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Supporting information for

Biosynthesis and characterization of medium-chain-length polyhydroxyalkanoate with enriched 3-hydroxydodecanoate monomer from a *Pseudomonas chlororaphis* cell factory

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Table Captions

Table S1. Primers used in this study.

Table S2. Cell dry weight (CDW), mcl-PHA content, and monomer composition of stepwise genetic engineering *P. chlororaphis* HT66 strains.

Table S3. Thermal and mechanical properties of mcl-PHAs synthesized by HT3Δ, HT4Δ, and HT4Δ::*C1C2J*, respectively.

Table S4. Comparison of mcl-PHA production using different carbon sources by *Pseudomonas*.

Figure captions

Figure S1. ^{13}C-NMR spectrum of mcl-PHA synthesized by HT4Δ::*C1C2J* at 600 MHz.

Figure S2. FTIR spectrum of mcl-PHA synthesized by HT4Δ::*C1C2J*.

Table S1.

Primers	Primer sequences (5'→3')	
phzE-F1	GGAATTCCCCCTGCGTGAACAAGTGGTGA	
phzE-R1	AGCACCACCTCGTCGGTGGT	
phzE-F2	ACCACCGACGAGGTGGTGCTCCATGCACCATTACGTCATCATC	*phzE* deletion
phzE-R2	CGGGATCCCGGCAGGCCGACAAACACATGA	
fadAB-F1	CCGGAATTCGTGCGTGCCCGTCTGAAGTG	
fadAB-R1	TCTTCGGCACGGTTTTCCTT	
fadAB-F2	GAAAACCGTGCCGAAGAGAAAGCCTGGCAGCCCTCAA	*fadAB* deletion
fadAB-R2	TGCTCTAGACGCACCCAGAAGCCGTAATC	
phaZ-F1	GGCGAATTCGCGGGCATATCCAAAGCATC	
phaZ-R1	CCTGGACGAACGGGAACACC	*phaZ*
phaZ-F2	TGTTCCCGTTCGTCCAGGACGACGGGCATCTGTTCC	deletion
phaZ-R2	GGCAAGCTTCTGTCCTGGGGGTTGGGCTT	
phaC1-sF	GAGGTCGACGGTATCGATAAGCTTATGAGTAACAAGAATAACGATGACCTG	
phaC1-sR	CACCGCGGTGGCGGCCGCTCTAGATTAACGTTCATGGACATAAGTACCC	
phaC2-sF	GAGGTCGACGGTATCGATAAGCTTATGCGAGACAAACCAACGGCG	
phaC2-sR	CACCGCGGTGGCGGCCGCTCTAGATCAGCGGACGCGCACGTAG	
phaG-sF	GAGGTCGACGGTATCGATAAGCTTATGAGGCCAGAAATCGCTGT	single gene
phaG-sR	CACCGCGGTGGCGGCCGCTCTAGATCAGATAGCCAGGGCATGGT	overexpression
phaJ4-sF	GAGGTCGACGGTATCGATAAGCTTATGCCGTATGTTCCTGTTGCAG	
phaJ4-sR	CACCGCGGTGGCGGCCGCTCTAGATCAGACAAAGCAGAGGGACAATG	
alkK-sF	GAGGTCGACGGTATCGATAAGCTTATGTTGCAGACTCGCGTTATTC	
alkK-sR	CACCGCGGTGGCGGCCGCTCTAGATTACAGCGTCGAAAGAAAGGTG	
phaC1-cF	CTGGGTACCGGGCCCCCCCTCGAGATGAGTAACAAGAATAACGATGACCTG	
phaC1-cR	CTGCAGGAATTCGATATCAAGCTTTTAACGTTCATGGACATAAGTACCC	multiple gene
phaC2-cF	ATACAGGTACTTGCAATTAAGCTTATGCGAGACAAACCAACGGCG	co-overexpression
phaC2-cR	CCCGGGCTGCAGGAATTCTCAGCGGACGCGCACGTAG	

phaG-cF	GAATTCCTGCAGCCCGGGGGATCCATGAGGCCAGAA ATCGCTGT
phaG-cR	CACCGCGGTGGCGGCCGCTCTAGATCAGATAGCCAG GGCATGGT
phaJ4-cF	GAATTCCTGCAGCCCGGGGGATCCATGCCGTATGTTC CTGTTGCAG
phaJ4-cR	CACCGCGGTGGCGGCCGCTCTAGATCAGACAAAGCA GAGGGACAATG
alkK-cF	GAATTCCTGCAGCCCGGGGGATCCATGTTGCAGACTC GCGTTATTC
alkK-cR	CACCGCGGTGGCGGCCGCTCTAGATTACAGCGTCGA AAGAAAGGTG

Table S2.

Strains	Medium	CDW (g/L)	Mcl-PHA content (wt%)	Monomer composition (mol%)				
				3HHx	3HO	3HD	3HDD	3HTD
HT66	KB, glucose, C12	12.71 ± 0.53	45.61 ± 0.87	0.32 ± 0.02	10.95 ± 0.49	39.78 ± 0.31	43.34 ± 2.74	5.61 ± 0.63
HT66Δ*phzE*	KB, glucose, C12	13.73 ± 1.05	45.73 ± 2.42	0.33 ± 0.07	12.51 ± 0.06	38.26 ± 0.46	43.55 ± 1.77	5.35 ± 0.03
HT66Δ*fadAB*	KB, glucose, C12	14.69 ± 0.37	51.53 ± 0.92	0.21 ± 0.03	4.53 ± 0.26	34.99 ± 0.50	55.53 ± 2.93	4.74 ± 0.19
HT3Δ	KB, glucose, C12	16.54 ± 0.43	67.82 ± 1.73	0.22 ± 0.01	3.41 ± 0.14	31.89 ± 0.45	58.22 ± 1.53	6.26 ± 0.21
HT4Δ	KB, glucose, C12	16.83 ± 0.20	72.44 ± 3.01	0.08 ± 0.01	3.33 ± 0.23	29.65 ± 0.51	62.17 ± 1.01	4.75 ± 0.19
HT4Δ::*C1*	KB, glucose, C12	18.42 ± 0.78	64.60 ± 1.66	0.42 ± 0.03	3.61 ± 0.17	25.82 ± 0.22	64.25 ± 0.55	5.90 ± 0.29
HT4Δ::*C1C2J*	KB, glucose, C12	18.21 ± 1.36	84.89 ± 1.15	0.05 ± 0.01	1.78 ± 0.11	17.75 ± 0.71	71.64 ± 1.02	8.78 ± 0.31

Table S3.

	Thermal property		Mechanical property		
	T_m(˚C)	T_g(˚C)	E (MPa)	σ_{mt} (MPa)	ε_b (%)
HT3Δ	59.2	-40.7	6.6	4.0	159.4
HT4Δ	60.7	-38.4	12.4	3.4	127.6
HT4Δ::*C1C2J*	67.3	-34.2	47.7	2.3	27.2

Note: T_m: melting temperature; T_g: glass transition temperature; E: Young's modulus; σ_{mt}: maximum tension strength; ε_b: elongation at break.

Table S4.

Strains	Carbon sources	Medium type	Method	Dry cell weight (g/L)	PHA content (wt%)	References
P. putida KTQQ20	Tetradecanoic acid	Rich, LB medium	Inhibition of β-oxidation and deletion of *phaG*	0.6	77.5	[1]
P. putida CA-3	Volatile fatty acids	Minimum, MS medium		1.6	39	[2]
P. mendocina NKU-Δβ5	Decanoate	Rich, LB medium	Inhibition of β-oxidation, deletion of *phaG* and *phaZ*	2.3	48.1	[3]
P. putida KTMQ01	Sodium octanoate	Minimum, MS medium	Deletion of *phaZ*	4	86.5	[4]
P. entomophila LAC03	Dodecanoic Acid	Rich, LB medium	Inhibition of β-oxidation	9.9	51.9	[5]
P. chlororaphis HT4Δ::*C1C2J*	Glucose, dodecanoic acid	Rich, KB medium	Inhibition of β-oxidation, deletion of *phzE* and *phaZ*, overexpression of *phaC1*, *phaC2* and *phaJ*	18.2	84.9	This study
P. mosselii TO7	Crude glycerol	Minimum, MS medium	Fed-batch	1.3	48.4	[6]
P. aeruginosa BP C1	Glucose	Minimum, MS medium		7	14.7	[7]
P. chlororaphis DSM19603	Glycerol	Minimum, medium E	Fed-batch	11.8	19	[8]
P. putida WJPP03	Dodecanoic acid	Rich, LB medium	Batch, genome reduction	6.2	63.1	[9]
Pseudomonas sp.	Corn oil	Minimum, MS medium	Batch	12.5	35.6	[10]
P. citronellolis	Tallow-based biodiesel	Minimum, MS medium	Fed-batch	42	26.6	[11]
Pseudomonas sp. ASC2	Crude glycerol	Minimum, MS medium	Batch	32.3	61.8	[12]

Figure S1.

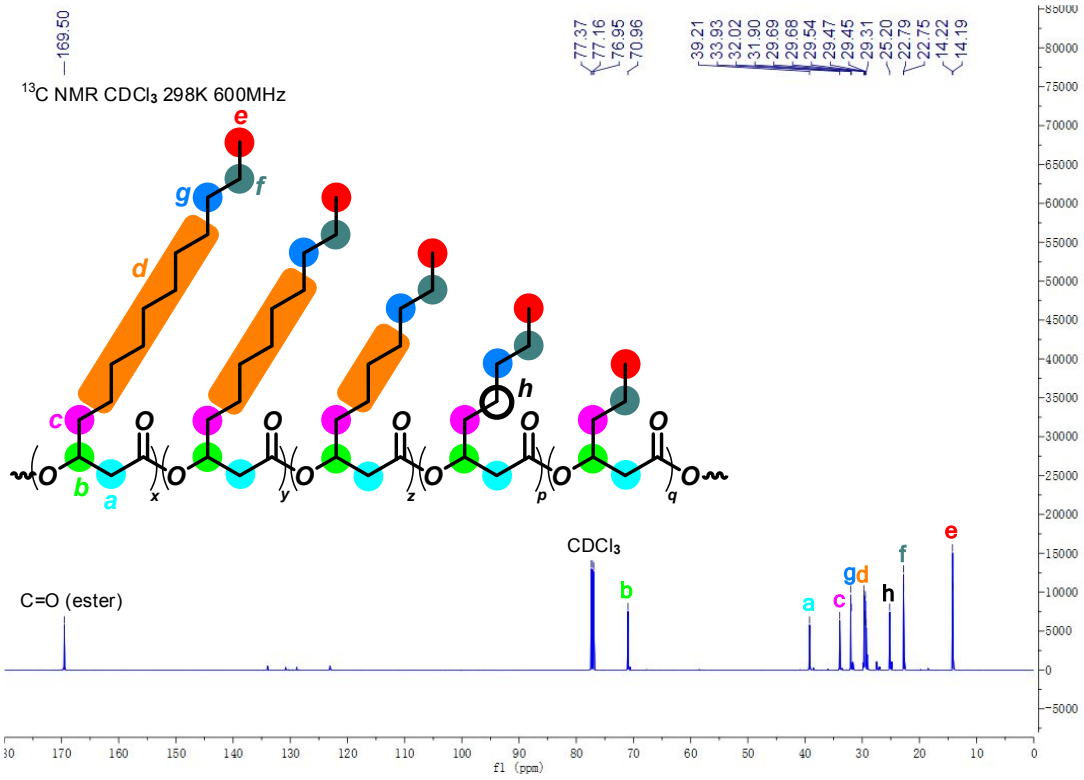
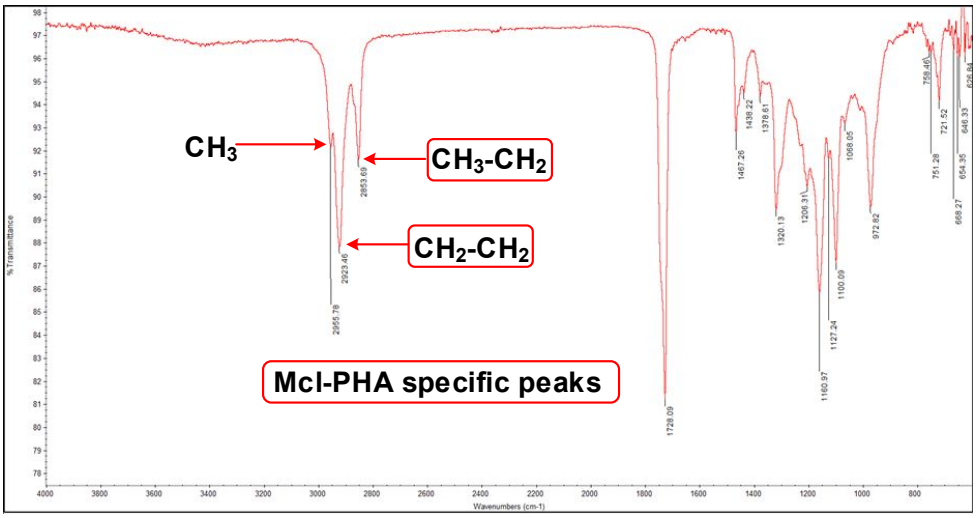

169.50
77.37
77.16
76.95
70.96
39.21
33.93
32.02
31.90
29.69
29.68
29.54
29.47
29.45
29.31
25.20
22.79
22.75
14.22
14.19
13C NMR CDCl3 298K 600MHz
e
g
f
d
c
h
b
a
x
y
z
p
q
CDCl3
C=O (ester)
b
a
c
g
d
h
f
e
f1 (ppm)

Figure S2.


CH3
CH3-CH2
CH2-CH2
Mcl-PHA specific peaks
% Transmittance
Wavenumbers (cm-1)

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