

Supplementary Information

Synthesis and Characterization of 3-Hydroxybutyrate and 3-Hydroxy-9-octadecenoate Copolymer from Engineered *Halomonas*

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Supplementary Tables

Supplementary Table S1: Strains and plasmids used in this study.

Strains/Plasmids	Description	Source/ Literature
Strains		
<i>E. coli</i> S17-1	Harboring the <i>tra</i> gene on the chromosome, used for conjugation	[1]
<i>Halomonas bluephagenesis</i> sp. TD01	Wild type isolated from Aydingkol Lake of Xinjiang, China	[2]
<i>H. bluephagenesis</i> sp. TD1.0	Carrying P _{T7-like} RNA polymerase expression module on <i>H. bluephagenesis</i> sp. TD01	[3]
TXY21	TD1.0Δ <i>phaC</i> Δ <i>fadA</i>	This study
TXY26	TD1.0Δ <i>phaC</i> Δ <i>fadA</i> Δ <i>phaZ</i> 123	This study
TXY27	Chromosome insertion of P _{Porin} - <i>phaC</i> _{Ps-STQKopt} in G51 sites of TXY26	This study
TXY28	Chromosome insertion of P _{Porin} - <i>phaC</i> _{Ps-STQKopt} in G49 sites of TXY27	This study
TXY29	Chromosome insertion of P _{Porin58} - <i>phaC</i> _{Ps-STQKopt} in GY10 sites of TXY28	This study
TXY30	Chromosome insertion of P _{Porin} - <i>phaC</i> _{Ps-STQKopt} in GY7 sites of TXY29	This study
TXY31	Chromosome insertion of P _{Porin} - <i>phaC</i> _{Ps-STQKopt} in G4 sites of TXY30	This study
TXY32	Chromosome insertion of P _{Porin} - <i>phaC</i> _{Ps-STQKopt} in G7 sites of TXY31	This study
Plasmids		
pSEVA321	An expression vector harboring RK2 replication origin, <i>oriT</i> sequence for conjugation in <i>H. bluephagenesis</i> , Cm ^R	[4]
pSEVA341	High copy number expression vector used for overexpression, pRO1600/ColE1 replication origin, <i>oriT</i> , Cm ^R	[4]
pQ08	pSEVA321 derivative, expressing <i>S. pyogenes</i> cas9, Cm ^R	[5]
pSEVA341- <i>phaC</i> _{Ps-STQK}	pSEVA341 derivative, expressing <i>phaC</i> _{Ps-STQK} (S325T, Q481K), Spe ^R	[6]
pSEVA341- <i>phaC</i> _{Ps}	pSEVA341 derivative, expressing <i>phaC</i> _{Ps} , Spe ^R	This study
pSEVA341- <i>phaC</i> _{Ps-STQKopt}	pSEVA341 derivative, expressing <i>phaC</i> _{Ps-STQKopt} , Spe ^R	This study
pXY216	pSEVA341 derivative <i>fadA</i> knockout, <i>fadA</i> sgRNA, homologous arm length 1000 bp, Km ^R and Spe ^R	This study
pXY266	pSEVA341 derivative <i>phaZ</i> 1 knockout, <i>phaZ</i> 1 sgRNA, homologous arm length 1000 bp, Km ^R and Spe ^R	This study

pXY269	pSEVA341 derivative <i>phaZ2</i> knockout, <i>phaZ2</i> sgRNA, homologous arm length 1000 bp, Km ^R and Spe ^R	This study
pXY272	pSEVA341 derivative <i>phaZ3</i> knockout, <i>phaZ3</i> sgRNA, homologous arm length 1000 bp, Km ^R and Spe ^R	This study
pXY354	pSEVA341 derivative, G43 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY355	pSEVA341 derivative, G49 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY356	pSEVA341 derivative, G51 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY357	pSEVA341 derivative, G52 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY358	pSEVA341 derivative, G57 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY359	pSEVA341 derivative, G58 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY360	pSEVA341 derivative, G61 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY361	pSEVA341 derivative, GY2 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY362	pSEVA341 derivative, GY3 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY363	pSEVA341 derivative, GY4 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY364	pSEVA341 derivative, GY7 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY365	pSEVA341 derivative, GY10 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY366	pSEVA341 derivative, GY11 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp	This study

	donor, Km ^R and Spe ^R	
pXY367	pSEVA341 derivative, GY12 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY368	pSEVA341 derivative, GY13 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study
pXY369	pSEVA341 derivative, GY14 chromosome site sgRNA with integrated <i>phaC</i> _{Ps-STQKopt} , 1000 bp donor, Km ^R and Spe ^R	This study

Supplementary Table S2: DNA sequences of genes used in the study.

No.	Gene	SEQUENCE	Source
1	<i>phaC_{TD}</i>	ATGGCGACCGCAAAGGCGCGGGCAGCTTCCACGCAGGAAGGCAAGTCCCAACCATCAAGGTCACGCCGGGGCCATTTCGATCCAGCCACATGGCTGGAATGGTCCCGC CAGTGGCAGGGCACTGAAGGCAACGGCCACGCGGCCGCTCCGGCATTCCGGGCTGGATGCGCTGGCAGGCGTCAAGATCGCGCCGGCGCAGCTGGGTGATATCCAGC AGCGCTACATGAAGGACTTCTCAGCGCTGTGGCAGGCCATGGCCGAGGGCAAGGCCGAGGCCACCGGTCCGCTGCACGACCGGCGCTTCGCCGGCGACGCATGGCGCAC CAACCTCCCATATCGCTTCGCTGCCGCGTTCTACCTGCTCAATGCGCGCGCCTTGACCGAGCTGGCCGATGCCGTCGAGGCCGATGCCAAGACCCGCCAGCGCATCCGCTT CGCGATCTCGCAATGGGTCGATGCGATGTGCCCCGCCAACTTCCTTGCCACCAATCCCGAGGCGCAGCGCCTGCTGATCGAGTCGGGCGGCGAATCGCTGCGTGCCGGCG TGCGCAACATGATGGAAGACCTGACACGCGGCAAGATCTCGCAGACCGACGAGAGCGCGTTTGAAGTCGGCCGCAATGTCGCGGTGACCGAAGGCGCCGTGGTCTCGA GAACGAGTACTTCCAGCTGTTGCAGTACAAGCCGTGACCGACAAGGTGCACGCGCGCCCGCTGCTGATGGTGCCCGGTGCATCAACAAGTACTACATCCTGGACCTGC AGCCGGAGAGCTCGCTGGTGCGCCATGTGGTGAGGAGGGACATACGGTGTTTTCTGGTGCTGCTGGCGCAATCCGGACGCCAGCATGGCCGGCAGCACCTGGGACGACTAC ATCGAGCACGCGGCCATCCGCGCCATCGAAGTCGCGCGCGACATCAGCGGCCAGGACAAGATCAACGTGCTCGGCTTCTGCGTGGGCGGCACCATTTGTCTCGACCGCGCT GGCGGTGCTGGCCGCGCGGCGAGCACCCGGCCGCCAGCGTACGCTGCTGACCACGCTGCTGGACTTTGCCGACACGGGCATCCTCGACGTCTTTGTCGACGAGGGCCAT GTGCAGTTGCGCGAGGCCACGCTGGGCGGCGCGCCGGCGCGCTGCGCGCTGCTGCGCGGCTTGAGCTGGCCAATACCTTCTCGTCTTTGCGCCCGAACGACCTGGT GTGGAATACTGGTTCGACAACTACCTGAAGGGCAACACGCCGGTGCCGTTTCGACCTGCTGTTCTGGAACGGCGACGCCACCAACCTGCCGGGGCGTGGTACTGCTGGTA CCTGCGCCACACCTACCTGCAGAACGAGCTCAAGGTACCGGGCAAGCTGACCGTGTGCGGCGTGCCGGTGGACCTGGCCAGCATCGACGTGCCGACCTATATCTACGGCT CGCGCGAAGACCATATCGTGCCGTGGACCGCGGCTATGCCTCGACCGCGCTGCTGGCGAACAAGCTGCGCTTCGTGCTGGGTGCGTCGGGCCATATCGCCGGTGTGATC AACCCGCCGGCCAAGAACAAGCGCAGCCACTGGACTAACGATGCGCTGCCGGAGTCGCCGACGAATGGCTGGCCGGCGCCATCGAGCATCACGGCAGCTGGTGGCCGG ACTGGACCGCATGGCTGGCCGGGAGGCCGGCGGCAACGCGCCGCGCCCGCCAATATGGCAATGCGCGCTATCGCGCAATCGAACC CGCCTGGGC GATACGTCA AAGCCAAGG CATGA	<i>Halomonas bluephagenesis</i>
2	<i>phaC_{Ps}</i>	ATGAGTAACAAGAATAGCGATGACTTGAATCGTCAAGCCTCGGAAAAACACCTTGGGGCTTAACCCTGTCATCGGCCTGCGTGGAAGATCTGCTGACTTCTGCCGAAT GGTTTAAACCAAGCCATCAAACAACCCATTACAGCGTCAAGCACGTCGCGCATTTTGGCATCGAGCTGAAGAACGTGATGTTTGGCAAAATCGAAGCTGCAACCGGAAA GCGATGACCGTCGTTTCAACGACCCCGCCTGGAGTCAGAACCCACTCTACAAACGTTATCTACAAACCTACCTGGCGTGGCGCAAGGAACTCCACGACTGGATCGGCAAC AGCAAAGTGTCCGAACAGGACATCAATCGCGTCACTTCGTGATCACCTGATGACCGAAGCCATGGCCCCGACCAACAGTGGCGCAATCCGGCGGCGGTCAAACGCTT CTTCGAAACGGCGGTAAAAAGCCTGCTCGACGGCTCACACATCTGGCCAAGGACCTGGTAAACAACGGCGGCATGCCGAGCCAGGTGGACATGGGCGCTTTCGAAGTCG GCAAGAGTCTGGGGACGACTGAAGGTGCAGTGGTTTTCCGCAACGACGTCCTCGAATTGATCCAGTACCGGGCCGACCACGAACAGGTGCATGAGCGACCGCTGCTGGTG GTCCACCGCAGATCAACAAGTTTATGTGTTTGACCTGAGCCCGGATAAAAGCCTGGCGCGCTTCTGCTGAGCAACAACAGCAAACTTTATCGTCAGCTGGCGCAAC	<i>Pseudomonas sp. 61-3</i>

3 *phaC*_{Ps-STQK}

CCGACCAAGGCCAGCGTGAGTGGGGTCTGTGCGACTTACATCGATGCGCTCAAAGAAGCCGTCGACGTAGTTTCCGCCATCACCGGCAGCAAAGACATCAACATGCTCGG
CGCCTGCTCCGGTGGCATTACCTGCACCGCGCTGCTGGGTCACTACGCCGCTCTCGGCGAGAAGAAGGTCAATGCCCTGACCCCTTTTGGTCAGCGTGCTCGACACCAACCT
CGACTCCCAGGTTGCACTGTTTCGTCGATGAGAAAAACCTGGAAGCTGCCAAGCGTCACTCGTATCAGGCCGGCGTGCTGGAAGGCCGCGACATGGCCAAAGTCTTCGCCT
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CTGCTGCGTTCCACGGCGATCTGATCGAAATGTTCAAAAATAACCCACTGGTGCGGCCAATGCCTCGAAGTGAGCGGCACGCCGATCGACCTCAAACAGGTCACTGCC
GACATCTACTCCCTGGCCGGCACCAACGATCACATCACGCCCTGGAAGTCTTGCTACAAGTCGGCGCAACTGTTTCGGTGGCAAGGTGCAATTCTGTGCTGTCCAGCAGTGGG
CATATCCAGAGCATTCTGAACCCGCCGGGCAATCCGAAATCACGTTACATGACCAGCACCGACATGCCAGCCACCGCAACGAGTGGCAAGAAAACCTCAACCAAGCACA
CCGACTCCTGGTGGCTGCACTGGCAGGCCTGGCAGGCCGAGCGCTCGGGCAAACTGAAAAAGTCCCCGACCAGCCTGGGCAACAAGGCCTATCCGTCAGGAGAAGCCGC
GCCGGGCACGTATGTGCATGAACGTAA

ATGAGTAACAAGAATAGCGATGACTTGAATCGTCAAGCCTCGGAAAACACCTTGGGGCTTAACCCCTGTCATCGGCCTGCGTGGAAGATCTGCTGACTTCTGCCGAAT
GGTTTTAACCAAGCCATCAAACAACCCATTACAGCGCTCAAGCACGTGCGCATTTTGGCATCGAGCTGAAGAACGTGATGTTTGGCAATCGAAGTGCAACCGGAAA
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CTTCGAAACCGGCGGTAAAAGCCTGCTCGACGGCCTCACACATCTGGCCAAAGGACCTGGTAAACAACGGCGGCATGCCGAGCCAGGTGGACATGGGCGCTTTTGAAGTCG
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GTCCACCGCAGATCAACAAGTTTTATGTGTTTGACCTGAGCCCGGATAAAAGCCTGGCGCGCTTCTGCCTGAGCAACAACAGCAAACCTTTATCGTCAGCTGGCGCAAC
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GCCGGGCACGTATGTGCATGAACGTAA

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ATGAGCAACAAAACAGCGACGACCTGAATCGTCAGGCCAGCGAAAATACTCTGGGCCTGAACCCGGTGATTGGCCTGCGTGGCAAGATCTGCTGACTAGCGCCGTAT

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GGTTCTTACCCAGGCCATCAAACAGCCGATCCACAGCGTTAAACACGTGGCCCACTTCGGCATCGAACTGAAAAACGTGATGTTTCGGCAAAAGCAAACCTGCAGCCGAAAA
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GTAAAGCCTGGGCACCACTGAAGGTGCTGTGGTGTTCGTAATGACGTGCTGGAACCTGATTAGTACCGCCCGACTACCGAACAAGTGCACGAACGTCCGCTGCTTGT
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GCTCCGGGTACTTATGTGCACGAACGCTAA

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ATGAGTAAACAAGAGTAACGATGAGTTGAAGTATCAAGCCTCTGAAAACACCTTGGGGCTTAATCCTGTGCTTGGGCTGCGTGGAAGGATCTACTGGCTTCTGCTCGAAT
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CCTGGATGCGCCCCAACGATCTGATCTGGAACCTACTGGGTCAACAATTACCTGCTAGGCAACGAACCGCGGTGTTTCGACATCCTGTTCTGGAACAACGACACCACACGG

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TTGCCCGCGGCTTCACGGCGACCTGGTCGAAGTGTTCAAAAATAACCCACTGATTCGCCCCGAATGCACTGGAAGTGTGCGGCACCCCATCGACCTCAAGCAGGTGAC
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TCTTCGAGACCGGCGGCAAGAGCCTGCTCGACGGCATGTGCGACCTGGCCAAGGACATGATCAACAACGGCGGCATGCCAGCCAGGTCAACATGGCCGCTTCGAAGTA
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ATGCGAGACAAGCCCAATAGCGGCGCCCTGCCACGCCTGCCACCTTCATGAACGCCAGAGCGCGTGGTCGGCGTGCAGCGGCCGCGACCTGCTCTCCACCGTGCSCCT
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GTTCAACAGTGGCGGCAGCAGCCTGTTCAAGGGCATGCGCCATCTGCTCGACGACCTGCTGCACAACGACGGCCTGCCAGCCAGGTGAGCAAGCACGCCTTCGAAGTGC

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phaC_{Bc}

GCCGCAACCTAGCCTGCACCCAGGTGCCGTGGTGTTCGCAACGAGCTGCTGGAGCTGATCCAGTACCGGCCGATGAGCGAGAAAAAGTACGTACAGGCCGCTGCTGATC GTGCCGCCACAGATCAACAAGTACTACATCTTCGATCTGTCCAACGACAAGAGCTTCGTCCAGTACGCCCTGAAGAACGGCTTGACAGACCTTCATGATCAGCTGGCGCAA CCCGGACGCGCGGCACCGCGAGTGGGGTCTGTCTGAGCTACGTGCAGGCGGTGAGCAGGCCGTGACGCCTGCCGCGGATCACCGGCAGCAAAGACGTCAACCTGCTC GGCGCTGCGCCGGTGGCTGACCATCGCCGCCCTGCAGGGCCACCTGCAGGCCAAGCGGCAGTTGCGCAAGGTGGCCAGCGCCACCTACATGGTCAGCCTGCTGGACAG CCAGATCGATAGCCCGGCCATGCTGTTGCGCCGACGAGCAGACCCTGGAGTCGGCCAAGCGCCGCTCCTACCAGCGCGGCGTGTGGACGGCCGCGACATGGCCAAGGTGT TCGCCTGGATGCGTCCCAATGACCTGATCTGGAATACTGGGTCAACAATACTACCTGCTCGGCAAGCAGCCGCCGGCCTTCGACATCCTCTACTGGAACAACGACAACGCC GCCTGCCGGCCGCTCTGCACGGCGACCTGATCGACTTCTTCAAGCACAAACCCACTCAGCCGCGCCGGCGGCCCTCGAGGTCTGCGGCACCCCGGTGGACTTGAGCAAGGTC GCGGTGGACAGCTTCAGCGTGGCCGGCATCAACGACCACATCACCCCTGGGACGCGGTGTACCGCTCGGCCCTGCTGCTCGGCGGCGAACGCCGCTTCATCCTGTCCAA CAGCGGACATATCCAGAGCATCCTCAACCCGCCAGGCAACCCCAAGGCCAACTACTACGAGAACGGCAAGCTGACCTCGGATCCGCGCGCTGGTACCACGACGCCCCG CGCGTGCAGGGCAGCTGGTGGCCGAGTGGCTGGAATGGATTAGGCACGCTCCGGCGAGCAGCGCGAAACCTGCTGGCACTGGGCGACCAAGAAGCATCCGCCGCAAG AGGCCGCGCCCGGCACCTACGTGCATATCCGCTGA	
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Bacillus cereus
strain MLY1

9

phaC_{4AK4}

Aeromonas
hydrophila 4AK4

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*Halomonas
bluephagenesis*

*Halomonas
bluephagenesis*

10 *fadA*

11 *phaZ1*

12	<i>phaZ2</i> atgatacgttccgccgttctacgtctgcctttttggcgcgcttttactgctgggcagcgcggctgttcacgctgaagcagaggacgtgcctggagattacctgcattaaagcgcagcgcagtgaccgcgctagcgtggtcggcgtgctcgtcggcgtacatggccgcacagttggcg gttcttggcctgaacgcttttagtggcgtagggcgtattagcggcggggccctggagctgtgcccaagggtgcgcttagtttggctcttagccagtgtatgagcaccgcagctgggttgccttctctggatgagctggatcgccggcgcgagcgttatgagtcgttgagcaggtgggtga gcgcgatgcgctaagccagctgcgagcctatgtatggcacgggtgatgaagatgacactgtttctccagagctgggtagcttgttggcagaacagtggcagggctggctggcatcttcagagcagctgcgtttcgtacgcagtgaagaactggtcatggttggccataaaactgccg aagcagggccaacgctagccccagacatttggcgggtgtcgcagcggcgggggcagccatgtgcttgccttgcgtatgagaatgtggctggcgatatgctttcatggctttaccctgagcgtgatgcaaatgctagcgcgaggcggaagtgtgctttgatcagtcggagttcgcgttaaa ggcttggccgacgtgggggatctgtttattcctgaggcgtgcgagcggggggcgtgcccgtcacgatagcgttgcagtgtgtcaaatgagtgtagacgccattggcgacacgtttatgcgccacagtggtttaaatcgtgggcagcagactatggtcaagtggtgctataccgc aggcggaaaagtagcatgctaatccgcaggggtgttgggatgtgtgggctttgccaaaagcactggcagctcaatccgctgcatgacacgcgtgatggcactcaagccagagcgcataatggcgatgcttgagcacctacaaaagtacgccttaa
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Supplementary Table S3: Genes related to β -oxidation in *H. bluephagenesis*.

Genes	Sequence ID	Description
<i>fadA</i>	TD01_03183	3-ketoacyl-CoA thiolase
<i>phaA1</i>	TD01_01557	beta-ketoadipyl CoA thiolase
<i>phaA2</i>	TD01_02102	acetyl-CoA acetyltransferase
<i>phaA3</i>	TD01_02197	acetyl-CoA acetyltransferase
<i>phaA4</i>	TD01_03154	acetyl-CoA acetyltransferase
<i>phaA5</i>	TD01_03174	acetyl-CoA acetyltransferase
<i>fadB</i>	TD01_03182	multifunctional fatty acid oxidation complex subunit alpha
<i>ech1</i>	TD01_00231	enoyl-CoA hydratase
<i>ech2</i>	TD01_00241	enoyl-CoA hydratase
<i>ech3</i>	TD01_01124	enoyl-CoA hydratase
<i>ech4</i>	TD01_01554	enoyl-CoA hydratase
<i>ech5</i>	TD01_01647	enoyl-CoA hydratase
<i>ech6</i>	TD01_02403	enoyl-CoA hydratase
<i>ech7</i>	TD01_02830	enoyl-CoA hydratase
<i>dcaE</i>	TD01_01553	2,3-dehydroadipyl-CoA hydratase
0120	TD01_00120	succinate-semialdehyde dehydrogenase
0462	TD01_00462	3-hydroxybutyryl-CoA dehydrogenase
1315	TD01_01315	aldehyde dehydrogenase
1555	TD01_01555	3-hydroxyacyl-CoA dehydrogenase
1564	TD01_01564	enoyl-CoA hydratase
2829	TD01_02829	3-hydroxybutyryl-CoA dehydrogenase

Supplementary Figures

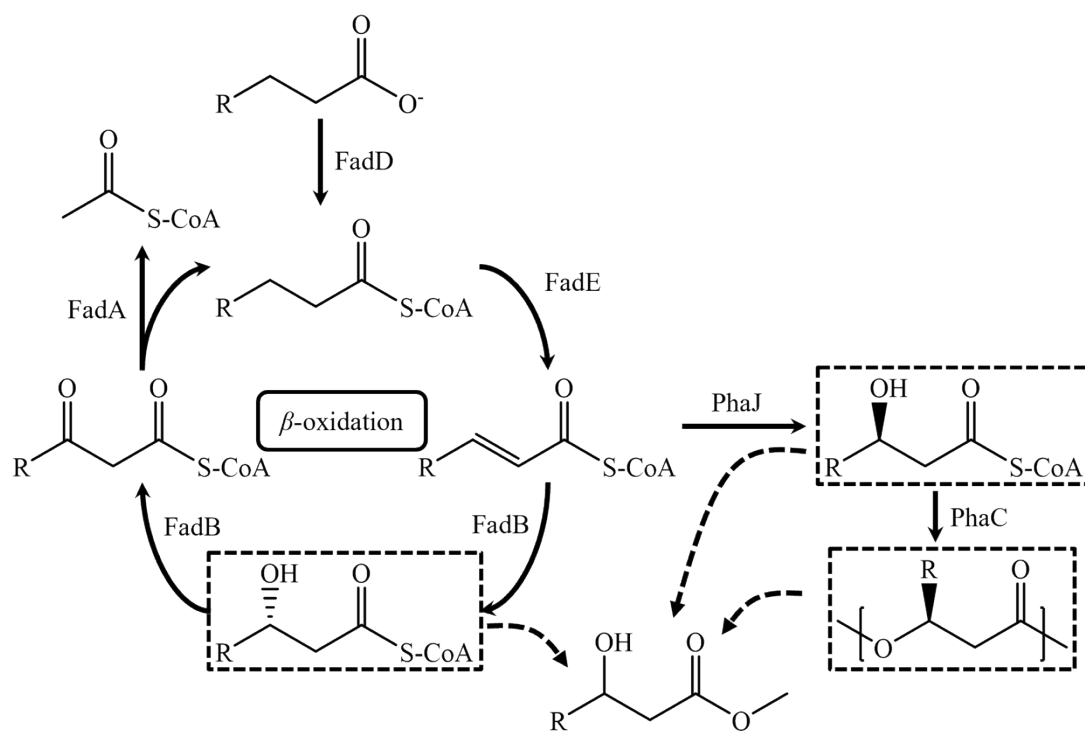


Fig. S1 Three possible sources of detected products

The substances in the dashed box may transform into the same product after methylation.

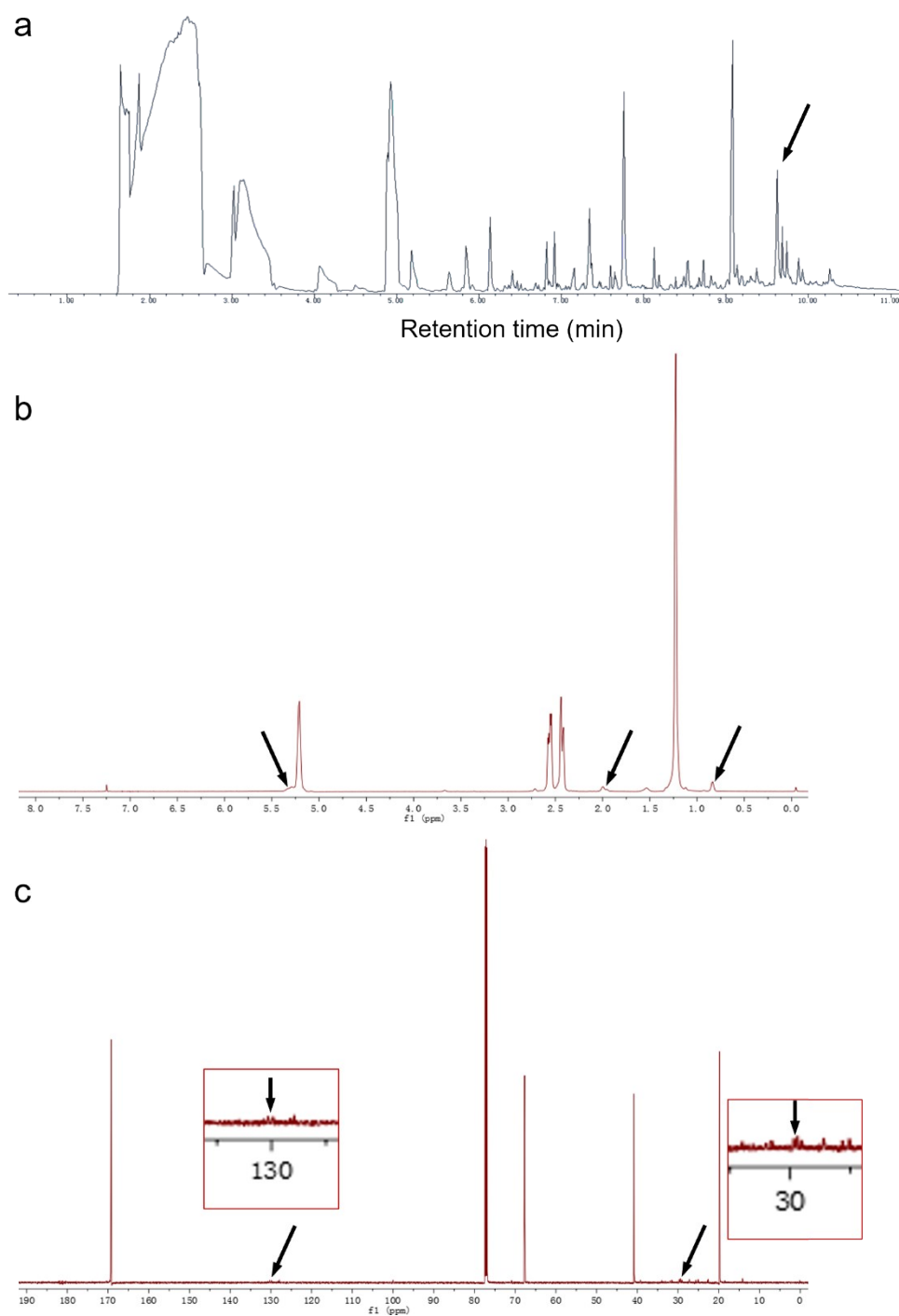


Fig. S2 Analysis of purified PHA material.

(a) GC-MS study of PHA material. (b) ^1H NMR study of the PHA material. (c) ^{13}C NMR study of the material. The arrows represent the characteristic peaks of P3H9Od.

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1      10      20      30      40
PhaC11317 . . . . . MSDKNNEDLKRQASENTLGLNPVIGIRGKDLLT[SARMVLAQALKQPFHS
PhaC1437   . . . . . MSNKSNDLKYQASENTLGLNPVVGRLGKDLLA[SARMVLRQAIKQPVHS
PhaC21317 . . . . . MRDKPNSGALPTPATFMNAQSAVVVGVRGDL[TVRLLAVQGLKHPVRS
PhaC4AK4   MSQPSYGP[LF[EA[LAHYNDKLLAMAKAQTERTAQALLQTNLDDL[QVLEQGSQQPWLIQA
PhaCBc     . . . . .
PhaCPs-STQK . . . . . MSNKNSSDDNRASNTGNVGRGKDT[SARMTAKHSVKHVAH

50     60     70     80     90     100
PhaC11317 AKHVAHFGLELKNVVFQSELK . . . . . PEDGDRRFADPAWSQ[NPLT[RRYL[QTYL[AWRK
PhaC1437   VKHVAHFGLELKNVLLGKSGLQ . . . . . FTSDDRRFADPAWSQ[NPLT[RRYL[QTYL[AWRK
PhaC21317 SRHL[LA[FGGLGRVLLGDTLHK . . . . . TNPQDARFADFTWQ[NPFT[RRSL[QAYL[AWRK
PhaC4AK4   QMNWWDQLKLMQHTLLKSAGQQSEFVITPERSDRRFKA[EWSE[QPIYDYLK[QSYLLTAR
PhaCBc     . . . . .
PhaCPs-STQK GKNVMGK . . . . . SKSDDRR[NDA[SNYKR[RYTY[AWRK

110     120     130     140     150     160
PhaC11317 E[LDHWI[E[HS[.LSEQDAS[RCH[FEVIN[LMT[EA[AP[SN[MAN.PAAVKRFFETGGKSLLDGMS
PhaC1437   E[LDHWI[D[ES[.LAPKDVAR[CH[FEVIN[LMTDAM[AP[NT[AN.PAAVKRFFETGGKSLLDGLS
PhaC21317 QLGAWI[D[SE[.LSADDRAR[AR[FLAA[ILSDAL[AP[SN[LLN.PLALKELFNSGGSSLFKGM
PhaC4AK4   HLLASV[DALGQVQKSRER[LR[FFTRQYVNAM[AP[SN[LATNPELLKLTLESQGQNLVRGLA
PhaCBc     QLELYP[EYR[. . . . . KAYRRV[KRASE[ILCEP[EPC[. . . . . MTTFATEWEK
PhaCPs-STQK HDWGN[S[SDN[. . . . . RAH[VTMT[AMATNS[AANAAV[KR[. . . . .

170     180     190     200     210
PhaC11317 HLA[KDMINNGMP[. . . . . SQVNMAAFEVGKN[LATTEGA[VVF[RNDVLE[LIQV[KPITE[SVHER[PL
PhaC1437   HLA[KDLVHNGMP[. . . . . SQVNMGAFEVGN[LGVT[EGA[VVF[RNDVLE[LIQV[KPITE[QVYER[PL
PhaC21317 HLLDDLLHNGLP[. . . . . SQVSKHAFEVGRN[LACT[PGA[VVF[RNELLE[LIQV[RPMSEKQYVR[PL
PhaC4AK4   LLAEDLERSADQLNIRLTDES[AFELGRD[LATTPGR[VVQ[RT[ELYELIQV[SPITTE[TVGKT[PV
PhaCBc     . . . . . VGLTPKE[VIT[KNKTKLYR[VIP[KO[KTORVPI
PhaCPs-STQK . . . . . TGGKS[DGTH[AKDVNN[GGMSV[DMGAVGKS[GT

220     230     240     250     260     270
PhaC11317 L[V]P[PQ[IN[KFYV[F]DLSPDK[S]LAR[F]LRSQV[QT[FVVS]WRN[PTKAQREWGLST[Y]IAA[.LKE[A
PhaC1437   L[V]P[PQ[IN[KFYV[F]DLSPDK[S]LAR[F]CLRN[V]QT[FIVS]WRN[PTKEQREWGLST[Y]IEA[.LKE[A
PhaC21317 L[V]P[PQ[IN[KYI[F]DL[SN]DK[SE]VQ[YALKNGL[QT[FMI]S]WRN[PDARHREWGLSS[Y]VQA[.VEQ[A
PhaC4AK4   L[V]P[P[F]IN[KYI[IM]DMRPQN[SLVA]WLVAQGG[TVFMI]S]WRN[PSVAQAQIDLD[DV]VDG[VIA[A
PhaCBc     LLLYAL[IN[KYI[IM]DLTPGN[SLVE]YLVD[RGFDV]YMLD[WGT]FGL[EDSHLKFDD[FV]FDY[IA[A
PhaCPs-STQK TGA[VVR[EDV]RT[V]HRVVN[KY]VDS[K]SARC[SNNTV]S]WRN[TKARWG[. . . . . STY[D

280     290     300     310     320     330
PhaC11317 T[EV]T[CAIT]G[S]K[DVN]ML[C]AC[S]G[LT]TAS[L]CHY[A]ALGEO[KV]HALTLLV[S]VLD[TC]LDTQVA
PhaC1437   VDVV[T]AIT[G]S[K]DVNML[C]AC[S]G[IT]TALLGHY[A]AIGEN[KV]NALTLLV[T]VLD[TC]LDSOVA
PhaC21317 VDA[CR]AIT[G]S[K]DVNML[C]AC[S]G[LT]TAA[LO]GHLOAKROL[KV]ASATYMV[S]LDSQIDSPAM
PhaC4AK4   LDG[VE]AAT[G]ER[E]VHGIC[YG]CIG[GT]ALS[L]AMG[WLA]RRQK[R]VRTATLFT[TL]DFFSOPGELG
PhaCBc     VKKVMRTAK[ED]EIS[LL]CYCMG[. . . . . GTLTSIY[A]ALHHPMP[IR]NLIFMT[SP]DFSETGLYG
PhaCPs-STQK A[K]V[VD]VVS[AT]G[S]KDN[M]CA[CS]G[. . . . . TCTAGHY[A]A[. . . . . GK[V]NATVTVDTTDS[. . . . .

340     350     360     370     380     390
PhaC11317 L[F]AD[E]K[LE]A[A]KRRSYQAG[V]IEGSDMAKVFA[W]MRP[N]DLI[W]N[Y]VWNNY[LL]GNE[. . . . . PPVFD
PhaC1437   L[F]VNE[Q]T[LE]A[A]KRRSYQAG[V]IEGRDMAKVFA[W]MRP[N]DLI[W]N[Y]VWNNY[LL]GNE[. . . . . PPVFD
PhaC21317 L[F]ADE[Q]T[LE]A[A]KRRSYQAG[V]IDGRDMAKVFA[W]MRP[N]DLI[W]N[Y]VWNNY[LL]GKQ[. . . . . PPAFD
PhaC4AK4   IFTH[E]P[IT]A[A]EAQNEAKG[IM]DGRQLAVSFS[L]RENSLY[W]N[Y]YID[S]YLGQS[. . . . . PPAFD
PhaCBc     PLLDE[KY]FNLD[KAVD]TFGN[IP]PEMID[F]GNK[MLK]PIT[IN]FVGP[V]VALVDRSENERFVESWRL
PhaCPs-STQK . . . . . V[AD]V[K]TAAKRHS[YA]GVGRD[MAKVFA[W]MRN[. . . . . D[W]N[Y]VWNNY[LL]G[. . . . .

400     410     420     430     440     450
PhaC11317 I[LY]WNNN[DT]TR[LP]AALHGEF[T]EMFQTNPLTRPGA[TE]VCGT[P]IDLKQV[TC]DFFC[V]AGTT[D]HI
PhaC1437   I[LY]WNNN[DT]TR[LP]AALHGD[L]VLFKNNPLIRPNA[LE]VCGT[P]IDLKQV[TAD]IFSLAGTND[DH]I
PhaC21317 I[LY]WNNN[NAR]LP[AL]HGD[L]IDFFKHNPLSRAGGLE[VC]GT[P]VDLSK[VAVDSFS]VAGIND[DH]I
PhaC4AK4   LLHWN[S]DSTNVAGKTHNSL[LR]LYLENQLVKGE[LK]IRN[TR]IDLKGVKTPVLL[VSAVD]DH[I]
PhaCBc     VQK[W]VG[G]IF[P]GESYRQW[IR]DFYQNNKLVKGEL[V]IRGQ[V]DLAN[IKANV]LN[ISGKR]DH[I]
PhaCPs-STQK N[V]D[W]NN[DT]TR[A]AHGDMKNN[VRAN[. . . . . AVSGT[DKVTAD]YSAGTNDH[TWKS]CYK

460     470     480     490     500     510
PhaC11317 TPWDS[CYKS]A[HL]FG[.GKCE[FVL]SN[SGHI]QS[TLN]PFGNPKARYMTNSEMPADPKAWQESS
PhaC1437   TPWKS[CYKS]A[HL]FG[.GNVE[FVL]SS[GGHI]KS[TLN]PFGNPKSRYMTST[EA]ENADEWQANAT
PhaC21317 TPWDA[VYRS]A[LL]LG[.GERR[FIL]SN[SGHI]QS[TLN]PFGNPKARYYENGKLTSDPRAWYHDAR
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PhaCBc     ALPQV[EA]LDHISST[DKQ]VVC[ET]GHS[IV]YGGTAVKQT[. . . . .
PhaCPs-STQK SAGGKVVS[SGH]XSN[GNKS]RYM[TS]TDMATAN[NS]TKHTDS[. . . . .

520     530     540     550
PhaC11317 KHADSW[WLHW]QS[W]LAE[R]SGGKTKNAPTALGNKKFPAGEA[APG]TYVHER[. . . . .
PhaC1437   KHTDSW[WLHW]QA[W]QAOR[SG]ELKKSPTKLGS[KAY]PAGEA[APG]TYVHER[. . . . .
PhaC21317 RVQGSW[WPQW]LE[W]IQA[RS]GEQRETLALGDQKHPQEA[APG]TYVHIR[. . . . .
PhaC4AK4   HQSGSW[WP]EMMG[F]IQSR[DE]GSEPVPARVP[EE]GLAP[. . . . . APGHYV[V]K[VRLNPVFASATEEDA
PhaCBc     . . . . . YPTIGN[WLE]ERSN[. . . . .
PhaCPs-STQK . . . . . WWH[W]A[WA]RS[GKKKS[. . . . . TSGNKAYSGA[AG]TYVHR[. . . . .

PhaC11317 .
PhaC1437 .
PhaC21317 .
PhaC4AK4 A
PhaCBc .
PhaCPs-STQK .

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Fig. S3 The protein sequences and homology of six PhaCs selected.
Amino acids with red highlights and white text are identical amino acids, and those in red text are amino acids with similar properties.

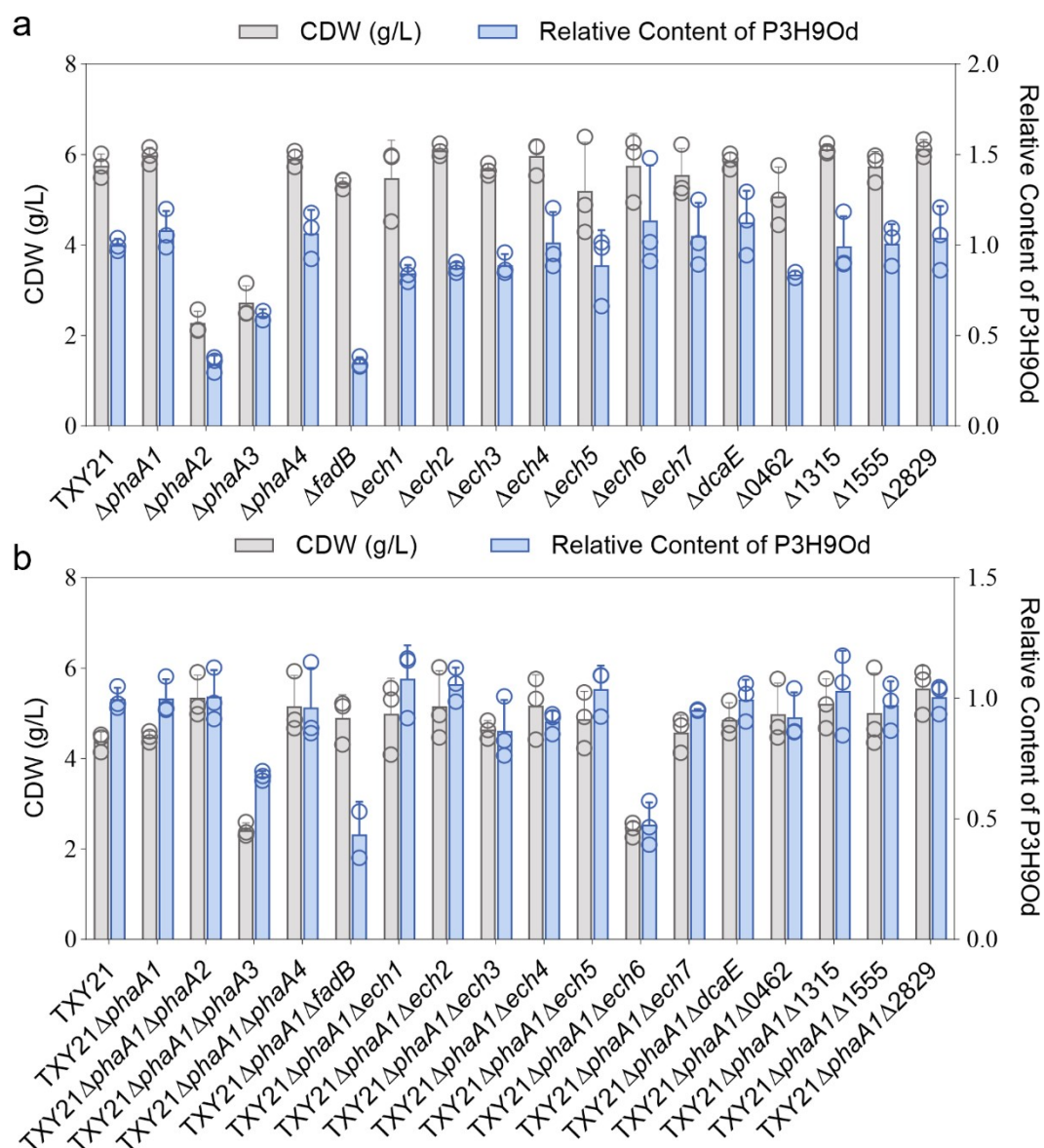


Fig. S4 Additional deletion of β -oxidation related genes.

(a) Gene deletion from TXY21. (b) Gene deletion from TXY21 Δ phaA1.

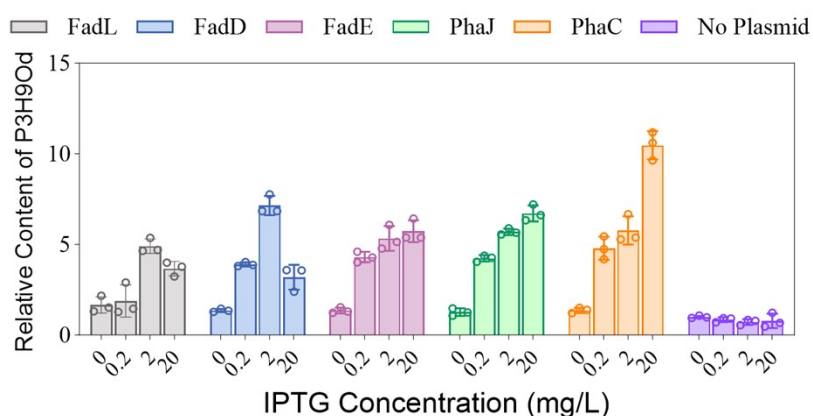


Fig. S5 Overexpression of enzymes involved in the P3HB3H9Od synthesis pathway in *H. bluephagenesis* TXY26 harboring pSEVA341- $P_{Re-phaC_{Ps-STQKopt}}$

References

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