Electronic Supplementary Information (ESI)

Bypassing the bottlenecks in the shikimate and methylerythritol phosphate pathways for enhancing the production of natural products from methane in Methylotuvimicrobium alcaliphilum 20Z

Diep Ngoc Pham,‡ Anh Duc Nguyen,‡ So Hyeon Oh, and Eun Yeol Lee*

Department of Chemical Engineering, Kyung Hee University, Yongin-si, Gyeonggi-do 17104, South Korea.
* Correspondence to: eunylee@khu.ac.kr (Tel: +82-31-201-3839; Fax: +82-31-204-8114)
‡ These authors contributed equally to this work.

Supplementary Figures

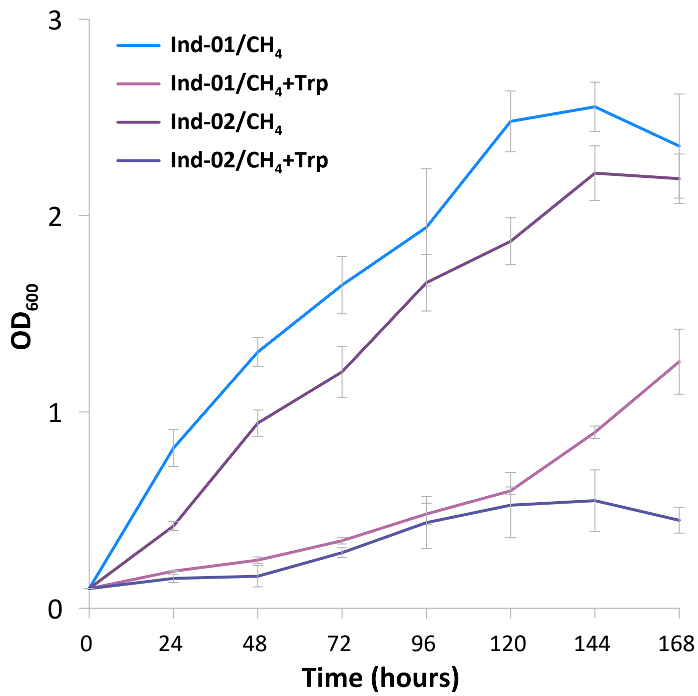

Figure S1. Growth profiles of Ind-01 and Ind-02 strains in medium supplied with methane or methane and 0.5 g/L of tryptophan.

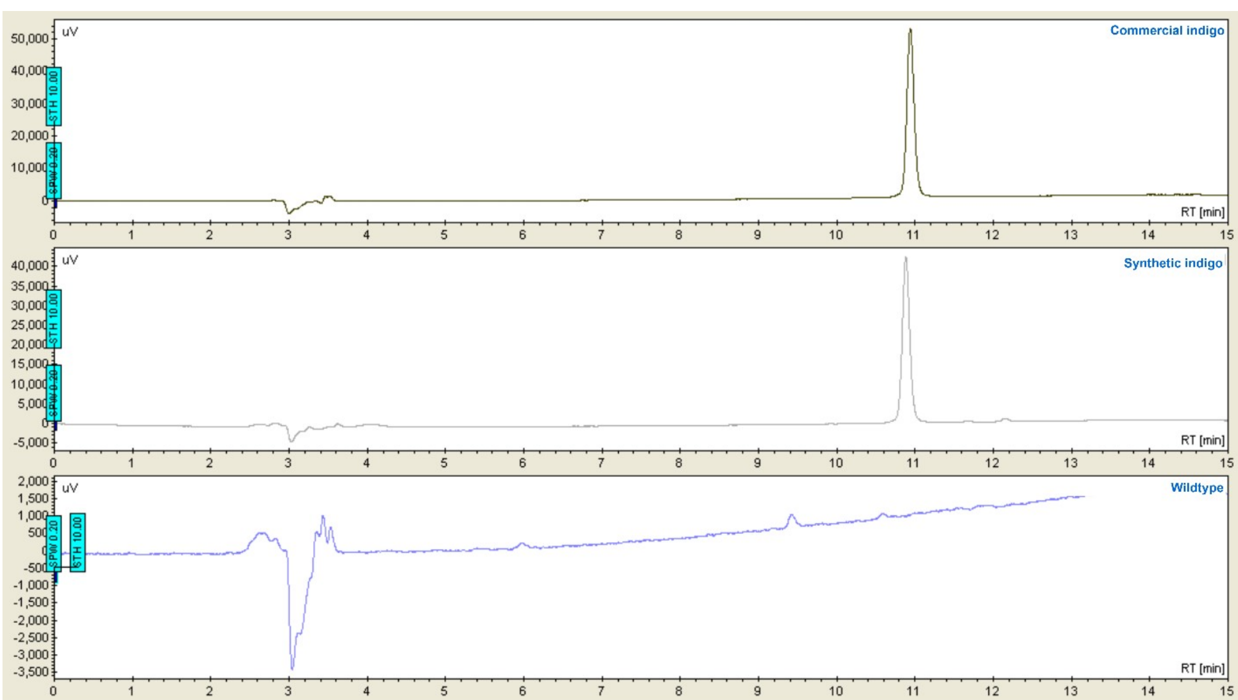

Figure S2. LC chromatograms of commercial indigo and synthetic indigo producedfrom Ind-03 strain.

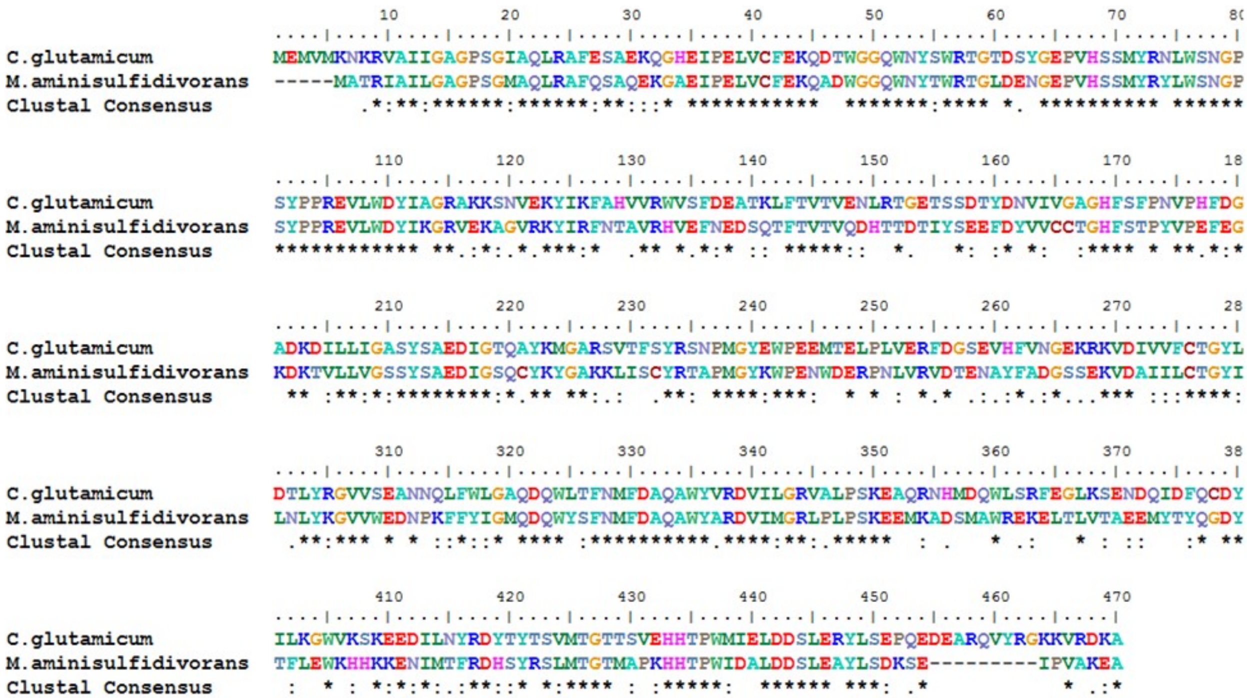

Figure S3. Sequence alignment of Fmo from *C. glutamicum* and *M. aminisulfidivorans*.

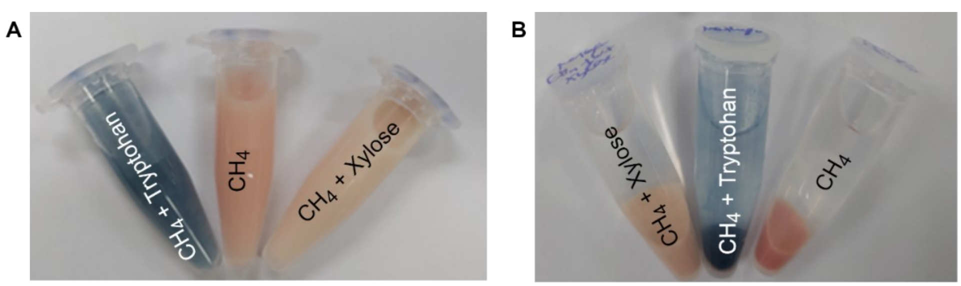

Figure S4. Culture broth of Ind-04 strain in methane, methane plus xylose and methane plus tryptophan before (A) and after (B) centrifugation.

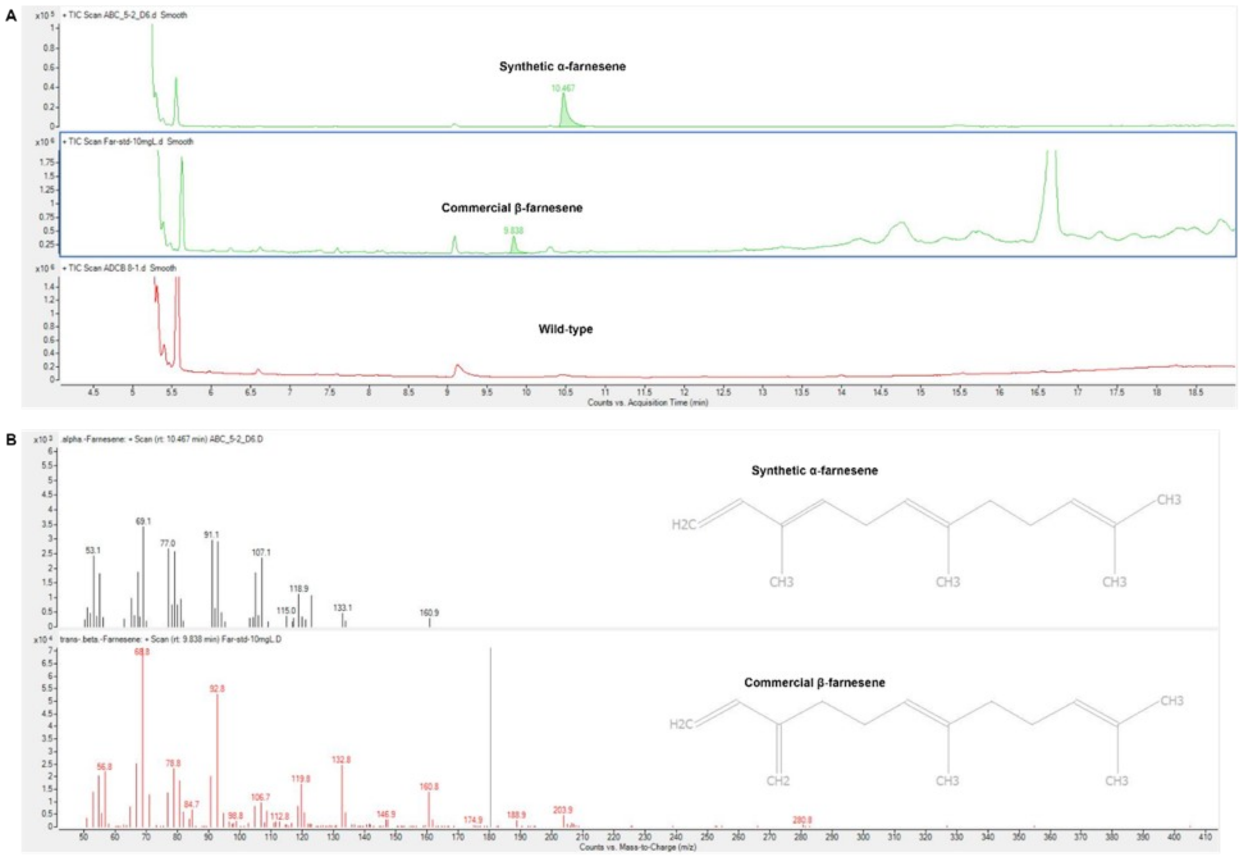

Figure S5. GC chromatograms (A) and mass spectrum chromatograms (B) of synthetic α-farnesene and commercial β-farnesene.

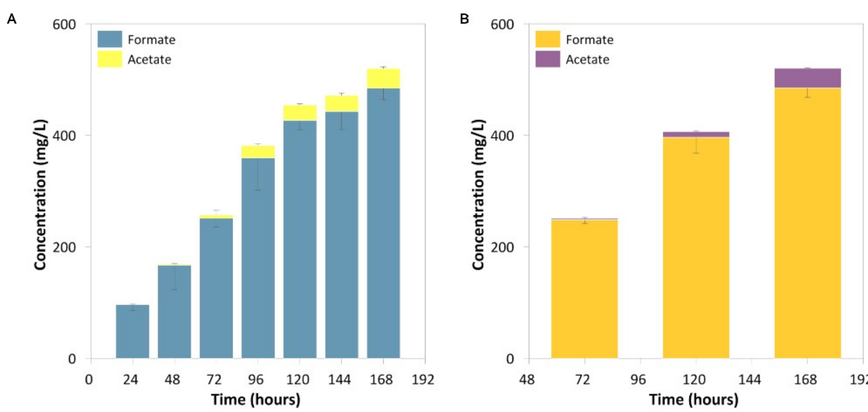

Figure S6. Exceretion of organic acids including formate and acetate during cultivation of engineered strains Ind-05 (A) and FAR-02 (B) on methane.

Supplementary Tables

Table S1. Strains and plasmids used in this study

Strain	Characteristic	Reference
Escherichia coli DH5α		Novagen
Corynebacterium glutamicum		KCTC
Methylophaga aminisulfidivorans		KCTC
Methylotuvimicrobium alcaliphilum 20Z	Used as host strain	DMSZ
Ind-01	*M. alcaliphilum* 20Z harboring pFMO-Cg plasmid	This study
Ind-02	*M. alcaliphilum* 20Z harboring pFMO-Ma plasmid	This study
Ind-03	*M. alcaliphilum* 20Z harboring pFMO-Ma-A plasmid	This study
Ind-04	*M. alcaliphilum* 20Z harboring pFMO-Ma-T plasmid	This study
Ind-05	*M. alcaliphilum* 20Z harboring pFMO-Ma-AT plasmid	This study
Ind-06	*M. alcaliphilum* 20Z harboring pFMO-Ma-AT plasmid with chromosomally integrated xylose-utilizing pathway genes	This study
FAR-01	*M. alcaliphilum* 20Z harboring pAFS plasmid	This study
FAR-02	*M. alcaliphilum* 20Z harboring pAFS-nDXP plasmid	This study

FAR-03	*M. alcaliphilum* 20Z harboring pAFS-nDXP plasmid with chromosomally integrated xylose-utilizing pathway gene	This study
Plasmid		
pAWP89	oriV oriT trfA ahp dTomato pTac	Puri et al. 2015[1]
pCM351-XYL	pCM351-(FR)glgA containing construct for integrating P_{tac}-*xylAB-rpe* into chromosome	Nguyen et al. 2021[2]
pBs-02	pAWP89-based backbone carrying *AgBS-ispA-dxs-ispG-ribB-DSAG-dxr*	Nguyen et al. 2021[3]
pET-28a(+)	Expression vector with N-terminal His-tag	Novagen
pET-tna	pET-28a carrying *tnaA*	This study
pET-tna-Mfmo	pET-28a carrying *tnaA* and *fmo* from *M. aminisulfidivorans*	This study
pET-tna-Cfmo	pET-28a carrying *tnaA* and *fmo* from *C. glutamicum*	This study
pET-Trp	pET-28a carrying *aroG*^{D146N} and *trpE*^{S40F}	Pham et al. 2022[4]
pET-indigo-trpE	pET-28a carrying *tna-Mfmo-trpE*^{S40F}	This study
pET-indigo-aroG-trpE	pET-28a carrying *tna-Mfmo-aroG*^{D146N}-*trpE*^{S40F}	This study
pFMO-Cg	pAWP89-based backbone carrying P_{tac} promoter and *tna-Cfmo* amplified from pET-28a-tna-Cfmo plasmid	This study
pFMO-Ma	pAWP89-based backbone carrying P_{tac} promoter and *tna-Mfmo* amplified from pET-28a-tna-Mfmo plasmid	This study

pFMO-Ma-A	pAWP89-based backbone carrying P_{tac} promoter and *tna-Mfmo-aroG*^{D146N} amplified from pET-28a-indigo-aroG-trpE plasmid	This study
pFMO-Ma-T	pAWP89-based backbone carrying P_{tac} promoter and *tna-Mfmo-trpE*^{S40F} amplified from pET-28a-indigo-trpE plasmid	This study
pFMO-Ma-AT	pAWP89-based backbone carrying P_{tac} promoter and *tna-Mfmo-aroG*^{D146N}-*trpE*^{S40F} amplified from pET-28a-indigo-aroG-trpE plasmid	This study
pAFS	pAWP89-based backbone carrying P_{tac} promoter *afs* and *ispA*	This study
pAFS-nDXP	pAWP89-based backbone carrying P_{tac} promoter *afs* and *ispA* and *ribB-DSAG-dxr* amplified from pBs-02 plasmid	This study

Table S2. Primers used in this study

Primers	**Primer sequences (5'-3')**	**Description**
pAWP89_fw	TAGTTGTCGGGAAGATGCG	For amplifying pAWP89 backbone, in which promoter P_{tac} and SD were kept.
pAWP89_rv	AGCTGTTTCCTGTGTGAATA	
afs_fw	aggtattcacacaggaaacagctATGGAATTTCGGGTTCAT	For amplifying *afs* gene from synthetic *aFS* fragment to ligate to *ispA* and pAWP89 backbone, resulting in pAFS plasmid.
afs_ rv	agaggtactcagagaCTAGTTAACCAACGGTTG	
ispA_ fw	caaccgttggttaactagGATCTGAGTACCTCTAGAAAATAAGGAGCAATCCAATGAGTAACGCACTGAAAG	For amplifying *ispA* gene from genomic DNA of *M. alcaliphilum* 20Z to ligate to *afs* and pAWP89 backbone, resulting in pAFS plasmid.
ispA_rv	gcatcttcccgacaactaTTAATGATCTCGCTGGAT	
pAWP89_fw	TAGTTGTCGGGAAGATGCG	For amplifying pAFS backbone, in which promoter P_{tac}, SD, *ispA*, and *afs* genes were kept.
pAWP89_Far_rv	TTAATGATCTCGCTGGATAATA	
ribBC_fw	atccagcgagatcattaaTTCACACAGGAAACAGCTATGAATCAGACGCTACTT	For amplifying *ribB-DSAG-dxr* fragment from pBs-02 plasmid, which was constructed by our group in previous study (Nguyen et al. 2021)[3], to ligate to pAFS backbone, resulting in pAFS-nDXP plasmid.
ribBC_rv	gcatcttcccgacaactaTTAACGCTTAAGTTCTTCGAC	
tnaA_fw	gacagcaaatgggtcgcgATGGAAAACTTTAAACATCTCC	For amplifying *tnaA* gene from genomic DNA of *E. coli* to ligate to pET-28a at BamHI restriction site, resulting in pET-28a-tna plasmid.

tnaA_rv	cgacggagctcgaattcgTTAAACTTCTTTAAGTTTTGCG	
Mfmo_fw	acttaaagaagtttaacgTATTCACACAGGAAACAGCTATGGCAACTCGTATTGCG	For amplifying *fmo* gene from genomic DNA of *M. aminisulfidivorans* to ligate to pET-28a-tna at SacI restriction site, resulting in pET-28a-tna-Mfmo plasmid.
Mfmo_rv	gcttgtcgacggagctcgTTAAGCTTCTTTAGCCACAG	
Cfmo_fw	acttaaagaagtttaacgTATTCACACAGGAAACAGCTATGAAGAATAAGCGCGTT	For amplifying *fmo* gene from genomic DNA of *C. glutamicum* to ligate to pET-28a-tna at SacI restriction site, resulting in pET-28a-tna-Cfmo plasmid.
Cfmo _rv	gcttgtcgacggagctcgTTAGGCTTTATCGCGGAC	
trpE_fw	gacagcaaatgggtcgcgTATTCACACAGGAAACAGCTATGCAAACACAAAAACC GACTCT	For amplifying *trpE*^{fbr} from pET-Trp to ligate to pET-tna-Mfmo at NotI restriction site, resulting in pET-28a-Ma-T plasmid.
trpE_rv	gcttgtcgacggagctcgTCAGAAAGTCTCCTGTGC	
aroG_fw	gacagcaaatgggtcgcgTATTCACACAGGAAACAGCTATGAATTATCAGAACGA CGA	For amplifying *aroG*^{fbr}-*trpE*^{fbr} from pET-Trp to ligate to pET-tna-Mfmo at NotI restriction site, resulting in pET-28a-Ma-AT plasmid.
trpE_rv	gcttgtcgacggagctcgTCAGAAAGTCTCCTGTGC	
tnaA_89_fw	ttcacacaggaaacagctATGGAAAACTTTAAACATCTCC	For amplifying *tnaA-Mfmo* cluster gene from pET-28a-tna-Mfmo plasmid to ligate to pAWP89 backbone, resulting in pFMO-Ma plasmid.
Mfmo_89_rv	gcatcttcccgacaactaTTAAGCTTCTTTAGCCACAG	
tnaA_89_fw	ttcacacaggaaacagctATGGAAAACTTTAAACATCTCC	For amplifying *tnaA-Cfmo* cluster gene from pET-28a-tna-Cfmo plasmid to ligate to pAWP89 backbone, resulting in pFMO-Cg plasmid.
Cfmo_89_rv	gcatcttcccgacaactaTTAGGCTTTATCGCGGAC	
tnaA_89_fw	ttcacacaggaaacagctATGGAAAACTTTAAACATCTCC	For amplifying *tnaA-Mfmo-aroG* cluster gene from pET-28a-indigo-aroG-trpE plasmid to ligate to pAWP89 backbone, resulting in pFMO-Ma-AT plasmid.
aroG_89_rv	gcatcttcccgacaactaTTACCCGCGACGCGCTTTTA	
tnaA_89_fw	ttcacacaggaaacagctATGGAAAACTTTAAACATCTCC	For amplifying tnaA-Mfmo-trpE cluster gene from pET-28a-indigo-trpE plasmid to ligate to pAWP89 backbone, resulting in pFMO-Ma-AT plasmid.
trpE_89_rv	gcatcttcccgacaactaTCAGAAAGTCTCCTGTGC	
tnaA_89_fw	ttcacacaggaaacagctATGGAAAACTTTAAACATCTCC	For amplifying *tnaA-Mfmo-aroG-trpE* cluster gene from pET-28a-indigo-aroG-trpE plasmid to ligate to pAWP89 backbone, resulting in pFMO-Ma-AT plasmid.
trpE_89_rv	gcatcttcccgacaactaTCAGAAAGTCTCCTGTGC	

Homologous sequences used for Gibson Assembly are indicated by lower-case. Ribosome binding sites are underlined.

Table S3. Sequence of homologous genes used in this study

Gene	Sequence
tnaA (E. coli K12)	ATGGAAAACTTTAAACATCTCCCTGAACCGTTCCGCATTCGTGTTATTGAGCCAGTAAAACGTACCACTCGCGCTTATCGTGAAGAGGCAATTATTAAATCCGGTAT GAACCCGTTCCTGCTGGATAGCGAAGATGTTTTTATCGATTTACTGACCGACAGCGGCACCGGGGCGGTGACGCAGAGCATGCAGGCTGCGATGATGCGCGGCG ACGAAGCCTACAGCGGCAGTCGTAGCTACTATGCGTTAGCCGAGTCAGTGAAAAATATCTTCGGTTATCAATACACCATTCCGACTCACCAGGGCCGTGGCGCAG AGCAAATCTATATTCCGGTACTGATTAAAAAACGCGAGCAGGAAAAAGGCCTGGATCGCAGCAAAATGGTGGCGTTCTCTAACTATTTCTTTGATACCACGCAGG GCCATAGCCAGATCAACGGCTGTACCGTGCGTAACGTCTATATCAAAGAAGCCTTCGATACGGGCGTGCGTTACGACTTTAAAGGCAACTTTGACCTTGAGGGAT TAGAACGCGGTATTGAAGAAGTTGGTCCGAATAACGTGCCGTATATCGTTGCAACCATCACCAGTAACTCTGCAGGTGGTCAGCCGGTTTCACTGGCAAACTTAA AAGCGATGTACAGCATCGCGAAGAAATACGATATTCCGGTGGTAATGGACTCCGCGCGCTTTGCTGAAAACGCCTATTTCATTAAGCAGCGTGAAGCAGAATACA AAGACTGGACCATCGAGCAGATCACCCGCGAAACCTACAAATATGCCGATATGCTGGCGATGTCCGCCAAGAAAGATGCGATGGTGCCGATGGGCGGCCTGCTG TGCATGAAAGACGACAGCTTCTTTGATGTGTACACCGAGTGCAGAACCCTTTGCGTGGTGCAGGAAGGCTTCCCGACATATGGCGGCCTAGAAGGCGGCGCGAT GGAGCGTCTGGCGGTAGGTCTGTATGACGGCATGAATCTCGACTGGCTGGCTTATCGTATCGCGCAGGTACAGTATCTGGTCGATGGTCTGGAAGAGATTGGCG TTGTCTGCCAGCAGGCGGGCGGTCACGCGGCATTCGTTGATGCCGGTAAACTGTTGCCGCATATCCCGGCAGACCAGTTCCCGGCAACAGGCCTGGCCTGCGAG CTGTATAAAGTCGCCGGTATCCGTGCGGTAGAAATTGGCTCTTTCCTGTTAGGCCGCGATCCGAAAACCGGTAAACAACTGCCATGCCCGGCTGAACTGCTGCGTT TAACCATTCCGCGCGCAACATATACTCAAACACATATGGACTTCATTATTGAAGCCTTTAAACATGTGAAAGAGAACGCGGCGAATATTAAAGGATTAACCTTTAC GTACGAACCGAAAGTATTGCGTCACTTCACCGCAAAACTTAAAGAAGTTTAA
Cfmo (C. glutamicum)	ATGGAGATGGTTATGAAGAATAAGCGCGTTGCGATTATTGGTGCAGGTCCGAGTGGTATCGCTCAGTTGAGGGCGTTTGAGTCTGCTGAAAAGCAGGGTCATGA GATCCCTGAGCTGGTGTGTTTTGAAAAGCAGGATACCTGGGGTGGGCAGTGGAATTACTCTTGGCGCACGGGAACAGACTCTTATGGTGAGCCTGTGCACTCAA GTATGTACCGAAACCTGTGGTCAAACGGTCCGAAGGAAGTTCTCGAATTTGCTGAGTACAGCTTCGATGAGCACTTCGGAAAGCCAATTTCTTCTTACCCTCCACG TGAAGTGTTGTGGGATTACATTGCAGGTCGTGCAAAGAAGTCGAACGTTGAGAAGTATATCAAGTTCGCGCATGTTGTTCGCTGGGTGAGTTTTGATGAGGCCAC CAAGCTGTTCACCGTGACGGTGGAGAACCTCCGCACCGGTGAGACCAGCAGTGATACTTATGACAACGTGATTGTTGGCGCTGGACACTTCAGTTTCCCGAACGT CCCTCACTTTGATGGTGTGGAGACTTTCCCAGGTCAGATCATGCATGCTCACGAGTTCCGTGGTGCAGAGGCTGTTGCTGACAAGGATATTTTGCTGATTGGTGCA AGTTATTCTGCGGAAGATATCGGTACCCAGGCGTACAAGATGGGTGCTCGTTCGGTGACTTTCTCTTACCGCTCAAACCCAATGGGGTATGAGTGGCCTGAAGAG ATGACTGAGCTTCCTTTGGTTGAGCGTTTCGATGGCTCCGAGGTTCACTTTGTCAATGGTGAAAAGCGCAAGGTTGACATCGTGGTGTTCTGTACTGGTTACTTAC ACCATTACCCATTTATGCCGTCTGAGCTGACTTTAAGCTCACCAAACAACCTGTACCCGGATACGCTTTATCGTGGCGTGGTGTCCGAGGCTAATAACCAGCTGTTC TGGTTGGGCGCTCAGGATCAGTGGCTGACGTTCAACATGTTTGATGCTCAGGCTTGGTATGTTCGCGATGTCATTTTGGGTCGCGTGGCTCTTCCTTCCAAGGAGG CGCAGCGCAATCATATGGATCAGTGGCTGTCACGTTTTGAGGGTTTGAAGTCTGAGAATGATCAGATTGATTTCCAGTGCGATTACGTTGAGGACCTCATTGACCA GACCGATTACCCTTCGTTTGATCTGAAGGAAGTTGCGAATATCTTGAAGGGCTGGGTGAAGTCGAAGGAGGAGGATATCCTCAACTACCGCGATTACACCTACAC GTCCGTGATGACTGGCACTACCTCTGTTGAGCACCACACTCCGTGGATGATTGAGTTGGATGATTCCTTGGAGCGTTACCTCAGCGAGCCTCAGGAAGATGAAGC TCGTCAGGTTTACCGTGGCAAGAAAGTCCGCGATAAAGCCTAA
Mfmo (M. aminisulfidivorans)	ATGGCAACTCGTATTGCGATACTTGGTGCAGGCCCAAGTGGTATGGCACAACTCAGAGCATTCCAATCCGCCCAGGAAAAAGGTGCTGAGATCCCTGAACTCGTT TGTTTTGAAAAACAAGCTGATTGGGGCGGCCAGTGGAATTACACATGGCGCACTGGTTTAGATGAAAATGGCGAACCTGTTCATAGCAGTATGTATCGCTATCTG TGGTCAAACGGCCCGAAAGAATGTCTTGAATTTGCTGATTACACGTTTGACGAACACTTTGGTAAGCCCATCGCCTCTTATCCACCCCGTGAAGTCTTATGGGACT ATATTAAAGGCCGTGTTGAAAAAGCCGGCGTCAGAAAATATATCCGTTTTAATACCGCTGTTCGTCATGTTGAATTCAACGAAGACAGCCAAACTTTTACCGTTAC CGTGCAGGACCATACTACTGACACAATTTACTCTGAAGAGTTTGACTATGTTGTCTGTTGTACCGGTCACTTCTCAACACCTTACGTGCCTGAATTTGAAGGCTTTG AAAAATTTGGTGGCCGCATTCTGCATGCCCATGACTTCCGTGACGCATTAGAATTTAAAGACAAAACTGTATTACTGGTCGGCAGCAGTTACTCAGCTGAAGATAT CGGCTCACAATGTTATAAATACGGCGCGAAAAAACTGATCAGCTGCTACCGTACCGCACCGATGGGTTATAAATGGCCTGAAAACTGGGATGAAAGACCCAACCT GGTTCGTGTTGATACTGAAAACGCTTATTTTGCCGATGGTTCATCAGAAAAAGTCGATGCGATTATTCTGTGTACCGGTTATATCCATCACTTCCCCTTCCTCAATGA

	CGATCTGCGTCTGGTCACCAATAACCGTTTATGGCCGCTCAACCTTTATAAAGGCGTGGTGTGGGAAGATAATCCAAAATTCTTCTACATTGGCATGCAGGATCAA TGGTACAGCTTCAATATGTTTGATGCCCAAGCCTGGTATGCCCGTGATGTGATTATGGGTCGACTGCCATTGCCATCAAAAGAAGAGATGAAAGCCGACAGCATG GCCTGGCGTGAAAAAGAACTGACGCTGGTTACGGCTGAAGAAATGTACACCTACCAGGGTGACTACATTCAGAATCTGATTGATATGACTGACTATCCGTCATTT GATATTCCGGCAACCAACAAAACTTTCCTGGAATGGAAACATCACAAAAAAGAAAACATCATGACTTTCCGTGACCACTCATACCGTTCACTGATGACTGGCACGA TGGCACCGAAACATCACACACCATGGATAGATGCACTGGATGATTCTCTGGAAGCCTATCTCTCTGATAAGAGCGAAATTCCTGTGGCTAAAGAAGCTTAA
aroG$^{fbr(D146N)}$	ATGAATTATCAGAACGACGATTTACGCATCAAAGAAATCAAAGAGTTACTTCCTCCTGTCGCATTGCTGGAAAAATTCCCCGCTACTGAAAATGCCGCGAATACGG TTGCCCATGCCCGAAAAGCGATCCATAAGATCCTGAAAGGTAATGATGATCGCCTGTTGGTTGTGATTGGCCCATGCTCAATTCATGATCCTGTCGCGGCAAAAGA GTATGCCACTCGCTTGCTGGCGCTGCGTGAAGAGCTGAAAGATGAGCTGGAAATCGTAATGCGCGTCTATTTTGAAAAGCCGCGTACCACGGTGGGCTGGAAAG GGCTGATTAACGATCCGCATATGGATAATAGCTTCCAGATCAACGACGGTCTGCGTATAGCCCGTAAATTGCTGCTTGATATTAACGACAGCGGTCTGCCAGCGG CAGGTGAGTTTCTCAATATGATCACCCCACAATATCTCGCTGACCTGATGAGCTGGGGCGCAATTGGCGCACGTACCACCGAATCGCAGGTGCACCGCGAACTGG CATCAGGGCTTTCTTGTCCGGTCGGCTTCAAAAATGGCACCGACGGTACGATTAAAGTGGCTATCGATGCCATTAATGCCGCCGGTGCGCCGCACTGCTTCCTGTC CGTAACGAAATGGGGGCATTCGGCGATTGTGAATACCAGCGGTAACGGCGATTGCCATATCATTCTGCGCGGCGGTAAAGAGCCTAACTACAGCGCGAAGCACG TTGCTGAAGTGAAAGAAGGGCTGAACAAAGCAGGCCTGCCAGCACAGGTGATGATCGATTTCAGCCATGCTAACTCGTCCAAACAATTCAAAAAGCAGATGGAT GTTTGTGCTGACGTTTGCCAGCAGATTGCCGGTGGCGAAAAGGCCATTATTGGCGTGATGGTGGAAAGCCATCTGGTGGAAGGCAATCAGAGCCTCGAGAGCG GGGAGCCGCTGGCCTACGGTAAGAGCATCACCGATGCCTGCATCGGCTGGGAAGATACCGATGCTCTGTTACGTCAACTGGCGAATGCAGTAAAAGCGCGTCGC GGGTAA
trpE$^{fbr(S40F)}$	ATGCAAACACAAAAACCGACTCTCGAACTGCTAACCTGCGAAGGCGCTTATCGCGACAATCCCACCGCGCTTTTTCACCAGTTGTGTGGGGATCGTCCGGCAACGC TGCTGCTGGAATTCGCAGATATCGACAGCAAAGATGATTTAAAAAGCCTGCTGCTGGTAGACAGTGCGCTGCGCATTACAGCTTTAGGTGACACTGTCACAATCC AGGCACTTTCCGGCAACGGCGAAGCCCTCCTGGCACTACTGGATAACGCCCTGCCTGCGGGTGTGGAAAGTGAACAATCACCAAACTGCCGTGTGCTGCGCTTCC CCCCTGTCAGTCCACTGCTGGATGAAGACGCCCGCTTATGCTCCCTTTCGGTTTTTGACGCTTTCCGTTTATTGCAGAATCTGTTGAATGTACCGAAGGAAGAACGA GAAGCCATGTTCTTCGGCGGCCTGTTCTCTTATGACCTTGTGGCGGGATTTGAAGATTTACCGCAACTGTCAGCGGAAAATAACTGCCCTGATTTCTGTTTTTATCT CGCTGAAACGCTGATGGTGATTGACCATCAGAAAAAAAGCACCCGTATTCAGGCCAGCCTGTTTGCTCCGAATGAAGAAGAAAAACAACGTCTCACTGCTCGCCT GAACGAACTACGTCAGCAACTGACCGAAGCCGCGCCGCCGCTGCCAGTGGTTTCCGTGCCGCATATGCGTTGTGAATGTAATCAGAGCGATGAAGAGTTCGGTG GCGTAGTGCGTTTGTTGCAAAAAGCGATTCGCGCTGGAGAAATTTTCCAGGTGGTGCCATCTCGCCGTTTCTCTCTGCCCTGCCCGTCACCGCTGGCGGCCTATTA CGTGCTGAAAAAGAGTAATCCCAGCCCGTACATGTTTTTTATGCAGGATAATGATTTCACCCTATTTGGCGCGTCGCCGGAAAGCTCGCTCAAGTATGATGCCACC AGCCGCCAGATTGAGATCTACCCGATTGCCGGAACACGCCCACGCGGTCGTCGCGCCGATGGTTCACTGGACAGAGATCTCGACAGCCGTATTGAACTGGAAAT GCGTACCGATCATAAAGAGCTGTCTGAACATCTGATGCTGGTTGATCTCGCCCGTAATGATCTGGCACGCATTTGCACCCCCGGCAGCCGCTACGTCGCCGATCTC ACCAAAGTTGACCGTTATTCCTATGTGATGCACCTCGTCTCTCGCGTAGTCGGCGAACTGCGTCACGATCTTGACGCCCTGCACGCTTATCGCGCCTGTATGAATAT GGGGACGTTAAGCGGTGCGCCGAAAGTACGCGCTATGCAGTTAATTGCCGAGGCGGAAGGTCGTCGCCGCGGCAGCTACGGCGGCGCGGTAGGTTATTTCACC GCGCATGGCGATCTCGACACCTGCATTGTGATCCGCTCGGCGCTGGTGGAAAACGGTATCGCCACCGTGCAAGCGGGTGCTGGTGTAGTCCTTGATTCTGTTCCG CAGTCGGAAGCCGACGAAACCCGTAACAAAGCCCGCGCTGTACTGCGCGCTATTGCCACCGCGCATCATGCACAGGAGACTTTCTGA
afs (codon-optimized)	ATGGAATTTCGGGTTCATTTGCAAGCCGATAACGAACAAAAAATCTTTCAAAACCAAATGAAACCGGAACCGGAAGCCTCGTATTTGATCAACCAACGGCGGTCG GCCAACTATAAACCGAACATCTGGAAAAACGATTTTTTGGATCAATCGTTGATCTCGAAATATGATGGCGATGAATATCGGAAATTGTCGGAAAAATTGATCGAA GAAGTTAAAATCTATATCTCGGCCGAAACCATGGATTTGGTTGCCAAATTGGAATTGATCGATTCGGTTCGGAAATTGGGCTTGGCCAACTTGTTTGAAAAAGAAA TCAAAGAAGCCTTGGATTCGATCGCCGCCATCGAATCGGATAACTTGGGCACCCGGGATGATTTGTATGGCACCGCCTTGCATTTTAAAATCTTGCGGCAACATGG CTATAAAGTTTCGCAAGATATCTTTGGCCGGTTTATGGATGAAAAAGGCACCTTGGAAAACCATCATTTTGCCCATTTGAAAGGCATGTTGGAATTGTTTGAAGCC TCGAACTTGGGCTTTGAAGGCGAAGATATCTTGGATGAAGCCAAAGCCTCGTTGACCTTGGCCTTGCGGGATTCGGGCCATATCTGCTATCCGGATTCGAACTTGT CGCGGGATGTTGTTCATTCGTTGGAATTGCCGTCGCATCGGCGGGTTCAATGGTTTGATGTTAAATGGCAAATCAACGCCTATGAAAAAGATATCTGCCGGGTTA ACGCCACCTTGTTGGAATTGGCCAAATTGAACTTTAACGTTGTTCAAGCCCAATTGCAAAAAAACTTGCGGGAAGCCTCGCGGTGGTGGGCCAACTTGGGCTTTG

	CCGATAACTTGAAATTTGCCCGGGATCGGTTGGTTGAATGCTTTTCGTGCGCCGTTGGCGTTGCCTTTGAACCGGAACATTCGTCGTTTCGGATCTGCTTGACCAA AGTTATCAACTTGGTTTTGATCATCGATGATGTTTATGATATCTATGGCTCGGAAGAAGAATTGAAACATTTTACCAACGCCGTTGATCGGTGGGATTCGCGGGAA ACCGAACAATTGCCGGAATGCATGAAAATGTGCTTTCAAGTTTTGTATAACACCACCTGCGAAATCGCCCGGGAAATCGAAGAAGAAAACGGCTGGAACCAAGTT TTGCCGCAATTGACCAAAGTTTGGGCCGATTTTTGCAAAGCCTTGTTGGTTGAAGCCGAATGGTATAACAAATCGCATATCCCGACCTTGGAAGAATATTTGCGGA ACGGCTGCATCTCGTCGTCGGTTTCGGTTTTGTTGGTTCATTCGTTTTTTTCGATCACCCATGAAGGCACCAAAGAAATGGCCGGCTTTTTGCATAAAAACGAAGAT TTGTTGTATAACATCTCGTTGATCGTTCGGTTGAACAACGATTTGGGCACCTCGGCCGCCGAACAAGAACGGGGCGATTCGCCGTCGTCGATCGTTTGCTATATGC GGGAAGTTAACGCCTCGGAAGAAACCGCCCGGAAAAACATCAAAGGCATGATCGATAACGCCTGGAAAAAAGTTAACGGCAAATGCTTTACCACCAACCAAGTT CCGTTTTTGTCGTCGTTTATGAACAACGCCACCAACATGGCCCGGGTTGCCCATTCGTTGTATAAAGATGGCGATGGCTTTGGCGATCAAGAAAAAGGCCCGCGG ACCCATATCTTGTCGTTGTTGTTTCAACCGTTGGTTAACTAG
ispA (M. alcaliphilum 20Z)	ATGAGTAACGCACTGAAAGACTATCTCTCCTTTTGTCAAAACCGTGTCGAAAGAGCCTTGGAAGCCCGACTGCCAAGCGAAAACCAAATTCCGACAAAATTGCAC GAAGCGATGCGCTATTGCGTGCTGGACGGCGGTAAACGCATGCGTCCGATGCTAACCTACTGTACAGGAAAAGCCGTGGGCATTGCACCGGAAGATTTAGATGG CGCGGCCTGTGCGGTTGAATTCATTCATGTTTATTCGTTGATACATGACGATTTGCCGGCCATGGACGACGACGACCTCAGACGCGGAAAGCCGACCTGTCATATC GCTTATGATGAAGCGACCGCCATTTTGACCGGCGACGCTTTACAAGCATTGGCATTCAAGGTCTTGGCTGACGACCCTACCATCCGAGCCGATGCCGAAAGCCGT CTAAAAATGATTACGTTGCTGGCTAAGGCTAGCGGCTCTCAAGGCATGGTCGGCGGCCAAGCCATCGATTTAGAATCGGTCGGCACGATGCTGACGCTGCCTCAG CTTGAAAATATGCATATCCACAAGACCGGAGCGTTAATTCGAGCCAGCGTCAACATGGCGACGTTAACGAGACCCGATATCGACCCGAAACAGGCCGAAGGGCT CGATCATTACGCAAAATGCATCGGCCTATCCTTCCAAGTCAAGGATGATATTTTGGACGAAGAAAGCGATACCGCAACACTCGGCAAAACCCAAGGCAAGGACAA AGACAACGACAAGCCGACTTACCCTGCCTTACTCGGCTTGGCCGGCGCAAAGCAAAAAGCTCAGGAACTTCATGAGCAAGCCATTGAAAGCTTAAACGGATTCGG CCCCGAAGCCGATCTGCTTCGTGACTTGTCGCTTTATATTATCCAGCGAGATCATTAA
ribB^{G113S} [3]	ATGAATAATGTCGAAAAGGCGATCGAAGCGTTGAAAAAAGGCGAGATCATTTTGGTTTACGATAGCGATGAACGCGAGGGCGAAACCGATATGGTGGTCGCGT CGCAATTTATCACGCCGGAACATATCCGCATCATGCGCAAAGATGCCGGCGGCTTGATTTGTACCGCATTGCATCCGGACATCTGCAATAAATTGGGCATTCCGTT CATGGTCGATATCTTGGAATTTGCCAGCCAAAAATTCAAAGTCTTGCGCGAACTGTATCCGAACGATATTCCTTATGACGAAAAATCGTCGTTTTCGATTACCATTA ATCATCGAAAAACCTTTACCTCGATCACCGACAACGACCGCGCGTTTACGATCAAAAAGCTGGCCGAGCTGGTCAAGGAAGGCCGCTTCAATGATTTCGGCAAAG AATTCCGCTCGCCGGGTCATGTCACCTTGCTGCGCGCCGCCGAAGGTTTGGTCAAAAATCGGCAAGGCCATACCGAAATGACCGTCGCCTTGGCGGAATTGGCCA ATTTGGTGCCGATTACGACGATTTGCGAAATGATGGGCGATGACGGCAATGCGATGTCGAAAAACGAAACGAAACGCTATGCCGAAAAACATAATCTGATTTATT TAAGCGGCGAAGAAATCATCAATTATTATTTGGATAAATATCTGAAAGAC
dxr (M. alcaliphilum 20Z)	ATGAAAGGTATTTGTATTTTGGGCGCGACCGGTTCTATCGGTGTCAGCACGCTGGATGTGGTTGCTCGCCATTCGAATCGGTATAGAGTCGTTGCGTTGACCGCG AACAATAATATCGACCTGCTGTACGACCAATGCATCGTCCATCGTCCTGACTATGTTGTCGTGGTTGATGAAAATAAGGCTAAACAATTTGCAGAGCGCATTGCTA CATCGCCGGTATCCGATATAAAGGTGTTATCGGGAGCCGAATCGTTGCAGCAAGTAGCTACACTGGATAGTGTTGATTCGGTAATGGCGGCAATCGTCGGCGCG GCTGGTCTATTACCAACTTTGGCGGCTGCTAAAGCCGGTAAAACCGTATTGCTGGCAAATAAAGAAGCGTTGGTGATGTCCGGCGATATTTTTATGAAAGCGGTT ACCGAGTCCGGTGCCCATTTGCTGCCGATCGATAGCGAGCATAATGCCATTTTTCAATGCATGCCGGCAGATTACTGTGCGGGTCAAGAGGCTAAGGAGGCGCG GCGAATTTTGTTGACTGCCTCGGGTGGCCCGTTCAGAACTAAGCCGGTAGAAGAGTTGGTCGATGTCACTCCGGATCAAGCCGTCGCGCATCCGAATTGGGATAT GGGGCGTAAGATTTCTGTCGATTCTGCGACGATGATGAATAAAGGGCTTGAATTGATTGAAGCCTGTTTATTGTTCAATATGTCGCCGGATAAAATTCAGGTGGT GATCCATCCGCAAAGCGTGATTCATTCAATGGTCGATTATGTCGACGGCACTGTGTTGGCGCAAATGGGTAACCCCGATATGCGAATACCCATTGCGCATGCGAT GGCGTGGCCGGAACGCTTCGACTCCGGTGCGGCGCCGCTGAATATATTCGACGTTAAGCACATGGATTTCGAACAACCCGATCTTCAGCGTTTCCCTTGTTTACGC TTGGCGATTGAAGCCGTCGAGGCAGGCGGTATTATGCCGGCTGTGTTAAATGCCGCTAATGAAATCGCGGTTGCTGCCTTTTTGGACGAGAAAGTCCGTTTTACC GACATCCCTTACATTATTGAACGGAGCATGCATCAATTCGAAGCCGATCCGGCGGATACGCTGGATATTGTATTGGCAGCCGATAGCAAAGCCCGGGAAGTGGCT GAGCGTATCGTCGAAGAACTTAAGCGTTAA

Table S4. The production of indigo and farnesene in previous studies. (n.d: not determined)

Host strain	Product	Substrate	Culture conditions	Titer	Production yield	References
E. coli	Indigo	Tryptophan (1.0 g/L)	Optimized medium (3.55 g/L NaCl, 5.12 g/L yeast extract); Batch culture	307.4 mg/L	n.d	Dai et al.[5]
E. coli	Indigo	Tryptophan	Tryptophan medium (2 g/L tryptophan, 5 g/L yeast extract, 10 g/L NaCl); Continuos fermentation, 3000 L	911 mg/L	0.234 mg/g tryptophan	Han et al.[6]
E. coli	Indigo	Glycerol (5 g/L) Tryptophan (0.5 g/L)	M9 medium; Consortium cultivation; Batch fermentation.	104.3 mg/L	n.d	Chen et al.[7]
M. alcaliphilum 20Z	Indigo	Methane (176 mg)	NMS medium; Fed-batch culture, 50 mL	3.5 µg/L	0.994 ug/g methane	This study
M. alcaliphilum 20Z	Indigo	Methane (135 mg) Xylose (641 mg)	NMS medium; Semi fed-batch culture, 50 mL	6.3 µg/L	0.421 ug/g substrates	This study
S. cerevisiae	Farnesene	Cane syrup	Culture medium (15 g/L $NH_4H_2PO_4$, trace element, vitamin); Fed-batch fermentation, 250 mL	130 g/L	143 mg/g glucose	Meadows et al.[8]
Yarrowia lipolytica	Farnesene	Glucose	Modified YPD medium (50 g/L glucose, 20 g/L tryptone, 10 g/L yeast extract); Fed-batch fermentation, 800 mL	25.55 g/L	n.d	Liu et al.[9]
S. elongatus PCC 7942	Farnesene	CO_2	BG-11 medium (10 mM MOPS, 5% CO_2, 100 µE/(m2 s) continuous fluorescent light); Fed-batch fermentation, 100 mL	12.99 mg/L	n.d	N.P. et al.[10]
M. alcaliphilum 20Z	Farnesene	Methane (140 mg)	NMS medium; Fed-batch culture, 50 mL	48.98 mg/L	17.49 mg/g methane	This study
M. alcaliphilum 20Z	Farnesene	Methane (130 mg) Xylose (740 mg)	NMS medium; Semi fed-batch culture, 50 mL	91.55 mg/L	5.26 mg/g substrates	This study

References

1. A. W. Puri, S. Owen, F. Chu, T. Chavkin, D. A. Beck, M. G. Kalyuzhnaya and M. E. Lidstrom, *Appl. Environ. Microbiol.*, 2015, **81**, 1775-1781.

2. A. D. Nguyen, T. H. T. Chau and E. Y. Lee, *Chemical Engineering Journal*, 2021, **420**, 127632.
3. A. D. Nguyen, D. N. Pham, T. H. T. Chau and E. Y. Lee, *Microorganisms*, 2021, **9**, 1236.
4. D. N. Pham, D. H. A. Mai, A. D. Nguyen, T. H. T. Chau and E. Y. Lee, *Chemical Engineering Journal*, 2022, **429**, 132522.
5. C. Dai, Q. Ma, Y. Li, D. Zhou, B. Yang and Y. Qu, *Bioprocess and biosystems engineering*, 2019, **42**, 1963-1971.
6. G. H. Han, S. E. Bang, B. K. Babu, M. Chang, H.-J. Shin and S. W. Kim, *Process biochemistry*, 2011, **46**, 788-791.
7. T. Chen, X. Wang, L. Zhuang, A. Shao, Y. Lu and H. Zhang, *Microbial cell factories*, 2021, **20**, 1-11.
8. A. L. Meadows, K. M. Hawkins, Y. Tsegaye, E. Antipov, Y. Kim, L. Raetz, R. H. Dahl, A. Tai, T. Mahatdejkul-Meadows and L. Xu, *Nature*, 2016, **537**, 694-697.
9. Y. Liu, X. Jiang, Z. Cui, Z. Wang, Q. Qi and J. Hou, *Biotechnology for biofuels*, 2019, **12**, 1-11.
10. N. Pattharaprachayakul, H. J. Lee, A. Incharoensakdi and H. M. Woo, *Journal of agricultural and food chemistry*, 2019, **67**, 13658-13664.