGGRSQYQHLLRLEDGTDIVKCRTEWRPKNPGANGAISTGKTSNGNSVS* 403
'CaFatB3-5             SVTAMDPTKVGGRSQYQHLLRLEDGTDIVKCRTEWRPKNPGANGAISTGKTSNGNSVS* 306
                       ***:***:***.* **:********:**:* ******** *:*********
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Fig. S1. Amino acid sequence alignments of CpFatB1_wild type, ‘CpFatB1, ‘CpFatB1-4, CaFatB3_ wild type, ‘CaFatB3 and ‘CaFatB3-5

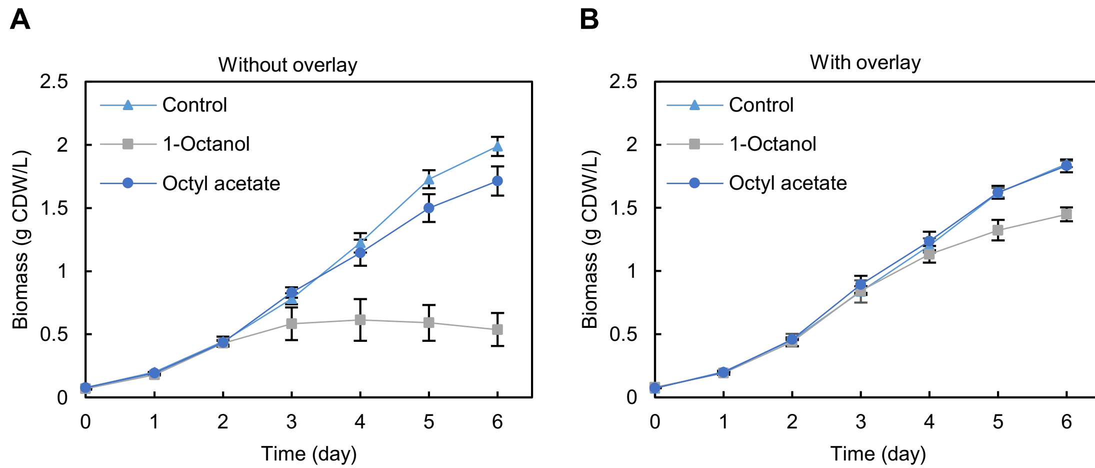


Fig. S2. Toxicity of externally added octyl acetate or 1-octanol to *Synechocystis* sp. PCC 6803. Twenty-five millilitre of *Synechocystis* sp. PCC 6803 lacking acyl-ACP synthetase was cultivated in a 100-ml Erlenmeyer flask with a starting OD_{730} 0.2 at 30°C, 180 rpm (60 μmol photons/m^2.s, 1% CO_2) in AlgaeTron AG 230 (Photon Systems Instruments). On day 2 onwards, 25 mg/L 1-octanol or octyl acetate was added exogenously every 24 h to the liquid culture in the (A) absence of 10% (v/v) hexadecane solvent overlay and in the (B) presence of 10% (v/v) hexadecane solvent overlay . OD_{730} was monitored every 24 h.

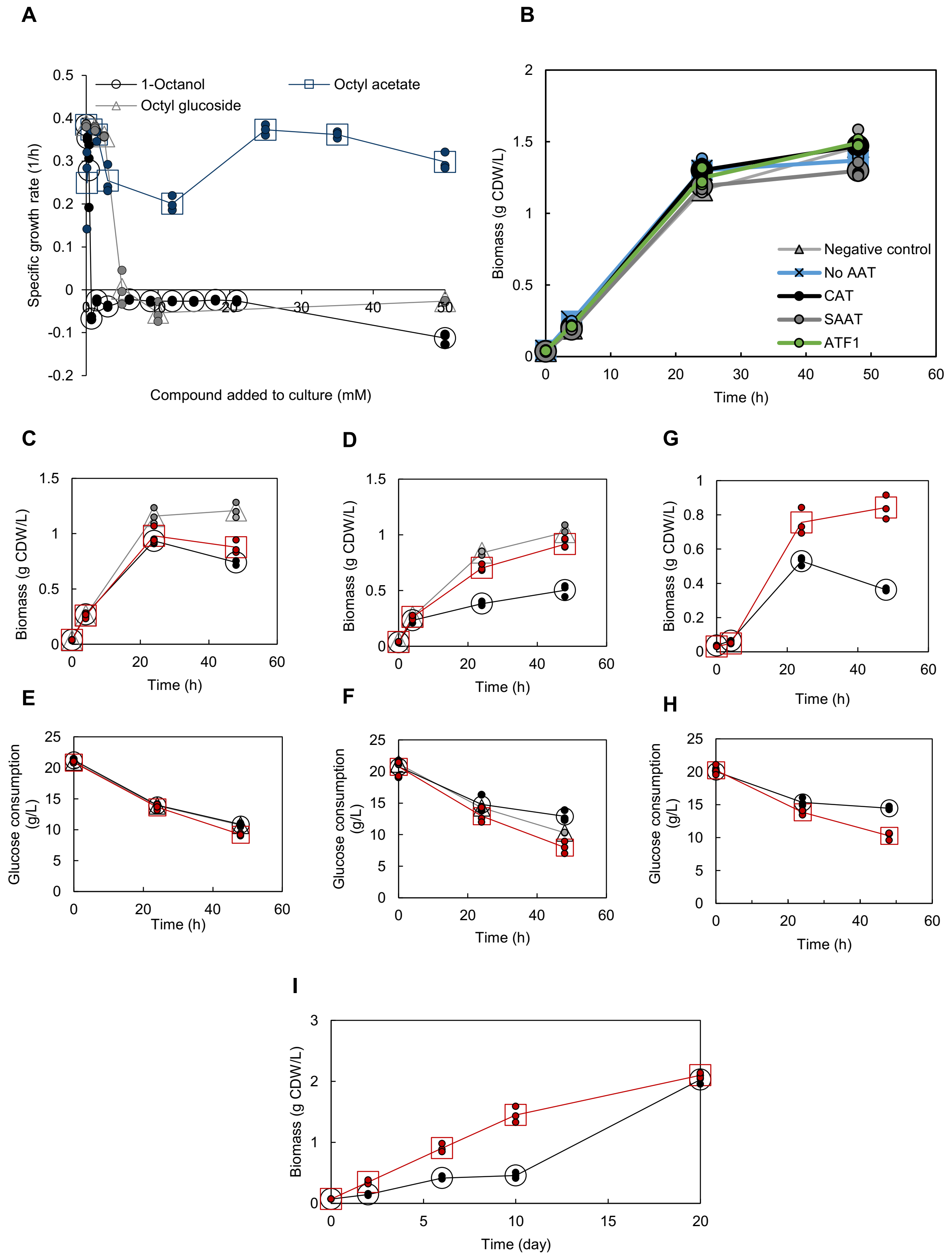


Fig. S3. Individual data points from (A) Fig 2 (B) Fig 3E (C) Fig 4C (D) Fig 4D (E) Fig 4E (F) Fig 4F (G) Fig 5E (H) Fig 5F (I) Fig 7A.

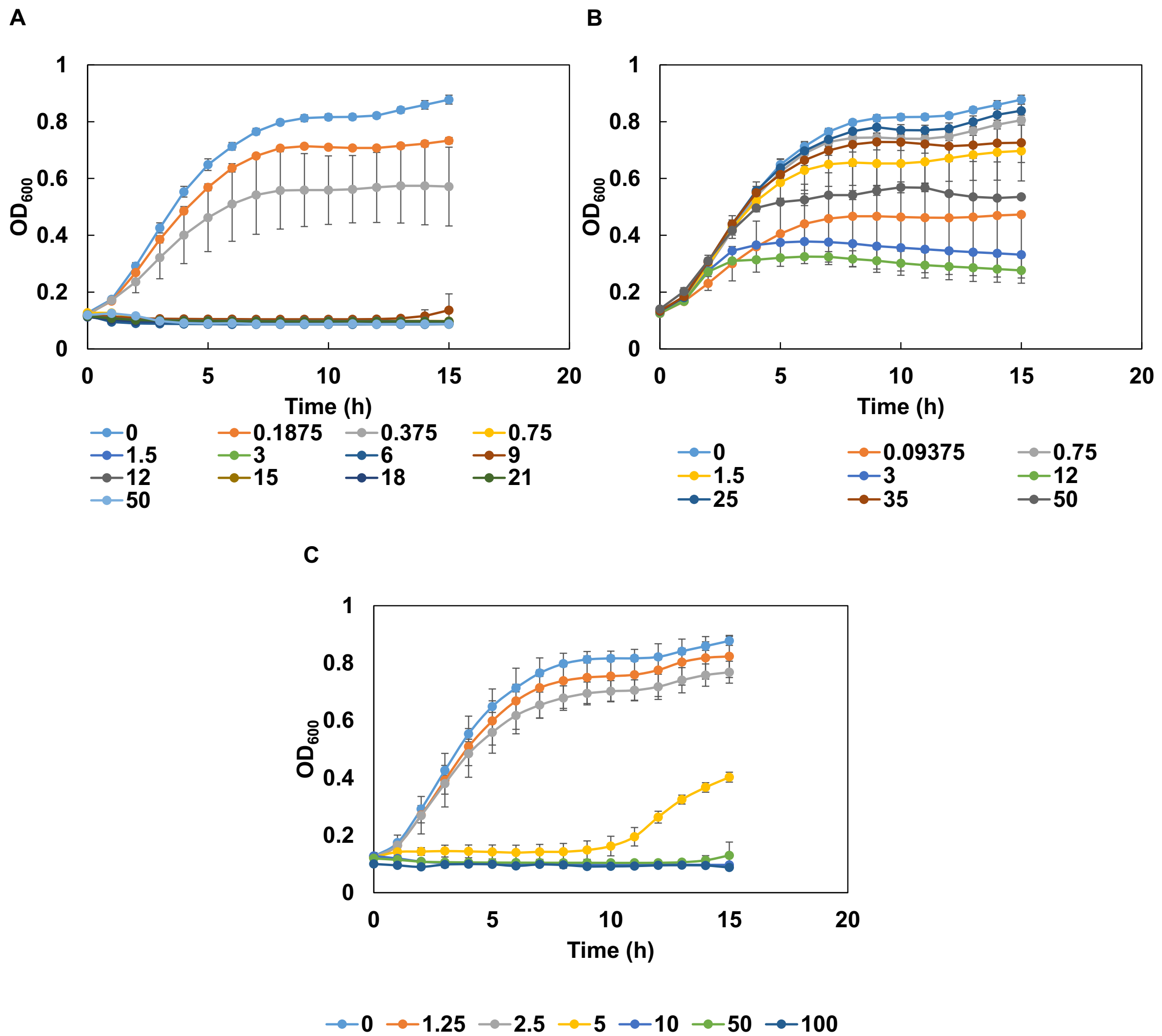


Fig. S4. Average growth curves of *E. coli* C43 (DE3) cultivated in M9 media with varying concentrations of (A) 1-octanol (0 – 50 mM), (B) octyl acetate (0 – 50 mM), and (C) octyl glucoside (0 – 100 mM). All cells were cultivated in 96-well microtiter plates at 37°C, and 432 rpm. Data are the average ± standard deviation from 3 replicates.

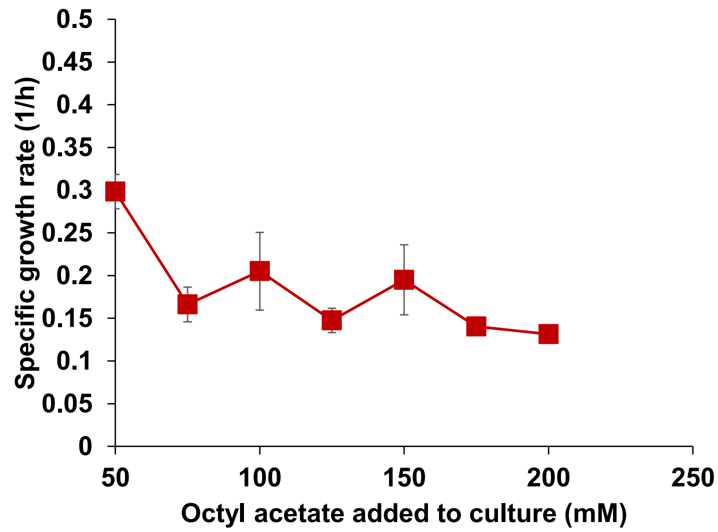


Fig. S5. Toxicity of octyl acetate. Specific growth rates of *E. coli* C43 (DE3) cultivated in M9 minimal media with 2% (w/v) glucose at different concentrations (50-200 mM) of octyl acetate added at the beginning of the cultivation. The specific growth rate was calculated using slopes from average growth curves and only considered data in the range of 1-4 h. Data are the average ± standard deviation from 3 replicates.

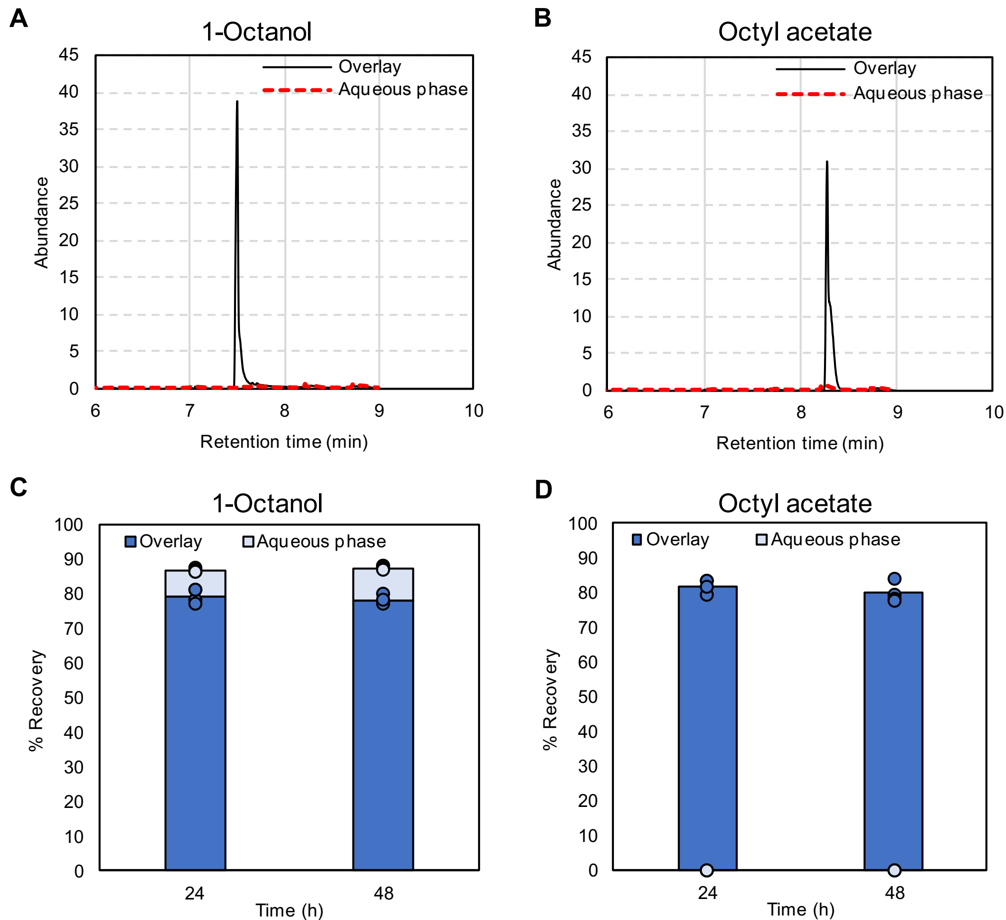


Fig. S6. Localization of 1-octanol and octyl acetate from mock experiments. GC-MS chromatograms obtained from hexadecane overlay (overlay) and M9 liquid medium (aqueous phase) when spiked with 1 mM of (A) 1-octanol and (B) octyl acetate after 24 h incubation. Percent recovery of (C) 1-octanol and (D) octyl acetate from hexadecane overlay (overlay) and M9 liquid medium (aqueous phase) when spiked with 500 mg/L of 1-octanol (3.84 mM) or octyl acetate (2.9 mM). The mock experiment used 25 ml M9 liquid media (with 2% (w/v) glucose) overlaid with 10% (v/v) hexadecane. Flasks were incubated at 30°C, 150 rpm for 24 h or 48 h. The M9 liquid medium was extracted with 10% (v/v) hexadecane prior to GC-MS analysis. Data were obtained from four independent replicates.

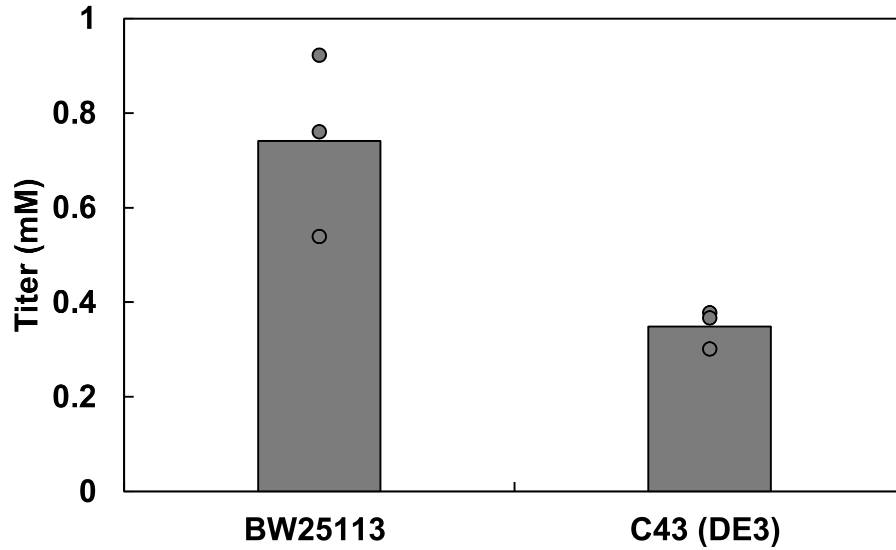
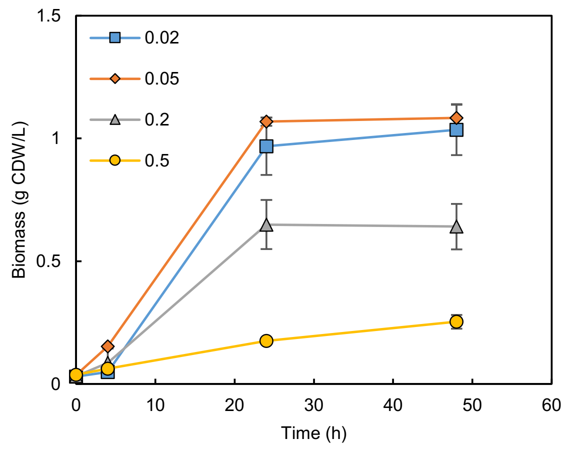


Fig. S7. 1-octanol production with different *E. coli* strain backgrounds. *E. coli* BW25113 and C43 (DE3) harboring pET-PA1lacO-1-TPC3 were cultivated in M9 minimal media with 2% (w/v) glucose. Cultures were induced with 0.5 mM IPTG and overlaid with 10% (v/v) hexadecane overlay for 48 h. Data are the average from 3 biological replicates.

A



B

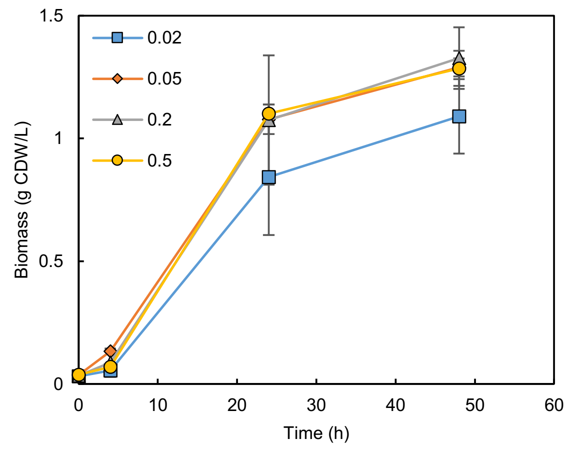


Fig. S8. Growth of *E. coli* strains harboring different thioesterases. Average growth of (A) Strain No. 10 ('CpFatB1-4) and (B) 11 ('CaFatB3-5) (Table S1) when cultivated in M9 media with 2% (w/v) glucose overlaid with 10% (v/v) hexadecane overlay with different IPTG used to induce the cultures (0.02, 0.05, 0.2 and 0.5 mM). Data are the average ± standard deviation from 3 replicates.

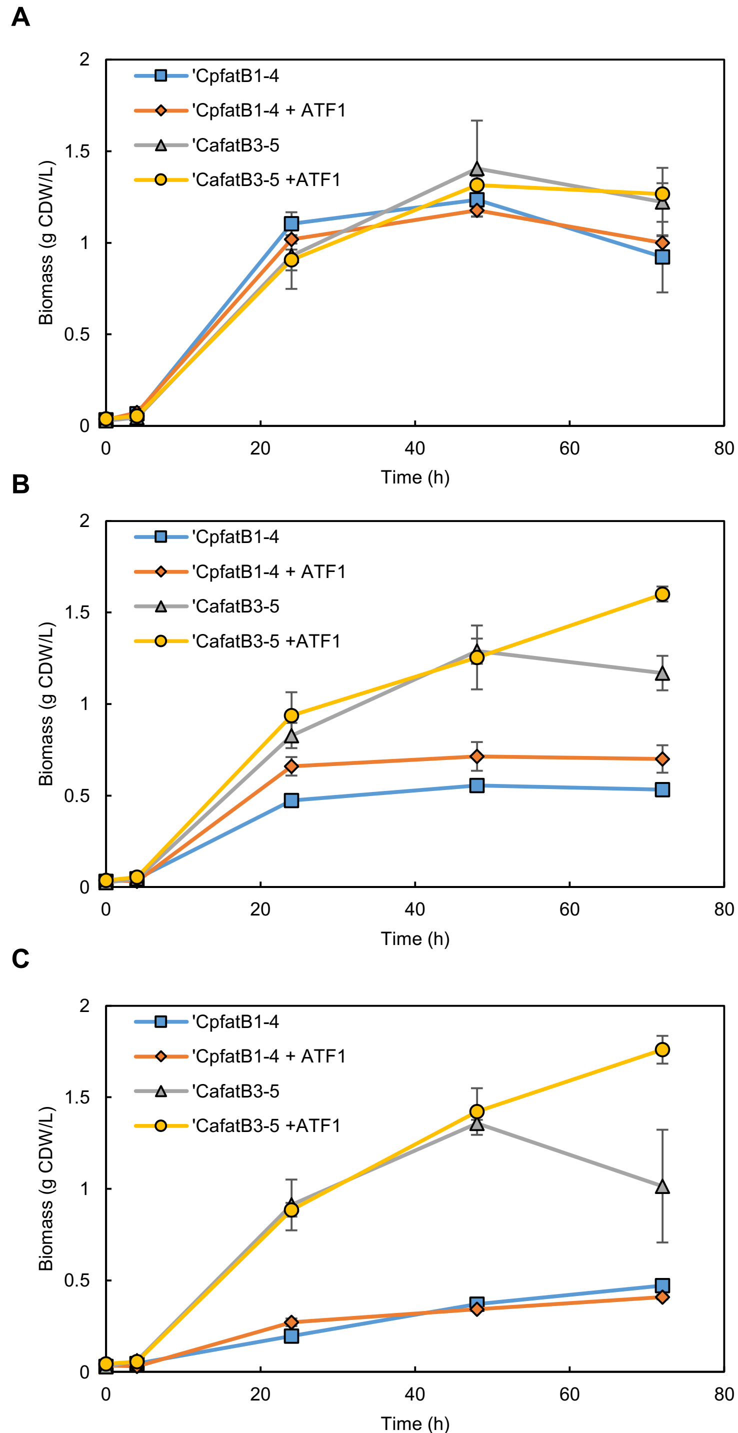


Fig. S9. Growth of octyl acetate and 1-octanol producing strains in the presence of solvent overlay. *E. coli* Strains no. 12 ('CpFatB1-4-Sfp-CAR), 14 ('CpFatB1-4-Sfp-CAR + ATF1), 13 ('CaFatB3-5-Sfp-CAR) and, 15 ('CaFatB3-5-Sfp-CAR + ATF1) (Table S1) were cultivated in M9 minimal media with 2% (w/v) glucose and 10% (v/v) hexadecane overlay. Different concentrations of IPTG were used to induce the cultures (A) 0.05, (B) 0.2 and, (C) 0.5 mM.

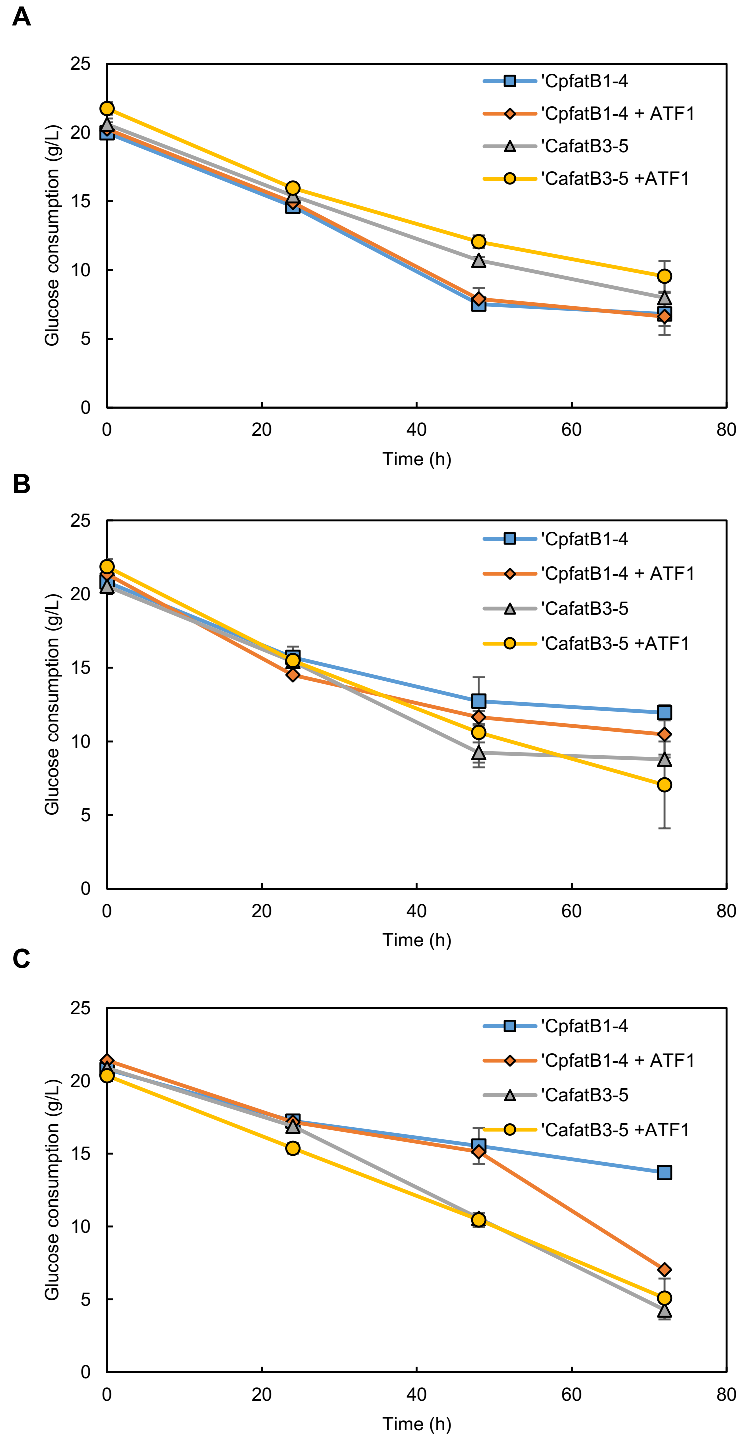


Fig. S10. Glucose consumption by octyl acetate and 1-octanol producing strains in the presence of solvent overlay. *E. coli* Strain No. 12 (‘CpFatB1-4-Sfp-CAR), 14 (‘CpFatB1-4-Sfp-CAR + ATF1), 13 (‘CaFatB3-5-Sfp-CAR) and, 15 (‘CaFatB3-5-Sfp-CAR + ATF1) (Table S1) were cultivated in M9 minimal media with 2% (w/v) glucose and 10% (v/v) hexadecane overlay. Different concentrations of IPTG were used to induce the cultures (A) 0.05, (B) 0.2 and, (C) 0.5 mM.

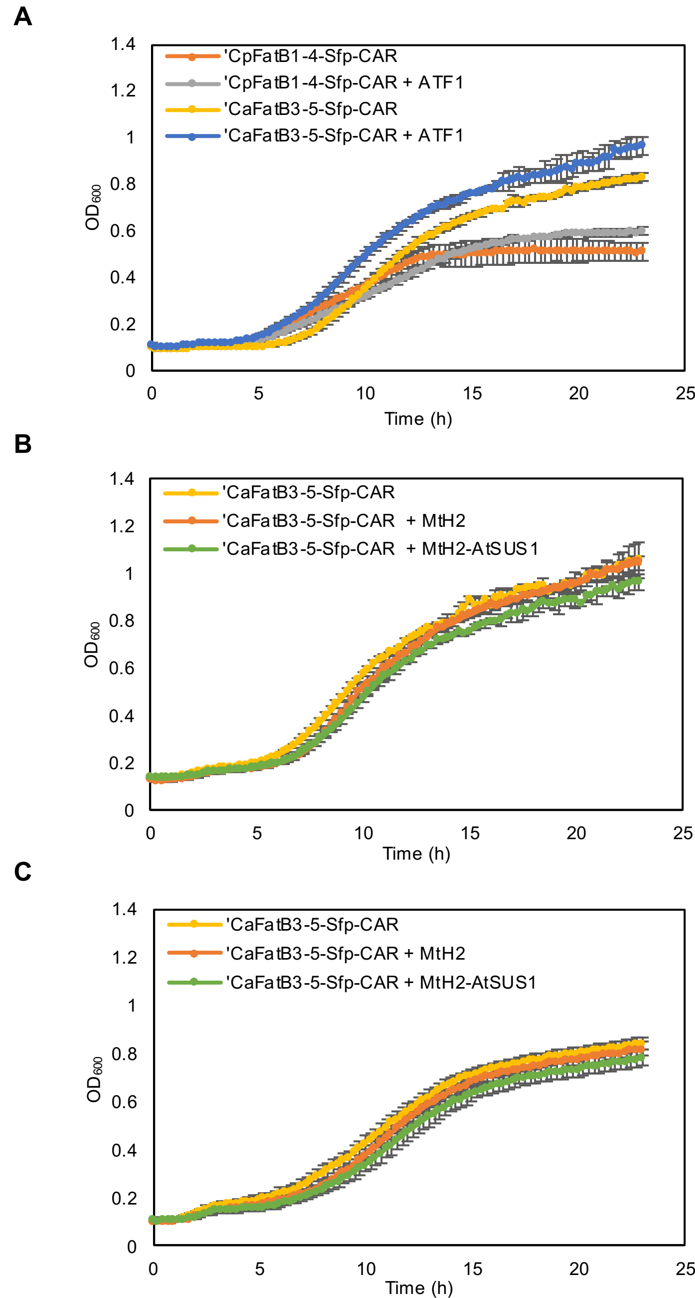
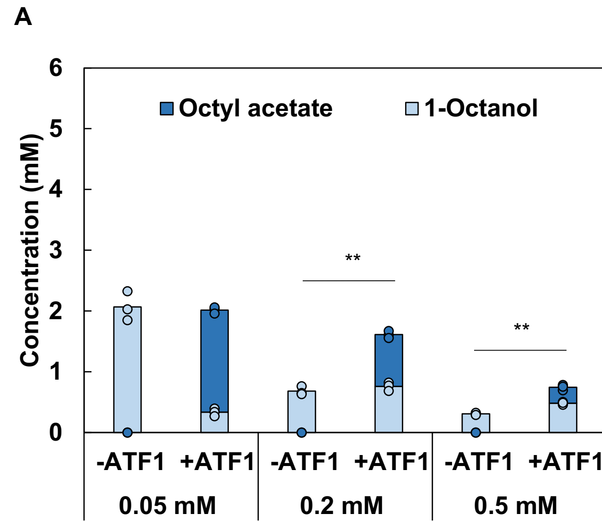
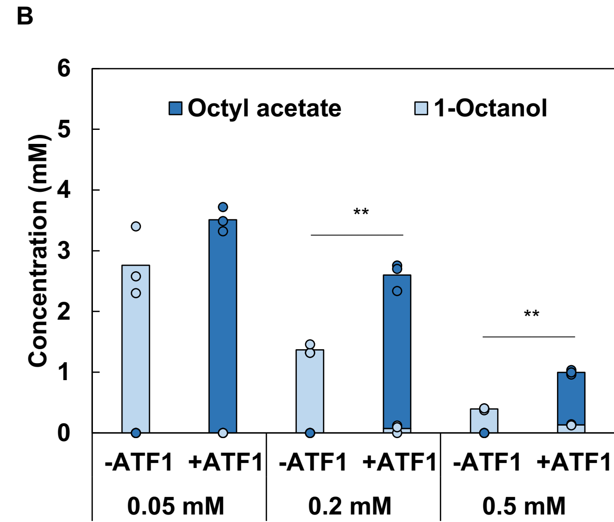
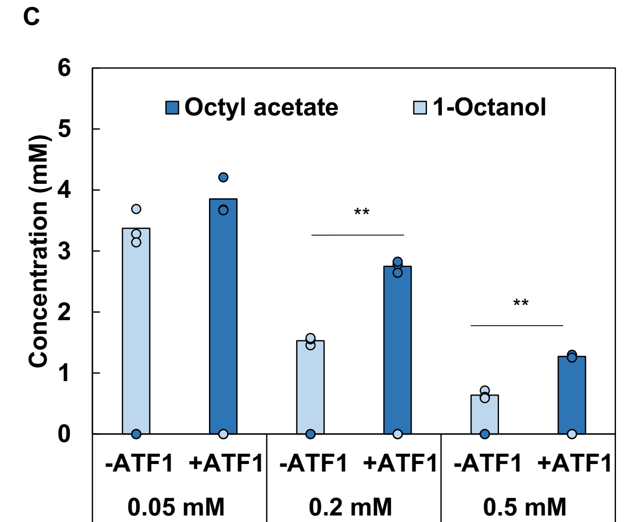
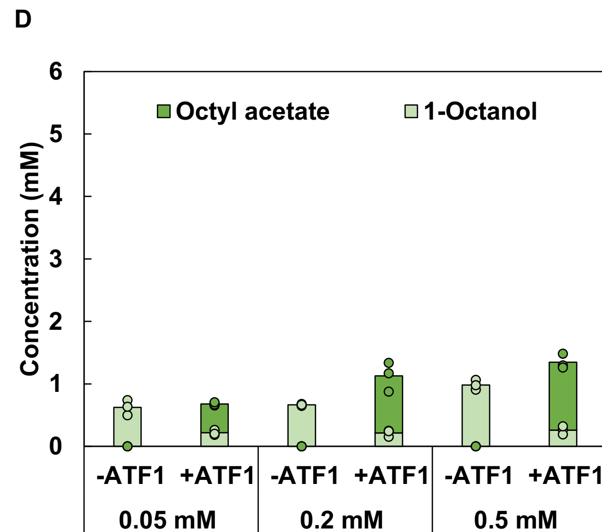


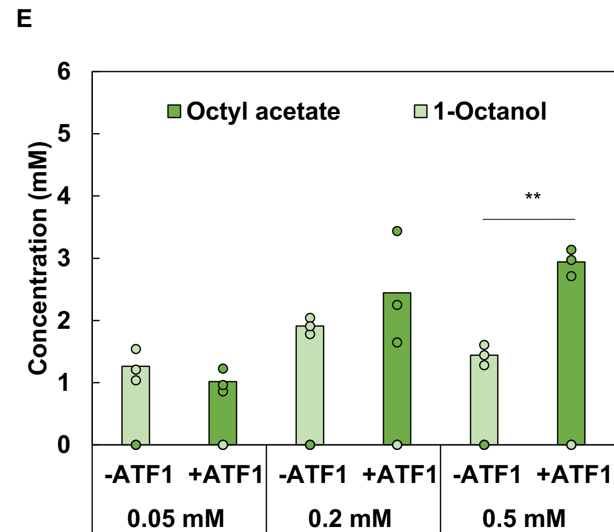
Fig. S11. Growth of 1-octanol, octyl acetate and octyl glucoside producing strains in a 24-well plate. (A) *E. coli* Strain No. 12 ('CpFatB1-4-Sfp-CAR), 14 ('CpFatB1-4-Sfp-CAR + ATF1), 13 ('CaFatB3-5-Sfp-CAR) and 15 ('CaFatB3-5-Sfp-CAR + ATF1) (Table S1) were studied in the presence of 10% (v/v) hexadecane. *E. coli* Strains No. 13 ('CaFatB3-5-Sfp-CAR), 19 ('CaFatB3-5-Sfp-CAR + MtH2) and 22 ('CaFatB3-5-Sfp-CAR + MtH2 + AtSUS1) (Table S1) were studied in the (B) presence and (C) absence of 10% (v/v) hexadecane and 15 mM sucrose. All strains were cultivated in M9 minimal media with 2% (w/v) glucose in 24-well microtiter plate at 30°C and 432 rpm. IPTG was used to induce the strains at (A) 0.2 mM and (B), (C) 0.5 mM and (A), (B) solvent overlay was applied at the beginning of the incubation. Data are the average ± standard deviation from 4 replicates.


A
Concentration (mM)
Octyl acetate
1-Octanol
-ATF1
+ATF1
0.05 mM
0.2 mM
0.5 mM
**


B
Concentration (mM)
Octyl acetate
1-Octanol
-ATF1
+ATF1
0.05 mM
0.2 mM
0.5 mM
**


C
Concentration (mM)
Octyl acetate
1-Octanol
-ATF1
+ATF1
0.05 mM
0.2 mM
0.5 mM
**


D
Concentration (mM)
Octyl acetate
1-Octanol
-ATF1
+ATF1
0.05 mM
0.2 mM
0.5 mM


E
Concentration (mM)
Octyl acetate
1-Octanol
-ATF1
+ATF1
0.05 mM
0.2 mM
0.5 mM
**

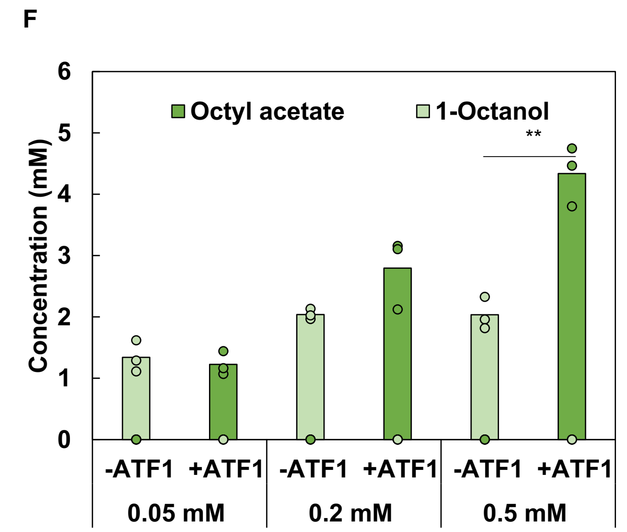

F
Concentration (mM)
Octyl acetate
1-Octanol
-ATF1
+ATF1
0.05 mM
0.2 mM
0.5 mM
**

Fig. S12. Comparison of 1-octanol and octyl acetate production titers. *E. coli* BW25113 strains 'CpFatB1-4-Sfp-CAR and 'CpFatB1-4-Sfp-CAR + ATF1 were sampled at (A) 24 h, (B) 48 h, (C) 72 h. Similarly, strains 'CaFatB3-5-Sfp-CAR and 'CaFatB3-5-Sfp-CAR + ATF1 were sampled at (D) 24 h, (E) 48 h and (F) 72 h. All strains were cultivated in M9 minimal media with 2% (w/v) glucose and 10% (v/v) hexadecane overlay. Different concentrations of IPTG were used to induce the cultures (0.02, 0.2 and, 0.5mM). ** indicates significant difference between 2 treatments (p≤0.01). Data are the mean from 3 biological replicates

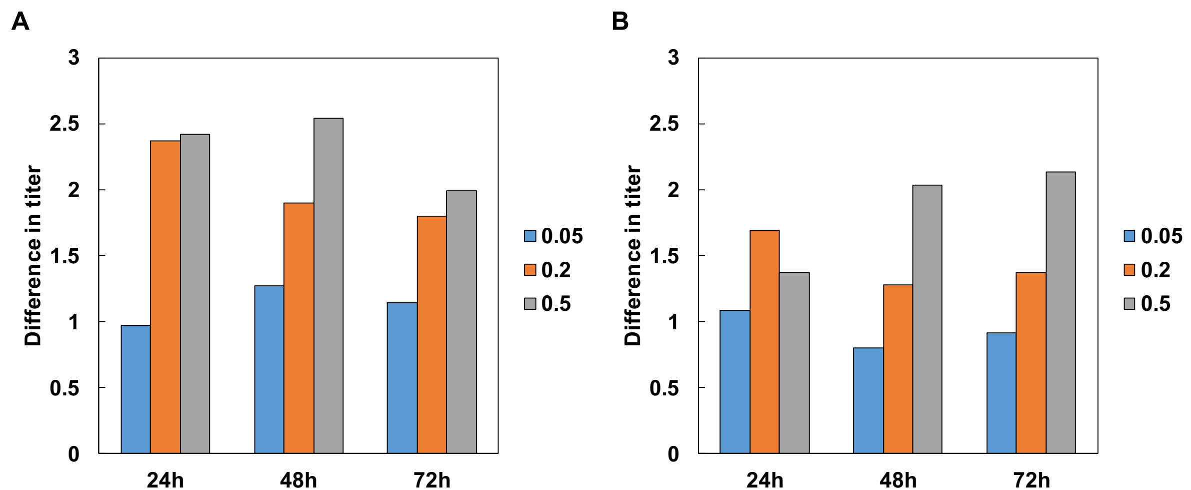


Fig. S13. The octyl acetate : 1-octanol titer ratio between the octyl acetate and 1-octanol producing strains. *E. coli* strains (A) 'CpFatB1-4-Sfp-CAR and 'CpFatB1-4-Sfp-CAR + ATF1 and (B) 'CaFatB3-5-Sfp-CAR and 'CaFatB3-5-Sfp-CAR + ATF1 were cultivated in M9 minimal media with 2% (w/v) glucose and 10% (v/v) hexadecane overlay. Different concentrations of IPTG were used to induce the cultures (0.05, 0.2 and 0.5 mM).

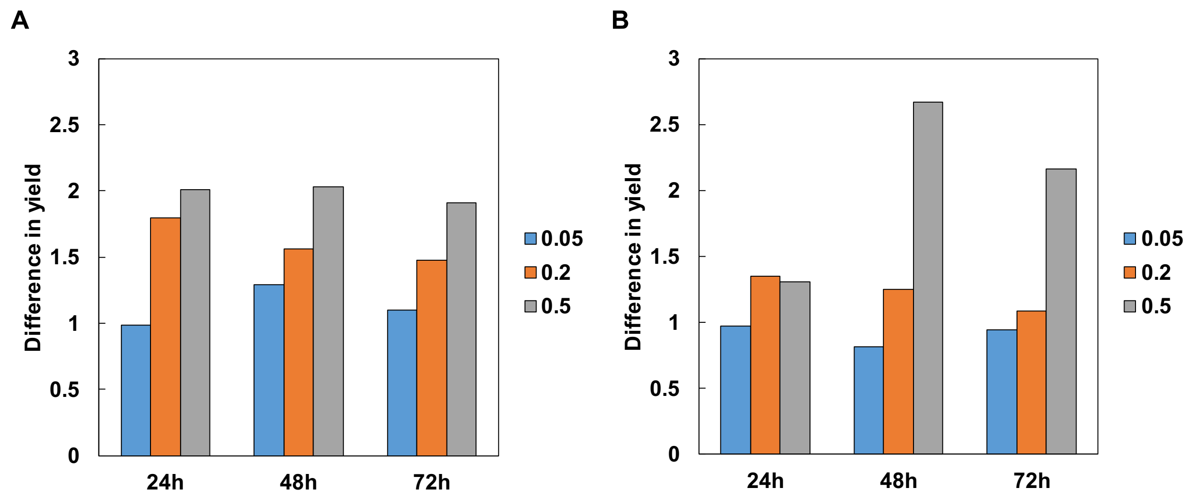


Fig. S14. The octyl acetate : 1-octanol yield ratio between the octyl acetate and 1-octanol producing strains . *E. coli* strains (A) 'CpFatB1-4-Sfp-CAR and 'CpFatB1-4-Sfp-CAR + ATF1 and (B) 'CaFatB3-5-Sfp-CAR and 'CaFatB3-5-Sfp-CAR + ATF1 were cultivated in M9 minimal media with 2% (w/v) glucose and 10% (v/v) hexadecane overlay. Different concentrations of IPTG were used to induce the cultures (0.05, 0.2 and 0.5 mM).

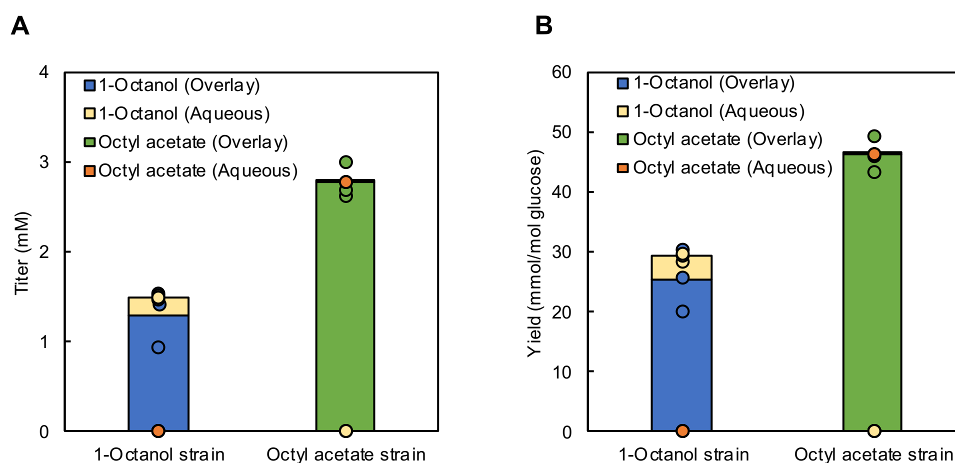


Fig. S15. Liquid phase partitioning of 1-octanol and octyl acetate accumulating in cultures with the engineered strains. (A) Titer and (B) yield of 1-octanol and octyl acetate obtained from Strains 'CaFatB3-5-Sfp-CAR and 'CaFatB3-5-Sfp-CAR + ATF1 (Table S1). The strains were cultivated in M9 media with 2% (w/v) glucose induced with 0.5 mM, overlaid with 10% (v/v) hexadecane, and incubated for 48 h. 1-Octanol or octyl acetate obtained from the M9 liquid medium was extracted with 10% (v/v) hexadecane prior to GC-MS analysis. Data were obtained from three biological replicates.

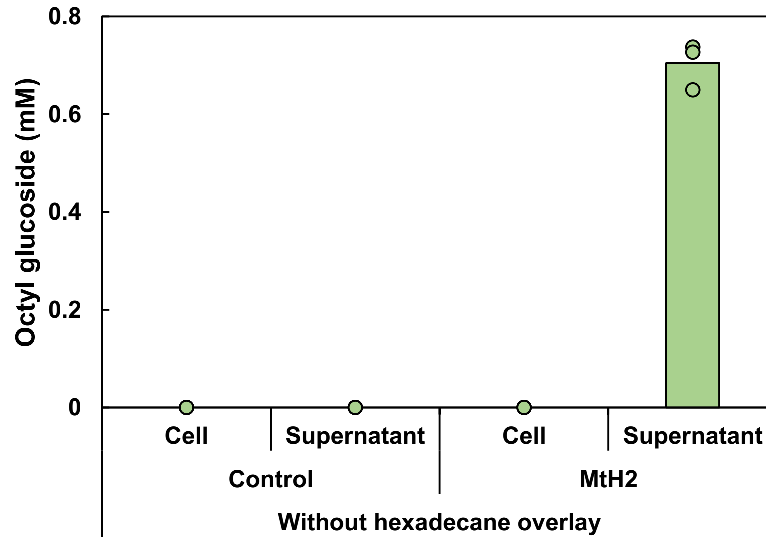


Fig. S16. Localization of octyl glucoside in the absence of solvent overlay. *E. coli* BW25113 strains 'CaFatB3-5-Sfp-CAR and 'CaFatB3-5-Sfp-CAR + MtH2 were cultivated in M9 media with 2% (w/v) glucose with 0.5 mM IPTG. The cultures were harvested at centrifuged (17,000 x g, 10 min) to separate culture media and cells. The supernatant was analysed with HPLC. The cell fraction was suspended and lyzed with glass beads (Sigma Aldrich) at 30 hertz for 6 min on Tissuelyser II (QIAGEN). After that, the liquid fraction was analysed with HPLC.

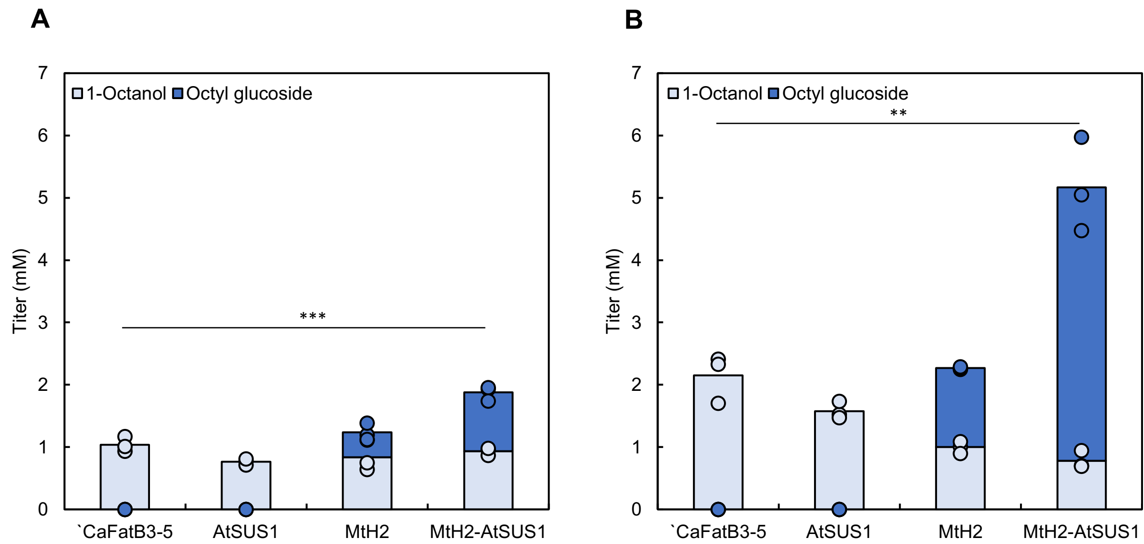


Fig. S17. Comparison of *in vivo* 1-octanol and octyl glucoside production from *E. coli* BW25113 (titers). *E. coli* strains 'CaFatB3-5-Sfp-CAR, 'CaFatB3-5-Sfp-CAR + AtSUS1, 'CaFatB3-5-Sfp-CAR + MtH2 and 'CaFatB3-5-Sfp-CAR + MtH2 + AtSUS1 were cultivated in M9 media with 10% (v/v) hexadecane, 2% (w/v) glucose and 15 mM sucrose, and sampled at (A) 24 h (B) 48 h. The asterisk indicates a significant difference by the Student's t-test (**, P≤0.01;***, P≤0.005). All data are shown as the average from 3 biological replicates and circles represent data from independent biological replicates.

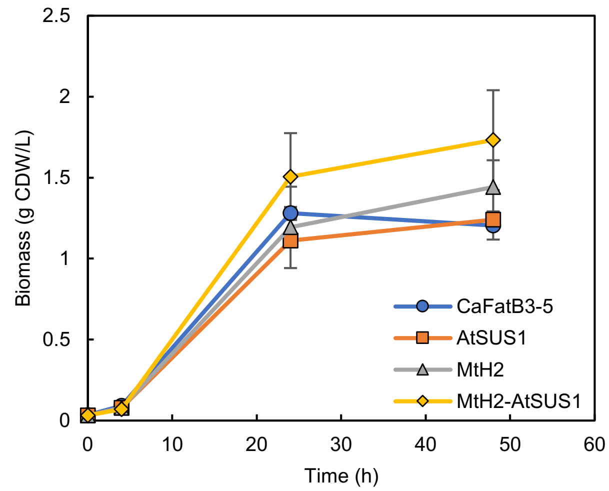


Fig. S18. Growth of octyl glucoside and 1-octanol producing strains in the presence of solvent overlay. *E. coli* strains 'CaFatB3-5-Sfp-CAR, 'CaFatB3-5-Sfp-CAR + MtH2, 'CaFatB3-5-Sfp-CAR + AtSUS1, and 'CaFatB3-5-Sfp-CAR + MtH2 + AtSUS1 were cultivated in M9 minimal media with 2% (w/v) glucose, 0.5 mM and 10% (v/v) hexadecane overlay.

Table S1. List of *E. coli* strains used in this study

Strain No.	*E. coli* strain	ColE1 plasmids	CloDF13 plasmids
1	C43(DE3)	P_{T7}: empty	P_{T7}: empty
2	C43(DE3)	P_{T7}: *tes3, sfp, car*	P_{T7}: empty
3	C43(DE3)	P_{T7}: *tes3, sfp, car*	P_{T7}: *cat*
4	C43(DE3)	P_{T7}: *tes3, sfp, car*	P_{T7}: *saat*
5	C43(DE3)	P_{T7}: *tes3, sfp, car*	P_{T7}: *atf1*
6	C43(DE3)	$P_{A1lacO-1}$: *tes3, sfp, car*	-
7	BW25113	$P_{A1lacO-1}$: *tes3, sfp, car*	-
8	BW25113	$P_{A1lacO-1}$: ‘*CpFatB1, sfp, car*	-
9	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3, sfp, car*	-
10	BW25113	$P_{A1lacO-1}$: ‘*CpFatB1-4, sfp, car*	-
11	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	-
12	BW25113	$P_{A1lacO-1}$: ‘*CpFatB1-4, sfp, car*	$P_{A1lacO-1}$: empty
13	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: empty
14	BW25113	$P_{A1lacO-1}$: ‘*CpFatB1-4, sfp, car*	$P_{A1lacO-1}$: *atf1*
15	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *atf1*
16	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *AdGT4*
17	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *AtGT1*
18	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *MtG1*
19	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *MtH2*
20	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *VvGT1*
21	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *AtSUS1*
22	BW25113	$P_{A1lacO-1}$: ‘*CaFatB3-5, sfp, car*	$P_{A1lacO-1}$: *MtH2, AtSus1*

Table S2. List of *Synechocystis* sp. PCC 6803 strains used in this study

Strain No.	Cyanobacterial strain	RSF1010-based plasmid
1	6803-Δ*aas*	-
2	6803-Δ*aas*-PnrsB-Sfp-CAR	-
3	6803-Δ*aas*-PnrsB-Sfp-CAR-Pcoa-‘CpFatB1-4	Pcoa: ‘*CpFatB1-4*
4	6803-Δ*aas*-PnrsB-Sfp-CAR-Pcoa-‘CpFatB1-4-SAAT	Pcoa: ‘*CpFatB1-4, saat*

Table S3. Source organisms of overexpressed genes in this study

Gene	Source organism	UniProtKB	Reference
tes3	*Anaerococcus tetradius*	C2CIR4	(Akhtar et al., 2015)
sfp	*Bacillus subtilis*	P39135	(Akhtar et al., 2015)
car	*Mycobacterium marinum*	B2HN69	(Akhtar et al., 2015)
cat	*Escherichia coli*	P62577	(Röttig and Steinbüchela, 2013)
saat	*Fragaria ananassa cv. Elsanta*	Q9FVF1	(Aharoni et al., 2000)
atf1	*Saccharomyces cerevisiae*	P40353	(Verstrepen et al., 2003)
CpFatB1 (wild type)	*Cuphea palustris*	Q39554	(Dehesh et al., 1996)
CaFatB3 (wild type)	*Cuphea avigera pulcherrima*	V9MHU7	(Tjellstrom et al., 2013)
'CpFatB1-4	*Cuphea palustris*	-	(Hernandez Lozada et al., 2018)
'CaFatB3-5	*Cuphea avigera pulcherrima*	-	This work
AdGT4	*Actinidia deliciosa*	A0A077EMP8	(Yauk et al., 2014)
VvGT1	*Vitis vinifera*	P51094	(Christopher M. Ford et al., 1998)
AtGT1	*Arabadopsis thaliana*	Q9M156	(Lim et al., 2002)
MtG1	*Medicago truncatula*	Q5IFH7	(Shao et al., 2005)
MtH2	*Medicago truncatula*	A6XNC5	(Li et al., 2007)
AtSUS1	*Arabidopsis thaliana*	P49040	(Zheng et al., 2011)

If the genes were from foreign organisms, codon optimization was carried out prior to synthesis in order to overexpress in bacteria.

Table S4. List of templates and primers used for gene(s) amplification

Gene	*Template*	*Primer sequence (5' – 3')*
cat	pACYC-petF-fpr[a]	Forward: TCTGGTGGGTCTCTGTCCatggagaaaaaaatcactggatataccaccg Reverse: CGATAGGTCTCCCGAGCCttacgccccgccctgc
saat	pJET-SAAT	Forward: TCTGGTGGGTCTCTGTCCATG Reverse: CGATAGGTCTCCCGAGCC
atf1	pJET-ATF1	Forward: TCTGGTGGGTCTCTGTCCATG Reverse: CGATAGGTCTCCCGAGCC
gfp	pJET-GFP	Forward: GGCCATGGTCTGGTGGGTCTCTGTCC Reverse: GGCCTAGGCGATAGGTCTCCCGAGCC
tes3	pET-TPC3	Forward: TCTGGTGGGTCTCTGTCCATGAAATTTAAAAAAAAATTTAAAATTGGGCGGATGCACG Reverse: CGATAGGTCTCCCGAGCCTTACACGTTAGTTTTAATTTTCCCCAAACAGTAGTCC
sfp-car	pET-TPC3	Forward: TCTGGTGGGTCTCTGTCCATGAAGATCTACGGCATATACATGGACC Reverse: CGATAGGTCTCCCGAGCCgtggcagcagcctagggaattcttacagc

[a]Kallio et al., 2014, *Nature communications*

Table S5. DNA linkers used to generate plasmid constructs by BASIC assembly

Name	Prefix linker	
	Linker sequence (5’ to 3’)	Adapter sequence (5’ to 3’)
1MP	GGACAGAGACCCACCAGATAATAGTGTTTCCACGAAGTG	TCTGGTGGGT/iMe-dC/TCT
2MP	GGACGATTCCGAAGTTACACCAGATTGGACTGTTATTAC	AACTTCGGAATC
1P	GGACTAGTTCAATAAATACCCTCTGACTGTCTCGGAG	TTTATTGAACTA
2P	GGACAGGTAATAAGAACTACACGACTGGATACTGACT	TTCTTATTACCT
3P	GGACTCTGTAATAACAATACCGATAAAGCAACGAGTG	TGTTATTACAGA
LRBS1-3P	GGACTATTTCTCCTCTTTTTACAACTGATACTTACCTGA	AAAGAGGAGAAATA
LRBS2-3P	GGACTATTTCTCCTCTTTTTTCTGCTACCCTTATCTCAG	AAAGAGGAGAAATA
Name	**Suffix linker**	
	Linker sequence (5’ to 3’)	**Adapter sequence (5’ to 3’)**
1MS	CTCGGGTAAGAACTCGCACTTCGTGGAAACACTATTA	CGAGTTCTTACC
2MS	nTATCGGTAATAACAGTCCAATCTGGTGT	CGATAGGT/iMe-dC/TCC
1S	CTCGTTACTTACGACACTCCGAGACAGTCAGAGGGTA	TGTCGTAAGTAA
2S	CTCGATCGGTGTGAAAAGTCAGTATCCAGTCGTGTAG	TTTCACACCGAT
3S	CTCGATCACGGCACTACACTCGTTGCTTTATCGGTAT	TAGTGCCGTGAT
LRBS1-XS	CTCGTTGAACACCGTCTCAGGTAAGTATCAGTTGTAA	GACGGTGTTCAA
LRBS2-XS	CTCGTGTTACTATTGGCTGAGATAAGGGTAGCAGAAA	CCAATAGTAACA

Table S6. Plasmids used for construct preparation in this study.

No.	Prefix	Plasmid	Suffix	Plasmid generated	Relevant information
1	3P	pJET-ColE1-rop-bom	1S	pET- PA1lacO-1-GFP	A plasmid used as a backbone for BASIC assembly with ColE1 origin of replication, pA1lacO1 promoter and kanamycin resistance.
	1P	pJET- PA1lacO-1	2MS		
	2MP	pJET-GFP	1MS		
	1MP	pJET-termB15	2S		
	2P	pJET-Kan	3S		
2	1P	pJET-CloDF-Spec	2S	pCDF- PA1lacO-1-GFP	A plasmid used as a backbone for BASIC assembly with CloDF origin of replication, pA1lacO1 promoter and spectinomycin resistance.
	2P	pJET- PA1lacO-1	2MS		
	2MP	pJET-GFP	1MS		
	1MP	pJET-termB15	1S		
3	2P	pET-GFP	LRBS1-XS	pET- PA1lacO-1-TPC3	A plasmid encoding Tes3, Sfp and CAR.
	LRBS1-3P	pJET-Tes3	LRBS2-XS		
	LRBS2-3P	pJET-Sfp-CAR	2S		
4	2P	pET-GFP	LRBS1-XS	pET- PA1lacO-1-‘CpFatB1-Sfp-CAR	A plasmid encoding ‘CpFatB1, Sfp and CAR.
	LRBS1-3P	pJET-‘CpFatB1	LRBS2-XS		
	LRBS2-3P	pJET-Sfp-CAR	2S		
5	2P	pET-GFP	LRBS1-XS	pET- PA1lacO-1-‘CaFatB3-Sfp-CAR	A plasmid encoding ‘CaFatB3, Sfp and CAR.
	LRBS1-3P	pJET-‘CaFatB3	LRBS2-XS		
	LRBS2-3P	pJET-Sfp-CAR	2S		
6	2P	pET-GFP	LRBS1-XS	pET- PA1lacO-1-‘CpFatB1-4-Sfp-CAR	A plasmid encoding ‘CpFatB1-4, Sfp and CAR.
	LRBS1-3P	pJET-‘CpFatB1-4	LRBS2-XS		
	LRBS2-3P	pJET-Sfp-CAR	2S		
7	2P	pET-GFP	LRBS1-XS	pET- PA1lacO-1-‘CaFatB3-5-Sfp-CAR	A plasmid encoding ‘CaFatB3-5, Sfp and CAR.
	LRBS1-3P	pJET-‘CaFatB3-5	LRBS2-XS		

	LRBS2-3P	pJET-Sfp-CAR	2S		
8	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-ATF1	A plasmid encoding ATF1
	LRBS1-3P	pJET-ATF1	2S		
9	2P	pCDF-GFP	2S	pCDF- PA1lacO-1-empty	A negative control plasmid
10	1P	pRSF1010-Ery-Pcoa-GFP	LRBS1-XS	pRSF1010-Ery-Pcoa-‘CpFatB1-4	A plasmid encoding ‘CpFatB1-4
	LRBS1-4P	pJET-‘CpFatB1-4	1S		
11	1P	pRSF1010-Ery-Pcoa-GFP	LRBS1-XS	pRSF1010-Ery-Pcoa-‘CpFatB1-4-SAAT	A plasmid encoding ‘CpFatB1-4 and SAAT
	LRBS1-4P	pJET-‘CpFatB1-4	LRBS2-XS		
	LRBS2-4P	pJET-SAAT	1S		
12	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-AdGT4	A plasmid encoding AdGT4
	LRBS1-3P	pJET-AdGT4	2S		
13	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-AtGT1	A plasmid encoding AtGT1
	LRBS1-3P	pJET-AtGT1	2S		
14	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-MtG1	A plasmid encoding MtG1
	LRBS1-3P	pJET-MtG1	2S		
15	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-MtH2	A plasmid encoding MtH2
	LRBS1-3P	pJET-MtH2	2S		
16	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-VvGT1	A plasmid encoding VvGT1
	LRBS1-3P	pJET-Vv	2S		
17	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-MtH2-AtSUS1	A plasmid encoding MtH2 and AtSUS1
	LRBS1-3P	pJET-MtH2	LRBS2-XS		
	LRBS2-3P	pACIDT-AtSUS1	2S		
18	2P	pCDF-GFP	LRBS1-XS	pCDF- PA1lacO-1-AtSUS1	A plasmid encoding AtSUS1
	LRBS1-3P	pACIDT-AtSUS1	2S		

Table S7. Summary of plasmid construction via traditional restriction cloning

Plasmid used	Ligated fragment		Relevant information
	Plasmid backbone	**Insert**	
pCDF-CAT	pCDF-GFP (BsaI)	Cat (BsaI)	A plasmid encoding CAT protein
pCDF-SAAT	pCDF-GFP (BsaI)	Saat (BsaI)	A plasmid encoding SAAT protein
pCDF-ATF1	pCDF-GFP (BsaI)	Atf1 (BsaI)	A plasmid encoding ATF1 protein
pCDF-empty	pCDF-GFP (BsaI)	-	A negative control plasmid
pET-empty	pET-TPC3 (NcoI and AvrII)	-	A negative control plasmid

The GFP encoding gene was amplified from pJET-GFP using oligonucleotides with NcoI and AvrII cutting sites for the construction of pCDF-GFP plasmid backbone. The amplified GFP was then cloned into pCDF-Ahr_{his} by replacing Ahr_{his} with *GFP*. *cat* was amplified from pACYC-petF-fpr with BsaI prefix and suffix, while SAAT and ATF1 were order as a gblock from IDT technology. All 3 genes were cloned into pCDF-GFP backbone. To construct empty plasmids, pET-TPC4 was digested with NcoI and AvrII and pCDF-GFP was digested with BsaI. Both backbones were recirculated using Quick Blunting™ and Quick Ligation™ Kits from NEB after restriction digestion.

Table S8. Plasmids used for production in this study

Plasmid Name	Genotype	Reference
pET-TPC3	ColE1; CmR; P_{T7}: *tes3, sfp, car*	(Akhtar et al., 2015)
pCDF-P_{T7}-empty	CloDF13; SpecR; P_{T7}:	This work
pET-P_{T7}-empty	ColE1; CmR; P_{T7}:	This work
pCDF-P_{T7}-CAT	CloDF13; SpecR; P_{T7}: *cat*	This work
pCDF-P_{T7}-SAAT	CloDF13; SpecR; P_{T7}: *saat*	This work
pCDF-P_{T7}-ATF1	CloDF13; SpecR; P_{T7}: *atf1*	This work
pET- PA1lacO-1-TPC3	ColE1; KanR; PA1lacO-1: *tes3, sfp, car*	This work
pET- PA1lacO-1-‘CpFatB1-Sfp-CAR	ColE1; KanR; PA1lacO-1: ‘*CpFatB1-3, sfp, car*	This work
pET- PA1lacO-1-‘CaFatB3-Sfp-CAR	ColE1; KanR; PA1lacO-1: ‘*CaFatB3, sfp, car*	This work
pET- PA1lacO-1-‘CpFatB1-4-Sfp-CAR	ColE1; KanR; PA1lacO-1: ‘*CpFatB1-4, sfp, car*	This work
pET- PA1lacO-1-‘CaFatB3-5-Sfp-CAR	ColE1; KanR; PA1lacO-1: ‘*CaFatB3-5, sfp, car*	This work
pCDF- PA1lacO-1-empty	CloDF13; SpecR; PA1lacO-1:	This work
pCDF- PA1lacO-1-ATF1	CloDF13; SpecR; PA1lacO-1: *atf1*	This work
pRSF1010-Ery-Pcoa-‘CpFatB1-4	RSF1010; EryR; Pcoa: ‘*CpFatB1-4*	This work
pRSF1010-Ery-Pcoa-‘CpFatB1-4-SAAT	RSF1010; EryR; Pcoa: ‘*CpFatB1-4*, *saat*	This work
pCDF- PA1lacO-1-AdGT4	CloDF13; SpecR; PA1lacO-1: *AdGT4*	This work
pCDF- PA1lacO-1-AtGT1	CloDF13; SpecR; PA1lacO-1: *AtGT1*	This work
pCDF- PA1lacO-1-MtG1	CloDF13; SpecR; PA1lacO-1: *MtG1*	This work
pCDF- PA1lacO-1-MtH2	CloDF13; SpecR; PA1lacO-1: *MtH2*	This work
pCDF- PA1lacO-1-VvGT1	CloDF13; SpecR; PA1lacO-1: *VvGT1*	This work
pCDF- PA1lacO-1-AtSUS1	CloDF13; SpecR; PA1lacO-1: *AtSUS1*	This work
pCDF- PA1lacO-1-MtH2-AtSUS1	CloDF13; SpecR; PA1lacO-1: *MtH2, AtSUS1*	This work

Table S9. List of DNA templates and primers used to generate genetic parts for the construction of the suicide plasmid targeting phaAB site

Template	Primer		Plasmid Generated	Plasmid Contained	Relevant information
	F	R			
Genetic Part 1 - Upstream homology region					
DNA chromosome[a]	IY224	IY225	pIY442	pJET::*pha*AB_UHR	Plasmid containing a *lacI* repressor gene and $P_{clac143}$ promoter.
Genetic Part 2 – Downstream homology region					
DNA chromosome[a]	IY226	IY227	pIY443	pJET::p*ha*AB_DHR	Plasmid containing P_{nrsB} promoter.
Genetic Part 3 – Promoter					
DNA chromosome[a]	PB210	PB211	pIY171	pJET::P_{nrsB}	Plasmid containing P_{nrsB} promoter.
Genetic Part 4 – GFP dropout gene					
-	-	-	pIY24	pJET::*gfp*	Superfold green fluorescence (GFP) gene ordered as a gBlock.
Genetic Part 5 – Spectinomycin resistance cassette					
-	PB29	PB30	pIY98	pJET::Sp^R	Spectinomycin resistance cassette was ordered as a gBlock.
Genetic Part 6 – Terminator					
-	-	-	pIY67	pJET::termB15	Plasmid storage containing rrnB T1 terminator and T7Te terminator.
Genetic Part 7 & 8 – Backbone					
-	-	-	pIY99	pColE1::*gfp* KanR	Plasmid backbone for plasmid propagation in *E. coli* with a kanamycin selection marker cassette and a GFP dropout gene
-	-	-	pIY23	pColE1::AmpR	Plasmid backbone for plasmid propagation in *E. coli* with a carbenicillin selection marker cassette (requested from Dr. Geoff Baldwin)
Gene of interest					
pET-TPC3[b]	IY234	IY155	pIY485	pJET-Sfp-CAR	Plasmid storage containing Sfp and CAR.

[a]*Synechocystis* sp. PCC 6803 wild-type strain

[b]Akhtar et al., *Metabolic Engineering Communications*, 2015

Table S10. List of genetic parts and linkers used to construct plasmid pIY453 and pIY454

Prefix Linker	Plasmid	Genetic part	Suffix Linker	Plasmid generated	Relevant Information
1MP	pIY442	phaAB_UHR	1S	pIY453	pIY453 is the plasmid carrying the upstream genetic parts for the construction of suicide vector targeting phaAB site
1P	pIY171	PnrsB	2MS		
2MP	pIY99	ColE1-Kan^R	1MS		
1MP	pIY67	TermB15	1S	pIY454	pIY454 is the plasmid carrying the downstream genetic parts for the construction of suicide vector targeting phaAB site
1P	pIY98	Sp^R	2S		
2P	pIY443	phaAB_DHR	2MS		
2MP	pIY99	ColE1-Kan^R	1MS		

Table S11. Linkers and genetic parts used to construct the suicide vector backbone pIY475 carrying a *gfp* dropout gene

Prefix Linker	Plasmid No.	Genetic part	Selectable Marker	Suffix Linker	Plasmid generated	Relevant Information
1P	pIY453	phaAB_UHR, PnrsB	Amp^R	2MS	pIY475	pIY475 is a suicide vector targeting *pha*AB site containing phaAB upstream homology region, *gfp* dropout gene, a terminator, a spectinomycin selection marker cassette, a phaAB downstream homology region, and an *E. coli* ori with an ampicillin selection marker
2MP	pIY24	GFP	GFP, Amp^R	1MS		
1MP	pIY454	termB15, Sp^R, phaAB_DHR	Sp^R, Kan^R	2S		
2P	pIY23	ColE1, Amp^R	Amp^R	1S		

Table S12. Linkers and genetic parts used to construct the suicide vector carrying *sfp* and *car* genes targeting *pha*AB site

Prefix Linker	Plasmid	Selectable Marker	Suffix Linker	Plasmid generated	Relevant Information
LRBS1-4P	pIY485	Amp^R	1S	pIY706	pIY706 is a suicide vector carrying *sfp* and *car* genes targeting *pha*AB site.
1P	pIY475	GFP, Kan^R, Amp^R	LRBS1-4S		

References

1. J. Elhai, A. Vepritskiy, A. M. Muro-Pastor, E. Flores, C. P. Wolk, Reduction of conjugal transfer efficiency by three restriction activities of Anabaena sp. strain PCC 7120. *J. Bacteriol.* **179**, 1998–2005 (1997).