



Title	Synergy of valine and threonine supplementation on poly(2-hydroxybutyrate-block-3-hydroxybutyrate) synthesis in engineered Escherichia coli expressing chimeric polyhydroxyalkanoate synthase
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Citation	Journal of Bioscience and Bioengineering, 129(3), 302-306 https://doi.org/10.1016/j.jbiosc.2019.09.018
Issue Date	2020-03
Doc URL	https://hdl.handle.net/2115/80504
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Type	journal article
File Information	Supplementary information-2 (Matsumoto).pdf



Supplementary information

to

Synergy of valine and threonine supplementation on poly(2-hydroxybutyrate-*block*-3-hydroxybutyrate) synthesis in engineered *Escherichia coli* expressing chimeric polyhydroxyalkanoate synthase

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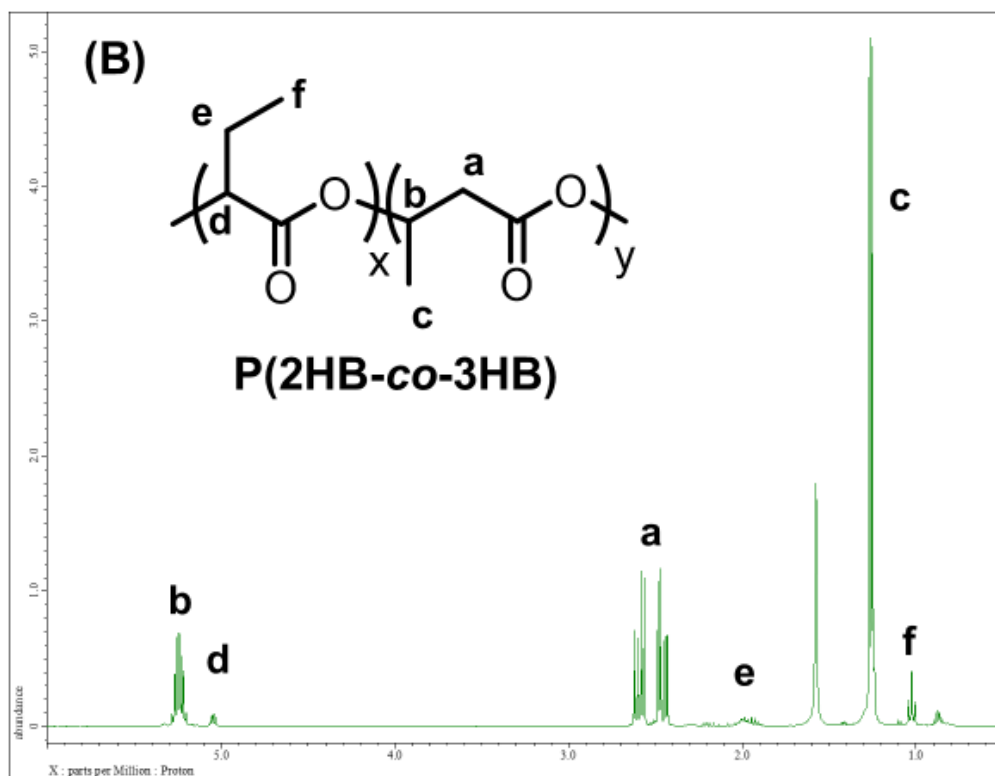
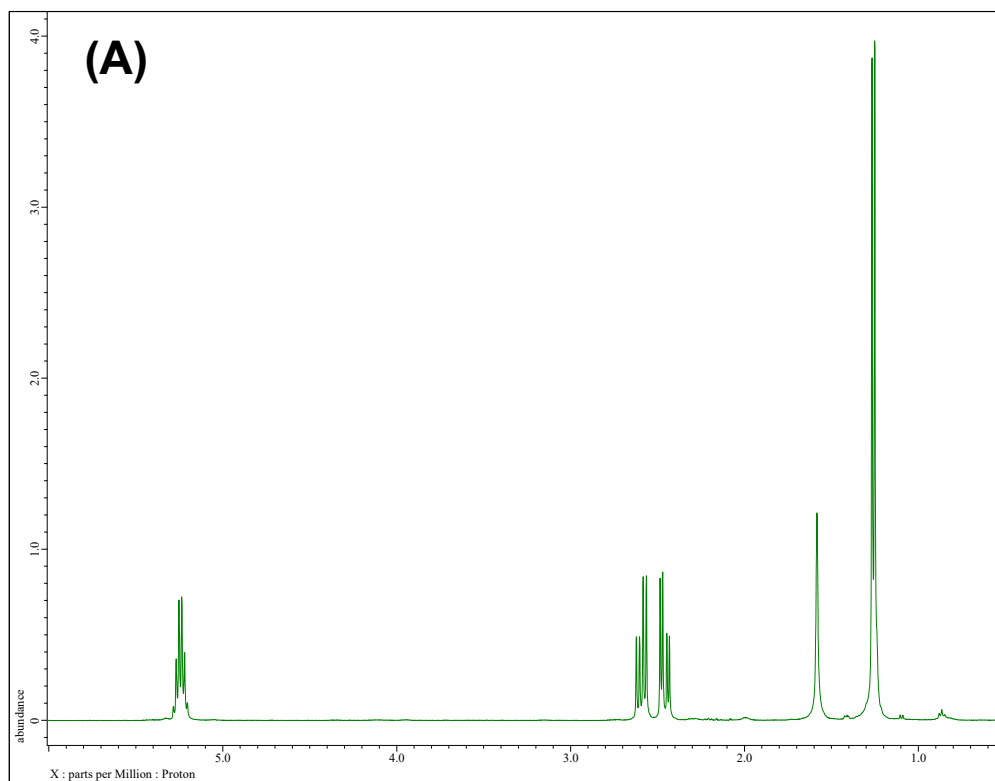
Ken'ichiro Matsumoto^{1,*}

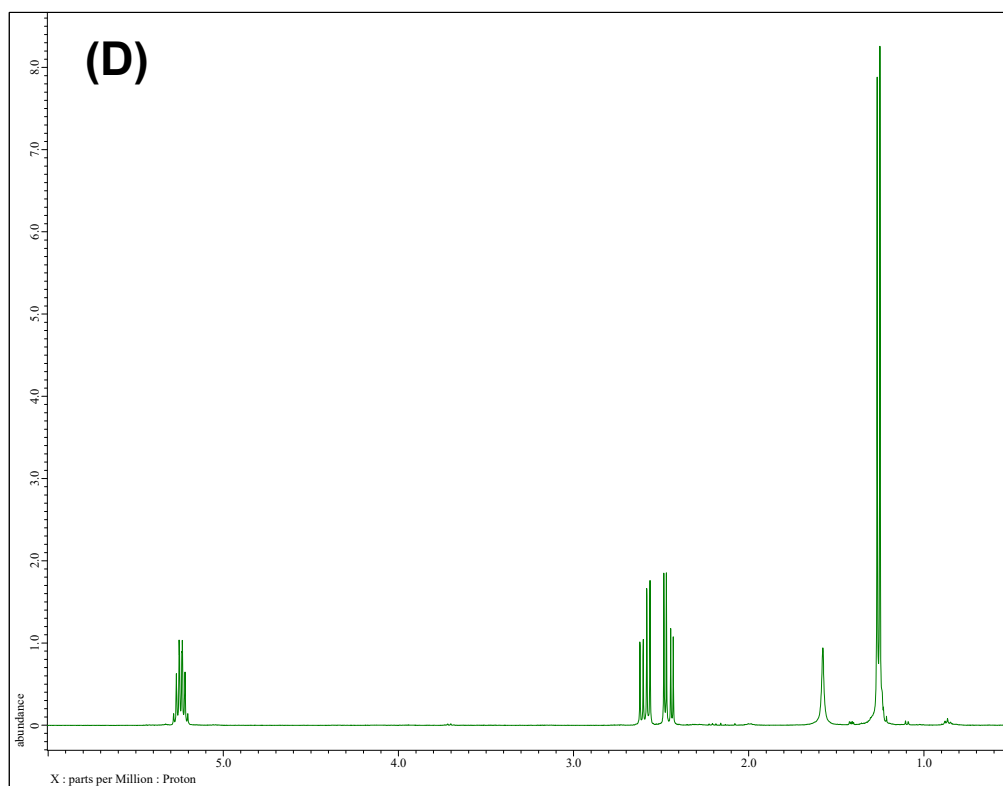
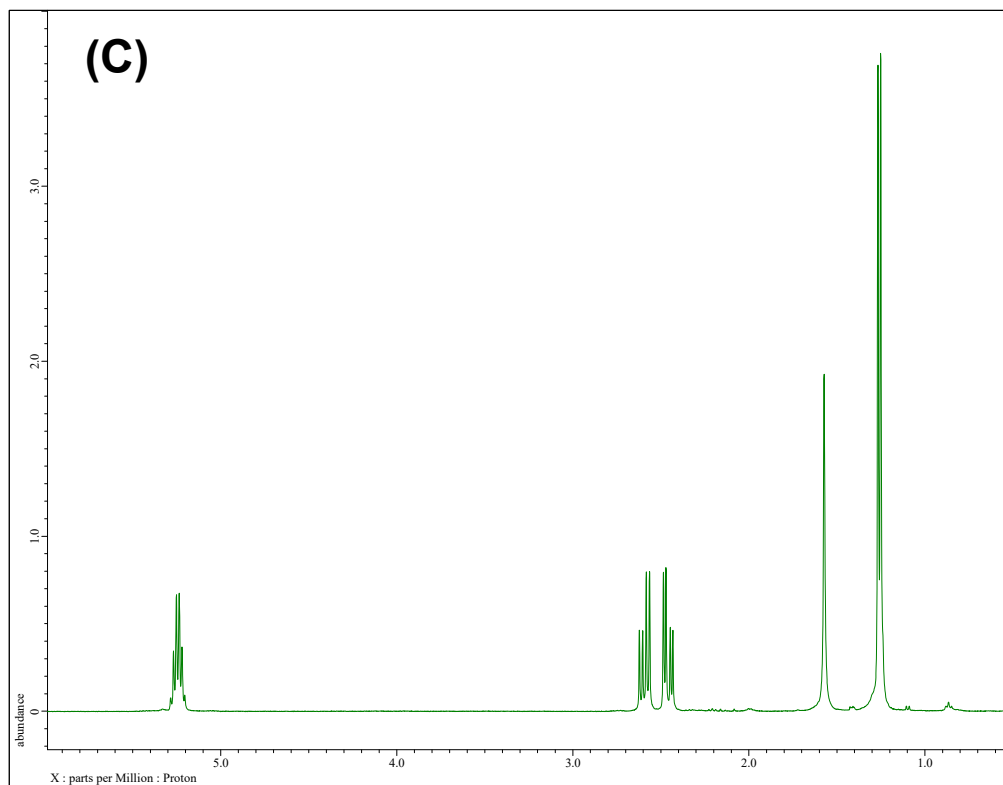
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Supplementary FIG. S1. Proton nuclear magnetic resonance (^1H NMR) analysis of the polymers synthesized in *Escherichia coli* harboring pBBR1MCS2C_{ARAB} and pTTQ19ldhAhadA_{Cd_opt}. The polymers were synthesized with supplementation of 1 g/L each of leucine (A), valine (B), isoleucine (C), and phenylalanine (D).

Supplementary Table S1. Polymer production after supplementation with four hydrophobic amino acids (leucine, valine, isoleucine, and phenylalanine) in a shake flask^a

Amino acid (1 g/L)	CDW (g/L)	Polymer production (g/L)	2HB production (g/L)	Monomer composition (mol%)	
				2HB	3HB
none	2.74	1.18	0.99	0.8	99.2
Leu	1.67	1.63	0.95	0.6	99.4
Val	0.40	0.18	1.52	8.3	91.7
Ile	2.72	1.34	0.04	0.03	99.97
Phe	2.24	0.94	0.59	0.6	99.4

^aPolymers were synthesized using recombinant *Escherichia coli* harboring pBBR1MCS2C_{ARAB} and pTTQ19ldhAhadA_{Cd_opt} with supplementation of 1 g/L of each amino acid in a shake flask. 2-Hydroxybutyrate (2HB) production and monomer composition in the cells were determined by ^1H NMR.

Supplementary Table S2. Polymer production in *Escherichia coli* with supplementation of hydroxy-4-methylvalerate (2H4MV), 2-hydroxy-3-phenylpropionate (2H3PhP), and 2-hydroxyhexanate (2HHx)^a

Precursor	precursor conc. (g/L) ^b	Polymer production (g/L)	Monomer composition (mol%) ^c			
			3HB	2H4MV	2H3PhP	2HHx
none	0	0.10	100	0	0	0
2HHx	1	0.02	100	0	0	trace
2H4MV	1	0.17	100	0	0	0
2H3PhP	1	0.10	100	0	0	0

^aPolymers were synthesized using *E. coli* harboring pBBR1MCS2C_{ARAB} and pTTQ19ldhAhadA_{Cd_opt} in a test tube. ^bThe weight of the sodium salts. ^cPolymer production and polymer composition were determined by gas chromatography–mass

spectrometry (GC–MS). 3HB, 3-hydroxybutyrate.