

**Muconic Acid Production from Methane using Rationally-engineered
Methanotrophic Biocatalysts**

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Supplemental Material

Table S1. Strains and Plasmids

Strains	Genotype/Description	References
<i>Methylovimicrobium buryatense</i> 5GB1S	Wild-type	(Puri et al. 2015)
<i>Methylovimicrobium alcaliphilum</i> 20Z ^R	Wild-type	(Akberdin et al. 2018)
<i>Methylococcus capsulatus</i> Bath	Wild-type	ATCC
<i>M. alcaliphilum</i> 20Z ^R Δ pdh::gm ^r	Δ MALCv4_1358	(Henard et al. 2018)
<i>M. alcaliphilum</i> 20Z ^R Δ aroE	Δ MALCv4_3465	This study
<i>E. coli</i> Zymo 10b	<i>F</i> - mcrA Δ (mrr-hsdRMS-mcrBC) Φ 80lacZ Δ M15 Δ lacX74 recA1 endA1 araD139 Δ (ara leu) 7697 galU galK rpsL nupG λ -	Zymo Research
<i>E. coli</i> S17-1	Tp ^r Sm ^r recA thi pro hsd(r ⁻ m ⁺)RP4-2-Tc::Mu::Km Tn7	ATCC
<i>E. coli</i> BW25113	Δ (araD-araB)567 Δ lacZ4787(::rrnB-3) rph-1 Δ (rhaD-rhaB)568 hsdR514 λ -	(Baba et al. 2006)
JW3242	Δ aroE721::kan BW25113	(Baba et al. 2006)

Plasmids

pCAH01	<i>P_{tetA} bla-tetR oriR_{CoEI} oriR_{RP4/RK2}, oriT_{RP4/RK2}, trfA ahp</i>	(Henard et al. 2016)
pMUC	Synthetic, codon- optimized <i>Bacillus thuringensis asbF</i> , <i>Enterobacter cloacae aroY</i> , and <i>Acinetobacter baylyi catAP76A operon</i> under <i>Ptet</i> promoter/operator inducible expression.	This study
pK18mobsacB	<i>oriR_{CoEI}, oriT_{RP4/RK2}, sacB, ahp</i>	
pK18mobsacBΔMALCv4_3465	<i>oriR_{CoEI}, oriT_{RP4/RK2}, MALCv4_3465 upstream, MALCv4_3465 downstream</i>	This study
pBluescript II KS+	High copy IPTG- inducible expression vector	Stratagene
pKS::Ec <i>aroE</i>		This study
pKS::MALCv4_3465		This study

Table S2. Primers

NAME	PRIMERS (5' TO 3')	USAGE
oCAH152 oCAH149	F: AAGCTTGACCTGTGAAGTG R: TTCACCTTTTCTCTATCACTGATAG	pCAH01 backbone for Gibson assembly
oCAH788 oCAH789	F:gtgatagagaaaagttaaATGAAATACAGTCTTTGTACC ATTTC R:cattctccctctctTTAGCTCGTTACAACCTTCC	<i>Bt asbF</i>
oCAH790 oCAH791	F:gttgtaacgagctaaAGAGGAGGGAGAATGCAAAATC R:attctacctcctaaCTATTTTTTGTCTACTAAACAATTCT GG	<i>Ec aroY</i>
oCAH792 oCAH793	F:agtgacaaaaaatagTTAGGAGGTAGAAATGGAAGTT AAAATC R:cttcacaggtcaagcttTTACACCGCTAGACGTGG	<i>Ab catAP76A</i>
oCAH056 oCAH053	F: GACTCTAGAGGATCCCCG R: ACCTGCAGGCATGCAAGC	Amplify pK18mobsacB backbone for MALCv4_3465 KO Gibson assembly
oCAH308 oCAH309	F:aagettgcatgcctgcaggtCCATGCCGCCAAGCTTGC R: gacaattataAAGGTGAAAGGTGAAAGGTGAAAGG	MALCv4_3465 1kb Upstream
oCAH310 oCAH311	F:ctttcaccttTATAATTGTCTTTTATATTGCCAAACTT CC R:cccggggatcctctagagtcAAATGGACCCGGCCAATAC	MALCv4_3465 1kb Downstream
oCAH890 oCAH891	F:AACGGtctagaAGAGGAGGGAGAATGGAAACCTATC TTTGGT R:GGCGGaagcttTCACGCGGACAATTCCTCC	<i>E.coli aroE</i>
oCAH896 oCAH897	F:AACGGtctagaAGAGGAGGGAGAATGACACAAATC TATGCCG R:GGCGGaagcttTCATCTTTCCGCATTCAATAACT	MALCv4_3465
oCAH952 oCAH953	F:CACCGATATTGTCCAGTTTGC R:GGTCGTTTCGTATTCTTGCATG	<i>Bt asbF RT</i>
oCAH954 oCAH955	F:GAATCCTGTAGCCCCTGTTG R:CATCGATAGGAGTGTTCTGTGG	<i>Ec aroY RT</i>
oCAH956 oCAH957	F:ATGAATACTGGGCAGGTGTG R:TCGGCATCCATACGCATATC	<i>Ab catAP76A RT</i>
oCAH097 oCAH098	F:TGCAGGGCTTAGAGAATCAAC R:AAACTGCTTACCGACCTCTTC	<i>Methylobacterium rpoD RT</i>

oCAH1074 F:TTCAAGGAACTGACCGATACG
oCAH1075 R:GACTCTATGAACTCCGCCTTG

Methylococcus rpoD RT

Homology tails for Gibson assembly are in lowercase; restriction enzyme sites are underlined

Table S3. Codon-optimized muconate biosynthetic pathway genes.

Gene	Optimized sequence
<i>Bacillus thuringensis asbF</i>	ATGAAATACAGTCTTTGTACCATTTCCTTCCGACATCAGCTG ATCTCTTTCACCGATATTGTCCAGTTTGCCTATGAAAATGGCT TCGAAGGAATTGAGCTGTGGGGCACTCATGCCCAAATTTAT ACATGCAAGAATACGAAACGACCGAACGCGAACTGAACTGC TAAAAAGATAAGACATTAGAAATCACGATGATATCTGACTAT TTGGACATCTCCCTTTCGGCAGATTTTGAAAAAACGATTGAG AAATGCGAACAGTTGGCAATCCTGGCAAACCTGGTTCAAGAC CAACAAAATCCGTACCTTTGCCGGACAGAAAGGCTCTGCAG ATTTCAAGTCAGCAAGAACGCCAAGAGTATGTGAACCGAATC CGCATGATCTGCGAATTATTCGCTCAACACAACATGTACGTC CTTCTAGAAACCCATCCTAATACTCTGACGGATACGCTGCCC TCGACCCTGGAGCTGTTGGGCGAGGTCGATCACCCGAATCTT AAAATCAATCTCGATTTCTTGACATCTGGGAGTCCGGCGCC GATCCCGTCGACAGCTTCCAGCAACTGCGCCCATGGATCCAA CATTACCATTTTAAAAATATAAGCTCGGCTGACTACCTACAC GTTTTTGAACCCAACAACGTTTATGCTGCGGCTGGAAATCGA ACTGGTATGGTTCCGTTGTTTGAAGGCATCGTTAACTATGAT GAAATTATACAGGAAGTTCGCGACACGGATCATTTTCGCCTCA CTAGAGTGGTTTCGGACATAATGCAAAAGACATACTCAAGGC TGAAATGAAAGTTCTCACTAACAGAAATTTGGAAGTTGTAAC GAGCTAA
<i>Enterobacter cloacae aroY</i>	AGAGGAGGGGAGAATGCAAAATCCCATTAACGACTTGCGCTC GGCAATTGCCTTGCTCCAACGTCATCCCGGTCATTACATCGA AACTGACCATCCCGTTGACCCGAATGCGGAACTAGCCGGCGT TTACCGACACATTGGCGCTGGCGGCACTGTCAAACGACCGAC TCGCACTGGCCCAGCGATGATGTTTAACTCGGTCAAAGGTTA TCCGGGCTCCCGCATTTTAGTAGGAATGCATGCCTCACGCGA AAGAGCCGCTTTGCTTCTGGGCTGCGTGCCATCGAAATTAGC GCAACATGTTGGGCAGGCCGTCAAGAATCCTGTAGCCCCTGT TGTAGTACCTGCATCTCAAGCGCCATGTCAAGAGCAGGTGTT CTATGCTGATGACCCCGATTTTCGACCTCCGTAAGCTGTTGCC GGCCCCCACGAACACTCCTATCGATGCCGGCCCGTTTTTCTG CTTGGGCTTAGTACTGGCCTCCGATCCGGAGGATACCAGCCT GACCGATGTAACCATCCATCGCCTTTGCGTGCAAGAGCGTGA TGAAGTGAAGCATGTTCTTAGCTGCAGGCCGCCATATCGAAGT ATTTAGAAAGAAAGCTGAAGCGGCCGGCAAACCGTTGCCCG TAACTATTAATATGGGCTTAGACCCTGCTATCTACATCGGGG CCTGTTTTGAGGCTCCTACCACCCCTTTCGGCTATAACGAGTT GGGAGTTGCTGGTGCCTTACGCCAGCAACCGGTGGAACCTCGT CCAGGGCGTGGCTGTAAAAGAAAAAGCCATCGCGCGTGCCG AAATCATTATCGAAGGTGAGCTCCTCCCGGGAGTCCGTGTGC GCGAAGACCAGCATACTAACACCGGCCACGCAATGCCGGAA TTTCCGGGCTACTGCGGCGAAGCTAACCCCTTCGCTTCCTGTC

	ATAAAAGTCAAAGCGGTGACCATGCGTAATCATGCGATCTTG CAAACGCTAGTAGGCCCCGGCGAAGAGCATAACCACGCTGGC TGGCCTGCCGACCGAAGCTTCGATTTCGTAATGCCGTTGAAGA GGCTATTCCTGGCTTTCTGCAAAACGTTTATGCCCATACTGC AGGGGGAGGCCAAATTCTTGGGCATTTTGCAAGTTAAGAAAC GCCAACCGTCAGACGAGGGCCGTCAGGGCCAAGCAGCCCTA ATAGCACTGGCAACCTACTCCGAGCTCAAAAACATCATACTG GTTGATGAAGATGTGGATATCTTCGACTCCGACGACATCCTA TGGGCTATGACCACCCGTATGCAAGGAGATGTGTGTCGATCACC ACCCTCCCCGGCATAACGCGGCCACCAACTCGATCCAAGCCAG TCACCAGACTACTCGACTTCAATTCGCGGCAATGGTATCTCT TGCAAAACCATTTTTCGACTGCACGGTTCCTGGGCGCTGAAA GCCCGGTTTGAACGTGCGCCATTTCATGGAGGTGGATCCCCT CCATGGGCTCCAGAATTGTTTAGTGACAAAAAATAG
<i>Acinetobacter</i> <i>baylyi</i> <i>catAP76A</i>	TTAGGAGGTAGAAATGGAAGTTAAAATCTTCAATACTCAGG ATGTGCAAGATTTTTTACGTGTTGCAAGCGGCCTTGAACAAG AAGGTGGCAATCCGCGTGTA AACAGATCATCCATCGTGTGC TTTCAGATTTATATAAAGCCATTGAAGATTTGAATATCACTT CAGATGAATACTGGGCAGGTGTGGCATATTTAAATCAGCTAG GTGCCAATCAAGAAGCTGGTTTATTGTCGGCAGGCTTGGGTT TTGACCATTACTTGGATATGCGTATGGATGCCGAAGATGCCG CACTAGGTATTGAAAATGCGACACCACGTACCATTGAAGGC CCGCTATACGTGGCAGGTGCGCCTGAATCGGTAGGTTATGCG CGCATGGATGACGGCAGTGATCCAAATGGTCATACCCTGATT CTACATGGCACGATCTTTGATGCAGATGGCAAACCTTTACCC AATGCCAAAGTTGAAATCTGGCATGCCAATACCAAAGGCTTT TATTCACACTTCGACCCAACAGGCGAACAGCAGGCGTTCAAT ATGCGCCGTAGTATTATTACCGATGAAAACGGTCAGTATCGC GTTCGTACCATTTTGCCTGCGGGTTATGGTTGCCACCAGAA GGTCCAACGCAACAGTTGCTGAATCAGTTGGGCCGTCATGGT AACCGCCCTGCGCACATTCATATTTTGTCTGCGCATGGCC ACCGCAAATACTACGCAAATTAATGTGGCTGGCGATCCGT ACACCTATGACGACTTTGCTTATGCAACCCGTGAAGGCTTGG TGGTTGATGCAGTGGAACACACCGATCCTGAAGCCATTAAA GCCAATGATGTTGAAGGCCCATTCGCTGAAATGGTTTTTCGAT CTAATAATTGACGCGTTTGGTTGATGGTGTAGATAACCAAGTT GTTGATCGTCCACGTCTAGCGGTGTAA

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