9. SUPPLEMENTARY MATERIAL

Metabolic engineering strategies to produce medium-chain oleochemicals via acyl-ACP:CoA transacylase activity

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Table S1: Strains and plasmids used in this study

Strain/Plasmid	Genotype	source
Strains		
CM23	MG1655*ΔaraBAD ΔFadABIJDRE ΔldhA ΔackApta ΔadhE ΔpoxB ΔfrdABCD ΔydiO ΔatoC*	[1]
RAI	MG1655 *ΔaraBAD ΔfadR ΔfadA ΔfadI*	[2]
RADI	MG1655 *ΔaraBAD ΔfadR ΔfadA ΔfadI ΔfadD*	This study
RABIJ	MG1655 *ΔaraBAD ΔfadR ΔfadA ΔfadB ΔfadI ΔfadJ*	[2]
CM23-*ΔlipB*	CM23 *ΔlipB*	This study
Plasmids		
pMP11	pKD46 with constitutively expressed Cas9 and an aTc gRNA targeting the ColE1 origin	[1]
pgRNA	Constitutively expressed sgRNA targeting a desired gene	[1]
pBTRCK	Trc promoter, pBRR1 origin, Kan^R	[1]
pBTRCK-MaTesB*	Trc promoter, containing ^{Ma}TesB A197D an acyl-CoA thioesterase from Mycobacterium avium	This study
pBTRCK-PkPhaG	Trc promoter, containing ^{PkPhaG} a 3-hydroxy-acyl-ACP:CoA transacylase from *Mycobacterium avium*	This study
pBTRCK-MaACR	Trc promoter, containing ^{Ma}ACR an acyl-CoA reductase from Mycobacterium avium	[1]

pACYC	Trc promoter, pACYC origin, CmR	[1]
pACYC-PhaJ1	Trc promoter, containing ^{Pa}PhaJ1 an (R)-specific enoyl-CoA hydratase from *Pseudomonas aeruginosa PAO1*	This study
pACYC-PhaJ2	Trc promoter, containing ^{Pa}PhaJ1 an (R)-specific enoyl-CoA hydratase from *Pseudomonas aeruginosa PAO1*	This study
pACYC-PhaJ3	Trc promoter, containing ^{Pa}PhaJ1 an (R)-specific enoyl-CoA hydratase from *Pseudomonas aeruginosa PAO1*	This study
pACYC-PhaJ4	Trc promoter, containing ^{Pa}PhaJ1 an (R)-specific enoyl-CoA hydratase from *Pseudomonas aeruginosa PAO1*	This study
pACYC-AbFadB	Trc promoter, containing ^{Ab}FadB a bi-functional 3-hydroxyacyl-CoA dehydrogenase from *Alcanivorax borkumensis*	[3]
pACYC-MaFadB	Trc promoter, containing ^{Ma}FadB a bi-functional 3-hydroxyacyl-CoA dehydrogenase from *Marinobacter aquaeolei*	[3]
pACYC-SeFadB	Trc promoter, containing ^{Se}FadB a bi-functional 3-hydroxyacyl-CoA dehydrogenase from *Salmonella enterica*	[3]
pACYC-PpFadB	Trc promoter, containing ^{Pp}FadB a bi-functional 3-hydroxyacyl-CoA dehydrogenase from *Pseudomona putida*	[3]
pACYC-EcFadB	Trc promoter, containing ^{Ec}FadB a bi-functional 3-hydroxyacyl-CoA dehydrogenase from *E. coli*	[3]
pACYC-VfFadB	Trc promoter, containing ^{Vf}FadB a bi-functional 3-hydroxyacyl-CoA dehydrogenase from *Vibrio fisheri*	[3]
pTRC99a	Trc promoter, pBR322 origin, AmpR	[1]
pTRC99a-^{Ppu}PhaG-^{Ec}fadM	Trc promoter, ^{Ec}FadM and ^{Ppu}PhaG (GI: WP_066852574) from *P. putida*	This study
pTRC99a-PmPhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Pm}PhaG (GI: WP_028690175) from *P. mosselii*	This study

pTRC99a-PsPhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Ps}PhaG (GI: WP_110604878) from *P. soli*	This study
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pTRC99a-PvPhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Pv}PhaG (GI: WP_088234671) from *Pseudomonas viridiflava*	This study
pTRC99a-PfPhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Pf}PhaG (GI: WP_105345860) from *Pseudomonas frederiksbergensis*	This study
pTRC99a-PaPhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Pa}PhaG (GI: WP_110664271) from *Pseudomonas aeruginosa*	This study
pTRC99a-CmPhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Cm}PhaG (GI: WP_047262589) from *Corynebacterium mustelae*	This study
pTRC99a-CePhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Ce}PhaG (GI: WP_006768596) from *Corynebacterium efficiens*	This study
pTRC99a-Mt1PhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Mt1}PhaG (GI: WP_103656228) from *Mycobacterium tuberculosis Mt1*	This study
pTRC99a-Mt2PhaG-EcfadM	Trc promoter, ^{Ec}FadM and ^{Mt2}PhaG (GI: PLV47039) from *Mycobacterium tuberculosis Mt2*	This study
pTRC99a-PkPhaG-TdTER	Trc promoter, ^{Td}TER and ^{Pk}PhaG (GI: WP_064586405) from *Pseudomonas koreensis*	This study
pTRC99a-'PkPhaG'-TdTER	Trc promoter, ^{Td}TER and ^{Pk}PhaG mutants	This study
pTRC99a-'PkPhaG'-PsFadM	Trc promoter, ^{Ps}FadM from *Providencia sneebia* and '^{Pk}PhaG'	This study

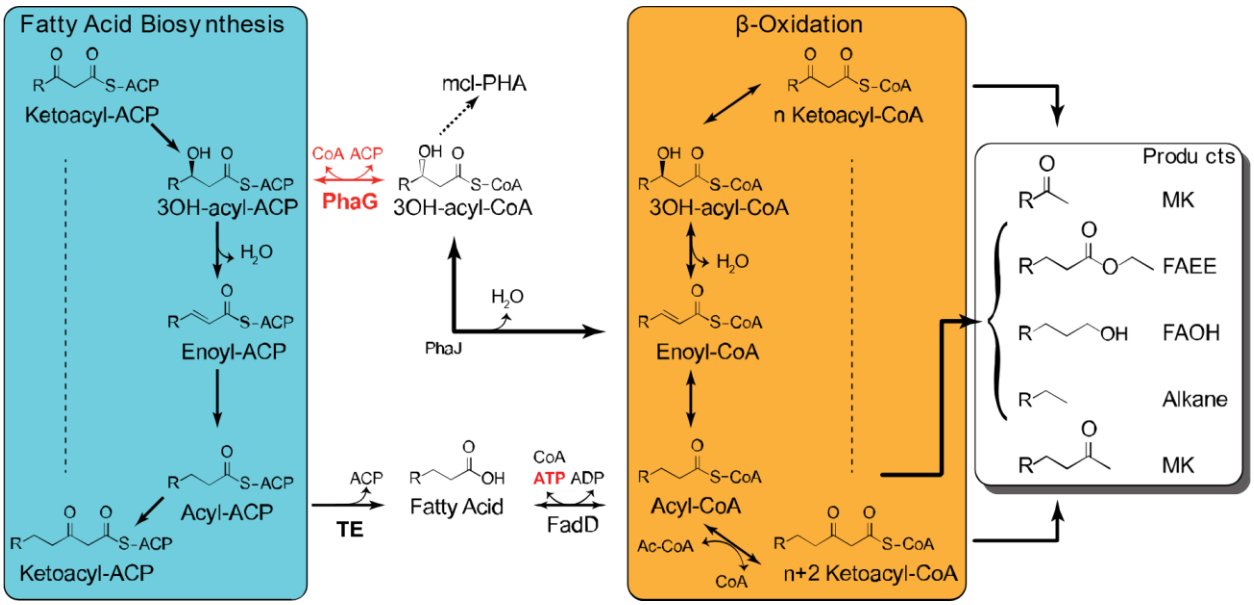


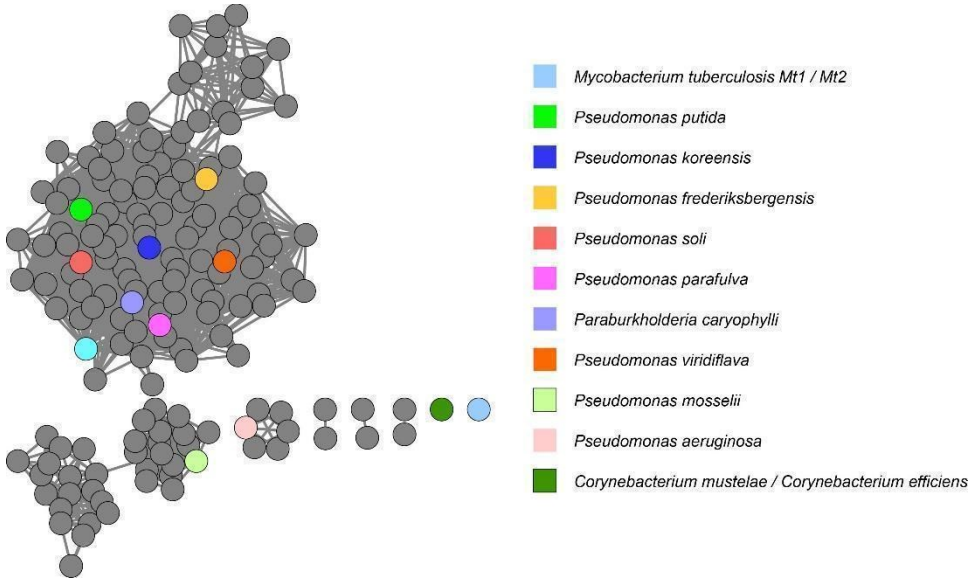
Figure S1 Detailed thioesterase and PhaG biosynthetic pathways for medium-chain oleochemical production

9.1. Genome scale metabolic model calculations

Maximum theoretical yields were calculated via flux balance analysis of a modified version of the iML1515 genome-scale metabolic model of *Escherichia coli* utilizing the cobrapy python package [4]. Mass and charge balanced reactions catalyzed by PhaG were added to the iML1515 model. Lower bounds for biomass production and non-growth associated maintenance requirements were set to 0 mmol gDW^{-1} h^{-1}, and each theoretical yield was calculated as the ratio of maximized product flux to glycerol uptake flux (**Table S2**). Flux was constrained to each described pathway through simulated gene knockouts, or individual reaction constraints. An IPython notebook containing the script used is included in supplemental file "PhaG yield analysis.ipynb", and the modified iML1515 model is included in supplemental file "Oleo_iML1515.xml".

Table S2 Theoretical yields of oleochemicals synthesis from glycerol as a carbon source using the thioesterase route or transacylase route.

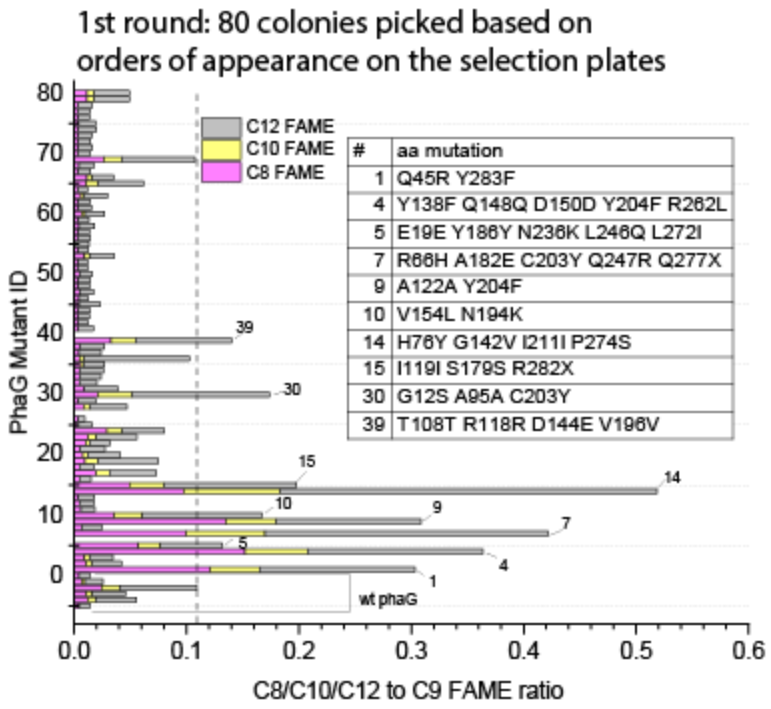
	Thioesterase Acyl-CoA Synthetase	Transacylase (PhaG)
methyl ketones		
2-heptanone	0.300	0.318
2-nonanone	0.236	0.247
2-undecanone	0.194	0.202
2-tridecanone	0.165	0.170
fatty alcohols		
1-octanol	0.267	0.276
1-decanol	0.215	0.221
1-dodecanol	0.180	0.184
1-tetradecanol	0.154	0.157
fatty acids		
octanoic acid	0.294	0.294
decanoic acid	0.232	0.232
dodecanoic acid	0.188	0.188
tetradecanoic acid	0.163	0.163



		PpuPhaG	PmPhaG	PsPhaG	PpaPhaG	PcPhaG	PkPhaG	PvPhaG	PfPhaG	PaPhaG	CmPhaG	CePhaG	Mt1PhaG	Mt2PhaG
Pseudomonas putida	PpuPhaG	100	88	87	83	78	68	66	62	60	28	25	22	24
Pseudomonas mosselii	PmPhaG	88	100	95	86	79	70	66	64	60	35	26	24	26
Pseudomonas soli	PsPhaG	87	95	100	87	78	69	66	64	62	38	26	24	26
Pseudomonas parafulva	PpaPhaG	83	86	87	100	76	69	66	64	62	31	27	27	25
Paraburkholderia caryophylli	PcPhaG	78	79	78	76	100	72	73	64	62	22	23	22	32
Pseudomonas koreensis	PkPhaG	68	70	69	69	72	100	72	65	58	27	24	22	23
Pseudomonas viridiflava	PvPhaG	66	66	66	66	73	72	100	62	57	27	22	28	40
Pseudomonas frederiksbergensis	PfPhaG	62	64	64	64	64	65	62	100	62	34	25	33	31
Pseudomonas aeruginosa	PaPhaG	60	60	62	62	62	58	57	62	100	28	26	22	30
Corynebacterium mustelae	CmPhaG	28	35	38	31	22	27	27	34	28	100	26	22	27
Corynebacterium efficiens	CePhaG	25	26	26	27	23	24	22	25	26	26	100	32	25
Mycobacterium tuberculosis Mt1	Mt1PhaG	22	24	24	27	22	22	28	33	22	22	32	100	25
Mycobacterium tuberculosis Mt2	Mt2PhaG	24	26	26	25	32	23	40	31	30	27	25	25	100

Figure S2 (Top) Sequencing similarity map of PhaG homologs. (Bottom) The quantitative relationship between the sequences. Matrices show the pairwise percent amino acid identity multiple alignment of each enzyme homolog.

A



#	aa mutation
1	Q45R Y283F
4	Y138F Q148Q D150D Y204F R262L
5	E19E Y186Y N236K L246Q L272I
7	R66H A182E C203Y Q247R Q277X
9	A122A Y204F
10	V154L N194K
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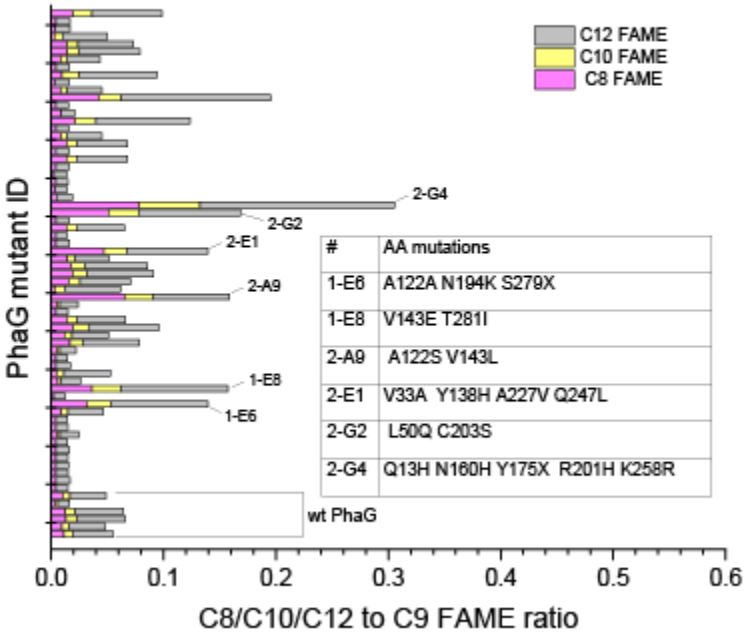

#	AA mutations
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1-E8	V143E T281I
2-A9	A122S V143L
2-E1	V33A Y138H A227V Q247L
2-G2	L50Q C203S
2-G4	Q13H N160H Y175X R201H K258R

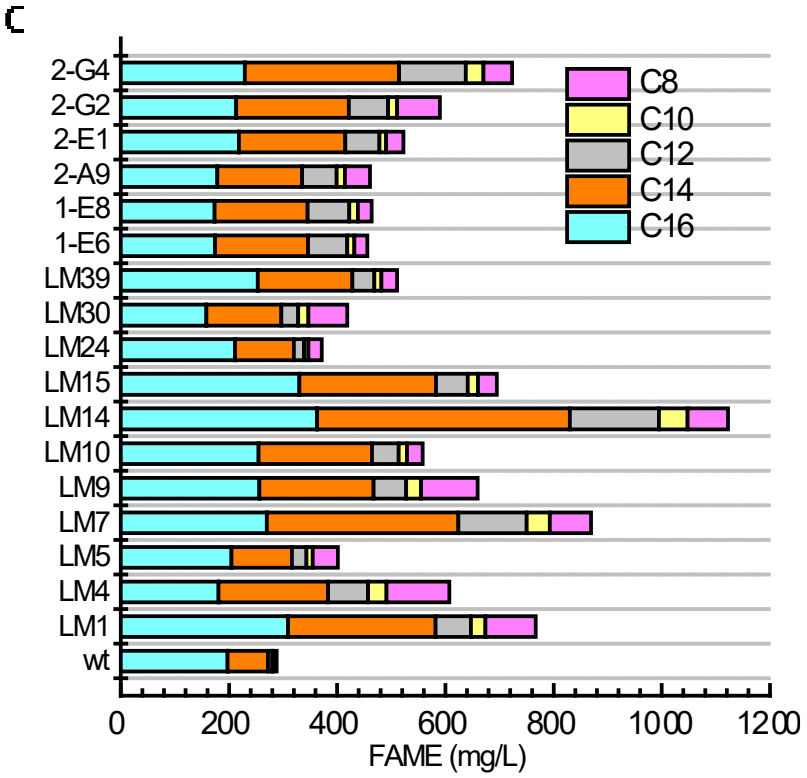

Figure S3 Evaluation of fatty acid production of PhaG mutant library. (**A-B**) We took two strategies to pick colonies of hits. First, we picked 80 colonies that first appeared on selection plates on day 4. We cultured them for fatty acid production in Clomburg liquid media 20 g/L glycerol at 30°C for 48 hrs. In the second strategy, on day 5, we cultured another 287 colonies in 96-well plates in Clomburg liquid media 20 g/L glycerol at 30°C for 48 hrs. Culture supernatant was stained by 2 ul 1 mM nile-red. Fluorescence intensity of each culture was used to prescreen active PhaG enzymes compared to that of wild-type PhaG. Then, 64 cultures were further cultured in test tubes for fatty acid production. (**C**) Evaluation of fatty acid production of 17 beneficial PhaG mutants.

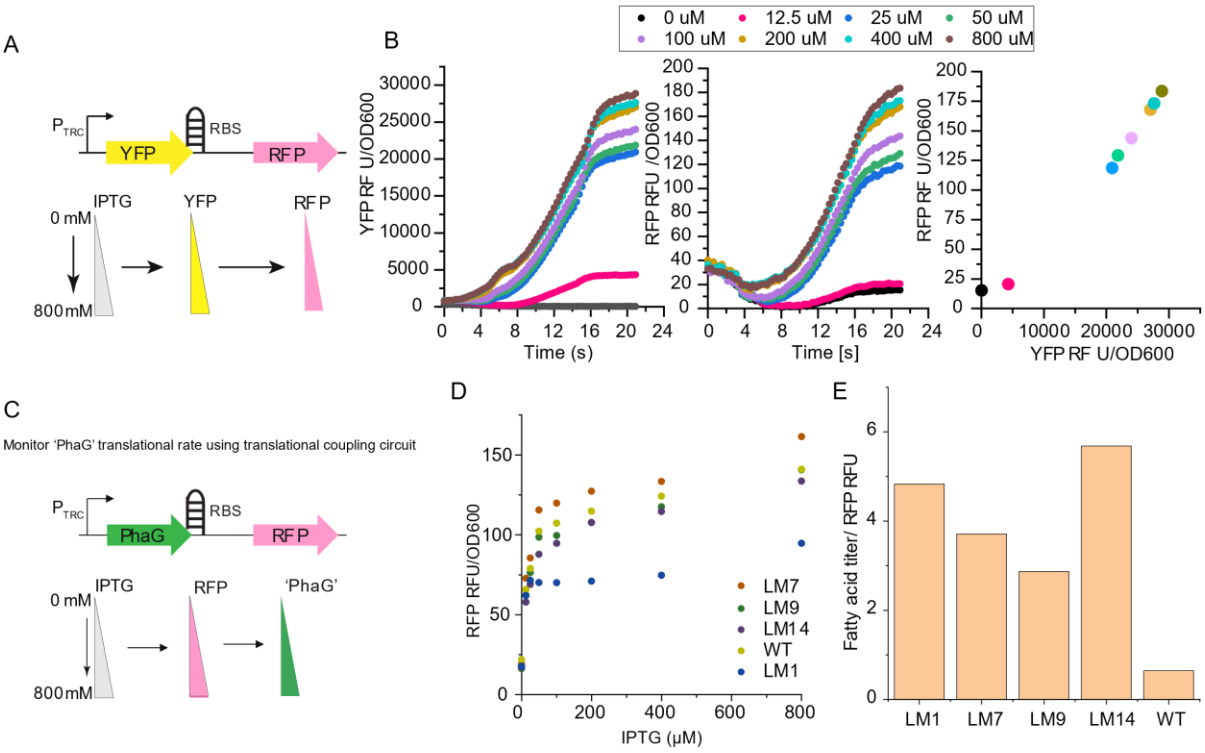

Figure S4 Evaluation of PhaG Variant Expression Via Translational Coupling. (A) Scheme outlining translational coupling circuit linking RFP expression to YFP expression. YFP expression was titrated by induction of transcription with IPTG. (B) Time-course of YFP fluorescence intensity per OD600, RFP fluorescence intensity per OD600, and correlation of YFP and RFP fluorescence taken at the experiment end-point (48 hrs) were plotted. (C) Scheme outlining translational coupling circuit linking RFP expression to PhaG expression. PhaG variant expression was titrated by induction of transcription with IPTG. (D) End-point (48 hrs) RFP fluorescence intensity per OD600 (RFU/OD600) as a function of IPTG concentration was plotted for five *E. coli* DH5a strain harboring one of the p'PhaG'-RFP variants. (E) Evaluation of C8-C14 total fatty acid titer over PhaG abundance (RFP RFU/OD600).

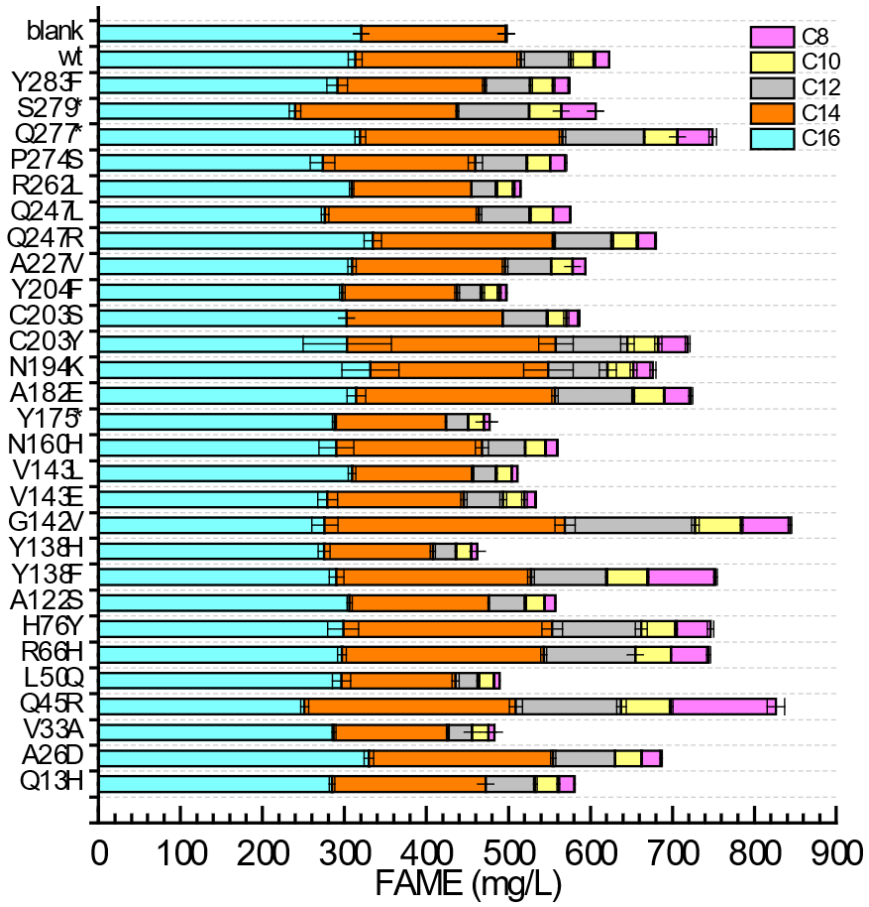

Figure S5. 28 ^{Pk}PhaG single point mutations were constructed and FAME profiles were evaluated using strain CM23 harboring pTRC99a-'^{Pk}*phaG*'-^{Td}*TER* + pACYC-^{Pa}*phaJ3* + pBTRCK-^{Ma}*tesB**. The strain was cultured in test tubes containing 5 mL Clomburg 20 g/L glycerol and 1 mM IPTG at 30°C for 72 hrs. (n=3)

9.2. Off Gas Methyl Ketone Capture and ASPEN Analysis

In effort to capture methyl ketones lost from shake flask experiments, we constructed a chilled condenser[3] to capture product lost in the off-gas stream of our bioreactors. This condenser provided only a modest increase in methyl ketone titer, suggesting that either there is not a significant loss of methyl ketones over time, or that the condenser was unable to condense the lost product. Aspen Plus V11 was used to estimate the feasibility of 2-heptanone capture using a condenser. The UNIFAC liquid-liquid (UNIF-LL) property method was used to model the liquid-liquid equilibrium between water, dodecane and 2-heptanone. The simulation consisted of a three-outlet flash drum and a two-outlet flash drum. The three-outlet flash modeled the bioreactor and served to separate the vapor, aqueous (water), and organic (dodecane) product/outlet streams. The operating pressure for all units was 1 bar and the operating temperature for the two-outlet flash was 5°C. The flowrates of water, dodecane, and air in the feed were 500 g/min, 133 g/min, and 1320 g/min, respectively to represent the ratio of these species in the bioreactor experiment. The flowrate of 2-heptanone was arbitrarily set to 1.0 g/min, as the focus of this analysis was the fractional recovery of 2-heptanone, which was not found to change significantly at different flowrates. The mass fraction of 2-heptanone recovered in the liquid outlet of the flash drum was 3.8%. The simulation was repeated to determine if the recovery could be improved by using an organic gas absorber instead of a condenser. The vapor product stream of the three-outlet flash drum was feed into a cooler (heat exchanger), cooled to 5°C, then fed into an absorber (modeled by a two-stage column). A secondary dodecane stream at a flowrate of 133 g/min was (also) fed into the absorber. The fractional recovery of 2-heptanone in the dodecane product stream was at least 90%. The recovery was not found to change significantly at different 2-heptanone initial

flowrates. These results suggest that the recovery of 2-heptanone from the bioreactor off-gas could be significantly improved if an absorber was used instead of a condenser.

To implement a gas absorber, the bioreactor off gas was fed directly into a jacketed gas dryer filled with 100mL of dodecane and chilled to 5°C with an external Fisher Scientific Isotemp water cooler (**Figure S6**). Over time water evaporated from the reactor collected in the absorber varying from 50-100 mL. Water collected was removed as needed to prevent dodecane overflow and combined and analyzed for methyl ketone concentration at the completion of the bioreactor run. Total methyl ketones in the absorber were calculated from a sample of dodecane and the collected water at the end of the fermentation and added to the total bioreactor titer.

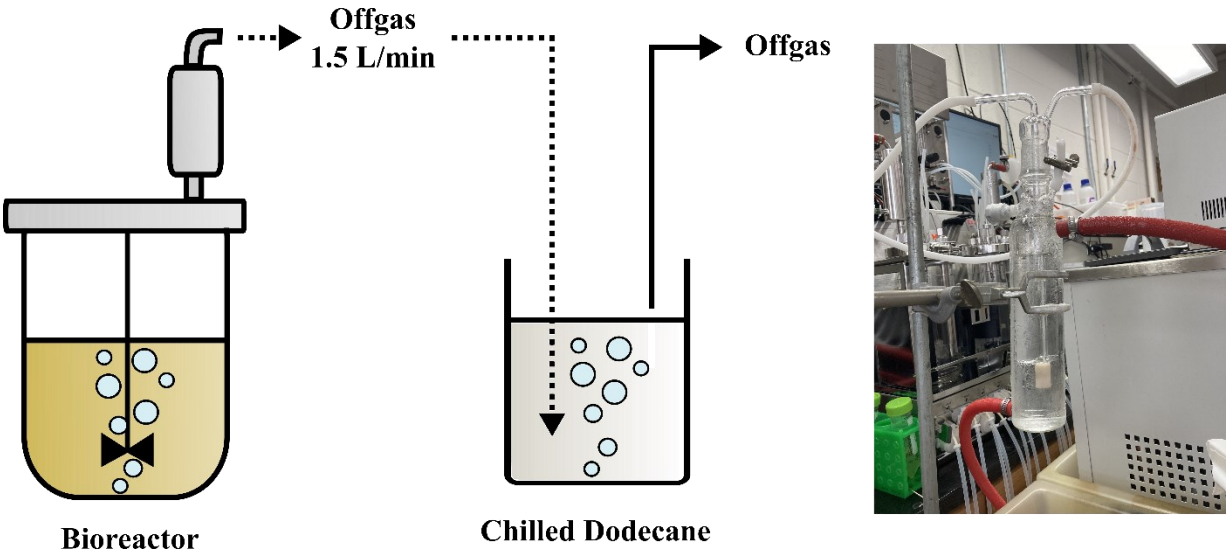

Figure S6. (A) (Left) Schematic of the bioreactor-absorber system, (B) (Right) Image of jacketed glass absorber filled with water and dodecane.

Jupiter Notebook Code for Analyzing Theoretical Yields


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\n",
       "2-Nonanone                    0.242331                      0.246875
\n",
       "2-Undecanone                  0.198492                      0.201531
\n",
       "2-Tridecanone                 0.168085                      0.170259
\n",
       "Octanoic Acid                 0.287273                       0.29368
\n",
       "Decanoic Acid                 0.227666                      0.231672
\n",
       "Dodecanoic Acid               0.188544                      0.191283
\n",
       "Tetradecanoic Acid            0.160896                      0.162887  "
      ]
     },
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   "source": [
    "# Define product names and corresponding model exchange reaction IDs as
a dictionary\n",
    "objectives = {'Octanol':'EX_OH_C080_LPAREN_e_RPAREN_', \n",
    "              'Decanol': 'EX_OH_C100_LPAREN_e_RPAREN_',\n",
    "              'Dodecanol': 'EX_OH_C120_LPAREN_e_RPAREN_',\n",
    "              'Tetradecanol': 'EX_OH_C140_LPAREN_e_RPAREN_',\n",
    "              '2-Heptanone': 'EX_MK_C080_LPAREN_e_RPAREN_',\n",
    "              '2-Nonanone': 'EX_MK_C100_LPAREN_e_RPAREN_',\n",
    "              '2-Undecanone': 'EX_MK_C120_LPAREN_e_RPAREN_',\n",
    "              '2-Tridecanone': 'EX_MK_C140_LPAREN_e_RPAREN_',\n",
    "              'Octanoic Acid': 'EX_octa_e',\n",
    "              'Decanoic Acid': 'EX_dca_e',\n",
    "              'Dodecanoic Acid': 'EX_ddca_e',\n",
    "              'Tetradecanoic Acid': 'EX_ttdca_e'}\n",
    "\n",
    "# Create DataFrames for storing the FBA solutions, and the theoretical
yields\n",
```

```
    "iML1515_solution_df = pd.DataFrame(index = objectives.keys(), columns =
['Reverse Beta Oxidation','Thioesterase (ΔfadAI)','PhaG PhaJ ΔfadDEK','PhaG
PhaJ tdTER BktB ΔfadBDK'])\n",
    "iML1515_theoretical_yeilds_df = pd.DataFrame(index = objectives.keys(),
columns = ['Reverse Beta Oxidation','Thioesterase (ΔfadAI)','PhaG PhaJ
ΔfadDEK','PhaG PhaJ tdTER BktB ΔfadBDK'])\n",
    "\n",
    "\n",
    "#########################################################\n",
    "##### Reverse Beta-Oxidation Pathway Simulations #####\n",
    "#########################################################\n",
    "\n",
    "# Load the oleochemical modified iML1515 model \n",
    "OleoiML1515 = cobra.io.read_sbml_model('Oleo_iML1515.xml')        \n",
    "OleoiML1515.solver = 'glpk'\n",
    "\n",
    "# Set the carbon source to glycerol, and remove the ATP maintenance
energy \n",
    "OleoiML1515.reactions.EX_glc__D_e.lower_bound = 0\n",
    "OleoiML1515.reactions.EX_glyc_e.lower_bound = -1\n",
    "OleoiML1515.reactions.ATPM.lower_bound = 0\n",
    "\n",
    "# Evaluate the theoretical yield of each product of interest. Store
solutions as Reverse Beta Oxidation.\n",
    "for o in objectives: \n",
    "    OleoiML1515.objective =
OleoiML1515.reactions.get_by_id(objectives[o])\n",
    "    FBA_Solution = OleoiML1515.optimize()\n",
    "    theoreticalYield = FBA_Solution.objective_value / -
FBA_Solution.fluxes['EX_glyc_e']\n",
    "    iML1515_solution_df.loc[o]['Reverse Beta Oxidation'] =
FBA_Solution\n",
    "    iML1515_theoretical_yeilds_df.loc[o]['Reverse Beta Oxidation'] =
theoreticalYield\n",
    "    \n",
    "    \n",
    "#############################################   \n",
    "##### Thioesterase Pathway Simulations #####\n",
    "#############################################\n",
    "\n",
    "# Reload the oleochemical modified iML1515 model \n",
    "OleoiML1515 = cobra.io.read_sbml_model('Oleo_iML1515.xml')
\n",
    "OleoiML1515.solver = 'glpk'\n",
    "\n",
    "# Set the carbon source to glycerol, and remove the ATP maintenance
energy \n",
    "OleoiML1515.reactions.EX_glc__D_e.lower_bound = 0\n",
    "OleoiML1515.reactions.EX_glyc_e.lower_bound = -1\n",
    "OleoiML1515.reactions.ATPM.lower_bound = 0    \n",
    "\n",
    "# Simulate fadA / fadI knockout - Prevent Reverse Beta Oxidation\n",
    "OleoiML1515.genes.b2342.knock_out()\n",
    "OleoiML1515.genes.b3845.knock_out()\n",
    "\n",
    "# Heterologous PhaG Reaction Removal - Prevent PhaG reactions\n",
    "OleoiML1515.reactions.PhaG6.delete()\n",
```

```
    "OleoiML1515.reactions.PhaG8.delete()\n",
    "OleoiML1515.reactions.PhaG10.delete()\n",
    "OleoiML1515.reactions.PhaG12.delete()\n",
    "OleoiML1515.reactions.PhaG14.delete()\n",
    "OleoiML1515.reactions.PhaG16.delete()\n",
    "OleoiML1515.reactions.CTECOAI6.delete()\n",
    "OleoiML1515.reactions.HXCT.delete()\n",
    "\n",
    "# Evaluate the theoretical yield of each product of interest.  Store
solutions as Thioesterase (ΔfadAI).\n",
    "for o in objectives:\n",
    "    OleoiML1515.objective =
OleoiML1515.reactions.get_by_id(objectives[o])\n",
    "    FBA_Solution = OleoiML1515.optimize()\n",
    "    theoreticalYield = FBA_Solution.objective_value / -
FBA_Solution.fluxes['EX_glyc_e']\n",
    "    iML1515_solution_df.loc[o]['Thioesterase (ΔfadAI)'] =
FBA_Solution\n",
    "    iML1515_theoretical_yeilds_df.loc[o]['Thioesterase (ΔfadAI)'] =
theoreticalYield\n",
    "     \n",
    "\n",
    "##########################################   \n",
    "##### PhaG -> TER Pathway Simulations ##### \n",
    "##########################################\n",
    "\n",
    "# Reload the oleochemical modified iML1515 model \n",
    "OleoiML1515 = cobra.io.read_sbml_model('Oleo_iML1515.xml')
\n",
    "OleoiML1515.solver = 'glpk'\n",
    "\n",
    "# Set the carbon source to glycerol, and remove the ATP maintenance
energy \n",
    "OleoiML1515.reactions.EX_glc__D_e.lower_bound = 0\n",
    "OleoiML1515.reactions.EX_glyc_e.lower_bound = -1\n",
    "OleoiML1515.reactions.ATPM.lower_bound = 0\n",
    "\n",
    "# Prevent Reverse Beta Oxidation cycle, but allow for single elongation
step via PhaG->FadB->TER->fadA\n",
    "OleoiML1515.reactions.HACD1.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD2.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD3.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD4.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD5.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD6.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD7.upper_bound = 0\n",
    "OleoiML1515.reactions.HACD8.upper_bound = 0\n",
    "\n",
    "# fadD / fadK knockout \n",
    "OleoiML1515.genes.b1805.knock_out()\n",
    "OleoiML1515.genes.b1701.knock_out()\n",
    "\n",
    "# Preventing Thioesterase Route for Free Fatty Acid Synthesis\n",
    "OleoiML1515.reactions.TE_C060_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C080_ACP.upper_bound = 0 \n",
    "OleoiML1515.reactions.TE_C100_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C120_ACP.upper_bound = 0\n",
```

```
    "OleoiML1515.reactions.TE_C140_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C160_ACP.upper_bound = 0\n",
    "\n",
    "#OleoiML1515.reactions.FA60ACPHi.upper_bound = 0 # Not in Model\n",
    "OleoiML1515.reactions.FA80ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA100ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA120ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA140ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA160ACPHi.upper_bound = 0\n",
    "\n",
    "# Prevent Acyl-(acyl carrier protein):phosphate acyltransferase for Free
Fatty Acid Synthesis\n",
    "OleoiML1515.reactions.ACPPAT120.upper_bound = 0\n",
    "OleoiML1515.reactions.ACPPAT140.upper_bound = 0\n",
    "\n",
    "\n",
    "# Evaluate the theoretical yield of each product of interest.  Store
solutions as PhaG PhaJ tdTER BktB ΔfadBDK.\n",
    "for o in objectives:\n",
    "    OleoiML1515.objective =
OleoiML1515.reactions.get_by_id(objectives[o])\n",
    "    FBA_Solution = OleoiML1515.optimize()\n",
    "    theoreticalYield = FBA_Solution.objective_value / -
FBA_Solution.fluxes['EX_glyc_e']\n",
    "    iML1515_solution_df.loc[o]['PhaG PhaJ tdTER BktB ΔfadBDK'] =
FBA_Solution\n",
    "    iML1515_theoretical_yeilds_df.loc[o]['PhaG PhaJ tdTER BktB ΔfadBDK']
= theoreticalYield\n",
    "    \n",
    "##########################################\n",
    "##### PhaG -> FadB / FadA solutions #####\n",
    "##########################################\n",
    "\n",
    "# Reload the oleochemical modified iML1515 model \n",
    "OleoiML1515 = cobra.io.load_json_model('Oleo_iML1515.json')        \n",
    "OleoiML1515.solver = 'glpk'\n",
    "\n",
    "# Set the carbon source to glycerol, and remove the ATP maintenance
energy \n",
    "OleoiML1515.reactions.EX_glc__D_e.lower_bound = 0\n",
    "OleoiML1515.reactions.EX_glyc_e.lower_bound = -1\n",
    "OleoiML1515.reactions.ATPM.lower_bound = 0\n",
    "\n",
    "# fadD / fadK knockouts \n",
    "OleoiML1515.genes.b1805.knock_out()\n",
    "OleoiML1515.genes.b1701.knock_out()\n",
    "\n",
    "# fadE and thioesterase knockouts\n",
    "OleoiML1515.genes.b0221.knock_out()\n",
    "OleoiML1515.reactions.TER04.delete()\n",
    "OleoiML1515.reactions.TER06.delete()\n",
    "OleoiML1515.reactions.TER08.delete() \n",
    "OleoiML1515.reactions.TER10.delete()\n",
    "OleoiML1515.reactions.TER12.delete()\n",
    "OleoiML1515.reactions.TER14.delete()\n",
    "\n",
    "# Preventing Thioesterase Route for Free Fatty Acid Synthesis\n",
```

```
    "OleoiML1515.reactions.TE_C060_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C080_ACP.upper_bound = 0 \n",
    "OleoiML1515.reactions.TE_C100_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C120_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C140_ACP.upper_bound = 0\n",
    "OleoiML1515.reactions.TE_C160_ACP.upper_bound = 0\n",
    "\n",
    "#OleoiML1515.reactions.FA60ACPHi.upper_bound = 0 # Not in Model\n",
    "OleoiML1515.reactions.FA80ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA100ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA120ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA140ACPHi.upper_bound = 0\n",
    "OleoiML1515.reactions.FA160ACPHi.upper_bound = 0\n",
    "\n",
    "# Prevent Acyl-(acyl carrier protein):phosphate acyltransferase for Free
Fatty Acid Synthesis\n",
    "OleoiML1515.reactions.ACPPAT120.upper_bound = 0\n",
    "OleoiML1515.reactions.ACPPAT140.upper_bound = 0\n",
    "\n",
    "# Evaluate the theoretical yield of each product of interest.  Store
solutions as PhaG PhaJ ΔfadDEK.\n",
    "for o in objectives:\n",
    "    OleoiML1515.objective =
OleoiML1515.reactions.get_by_id(objectives[o])\n",
    "    FBA_Solution = OleoiML1515.optimize()\n",
    "    theoreticalYield = FBA_Solution.objective_value / -
FBA_Solution.fluxes['EX_glyc_e']\n",
    "    iML1515_solution_df.loc[o]['PhaG PhaJ ΔfadDEK'] = FBA_Solution\n",
    "    iML1515_theoretical_yeilds_df.loc[o]['PhaG PhaJ ΔfadDEK'] =
theoreticalYield\n",
    "    \n",
    "\n",
    "# Display the theoretical yield results    \n",
    "iML1515_theoretical_yeilds_df"
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9.3. Supplementary References

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2. Youngquist, J. T. *et al.* Production of medium chain length fatty alcohols from glucose in Escherichia coli. *Metab. Eng.* **20**, 177–186 (2013).
3. Yan, Q. *et al.* Metabolic engineering of β-oxidation to leverage thioesterases for production of 2-heptanone, 2-nonanone and 2-undecanone. *Metab. Eng.* **61**, 335–343 (2020).
4. Monk, J. M. *et al.* iML1515, a knowledgebase that computes Escherichia coli traits. *Nat. Biotechnol.* **35**, 904–908 (2017).