

SUPPLEMENTAL INFORMATION

Engineering of *Ralstonia eutropha* H16 for Autotrophic and Heterotrophic Production of Methyl Ketones

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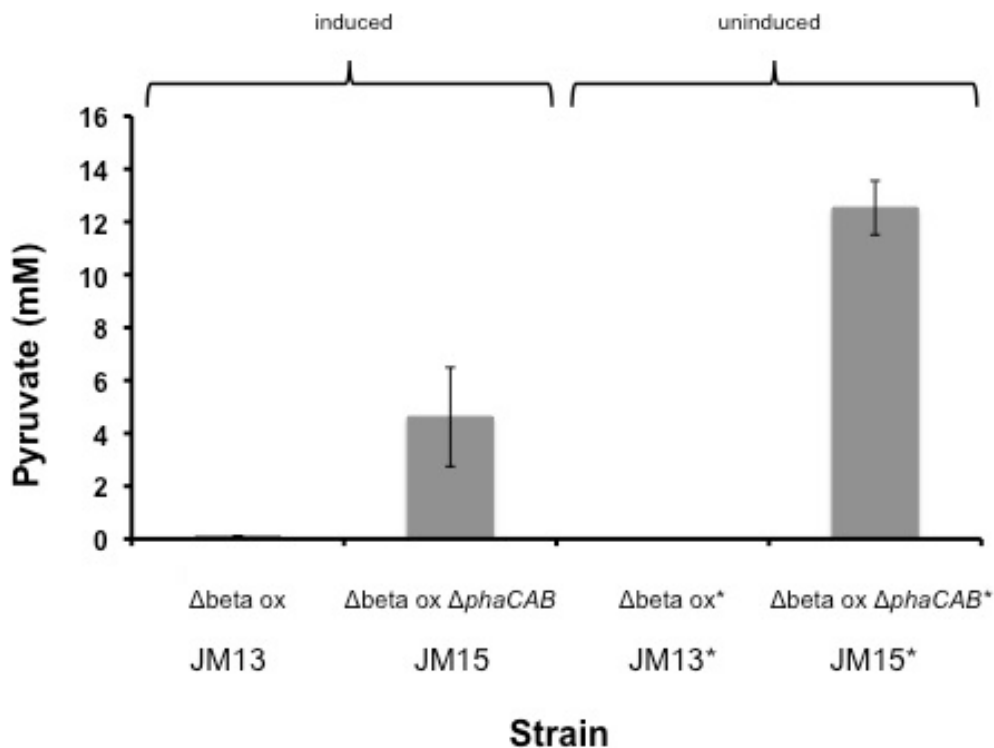


FIGURE S1. Pyruvate excreted into culture medium by *R. eutropha* mutant strains bearing the methyl ketone pathway under induced and uninduced heterotrophic conditions (mutants with β -oxidation and/or *phaCAB* chromosomal deletions; Table 1). Bar heights represent the average of at least three independent biological replicates and error bars represent one standard deviation at 96 h post-induction.

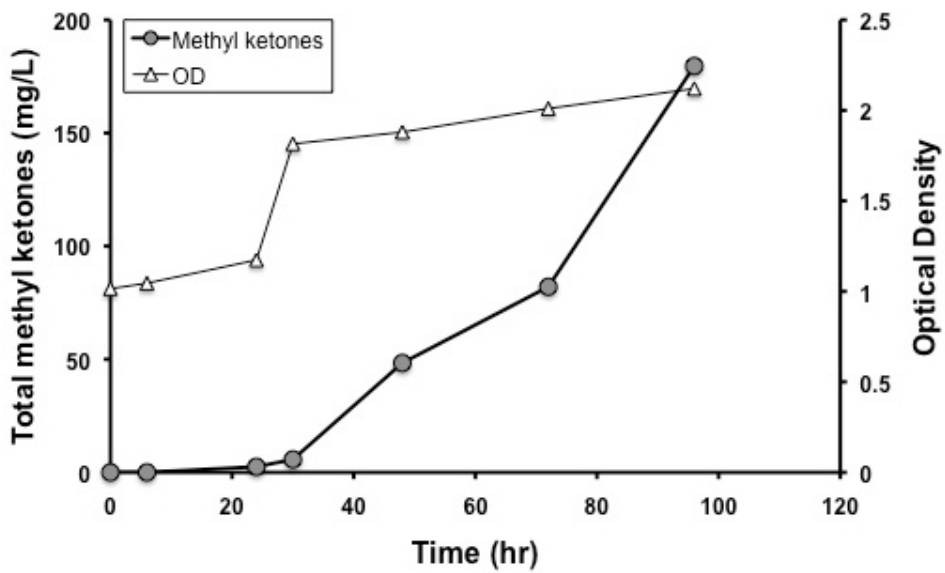


FIGURE S2. Autotrophic methyl ketone production in *R. eutropha* strain JM13 (β -oxidation mutant) under a $\text{H}_2:\text{O}_2:\text{CO}_2$ atmosphere (80:4:16).

TABLE S1. Primers used in this study

Primer name	Target	Primer Sequence (5' – 3') ^a
pha KO up fw_BamHI	H16 A1437- 1439	<u>ACTGCAGGATCCCGACGCCGGTCGTTCTACTC</u> CTAT
pha KO up rv	H16 A1437- 1439	<u>CTCATGCACTTGGTTCCGAGGTTTGCCGGTCGC</u> CATGATTT
pha KO down fw	H16 A1437- 1439	<u>CCTCGGAACCAAGTGCATGAGCTCAACGGCGG</u> CCTGCATAT
pha KO down rv_BamHI	H16 A1437- 1439	<u>ACTGCAGGATCC</u> TTTCCAGGTAGGTGCCCATC
phaC1AB1_check_fw	H16 A1437- 1439	AAGTGCGCGATGCCTTCCTG
phaC1AB1_check_rv	H16 A1437- 1439	ATCATGCCCTGCATCATCGG
fadE_A0460 KO up fw_BamHI	H16 A0460	<u>ACTGCAGGATCCCCAGTCAGCCCGTCTCGAAT</u>
fadE_A0460 KO up rv	H16 A0460	<u>CGTTCGGAGTCGAAGAAGCCCGTACTGGCCCA</u> TCTGAGACT
fadE_A0460 KO down fw	H16 A0460	<u>GGGCTTCTTCGACTCCGAACGTTCTGAGTCGAC</u> TCTACCCGTC
fadE_A0460 KO down rv_BamHI	H16 A0460	<u>ACTGCAGGATCCCGATGAAGTTGGGCGTGTCC</u>
fadE_A0460 KO check_fw	H16 A0460	CGGTTTCGGCCGCAAGCAAATA
fadE_A0460 KO check_rv	H16 A0460	ACGTCGAACGGGATGCCGAA
fadE_A1530 KO up fw_BamHI	H16 A1530	<u>ACTGCAGGATCCATCGCCGTGGGCCACCCCTA</u>
fadE_A1530KO up rv	H16 A1530	<u>GAGCTAGAGCGGGGCTGTTGACTCGCGGCGGG</u> ATAGGATCAT
fadE_A1530 KO down fw	H16 A1530	<u>GTCAACAGCCCCGCTCTAGCTCTGAGCGGCGC</u> TTGCCATCCCCGA
fadE_A1530 KO down rv_BamHI	H16 A1530	<u>ACTGCAGGATCCTCGTCGCCAGGCGGTGCAG</u>
fadE_A1530 KO check_fw_2	H16 A1530	GGCCTGAAGGTCGACGACAT

fadE_A1530 KO check_rv_2	H16	CCAGGATCTGGCCGGTGATG
ltesA_fw	A1530 'tesA	TGTGGAATTGTGAGCGGATA
ltesA_rv_BamHI	'tesA	ACTGCAGGATCCGGCGAATTGGAGCTCTTATG
pJM_pBAD_backbone_fw _NheI	pJM9	CAGACTGCAGCTAGCGGATCCAAACTCGAGTA AGG
pJM_pBAD_backbone_rv _EcoRI	pJM9	ACTGCAGAATTCCCAAAAAACGGGTATGGAG
pEG530_for_RBS_EcoRI	pEG530 [5]	ACTGCAGAATTCAAAGGAGGCCATCCTATGGC GGACACGTTATTGAT
fadM_rev_NheI	pEG530 [5]	CAGACTGCAGCTAGCCTCGAGTTACTTAACCAT CTGC

^a restriction sites and tags are underlined