Supplementary Material

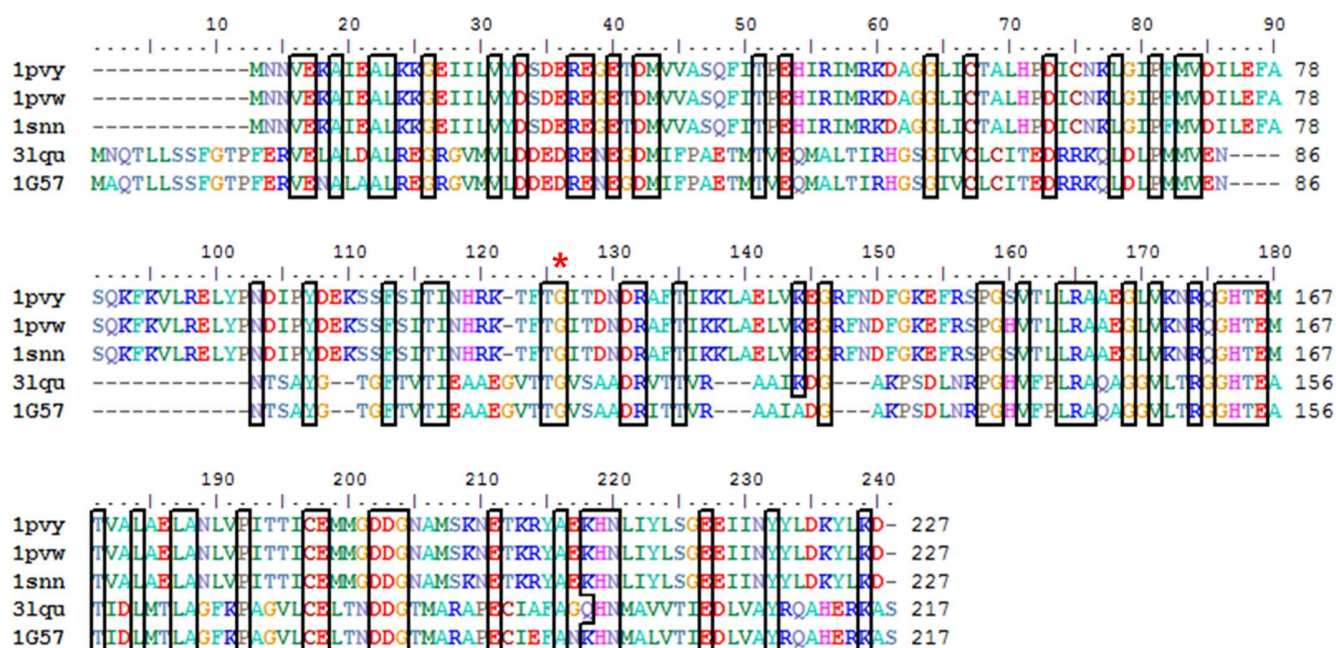# Enhancing Sesquiterpenoid Production from Methane via Synergy of the Methylerythritol Phosphate Pathway and a Short-Cut Route to 1-Deoxy-D-xylulose 5-Phosphate in Methanotrophic Bacteria

Anh Duc Nguyen, Diep Ngoc Pham, Tin Hoang Trung Chau, Eun Yeol Lee*

Department of Chemical Engineering, Kyung Hee University (Integrated Engineering), Yongin-si, Gyeonggi-do 17104, South Korea

Corresponding author: eunylee@khu.ac.kr;

Tel: +82-31-201-3839; Fax: +82-31-204-8114

1G57 : RibB of *E. coli* strain K12
1PVW : RibB of *Methanococcus jannaschii*
1PVY, 1SNN : RibB S147H *mutant of Methanococcus jannaschii*
3LQU : RibB of *Salmonella typhimurium*

Figure S1. Multiple alignment of some *ribB* variants from different species.

Table S1. Strains and plasmids used in this study

Strain	Characteristic(s)	Reference
Plasmids		
pHM03	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z); oriV oriT trfA; KanR	[1]
pDXP-01	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)-*yajO*(*E. coli*); oriV oriT trfA; KanR	This work
pDXP-02	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)- *ribBG108S*(*E. coli*); oriV oriT trfA; KanR	This work
pDXP-03	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)-*ribBG108S*(*Methanococcus jannaschii*); oriV oriT trfA; KanR	This work
pDXP-04	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)- fused *dxr-G2-ribB*; oriV oriT trfA; KanR	This work
pDXP-05	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)- fused *ribB-G2-dxr*; oriV oriT trfA; KanR	This work
pDXP-06	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)- fused *dxr-DSAG-ribB*; oriV oriT trfA; KanR	This work
pDXP-07	P*tac*:: *zssI*(opt)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)- fused *ribB-DSAG-dxr*; oriV oriT trfA; KanR	This work
pTrcAgBIS (CO)	pTrc99a backbone, Amp^R, containing bisabolene synthase from *Abies grandis*	[2]
pBs-01	P*tac*:: *AgBs*(*Abies grandis*)-*ispA*(20Z); oriV oriT trfA; KanR	This work
pBs-02	P*tac*:: *AgBs*(*Abies grandis*)-*ispA*(20Z)-*dxs*(20Z)-*ispG*(20Z)- fused *ribB-DSAG-dxr*; oriV oriT trfA; KanR	This work

Strains		
Escherichia coli DH5α		Novagen
Methylotuvimicrobium alcaliphilum 20Z	Used as the host strain	DMSZ
pDXP-02	*M. alcaliphilum* 20Z harboring pDXP-02 vector	This work
pDXP-03	*M. alcaliphilum* 20Z harboring pDXP-03 vector	This work
pDXP-04	*M. alcaliphilum* 20Z harboring pDXP-04 vector	This work
pDXP-05	*M. alcaliphilum* 20Z harboring pDXP-05 vector	This work
pDXP-06	*M. alcaliphilum* 20Z harboring pDXP-06 vector	This work
pDXP-07	*M. alcaliphilum* 20Z harboring pDXP-07 vector	This work
pBs-01	*M. alcaliphilum* 20Z harboring pBs-01 vector	This work
pBs-02	*M. alcaliphilum* 20Z harboring pBs-02 vector	This work

Table S2. Primers used and procedures for plasmids construction in this study

Primer	Sequence	Description
pAWP89-For	TAGTTGTCGGGAAGATGCGT	For amplifying pAWP89 backbone which contained P_{tac} promoter and SD sequence
pAWP89-Rev	AGCTGTTTCCTGTGTGAATA	
ispG-invert-Rev	CTACTGAGCTGTGGTTCCGT	For linear pHM03 vector
pAWP89-For	TAGTTGTCGGGAAGATGCGT	
agB.s-For	TTCACACAGGAAACAGCTATGGCGGGTGTTTCTGCG	For construction of pBs-01 vector. AgB.s and ispA from 20Z were amplified and ligated into pAWP89 backbone.
agB.s-Rev	ACTCAGATCTTACAGCGGCAGCGGTTC	
ispA-20Z-For	CCGCTGTAAGATCTGAGTACCTCTAGAAAATAAGGA	
ispA-20Z-Rev	GCATCTTCCCGACAACTATTAATGATCTCGCTGGATAA	
ribB-KpnI-For	CTGGTCTGTTTGTGGTACAGGAAACAGCTATGAATCAGACGCTACTTTCCTCT	For construction of ribB-G108S from *via* site-directed mutagenesis. RibB-G108S and yajO were first cloned into pLC291 vector at KpnI and SacI restriction sites.
ribB-KpnI-Rev	CGTAGATCTTCTAGAGGTACTCAGCTGGCTTTACGCTCAT	
ribB-G108S-Rev	GGCAGAAACCGAGGTAGTCA	
ribB-G108S-For	TGACTACCTCGGTTTCTGCC	
yajO-SacI-For	TACTAGTGCATGCGAGCTAAGGAGATATAATGCAATACAACCCCTTAGGA	
yajO-SacI-Rev	TGAATTCACCGGTGAGCTTTATTTAAATCCTACGACAGGATGCG	
ribB-89-For	CGGAACCACAGCCAGTAGAGGAAACAGCTATGAATCAGACGCTACTTTC	For construction of pDXP-06 and pDXP-07 vectors
ribB-DSAG-Rev	ACCAGCACTACCAGCACTACCAGCACTATCGCTGGCTTTACGCTCATGTG	
ispC-DSGA-For	GATAGTGCTGGTAGTGCTGGTAGTGCTGGTAAAGGTATTTGTATTTTGGGCGC	
ispC-89-Rev	GCATCTTCCCGACAACTATTAACGCTTAAGTTCTTCGACGA	
ispC-DSAG-ribB-Rev	ACCAGCACTACCAGCACTACCAGCACTATCACGCTTAAGTTCTTCGACGA	
ispC-DSAG-ribB-For	GATAGTGCTGGTAGTGCTGGTAGTGCTGGTAATCAGACGCTACTTTCCTCT	
ispC-89-For	CGGAACCACAGCTCAGTAGAGGAAACAGCTATGAAAGGT	

5

ribB-89-Rev	GCATCTTCCCGACAACTATCAGCTGGCTTTACGCTCAT	
yajO-89-For	CGGAACCACAGCTCAGTAGAAGGAGATATAATGCAATACAACCCC	For construction of pDXP-02 vector
yajO-89-Rev	GCATCTTCCCGACAACTATTATTTAAATCCTACGACAGGATGCG	
ribB-89-Rev	GCATCTTCCCGACAACTATCAGCTGGCTTTACGCTCAT	For construction of pDXP-01 vector
ribB-89-For	CGGAACCACAGCCAGTAGAGGAAACAGCTATGAATCAGACGCTACTTTC	
ribB-89-For	CGGAACCACAGCCAGTAGAGGAAACAGCTATGAATCAGACGCTACTTTC	For construction of pDXP-04 and pDXP-05 vectors
ribBG2ispC-Rev (G2 fused)	ACCGGAACCGCCAGAACCGCTGGCTTTACGCTCATGTG	
ribBG2ispC-For (G2 fused)	GGTTCTGGCGGTTCCGGTAAAGGTATTTGTATTTTGGGCGC	
ispC-89-Rev	GCATCTTCCCGACAACTATTAACGCTTAAGTTCTTCGACGA	
ispC-89-For	CGGAACCACAGCTCAGTAGAGGAAACAGCTATGAAAGGT	
ribB-89-Rev	GCATCTTCCCGACAACTATCAGCTGGCTTTACGCTCAT	
CO_ribB_G113S_For	CGGAACCACAGCTCAGTAGAATAAGGAGGAATAAACCATGAATA ATGTCGAAAAGGCGATCG	For construction of pDXP-03 vector
CO_ribB_G113S_Rev	GCATCTTCCCGACAACTAGTCTTTCAGATATTTATCCAAA	

APPENDIX B. Codon-optimized *ribB* from *Methanococcus jannaschii*

>*ribBG113S*
ATGAATAATGTCGAAAAGGCGATCGAAGCGTTGAAAAAAGGCGAGATCATTTTGGTTTACGATAGCGATG
AACGCGAGGGCGAAACCGATATGGTGGTCGCGTCGCAATTTATCACGCCGGAACATATCCGCATCATGCG
CAAAGATGCCGGCGGCTTGATTTGTACCGCATTGCATCCGGACATCTGCAATAAATTGGGCATTCCGTTCA
TGGTCGATATCTTGGAATTTGCCAGCCAAAAATTCAAAGTCTTGCGCGAACTGTATCCGAACGATATTCCT
TATGACGAAAAATCGTCGTTTTCGATTACCATTAATCATCGAAAAACCTTTACCTCGATCACCGACAACGA
CCGCGCGTTTACGATCAAAAAGCTGGCCGAGCTGGTCAAGGAAGGCCGCTTCAATGATTTCGGCAAAGA
ATTCCGCTCGCCGGGTCATGTCACCTTGCTGCGCGCCGCCGAAGGTTTGGTCAAAAATCGGCAAGGCCAT
ACCGAAATGACCGTCGCCTTGGCGGAATTGGCCAATTTGGTGCCGATTACGACGATTTGCGAAATGATGG
GCGATGACGGCAATGCGATGTCGAAAAACGAAACGAAACGCTATGCCGAAAAACATAATCTGATTTATT
TAAGCGGCGAAGAAATCATCAATTATTATTTGGATAAATATCTGAAAGAC

References

[1] Nguyen AD, Kim D, Lee EY. Unlocking the biosynthesis of sesquiterpenoids from methane via the methylerythritol phosphate pathway in methanotrophic bacteria, using α-humulene as a model compound. Metab Eng 2020;61:69-78.

[2] Peralta-Yahya PP, Ouellet M, Chan R, Mukhopadhyay A, Keasling JD, Lee TS. Identification and microbial production of a terpene-based advanced biofuel. Nat Commun 2011;2:1-8. 10.1038/ncomms1494