Supplementary Information for:

Biological conversion of cyclic ketones from catalytic fast pyrolysis with *Pseudomonas putida* KT2440

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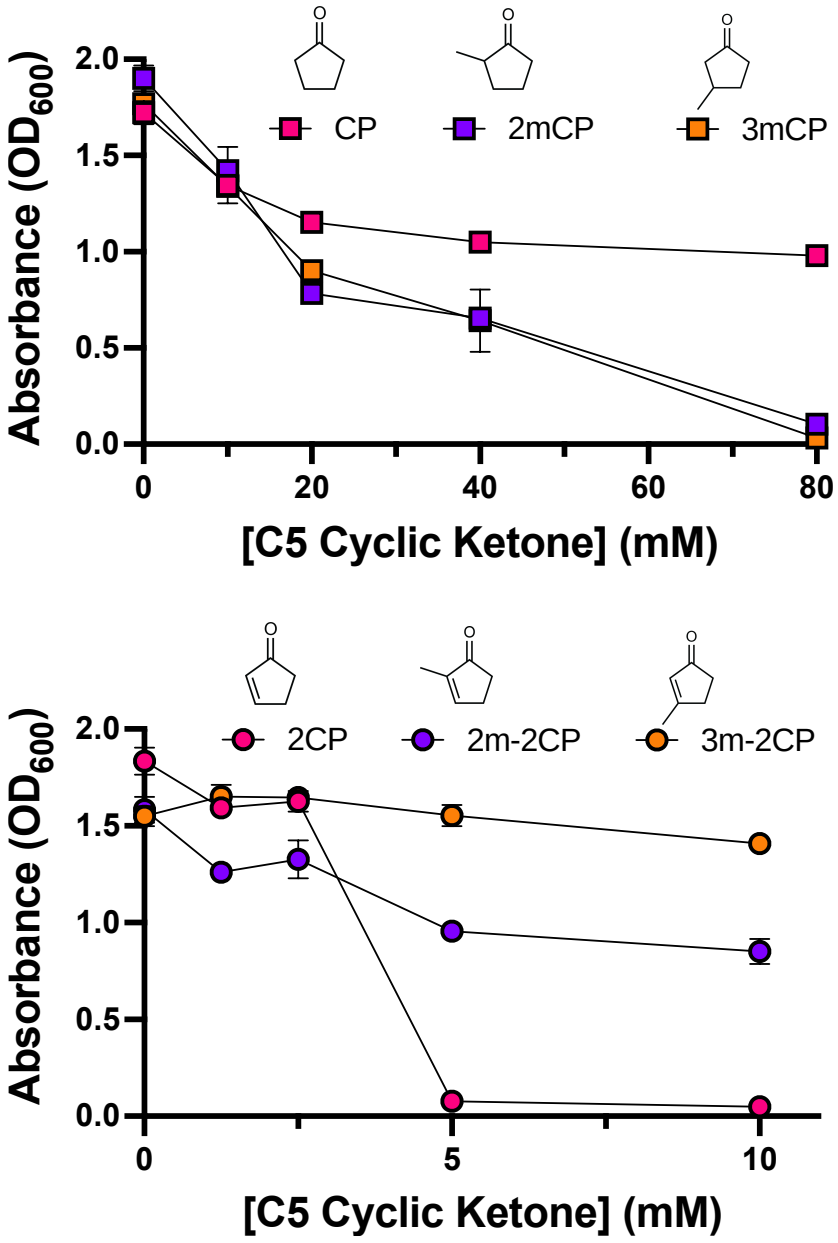
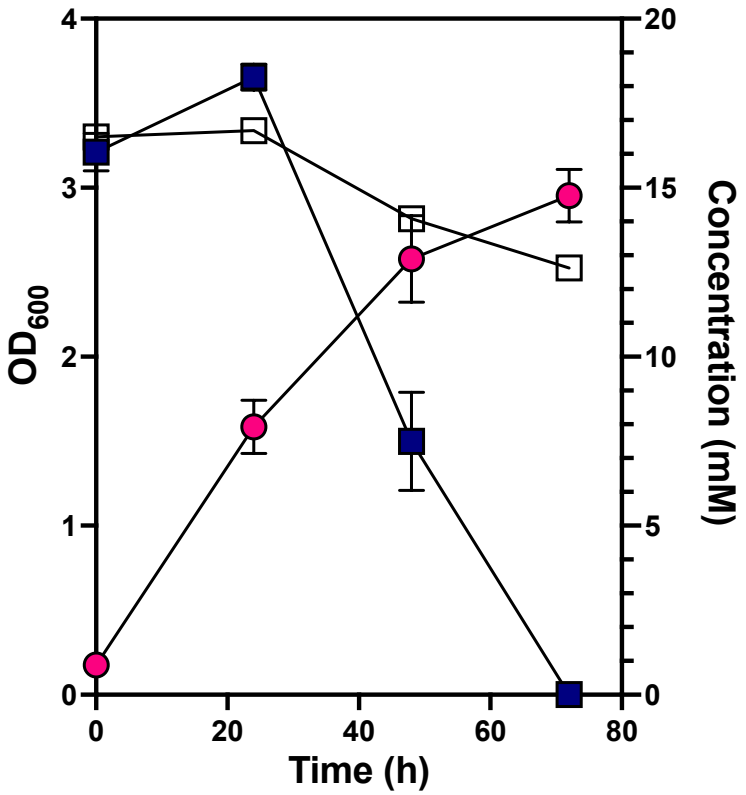
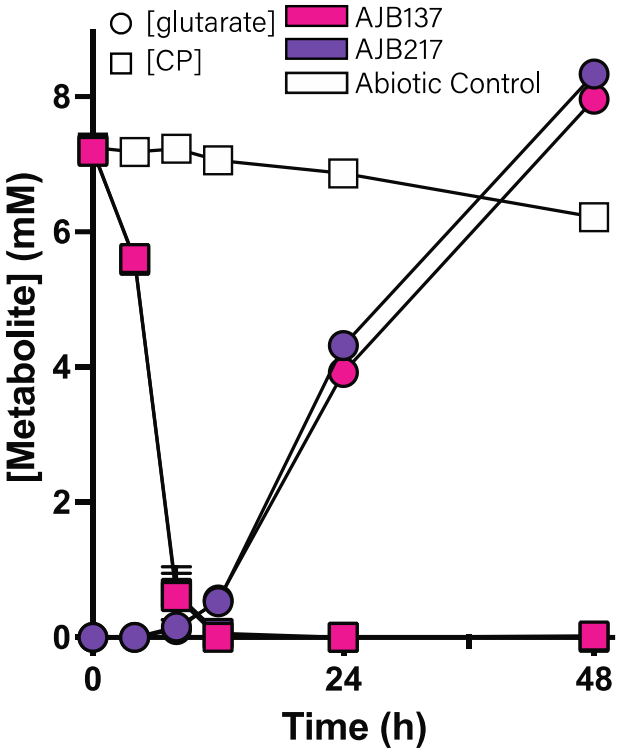


Supplementary Figure 1. Tolerance of AJB137 Against Different Cyclic Ketones. AJB137 was grown in M9 medium containing 10 mM D-glucose and supplemented with varying concentrations of cyclopentanone (CP), 2-methylcyclopentanone (2mCP), 3-methylcyclopentanone (3mCP), 2-cyclopenten-1-one (2CP), 2-methyl-2-cyclopenten-1-one (2m-2CP), and 3-methyl-2-cyclopenten-1-one (3m-2CP). AJB137 cultures were inoculated to an initial absorbance at 600 nm (OD600) of ~ 0.15 and growth was assessed by measuring the OD600 after 24 h. Data represent the mean from three biological replicates and error bars denote the standard error of the mean.



Supplementary Figure 2. Consumption of methanol by *P. putida* RH300. RH300 was grown in M9 medium containing 10 mM D-glucose and supplemented with the reduced and methanol-depleted cyclic ketone rich CFP fraction to an approximate initial cyclopentanone concentration of ~ 10 mM at 30 °C for 72 h. Methanol (blue squares) concentration was tracked for biological samples (filled shapes) as well as an abiotic control (empty shapes). Growth of RH300 was measured using absorbance at 600 nm (OD_{600}, pink circles). Data represent the mean from three biological replicates and error bars denote the standard error of the mean.



Supplementary Figure 3. Loss of the *paaZ* operon does not alter glutaric acid production in the AJB137 background. AJB137 and AJB217 (AJB137 ΔPP_3270-PP_3273) were grown in M9 medium containing 10 mM D-glucose and supplemented with 8 mM CP at 30 °C for 48 h. CP and glutaric acid concentrations were tracked for biological samples and an abiotic control. The data represent the mean from three biological replicates (triplicate abiotic controls) and error bars denote the standard error of the mean.

Supplementary Table 1. Strains and Plasmids Used in This Study

S.Table 3. Strains and Plasmids used in this study

Strains	Genotype	Description
EM42	*Pseudomonas putida* KT2440 Δ*prophage1* Δ*prophage4* Δ*prophage3* Δ*prophage2* ΔTn7 Δ*endA-1* Δ*endA-2* Δ*hsdRMS* Δ*flagellum* Δ*Tn4652*	Construction of this strain is described in Martinez-Garcia E, Nikel PI, Aparicio T, de Lorenzo V. *Pseudomonas* 2.0: genetic upgrading of *P. putida* KT2440 as an enhanced host for heterologous gene expression. Microb Cell Fact 13, 159 (2014).
RH300	EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE*	*Pseudomonas veronii* MK700 *mekAB* and *Comamonas sp.* NCIMB 9872 *cpnDE* were expressed in the *P. putida* chromosome using the P_{tac} promoter downstream of the PP_5042 locus in the EM42 strain background. This construct was made using pRH83 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oRH328/oRH329 was used to confirm correct insertion of the synthetic operon (9000 bp band). oRH328/oRH329, oRH293-oRH295, and oRH322-oRH327 were used for sequence verification by Sanger sequencing (Genewiz).
AJB87-AJB91	Parental- EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE*	RH300 isolates following growth in M9 + 10 mM CP
AJB137	EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*gcdH*:*gcoT* Δ*csiD*	The native *csiD* gene was eliminated from the RH300 backgroud using pAJB04via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB071/oAJB072 was used to confirm correct deletion of the *csiD* gene (1125 bp band). Then the native gcdH:gcoT operon was eliminated from the this backgroud using pAJB010 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB069/oAJB096 was used to confirm correct deletion of the *gcdH*:*gcoT* genes (544 bp band). The inability for the Δ*gcdH*:*gcoT* Δ*csiD* double mutant to grow on glutaric acid as the sole carbon source was confirmed by screening for no growth on M9 + 30 mM glutaric acid plates.
AJB171-172, 175-176, 179-180	Parental- EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*gcdH*:*gcoT* Δ*csiD*	AJB137 TALE isolates tolerated to 2-cyclopenten-1-one via serial passages in M9 + 10 mM D-glucose, 2.5 mM 2CP up to M9 + 10 mM D-glucose, 5.5 mM 2CP (0.5 mM increased 2CP between passages for 7 passages)
AJB185	EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*paaYX*	The native *paaYX* operon was eliminated from the RH300 backgroud using pAJB26 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB195/oAJB196 was used to confirm correct deletion of the *paaYX* operon (131 bp band).
AJB186	EM42 Δ*gcdH*:*gcoT* Δ*csiD* PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*paaYX*	The native *paaYX* operon was eliminated from the AJB137 backgroud using pAJB26via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB195/oAJB196 was used to confirm correct deletion of the *paaYX* operon (131 bp band).
AJB195	EM42 Δ*gcdH*:*gcoT* Δ*csiD* PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*nemA*	The native *nemA* gene was eliminated from the AJB137 backgroud using pAJB32 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB226/oAJB227 was used to confirm correct deletion of the *nemA* gene (626 bp band).
AJB196	EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*paaYX* Δ*paaFGHIJKABCDE*	The native *paaFGHIJKABCDE* operon was eliminated from the AJB186 backgroud using pAJB029 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB211/oAJB212 was used to confirm correct deletion of the *paaFGHIJKABCDE* operon (284 bp band).
AJB197	EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*paaYX* ΔPP_3270:PP_3274	The native PP_3270:PP_3274 operon was eliminated from the AJB186 backgroud using pAJB030 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB217/oAJB218 was used to confirm correct deletion of the PP_3270:PP_3274 operon (231 bp band).
AJB217	EM42 PP_5042:P_{tac}:*mekAB*:*cpnDE* Δ*gcdH*:*gcoT* Δ*csiD* ΔPP_3270:PP_3274	The PP_3270:PP_3274 operon was eliminated from the AJB137 backgroud using pAJB30 via a selection (*nptII*, kanamycin)/counterselection (*sacB*, sucrose) approach. Colony PCR using 2x MyTaq (Bioline) with oAJB217/oAJB218 was used to confirm correct deletion of thePP_3270:PP_3274 operon (231 bp band).
Plasmids	**Genotype**	**Description**
pRH83	pK18sB-PP_5042:P_{tac}:*mekAB*:*cpnDE*	For chromosomal heterologous expression of *Pseudomonas veronii* MK700 *mekAB* and *Comamonas sp*. NCIMB 9872 *cpnDE* in *P.putida* KT2440. Used Q5 Hot Start High-Fidelity 2X Master mix to amplify *mekAB* and the pK18sB backbone (8,081 bp) encoded as pAM032 (W.R. Henson, 2021, *Metabolic Engineering*) with oRH318/oRH319 and also amplify *cpnDE* from a *cpnABCDE* gene fragment synthesized by TWIST Biosciences (San Francisco) using oRH320/oRH321. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. HiFi assembly using the two fragments with 100 ng vector and a 3:1 molar ratio of the *cpnDE* fragment yielded a 10,720 bp plasmid that was confirmed by PCR amplification with oRH232/oRH233 and Sanger sequencing using oRH232/oRH233, oRH293-oRH295 and oRH322-oRH327 (Genewiz).
pAJB04	pK18sB-Δ*csiD*	For deletion of *P. putida* KT2440 *csiD* (PP_2909). Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 1000 bp upstream of PP_2909 (including the first 10 amino acids from *csiD*) with oAJB033/oAJB034 and 1000 bp downstream (including the *csiD* stop codon) with oAJB035/oAJB036. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB vector was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,993 bp plasmid that was confirmed by restriction digest and oCJ680/oCJ681 were used for PCR amplification and Sanger sequencing (Genewiz).
pAJB10	pK18sB-Δ*gcdH*:*gcoT*	For deletion of *P. putida* KT2440 *gcdH*:*gcoT*. Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 587 bp upstream of the *gcdH* start codon with oAJB137/oAJB093 and 560 bp downstream after the *gcoT* stop codon with oAJB094/oAJB138. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,140 bp plasmid that was confirmed by restriction digest and oCJ680/oCJ681 were used for PCR amplification and Sanger sequencing (Genewiz).
pAJB26	pK18sB-Δ*paaYX*	For deletion of *P. putida* KT2440 *paaYX* (PP_3285-PP_3286). Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 800 bp upstream of the *paaY* start codon with oAJB191/oAJB192 and 800 bp downstream starting after the *paaX* stop codon with oAJB193/oAJB194. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB vector was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,593 bp plasmid that was confirmed by PCR amplification with oCJ680/oCJ681 and these primers were also used for sequence confirmation using Sanger sequencing (Genewiz).
pAJB29	pK18sB-Δ*paaFGHIJKABCDE*	For deletion of the *P. putida* KT2440 *paaFGHIJKABCDE* operon (PP_3284-PP_3274). Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 800 bp upstream of *paaF* (including the first 10 amino acids from *paaF*) with oAJB207/oAJB208 and 800 bp downstream (including the *paaE* stop codon) with oAJB209/oAJB210. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB vector was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,993 bp plasmid that was confirmed by restriction digest and oCJ680/oCJ681 were used for PCR amplification and Sanger sequencing (Genewiz).
pAJB30	pK18sB-ΔPP_3270:PP_3273	For deletion of the *P. putida* KT2440 PP_3273-PP_3270 operon (PP_3270 also called *paaZ*, PP_3271 also called *phaK*, PP_3272 also called *actP*-III). Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 800 bp upstream of PP_3273 (including the first 10 amino acids from PP_3273) with oAJB213/oAJB214 and 800 bp downstream (including the *paaZ* stop codon) with oAJB215/oAJB216. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB vector was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,993 bp plasmid that was confirmed by restriction digest and oCJ680/oCJ681 were used for PCR amplification and Sanger sequencing (Genewiz).
pAJB31	pK18sB-Δ*mekB*:*cpnDE*	For deletion of *mekB* and *cpnDE* from *P. 0* RH300. Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 800 bp upstream of the *mekB* start codon with oAJB219/oAJB220 and 800 bp downstream starting after the *cpnE* stop codon with oAJB221/oAJB204. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB vector was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,593 bp plasmid that was confirmed by PCR amplification with oCJ680/oCJ681 and these primers were also used for sequence confirmation using Sanger sequencing (Genewiz).
pAJB32	pK18sB-Δ*nemA*	For deletion of *nemA* from *P. putida* KT2440. Used Q5 Hot Start High-Fidelity 2X Master mix to amplify 800 bp upstream of the *nemA* start codon with oAJB222/oAJB223 and 800 bp downstream starting after the *cpnE* stop codon with oAJB224/oAJB225. Fragments were gel purified using the Zymoclean Gel DNA Recovery kit. 2 µg pK18sB vector was digested using EcoRI and HindIII and a 2,993 bp band was gel purified. HiFi assembly using the three fragments with 100 ng vector and a 3:1 molar ratio of each fragment yielded a 4,593 bp plasmid that was confirmed by PCR amplification with oCJ680/oCJ681 and these primers were also used for sequence confirmation using Sanger sequencing (Genewiz).

Supplementary Table 2. Oligonucleotides Used in This Study

Primer ID	Sequence	Primer ID	Sequence
oAJB033	ACAGCTATGACATGATTACGAATTCGGCATGCTGGTTCCTGG	oAJB215	TTCTACACATCAGGCGATGCTTTCTATGCGTTCG
oAJB034	CCTGTCATTAAAGTTCGTCGATCTGCGTAAAGG	oAJB216	CGTTGTAAAACGACGGCCAGTGCCAGCTCCAGTTCGGGGG
oAJB035	CGACGAACTTTAATGACAGGGCGCGGG	oAJB217	AAGTGCCTTGAGCACC
oAJB036	TAAAACGACGGCCAGTGCCAAGCTTGCGTGGTCAGGGTCTGG	oAJB218	CTTACCTGCGAAGAGG
oAJB069	TGCTGGCGCATTACCGACAA	oAJB219	ACAGCTATGACATGATTACGAATTCGGAAGGACGGTAGCCTGG
oAJB071	GGCAGTATGGAACGCCTTGTGC	oAJB220	AGTGCTTCATGCCTCAGGCCATTTCGAAGCCC
oAJB072	CCAGTAGCGTTCCAGGCCATTG	oAJB221	GAAATGGCCTGAGGCATGAAGCACTGAACCCAC
oAJB093	GCCTGACGGCCTCCCCGCTTTTCATGAGCGAATCCTCGAAATAGGGG	oAJB222	ACAGCTATGACATGATTACGAATTCTGGGGATGGTATTCACCGGC
oAJB094	ATGAAAAGCGGGGAGGC	oAJB223	CTCTGGAGCTGCATGCAAAAACGGGCGCTCTTG
oAJB096	AAGTGGGGCGCATTATAAGGGG	oAJB224	CCCGTTTTTGCATGCAGCTCCAGAGGTGGAAAAC
oAJB137	ACAGCTATGACATGATTACGAATTCCATCGAAGGTAGTGTCGG	oAJB225	TAAAACGACGGCCAGTGCCAAGCTTGATGCCACGCTGACCC
oAJB138	TAAAACGACGGCCAGTGCCAAGCTTCAGTTCAATACTGCTTGGGT	oAJB226	GTAGCACAATGGCAAACGT
oAJB191	ACAGCTATGACATGATTACGAATTCTACCGACGGCACGTAACAG	oAJB227	GAATAACCAATGGGTCAGTTCC
oAJB192	GCGAGCACTCCCCGGCAACACTCTCGAAATGATTTGG	oCJ680	TTTGTGATGCTCGTCAGGG
oAJB193	GAGAGTGTTGCCGGGGAGTGCTCGCCTC	oCJ681	CTTCCCAACCTTACCAGAG
oAJB194	TAAAACGACGGCCAGTGCCAAGCTTAAACCCTATTCACCTGAAACCGCTG	oRH293	CTCGGTGTGCGGTTTCACGTG
oAJB195	CAACACAAGTGATACACGAT	oRH294	TCTGCTGCCGGCTATTCGTC
oAJB196	TCGACGCCGTGAATGTT	oRH295	CGGACAATTCGATGCGCATTAATCG
oAJB201	AGGAAACAGCTATGACATGATTACGAATTCTCAACCAGATCGGCAACGG	oRH318	AGTCAAAAGCCTCCGACCGG
oAJB202	AGTGCTTCATGCCTCAGTATGCCGGGCTCGA	oRH319	TCAGTATGCCGGGCTCGAG
oAJB203	CCGGCATACTGAGGCATGAAGCACTGAACCCAC	oRH320	GCCGAGCTCCTCTCGAGCCCGGCATACTGAACAACAAATAGGACAGACCAAAGACTAGG
oAJB204	TAAAACGACGGCCAGTGCCAAGCTTGCTGACGGCCATGGC	oRH321	ATGGGAGCGGTGGGTTCAGTGCTTCATGCCTCAACCTGCGCTCGTGTAGG
oAJB205	CCATCGAAGTACTGCATCAT	oRH322	TTCATGCCCACTCTGCGATTCCGG
oAJB206	TACGCCTCGAGCAGTTCA	oRH323	TGCGTGAAATGGGTCTGACCG
oAJB207	ACAGCTATGACATGATTACGAATTCGCGCACTGGGGTTTGC	oRH324	GTTCCCGGAGATGTTCTTCGATGAAC
oAJB208	TGTGCAGGCGCCCTAGACCGAGTCGGCTGCTTC	oRH325	GAAAGGCCAAGAAGGTCATAATGAACTCC
oAJB209	CGACTCGGTCTAGGGCGCCTGCACATCGATATAT	oRH326	CTGCTGACCATGGCCTGGAAGTG
oAJB210	TAAAACGACGGCCAGTGCCAAGCTTGTTTGACCTGGGCTGGCGA	oRH327	CGGTGGTGACCACCCCTAC
oAJB211	CGCATAGAGCATATACTG	oRH328	AAATCCAGCCAGTTCTCGG
oAJB212	GCGATCTCTTGGTTTTGT	oRH329	AACTGTTCGTGCCAGACGT
oAJB213	AGGAAACAGCTATGACATGATTACGTCCATGGAGAAGAGGAGGC	oRH332	GCAAACGCAGTTAAGGCTCGTCGCGGCTGA TCCGCGTTAAAATACCAGGTCAACAC
oAJB214	AGAAAGCATCGCCTGATGTGTAGAAGCCACTGGC	oRH333	ATGCCTGCAGGTCGACTCTAGAGGATCCCC GTTTCGCCGTCCTCGTTGG

Supplementary Table 3. Gene Sequences for Heterologously Expressed Genes.

Gene	Description	Sequence
P_{tac}	Promoter sequence for P_{tac}-controlled constructs used in this work	GAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACT
mekA	*mekA* from *Pseudomonas veronii* CF600 was codonoptimized for expression in *P. putida* KT2440 using OPTIMIZER (Puigbo *et al.*, 2007). A synthetic ribosome of 8097 arbitrary units was designed using the Salis RBS Calculator (https://salis.psu.edu/software/) (Salis *et al.*,2009).	*TTAAGGAGGTTTTTTT***ATG**TCCGCACAGAGCAAGCTCGCGGCCGGCAGCTGCGCTTACGGCAACGTTACCTCTCTG GACGCTATGGTTATCGGTGCCGGCGTCGCCGGCCTCTACCAGCTGTACCGCCTGCGTGAAATGGGTCTGACCGTGC GCGCATACGACACCGCCAGCGGTGTCGGTGGCACCTGGTACTGGAACCGTTACCCGGGCGCGCGTTTCGACAGCC AGGCCGAAATCTACCAGTATTGGTTCTCGGAGGAGCTGTACAAATCCTGGCAGCCTACCGAACGTTTCCCAGCCCA GCCAGAGACCGAAGAATGGCTGAACTTCGTGGCCAACCGTTTGAACCTGAAGAAAGACATTCAGTTCAACACCCG TATTGCATCGGCCCACTTCTGCGAGGACAGCGGCCGTTGGGTTGTGACCACCGCCGCCGGCGAAACCATCAACAC CCAGTATCTGATCTCGTGCTGCGGCATGCTGTCGGCTCCGCTGTCCGACCGCTTCCCAGGCCAAGCGGACTTCCAG GGCCAGATCTACCACACCGGCCTGTGGCCGAAGGACCCGGTCGACTTCAACGGAAAACGTGTTGCAGTTGTCGGT ACCGGCGCTACCGGTATCCAAGTTATCCAGACCATCGCCCCGACGGTAGGCAGCATGACTGTATTCGTTCGCACCC CGCAGTACGTCATCCCGATGCGCAACCCGAAGTACAGCAAGGCCGACTGGGAAAAGTGGGGTACCCAGTTCCACC AGCTGAAGAAGCGTGTCCGTGAGACCTTCGCTGGCTTCGACTACGATTTCGACGCGGGCCCATGGGCCGAAAAGA CCCCGGACGAACGCCAGGCTGTCCTGGAACAGCTGTGGAAGGACGGTAGCCTGGCCATGTGGCTGGCCTCGTTCC CGGAGATGTTCTTCGATGAACAGGTGAACGAGGTCGTCTCCCAGTTCGTGCGCATCAAGATGCGTGAACGCCTGCG TTCCCGCCCGGACCTGTGCGACTTGCTGATCCCTACCGACTACGGCTTCGGTACTCACCGCGTCCCGCTGGAAAAC AACTACCTGGAAGTGTACCTGCAGTCGAACGTTAAGGCAGTTGATTGCAAACAGAGCCCTATCGAACGTATCGTAC CACAGGGTATCCAGACCGCCGACGGCAAAATCCACGAGGTGGACATCATCGTTCTCGCGGTCGGCTTTGACGCCG GTTCGGGCGCCCTGTCCCGCATTGACATCCGTGGCCGTGACTCGCGTTCCCTGAAAGAACAGTGGCAGCAGGAAAT CCGTACCGCGATGGGTCTGCAGATCCACGGCTACCCAAACCTGTTCACTACCGGCGCCCCTCTGGCGCCATCCGC GGCGCTGTGCAACATGACCACCTGCCTGCAGCAGCAGGTAGACTGGATCACCGGTTGCATCGAATTCGCCGCTGA ACACGGTAAACACGTAGTTGAAGCCAGCAAGGCCCTGGAAGACAACTGGGTGCAGCACCACGATGAAACCGCCGC CAAGACCCTGGTTGTCAAGACCGACAGCTGGTACATGGGGAGCAACGTGGACGGCAAGCCTCGCCGTTTACTGAG CTACATCGGTGGCGCTGGCGACTACCACCGTCGCTGCGCTGAGATCGCTGCCCAAGGTTATCCGGGCTTCGAAATG GCCTGA
mekB	*mekB* from *Pseudomonas veronii* CF600 was codonoptimized for expression in *P. putida* KT2440 using OPTIMIZER (Puigbo *et al.*, 2007). The native RBS (Italics) was used.	*GCCCGCCACCTAAGAAAGGCCAAGAAGGTCATA***ATG**AACTCCTACTACACCGAAGAGAACCACGGTCCGTTCGAGC TGATCAACATCGGCCCACTGCCACTGGAAGAGGGCCGTTGCATGCCAGAGTGCCTGCTCGCTGTGGCGGTCCACG GCGCCCTGAATGCCGACAAGTCCAACGCTATCCTGGTTCCGACCTGGTACAGCGGTACCTCGAAGGCCATGGAAC AGATCTACATCGGCGAGGGTCGTGCCCTCGACCCATCGAAGTACTGCATCATCGTGGTCAACCAGATCGGCAACG GCCTGAGCTCTTCGGCTTCGAACACCGGTGGTTCCCTGGCTGGCCCAGGCTTCGCGAACGTACGCATCGGTGACGA CGTTTCCGCCCAGCACACGCTCCTGACTGAGTACTTCGGCATCGAGAGCCTGGCTCTGGTCGTCGGGGGCTCGATG GGTGCTCAGCAAACCTACGAATGGGCAGTTCGTTACCCAGACTTCGTGAAGCGTGCAGCCGCCATCGCCGGCACC GCGCGTAACTCCGAACACGACTTCCTGTTCACCGAGATCCTGATCGAGGCTATCACGACCGATCCGGCCTTCCAGG CCGGCCTGTATCGTTCGAGCTCGGCCGTCGCAGCGGGCCTGGAACGTCACGCTAAGCTGTGGACCCTGATGGGCTG GTCCCCGGAGTTCTTCCGCACCGGCCGCCACAAAGCCCTGGGCTTCGAATCCATGCAGATGTTCGTTGACGGTTTC ATGAAGCGTTACTTCGCCCCGATGGACCCAAACAATCTGCTGACCATGGCCTGGAAGTGGCAGCGTGGCGATGTCT CGCGCCACACCGGCGGTGACCTGGCCAAGGCCCTGGGCCGCATCAAAGCCAAGACCTACGTCATGCCTATCTCGC ACGACCAGTTCTTCACTGTTGACGACTGCCTGTCCGAACAAAAGATGATCCCGAACTCCGAGTTCCGCCCGCTGCG TTCGATTGACGGTCACCTCGGCCTGTTCGGTACCGACGCCCAGATGCTGGACCAACTGGACGCACACCTGGCCGA GCTCCTCTCGAGCCCGGCATACTGA
cpnD	*cpnD* from *Comamonas sp. NCIMB 9872* was codonoptimized for expression in *P. putida* KT2440 using OPTIMIZER (Puigbo *et al.*, 2007). A synthetic ribosome of 8986 arbitrary units was designed using the Salis RBS Calculator (https://salis.psu.edu/software/) (Salis *et al.*,2009).	*ACAACAAATAGGACAGACCAAAGACTAGGTTATC***ATG**AATCCGTTTCATTTCGAGACCACGCCCCGCATCATTTGTCA CAACGGTGCTAGCACGAAATTGGGGGAGGCCGCCCGCAATCTGGGCATCCGGCACGCATTCTTTGTCACGGATAA AGGTGTCCACCAAGCGGGTTTGACGAGCGCAGCGATGGATTCGTTGAAAGCTGCTGATATTGCTGTGACCATTTTC AGCGAGGTGCAAGCGGATCCTCCGGAGAGCGTCATTGTCCAGGCAGCAGCAGCTGCGCGGGAGGCTGGGGCCAAC GGCATTATTGGGTTTGGTGGGGGGTCCAGCATGGACACCGCAAAGTTGGTCGCATTGCTCGTCAAATCGCCCCAGG AGCTGTCGGCAATTTACGGTGCTGGCCTGGCACGCGGTCCTCGTCTCCCGCTGATCCAGATCCCAACCACCGCGGG TACCGGCAGCGAAGTGACCCCAATTTCGGTGGTGACCACCCCTACGGACGAAAAGAAGCCAGTGGTCTCCAGCCT CCTCTTGCCGGATATTGCCTTGTTGGACGCACAGCTCACGATCGCGTTGCCACCCGCGGTGACCGGCGCCACGGGG GTCGATGCTCTCGTGCATGCGATCGAAGCACTCTCGACGCGGCTGAAACGGAACCCGTTGTCCGACTTGCTGGCCA TTAAGGCAGTCCAAATGATCTACGATCATTTGCCACTCGCAGTCAAGGAAGGGTCGAATCTCGAAGTCCGTGAACA GATGCTGCTGGCCTCCCTGTTTGCAGGGATGGCATTCAGCAATGCATCCGTCGGGGCAGTGCACGCGTTCGCGTAC CCGCTCGGTGTGCGGTTTCACGTGCCCCATGGCTTGTCCAACAGCCTCATGCTGCCACACATCCTCCGGTTTAATC AACAAGCTGCCGAGTCCACGTATGCCTTGTTGGGCCGTTCCATTCGTCCGGAACTGGAGTCGGCCTCGGACGCGGC CGCGTCGGCCGCATTTGTGGATGCCATGTCCGAGCTCGTGGCTCAGATGCCATATGCTCAATCGCTGCGCGAACTG GGGGTCGAACGTGATGACTTGGCTATGTTGGCGAAAGACGTCATGGGTATCCAGCGTCTGCTGGATCACAACCCTT GCCCAATCACGCATGAGGACGCCATGAAATTGTACGCACGGGCATTTTGA
cpnE	*cpnE* from *Comamonas sp. NCIMB 9872* was codonoptimized for expression in *P. putida* KT2440 using OPTIMIZER (Puigbo *et al.*, 2007). A synthetic ribosome of 9994 arbitrary units was designed using the Salis RBS Calculator (https://salis.psu.edu/software/) (Salis *et al.*,2009).	*TCGGGCGGACGGACACATTTAAGGAGTAATTTT***ATG**AACAGCTCCTTGCATTACAACTACGTGGGGGGTGACTGGGT CAATGGGGCGACCGAACGCCACAACTTGAATCCTTCCGACCTCTCGGATTCGATCGGCATTTACGTCGGTGCCAAT GAGGCCCAAACGCAGGCCGCTATTGACGCAGCGTTCGATGCACGTACGGCTTGGGCTTGTTGGACCGCCGAACAA CGTGCCGACGCTTTGGACCGTATCGGCACCTCCATTTTGGCGTCGGCTAAGGAGCTGGGCCACCTCTTGTCGCGGG AGGAGGGGAAGACGCTGGCCGAGGGGATTGGCGAAGCAGCACGTGCTGGCCGCATCTTCAAGTACTTCGCCGCTG AAGCCCTCCGTTGCGGTGGTGATCTGCTGCCGGCTATTCGTCCCGGGGTCTCCGTCGAAGTGACGCGTGAACCCGA GGGTGTGGTGGGTATCATTACGCCATGGAATTTCCCCCTCGCCATCCCTGCATGGAAGATCGCCCCTGCTTTGGCA TACGGGAATACCGTCGTCTTCAAGCCTGCTGACTTGGTCCCTGGTAGCGCGTGGGCGCTCACCGAAATCATCCATC GGGTCGGTGGCCTGCCTCCAGGGGTCTTTAACCTCGTGATGGGTCCGGGCTCCAAGGTGGGCAACACGCTCGCTAA TGCTCCAAAAGTCCGTGCGTTGTCCTTCACGGGTAGCGCAACCACGGGGCGGCAGGTGGGTCTGGCATGTATGGAG CGCGGCGCTAAGGTCCAGATGGAGATGGGGGGGAAAAACCCCCTCGTGGTGTTGAATGATGCTGACCTGCAATCG GCCGCATCGGTGGCAATCCAAGGCTCGTTCTACTCGACGGGCCAGCGGTGTACCGGCTCCTCCCGGCTCATTGTCG AAGCGGGCATCCACGATGCTTTCGTCGAAGCAATGCGGCAGCAAATGTCCGCACTCTCGATTGGGCACGCCCTCA AGCAGGGCACGGATATCGGGCCGGTCGCATCCGAGGACCAGCTGCAACAGGATCTCGAATATATCCGCATCGGTG TCGCCGAAGGCGGGCGCCTGTACTGCGGCGGGTCGCAGCTCGAGCGGGAGACCGAGGGGTTTTATCTGTCGCCTG CACTCTTGACCGAAACGGACAATTCGATGCGCATTAATCGTGAGGAAATCTTTGGTCCCGTCGCCTCCGTCATCCG GGTCGGTTCGTATGAGGAGGCCCTGCACACCGCTAATGACACCGTCTTTGGGCTCTCCTCCGGCATTTGCACCACC AGCCTCAAGTACGCATCCGATTTCAAGCGCCGTTCGGCTGCTGGCATGGTCATGGTGAATGTCCCGACCGCAGGCG TGGACTATCACGCACCCTTCGGCGGCCGGAAGGGGAGCTCCTACGGGGCACGCGAACAGGGTGCTTATGCGCGGG AGTTCTATACCGTCATCAAGACCGCCTACACGAGCGCAGGTTGA

Supplementary Table 4. Mutations in RH300 vs. the Predicted RH300 Sequence (from NC_002947)

Genome Position	Nucleotide Change	Locus Effected	CDS Position	Protein Effect	Polymorphism Type	Amino Acid Change	Codon Change	Read Coverage	Variant Frequency	Notes
172966	A -> G	Intergenic	-	-	SNP (transition)	-	-	587	97.80%	Upstream of 23S ribosomal RNA (PP_RS00830)
178396	A -> G	Intergenic	-	-	SNP (transition)	-	-	485	98.40%	Upstream of 23S ribosomal RNA (PP_RS00845)
278745	(+) A	Intergenic	-	-	Insertion	-	-	269	95.90%	Upstream of SfnB family sulfur acquisition oxidoreductase (PP_RS01190)
307867	(+) C	PP_RS01335	738	Frameshift	Insertion	-	-	776	99.60%	PP_RS01335 annotated as a pseudogene (phosphoenolpyruvate carboxykinase fragment)
336126	(+) T	PP_RS01475	211	Frameshift	Insertion	-	-	559	99.80%	PP_RS01475 annotated as a DUF3077 domain-containing protein
353067	A -> G	Intergenic	-	-	SNP (transition)	-	-	283	98.20%	Between the convergently encoded genes *hisF* and *choV*
499203	A -> G	Intergenic	-	-	SNP (transition)	-	-	389	99.70%	Upstream of an ABC transporter ATP-binding protein (PP_RS02170)
1070248	(+) GA	Intergenic	-	-	Insertion	-	-	532 -> 542	95.1% -> 95.2%	Between the convergently encoded genes PP_RS0487 and PP_RS04880
1126648	(+) C	Intergenic	-	-	Insertion	-	-	310	98.40%	Upstream of L-serine ammonia-lyase (PP_RS05165)
1419550	(+) C	Intergenic	-	-	Insertion	-	-	588	98.10%	Upstream of a DUF3077 domain-containing protein (PP_RS06445)
1499480 -> 1499481	CA -> AC	Intergenic	-	-	2 SNPs (transversions)	-	-	215 -> 214	78.60% -> 94.40%	Upstream of cell division protein ZapE (PP_RS06790)
1499498 -> 1499499	T -> CG	Intergenic	-	-	SNP (transition) and Insertion	-	-	226 -> 219	83.60% -> 93.20%	Upstream of cell division protein ZapE (PP_RS06790)
1499509	(+) C	Intergenic	-	-	Insertion	-	-	223	94.20%	Upstream of cell division protein ZapE (PP_RS06790)
1892582	(+) C	Intergenic	-	-	Insertion	-	-	459	99.80%	Upstream of RluA family pseudouridine synthase (PP_RS08900)
2490804 -> 2490827	(-) GATCAACGGCAACGGCAACGGCAA	PP_RS28385	102	Deletion	Deletion	KINGNGNGN -> N	AAG,ATC,AAC,GGC, AAC,GGC,AAC,GGC, AAC -> AAC	419	99.80%	PP_RS28385 annotated as a hypothetical protein
2546570	(-) T	Intergenic	-	-	Deletion	-	-	602	100.00%	Upstream of tRNA-His (PP_RS12000)
3713411	(+) G	Intergenic	-	-	Insertion	-	-	315	98.70%	Upstream of hydroxymethylglutaryl-CoA lyase (PP_RS17675)
3818865 -> 3818867	TTA -> CTT	PP_RS18130	67 -> 65	Substitution	SNP (transition) -> SNP (transversion)	IR -> KG	ATA, AGA -> AAA, GGA	220 -> 220	94.10% -> 83.20%	PP_RS18130 annotated as an ABC transporter substrate-binding protein
4398970 -> 4398971	CT -> TC	Intergenic	-	-	2 SNPs (transitions)	-	-	356 -> 357	96.90% -> 86.00%	Between the convergently encoded genes PP_RS28355 and PP_RS21100
4398976	(+) G	Intergenic	-	-	Insertion	-	-	362	99.40%	Between the convergently encoded genes PP_RS28355 and PP_RS21100
4398998	(+) C	Intergenic	-	-	Insertion	-	-	364	94.50%	Between the convergently encoded genes PP_RS28355 and PP_RS21100
4549137	A -> T	PP_RS21755	389	Substitution	SNP (transversion)	L -> Q	CTG -> CAG	738	89.00%	PP_RS21755 annotated as a succinate dehydrogenase iron-sulfur subunit
4553751 -> 4553760	TATTTTTCTG -> ATTTGTCCT	Intergenic	-	-	Deletion -> SNP (transversion) -> Substitution	-	-	332 -> 295 -> 269	100.00% -> 98.30% -> 89.0%	Between the convergently encoded genes *gltA* and PP_RS21780
4554169 -> 4554238	AACCTCCGCCAACTGCA ATTCGTGAACCAAGCA AAAGGCCCCGAAAAGG CCCTCCGCAGCCCCCCC AACC -> CAGCTTCTGCGACTTGC ATTCGTGAATCCAGGCA CAGGCCCCGGACACGT CCTCCTGCAGCGCCTCG ACCT	PP_RS21780	254 -> 185	Disruption	Multiple Mutations	-	-	> 793	> 99.40%	PP_RS21780 is annotated as a START domain-containing pseudogene
4621120 -> 4621128	(-) AAGCGCCGC	Intergenic	-	-	Deletion	-	-	307	80.10%	Between the divergently encoded genes PP_RS21985 and *dsbD* *Note, this mutation is not present in strains made from this background. Likely unique to the isolate sequenced and not the background
5293712	(+) GCC	Intergenic	-	-	Insertion	-	-	405 -> 421	90.1% -> 90.4%	Upstream of a DUF2867 domain-containing protein (PP_RS25515)
5412855 -> 5412856	CG -> GCC	Intergenic	-	-	Insertion -> SNP (transversion)	-	-	507 -> 504	99.80% -> 99.60%	Between the convergently encoded genes PP_RS25985 and PP_RS25990
5419519	(+) CGGG	Intergenic	-	-	Insertion	-	-	> 291	80.70% -> 89.7%	Upstream of a hemolysin III family protein (PP_RS26015)
5757466	T -> C	Intergenic	-	-	SNP (transition)	-	-	256	99.20%	Between the convergently encoded genes PP_RS27440 and PP_RS27445

Supplementary Table 5. Mutations in AJB137 Background vs. the RH300 Background.

Change	CDS Nucleotide Position	Protein Effect	Genes Effected	Polymorphism Type	Amino Acid Change	Codon Change	Coverage	Variant Frequency
Deletion	*gcdH*$_{1}$ -> *gcoT*$_{1135}$	PP_RS00815 (*gcdH*)-PP_RS00820 (*gcoT*) Deletion	PP_RS00815 (gcdH) and PP_RS00820 (gcoT)	-	-	-	-	-
SNP (transition)	-	-	Reversion of the Intergenic mutation at position 178396 in RH300	G -> A (Transition)	-	-	8	100%
SNP (transition)	328 bp upstream PP_RS10035	-	Intergenic mutation upstream of PP_RS10035	C ->T (Transition)	-	-	65	100%
Deletion	*csiD*$_{30}$ -> *csiD*$_{978}$	PP_RS15090 (*csiD*) Deletion	PP_RS15090 (*csiD*)	-	-	-	-	-
SNP (transition)	PP_RS18045$_{610}$	A -> T Substitution	PP_RS18045	G -> A (Transition)	A -> T	GCT -> ACT	62	100%
SNP (transversion)	PP_RS21755$_{389}$	Substitution	Reversion of the mutation at position 4549137 in RH300	T -> A (Transversion)	Q -> L	CAG -> CTG	50	100%
SNP (transition)	39 bp upstream PP_RS28365	-	Intergenic mutation upstream of PP_RS28365	G -> A (Transition)	-	-	41	95%

Supplementary Table 6. Percent Bio-oil Input Analysis.

Distillation_1		
fraction	**weight (g)**	**weight_percent**
Initial	1684.04	1.000
1	86.72	0.051
2	15.89	0.009
3	103.93	0.062
4	247.76	0.147
5	98.81	0.059
Boiler Final	1077.25	0.640
Losses	53.68	0.032
Distillation_2		
fraction	**weight (g)**	**weight_percent**
Initial	86.97	1.000
1	9.45	0.109
2	9.9	0.114
3	36.88	0.424
4	14.47	0.166
Boiler Final	13.04	0.150
Losses	3.23	0.037
Distillation_3		
fraction	**weight (g)**	**weight_percent**
Initial	35.65	1.000
1	3.93	0.110
2	3.48	0.098
3	6.79	0.190
4	8.14	0.228
5	7.61	0.213
Boiler Final	4.58	0.128
Losses	1.12	0.031