

Supporting Information

for

Production of the sesquiterpene bisabolene from one- and two-carbon compounds in engineered *Methanosarcina acetivorans*

Andrea Mentrup, Luca V. Scheitz, Theo Wallenfang and Michael Rother

Fakultät Biologie, Technische Universität Dresden, 01062 Dresden, Germany

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Supplementary Tables S1-S4

Table S1: *M. acetivorans* strains used in this study.

Strain	Genotype	Reference
WWM73	$\Delta hpt::P_{mcrB}$ - <i>tetR</i> - $\Phi C31$ -int-attP	(Guss et al., 2008)
MVA I	WWM73, $\Delta ssu::P_{mcrB}$ (tetO1)-MM0870-MM0871-MM0335	This study
MVA II	WWM73, $\Delta MA3852::P_{mcrB}$ (tetO3)-MM1526-MM1525-MM1524-MM1871	This study
MVA III	WWM73, $\Delta MA2965::P_{mcrB}$ (tetO3)-MM1762-MM1763-MM1764	This study
MVA12	MVA I, $\Delta MA3852::P_{mcrB}$ (tetO3)-MM1526-MM1525-MM1524-MM1871	This study
MVA13	MVA I, $\Delta MA2965::P_{mcrB}$ (tetO3)-MM1762-MM1763(D ₃₁ N) -MM1764	This study
MVA23	MVA II, $\Delta MA2965::P_{mcrB}$ (tetO3)-MM1762-MM1763(D ₃₁ N)-MM1764	This study
MVA231	MVA23, $\Delta ssu::P_{mcrB}$ (tetO1)-MM0870-MM0871-MM0335	This study

Table S2: Plasmids used in this study^a.

Plasmid	Genotype and/or construction	Reference
pWM321	<i>M. acetivorans</i> / <i>E. coli</i> shuttle vector	(Metcalf et al., 1997)
pMR08	pWM321, <i>P_{mcrB}</i> - <i>tetR</i>	(Oelgeschläger and Rother 2009)
pJK028a	$\phi C31$ - <i>attB</i> vector (for genomic integration) with <i>P_{mcrB}(tetO3) promoter fusion to <i>uidA</i></i>	(Guss et al., 2008)
pMssu	Platform vector for chromosomal integration into the <i>ssu</i> locus (MA0063-MA0065)	(Sattler et al., 2024)
pMA2965	Platform vector for chromosomal integration into MA2965	(Sattler et al., 2024)
pMA3852	Platform vector for chromosomal integration into MA3852	(Sattler et al., 2024)
pUC57-BsaI-free	General cloning	Biocat GmbH, Heidelberg, Germany
pUC57_tetO1_Pt_APS	Codon optimized α -pinene synthase gene from <i>Pinus taeda</i> fused to <i>P_{mcrB}(tetO1) promoter</i>	Biocat

pUC57_tetO1_Ms_LMS	Codon optimized limonene synthase gene of <i>Mentha spicata</i> fused to PmcB(tetO1) promoter	Biocat
pUC57_tetO1_Sc_LIS	Codon optimized linalool synthase gene of <i>Streptomyces clavuligerus</i> fused to PmcB(tetO1) promoter	Biocat
pUC57/tetO1_AgBIS_His ₆	Codon optimized bisabolene synthase gene (with 3' His ₆ -tag encoded) of <i>Abies grandis</i> fused to PmcB(tetO1) promoter	Biocat
pUC57_tetO4_MVA I	Artificial operon of the <i>M. mazei</i> genes MM_0335, MM_0870 and MM_0871 fused to PmcB(tetO4) promoter	Biocat
pUC57_tetO3_MVA II	Artificial operon of the <i>M. mazei</i> genes MM_1526, MM_1525, MM_1524 and MM_1871 fused to PmcB(tetO3) promoter	Biocat
pJK028a_MVA III	MM_1762, MM_1763 and MM_1764 from <i>M. mazei</i> (PCR amplified) fused to PmcB(tetO3)	This study
pAPS	PmcB(tetO1)-APS fusion (pUC57_tetO1_Pt_APS) moved to pMR08	This study
pLMS	PmcB(tetO1)-LMS fusion (pUC57_tetO1_Ms_LMS) moved to pMR08	This study
pLIS	PmcB(tetO1)-LIS fusion (pUC57_tetO1_Sc_LIS) moved to pMR08	This study
pAgBIS	PmcB(tetO1)-AgBIS fusion PCR amplified, moved to pMR08	This study
pMssu_MVA I	PmcB(tetO4)_MVA I fusion (pUC57_tetO4_MVA I) moved to pMssu	This study
pMA3852_MVA II	PmcB(tetO3)_MVA II fusion (pUC57_tetO3_MVA II) moved to pMA3852	This study
pMA2965_MVA III	PmcB(tetO3)_MVA III fusion (pJK028a_MVA III) moved to pMA2965	This study
pMA2965_MVA III D ₃₁ N MM1763	PmcB(tetO3)_MVA III D ₃₁ N MM1763 fusion (pJK028a_MVA III D ₃₁ N MM1763) moved to pMA2965 (via PCR)	This study

a: sequences are available upon request.

Table S3: Oligonucleotides used in this study.

Oligonucleotide	Sequence (5'→3')
o_SphI_pmcrB_tetO1_for	AAAGCATGCTTCATTTATCGGAGAACAC
o_SpeI_HindIII_AgBIS_rev	AAAAC TAGTAAGCTTAAAGCGGAAGCG
o_NdeI_Operon_MM1762_1764_for	TTTCATATGGTTTCATGTTCTGCGC
o_Operon_MM1762_1764_BamHI_rev	AAAGGATCCTTAAAGAGCGTTCC
o_NotI_pmcrB_tetO3_Operon_MM1762_64_for	TTTGCGGCCGCGCATGCTTCATTTATCG
o_pmcrB_tetO3_Operon_MM1762_64_NotI_rev	TTTGCGGCCGCCTTAAAGAGCGTTCC

Table S4: Summary of the *trans* genes used in this study.

Name	Encoded enzyme	ORF/accession number ^a	Organism	Comment	Reference
APS	α -pinene synthase	AF543530	<i>Pinus taeda</i>	Without plastid transport sequence, codon usage optimized	(Tashiro et al., 2016)
LMS	limonene synthase	L13459	<i>Mentha spicata</i>	Without plastid transport sequence, codon usage optimized	(Wu et al., 2019)
LIS	Linalool synthase	CP016560	<i>Streptomyces clavuligerus</i>	Codon usage optimized	(Nakano et al., 2011)
AgBIS	bisabolene synthase	AF006195	<i>Abies grandis</i>	Without plastid transport sequence, codon usage optimized	(Sebesta and Peebles 2020)
MVA I	acetoacetyl-CoA thiolase, HMG-CoA synthase, HMG-CoA reductase	MM_0870, MM_0871, MM_0335 (AE008384)	<i>Methanosarcina mazei</i>	Synthetic operon fused to PmcRB(tetO4)	(Yoshida et al., 2020)
MVA II	Pphosphomevalonate dehydratase, anhydromevalonate phosphate decarboxylase, prenylated flavin mononucleotide synthase	MM_1524, MM_1525, MM_1526, MM_1871 (AE008384)	<i>Methanosarcina mazei</i>	Synthetic operon fused to PmcRB(tetO3)	(Yoshida et al., 2020)

MVA III	mevalonate kinase, isopentenyl phosphate kinase, IPP:DMAPP isomerase	MM_1762, MM_1763, MM_1764 (AE008384)	<i>Methanosarcina mazei</i>	Operon fused to PmcrB(tetO3)	(Yoshida et al., 2020)
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a: www.ncbi.nlm.nih.gov

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