Supporting Information

Biosynthesis of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) from glucose by *Escherichia coli* through butyryl-CoA formation driven by Ccr-Emd combination

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

Strain or plasmid	Relevant marker	Source or reference
Escherichia coli		
DH5α	F$^-$, φ80*lacZ*ΔM15, Δ(*lacZYA-argF*) U169, *deoR*, *recA*1, *endA*1, *hsdR*17(r_K^-, m_K^+), *phoA*, *supE*44, λ^-, *thi*-1, *gyrA*96, *relA*1	Lab stock
JM109	*endA*1, *recA*1, *gyrA*96, *thi*-1, *hsdR*17(r_K^- m_K^+), *relA*1, *supE*44, Δ(*lac-proAB*), F'[*traD*36, *proAB*, *lacI^q*, *lacZ*ΔM15]	Lab stock
BW25113	*rrnB*, Δ*lacZ*4787, *hsdR*514, Δ*araBAD*567, Δ*rhaBAD568*, *rph*-1	Datsenko and Wanner (2000)
JWΔcra	JW0078-KC (BW25113 Δ*cra*::Km^r) derivative, Δ*cra*::FRT	This study
JWΔpdhR	JW0109-KC (BW25113 Δ*pdhR*::Km^r) derivative, Δ*pdhR*::FRT	This study
JWΔrng	JW3216-KC (BW25113 Δ*rng*::Km^r) derivative, Δ*rng*::FRT	This study
JWΔcraΔrng	JWΔcra derivative, Δ*rng*::FRT	This study
JWΔpgi	JW3985-KC (BW25113 Δ*pgi*::Km^r) derivative, Δ*pgi*::FRT	This study
JWΔpgiΔrng	JWΔpgi derivative, Δ*rng*::FRT	This study
JWΔpta	JW2294-KC (BW25113 Δ*pta*::Km^r) derivative, Δ*pta*::FRT	This study
JWΔptaΔpoxB	JWΔpta derivative, Δ*poxB*::FRT	This study
Plasmids		
pBKS-PCJAB	pBluescript II KS(+) derivative; P_{lac}-P_{Ac}-*phaP*$_{D4N}$*C*$_{NSDG}$*J*$_{Ac}$, P_{Re}-*phaAB1*$_{Re}$	This study
pBKS-PCJA	pBluescript II KS(+) derivative; P_{lac}-P_{Ac}-*phaP*$_{D4N}$*C*$_{NSDG}$*J*$_{Ac}$, P_{Re}-*phaA*$_{Re}$	This study
pBBRtac	pBBR1-MCS2 derivative; P_{tac}	Fukui et al. 2009
pBtac-CJ$_{Re}$E	pBBRtac derivative, P_{tac}-*ccr*$_{Me}$-*phaJ4a*$_{Re}$-*emd*$_{Mm}$	This study
pBtac-CJ$_{Re}$EB	pBBRtac derivative, P_{tac}-*ccr*$_{Me}$-*phaJ4a*$_{Re}$-*emd*$_{Mm}$-*bktB*$_{Re}$	This study
pBtac-CJ4$_{Pa}$E	pBBRtac derivative, P_{tac}-*ccr*$_{Me}$-*phaJ4*$_{Pa}$-*emd*$_{Mm}$-*bktB*$_{Re}$	This study
pSTV-HC	pSTV28 derivative; P_{tac}-*had*$_{Re}$-*crt2*$_{Re}$	This study
pSTV-HCB	pSTV28 derivative; P_{tac}-*had*$_{Re}$-*crt2*$_{Re}$-*bktB*$_{Re}$	This study
pSTV-PCB	pSTV28 derivative; P_{tac}-*paaH1*$_{Re}$-*crt2*$_{Re}$-*bktB*$_{Re}$	This study
pMW-Gm-ptxD$_{EAAR}$ABC	pMW218 derivative, ΔKm^r::Gm^r, *ptxD*$_{EAAR}$-*ptxABC*	This study

FRT, FLP recombinase target*; Ac*, *Aeromonas caviae*; *Me*, *Methylorubrum extorquen*s; *Mm*, *Mus musculus*; *Re*, *Ralstonia eutropha*.

Supplementary Table S2. The sequences of primers used in this study.

Primer	Sequence (5'-3')	Note
Construction of plasmids		
del-phaB1-inv5	CCTGCCGGCCTGGTTCAACCAGTCG	For construction of pBKS-PCJA
del-phaB1-inv3	GTCCACTCCTTGATTGGCTTCGTTA	
del-PphaP-inv5	AAAGAGGAGAAATTAACTATGGCTGCAAGCGCAGCACCGGCCT	For construction of pBtac-CJ$_{Re}$E
del-PphaP-inv3	GGGGGTGGGCGAAGAACTCCAGCAT	
tac-5	TTGAATTCGAGCTCAATATTCTGAAATGAGCTGTTGA	
tac-Rv	GGTTCAATTCTGTTTCCTGTG	
bktB-Fw1	CGCTTCGAATCTAGAAAGGAGGC	For construction of pBtac-CJ$_{Re}$EB
bktB-Rv1	ATCCACCCCTTCCTCAGATACGC	
phaJ4a-Inv1	GTCGATAGTCTCCTCTTGACGATAAAGC	For construction of pBtac-CJ4$_{Pa}$E
phaJ4a-Inv2	GGATCCGTTTTTTTGGGCTAGCAGGAGGA	
phaJ4Pa_N	ATGCCATTCGTACCCGTAGCA	
phaJ4Pa_C	TCAGACGAAGCAGAGGCTGA	
A0602-F	ATGCAAATCCAAGGCAACGTATTCA	For construction of pSTV-HC
A0602-R-Fus	ATGTATTTGCCTTTACTTGGGCTGCATCCGGA	
A3307-R	GCCTTAGCGATGCTGGAAATT	
A3307-F-Fus	CCAAGTAAAGGCAAATACATAGGAGAAGACA	
pSTV-inv5	TATCGATGATAAGCTGTCAAACA	
pSTV-inv3	CATTAATGAATCGGCCAACGCGC	
pSTV-inv5_2	GGCTTAAGCCAGCCCCGACACC	For construction of pSTV-HCB
pSTV-inv3_2	TTAGCGATGCTGGAAATTCGGG	
Ptac-rrnB-Inv3	ATGATAATCCTCCTGAATTCAATTGTTATCCGCTCACAATTCCACACATT	For construction of pSTV-PCB
pSTV-AA-Inv_3307N	GGCAAATACATAGGAGAAGACAT	
A0282_N	ATGAGCATCAGGACAGTGGGCA	
A0282_C	TTACTTGCTATAGACGTACACG	
Construction of double-knockout strains of *E. coli*		
rng-KC-Fw	TGAATTATTAAGTAATTTTAACGCACTGCG	For construction of JWΔcraΔrng and JWΔpgiΔrng
rng-KC-Rv	CCTTTGCCGGATGGCGGCCCAGCATCTG	
poxB-KC-Fw	CCTTATTATGACGGGAAATGCCACCC	For construction of JWΔptaΔpoxB
poxB-KC-Rv	GATGAACTAAACTTGTTACCGTTATC	

Supplementary Table S3. P(3HB-*co*-3HHx) biosynthesis from glucose by *E. coli* JM109-derived recombinant strains.

Entry	Plasmid 1	Plasmid 2	Plasmid 3	Dry cell mass (g/L)	PHA [g/L]	Residual cell mass (g/L)	PHA content [wt%]	3HHx [mol%]	Monomer amount in PHA [mmol/L-culture]	
									3HB	3HHx
1	pBKS-PCJAB			9.86 ±0.05	5.64 ±0.18	4.22 ±0.19	57.2 ±1.8	0	65.6 ±2.0	0
2	pBKS-PCJAB	pBtac-CJ$_{Re}$E		6.29 ±0.59	2.37 ±0.41	3.92 ±0.27	37.5 ±3.4	0	27.6 ±4.8	0
3	pBKS-PCJAB	pBtac-CJ$_{Re}$E	pSTV-HCB	6.87 ±0.26	2.82 ±0.30	4.05 ±0.05	41.0 ±2.8	14.0 ±0.3	27.0 ±2.9	4.4 ±0.5
4	pBKS-PCJA	pBtac-CJ$_{Re}$E	pSTV-HCB	3.51 ±0.04	0.14 ±0.02	3.38 ±0.03	3.9 ±0.52	21.5 ±0.8	1.2 ±0.2	0.32 ±0.05
5	pBKS-PCJAB	pBtac-CJ$_{Re}$E	pSTV-HC	4.49 ±0.05	0.59 ±0.08	3.91 ±0.12	13.0 ±1.8	0	6.8 ±0.9	0
6	pBKS-PCJAB	pBtac-CJ$_{Re}$EB		8.06 ±0.15	3.39 ±0.13	4.66 ±0.17	42.1 ±1.7	1.2 ±0.1	38.8 ±1.4	0.49 ±0.06

The cells were cultivated in a 100 ml LB medium containing 2% (w/v) glucose and 1 mM IPTG for 48 h at 30˚C (*n*=3).

pBSKS-PCJAB (*phaP*$_{D4N}$*C*$_{NSDG}$*J*$_{Ac}$-*phaAB*$_{Re}$), pBKS-PCJA (*phaP*$_{D4N}$*C*$_{NSDG}$*J*$_{Ac}$-*phaA*$_{Re}$)

pBtac-CJ$_{Re}$E (*ccr*$_{Me}$-*phaJ4a*$_{Re}$-*emd*$_{Mm}$), pBtac-CJ$_{Re}$EB (*ccr*$_{Me}$-*phaJ4a*$_{Re}$-*emd*$_{Mm}$-*bktB*$_{Re}$)

pSTV-HCB (*had*$_{Re}$-*crt2*$_{Re}$-*bktB*$_{Re}$), pSTV-HC, (*had*$_{Re}$-*crt2*$_{Re}$)

Supplementary Table S4. Effects of replacements of (*R*)-hydratase gene and/or 3HB-CoA dehydrogenase gene on P(3HB-*co*-3HHx) biosynthesis from glucose by *E. coli* JM109-derived recombinant strains.

Plasmid 1	Plasmid 2 (*R*-hydratase gene)	Plasmid 3 (3HB-CoA DH gene)	Dry cell mass (g/L)	PHA [g/L]	Residual cell mass (g/L)	PHA content [wt%]	3HHx comp. [mol%]	Monomer amount in PHA [mmol/L-culture]	
								3HB	3HHx
pBKS-PCJAB	pBtac-CJ$_{Re}$E (*phaJ4a$_{Re}$*)	pSTV-HCB (*had$_{Re}$*)	6.55 ±0.18	2.06 ±0.19	4.48 ±0.04	31.5 ±2.0	15.4 ±1.5	19.4 ±1.4	3.5 ±0.1
		pSTV-PCB (*paaH1$_{Re}$*)	6.68 ±0.13	2.05 ±0.14	4.63 ±0.05	30.7 ±1.5	14.2 ±0.4	19.6 ±1.4	3.2 ±0.2
	pBtac-CJ4$_{Pa}$E (*phaJ4$_{Pa}$*)	pSTV-HCB (*had$_{Re}$*)	6.58 ±0.04	1.93 ±0.03	4.66 ±0.06	29.3 ±0.5	16.1 ±0.2	17.9 ±0.3	3.4 ±0.04
		pSTV-PCB (*paaH1$_{Re}$*)	6.78 ±0.05	2.09 ±0.22	4.69 ±0.21	30.8 ±3.2	14.3 ±1.0	19.9 ±1.8	3.3 ±0.6

The cells were cultivated in a 100 ml LB medium containing 2% (w/v) glucose and 1 mM IPTG for 48 h at 30˚C (*n*=3).

pBKS-PCJAB (*phaP*$_{D4N}$*C*$_{NSDG}$*J$_{Ac}$-phaAB$_{Re}$*)

pBtac-CJ$_{Re}$E (*ccr$_{Me}$-phaJ4a$_{Re}$-emd$_{Mm}$*), pBtac-CJ4$_{Pa}$EB (*ccr$_{Me}$-phaJ4$_{Pa}$-emd$_{Mm}$-bktB$_{Re}$*)

pSTV-HCB (*had$_{Re}$-crt2$_{Re}$-bktB$_{Re}$*), pSTV-PCB (*paaH1$_{Re}$-crt2$_{Re}$-bktB$_{Re}$*)

Supplementary Table S5. Effects of mutation(s) in sugar metabolism-regulating genes and acetate formation on P(3HB-*co*-3HHx) biosynthesis from glucose by *E. coli* BW25113-derived recombinant strains harboring pBKS-PCJAB/pBtac-cJ$_{Re}$e/pSTV-HCB.

Entry	Host strain	Dry cell mass (g/L)	PHA [g/L]	Residual cell mass (g/L)	PHA content [wt%]	3HHx comp. [mol%]	Monomer amount in PHA [mmol/L-culture]		Glucose consumption [g/L]	Acetate formation [g/L]
							3HB	3HHx		
7	BW25113 (control)	6.10 ±0.17	1.97 ±0.14	4.13 ±0.03	32.2 ±1.4	15.7 ±0.24	18.3 ±1.4	3.42 ±0.21	17.0 ±0.5	0.73 ±0.11
Sugar metabolisms										
8	JWΔcra	1.60 ±0.01	0.07 ±0.00	1.53 ±0.01	4.5 ±0.1	3.1 ±0.2	0.80 ±0.02	0.03 ±0.01	6.8 ±0.5	2.1 ±0.2
9	JWΔpdhR	3.02 ±0.18	0.36 ±0.01	2.66 ±0.17	11.8 ±0.4	trace	4.1 ±0.1	trace	9.8 ±0.3	0.56 ±0.14
10	JWΔrng	8.45 ±0.24	3.63 ±0.33	4.82 ±0.13	42.9 ±2.8	trace	42.2 ±3.9	trace	19.9 ±0.1	0.17 ±0.02
11	JWΔcraΔrng	8.23 ±0.12	3.28 ±0.08	4.95 ±0.08	39.8 ±0.6	trace	38.1 ±0.9	trace	19.9 ±0.1	0.16 ±0.02
12	JWΔpgi	4.01 ±0.18	0.75 ±0.06	3.27 ±0.12	18.6 ±0.7	22.2 ±1.1	6.3 ±0.4	1.8 ±0.2	12.9 ±0.6	1.3 ±0.1
13	JWΔpgiΔrng	3.82 ±0.06	0.66 ±0.05	3.10 ±0.02	18.8 ±0.7	6.5 ±0.1	7.6 ±0.5	0.54 ±0.03	11.2 ±0.2	1.2 ±0.1
Acetate formation										
14	JWΔpta	5.29 ±0.09	2.19 ±0.06	3.10 ±0.03	41.4 ±0.5	1.9 ±0.2	24.9 ±0.6	0.47 ±0.1	14.2 ±0.3	0.51 ±0.03
15	JWΔptaΔpoxB	1.65 ±0.12	trace	1.65 ±0.12	trace	0	trace	0	6.9 ±0.1	1.4 ±0.1 (pyruvate)

The cells were cultivated in a 100 ml LB medium containing 2% (w/v) glucose and 1mM IPTG for 48 h at 30˚C (*n*=3).
pBKS-PCJAB (*phaP*$_{D4N}$*C*$_{NSDG}$*J*$_{Ac}$-*phaAB*$_{Re}$), pBtac-CJ$_{Re}$E (*ccr*$_{Me}$-*phaJ4a*$_{Re}$-*emd*$_{Mm}$), pSTV-HCB (*had*$_{Re}$-*crt2*$_{Re}$-*bktB*$_{Re}$)