Table S1 Strains and plasmids used in this study.

	Description	Source
Plasmids		
pEZ15Asp	Shuttle vector contains *Z. mobilis* origin and *E. coli* origin p15A; Biobrick-compatible, *Spe*^{R}	Yang et al., 2016b
pEZ-Pt	pEZ15A containing a *phaCAB* operon from *C. necator* H16 driven by inducible promoter P*tet*, *Spe*^{R}	This work
pEZ-Pg	pEZ15A containing *phaCAB* operon driven by P*gap*, *Spe*^{R}	This work
pEZ-Pg^{N1}	pEZ15A containing *ZMO1329* (*ppnK*) gene and *phaCAB* operon driven by promoter P*gap*, *Spe*^{R}	This work
pEZ-Pg^{N2}	pEZ15A containing *ZMO0367* (*zwf*) gene and *phaCAB* operon driven by promoter P*gap*, *Spe*^{R}	This work
pE39p-Pe^{EUP}	pEZ15A containing *ada* from *Dickeya zeae* and *adh2* gene from *Saccharomyces cerevisiae* driven by stronger promoter P*eno*, *Kana*^{R}	This work
pL2R-Pt	The P*tet*-driven p*haCAB* operon was inserted into ZM4 chromosome *ZMO0038* using pL2R editing plasmid, *Spe*^{R}	This work
pL2R-Pg	The P*gap*-driven p*haCAB* operon was inserted into ZM4 chromosome *ZMO0038* using pL2R editing plasmid, *Spe*^{R}	This work
pL2R-Flo	pL2R with the deletion of a nucleotide thymine in *ZMO1082* result the integration of *ZMO1082* with *ZMO1083*, *Spe*^{R}	This work
Strains		
DH5α	*E. coli* for plasmid construction	Lab stock
Trans110	*E. coli* for plasmid demethylation	Lab stock
ZM4	*Zymomonas mobilis* subsp. *mobilis* ZM4 strain	Lab stock
ZM4Pt	*Z. mobilis* ZM4 containing plasmid pEZ-Pt, *Spe*^{R}	This work
ZM4Pg	*Z. mobilis* ZM4 containing plasmid pEZ-Pg, *Spe*^{R}	This work
ZM4Pg^{N1}	*Z. mobilis* ZM4 containing plasmid pEZ-Pg^{N1}, *Spe*^{R}	This work
ZM4Pg^{N2}	*Z. mobilis* ZM4 containing plasmid pEZ-Pg^{N2}, *Spe*^{R}	This work
ZMPt	P*tet*-driven *phaCAB* operon inserted into ZM4 chromosome	This work
ZMPg	*Pgap*-driven *phaCAB* operon inserted into ZM4 chromosome	This work
ZMPt^{g}	ZMPt containing plasmid pEZ-Pg, *Spe*^{R}	This work
ZMPg^{g}	ZMPg containing plasmid pEZ-Pg, *Spe*^{R}	This work
ZMPt^{N1}	ZMPt containing plasmid pEZ-Pg^{N1}, *Spe*^{R}	This work
ZMPt^{N2}	ZMPt containing plasmid pEZ-Pg^{N2}, *Spe*^{R}	This work
ZMPt^{EUP}	ZMPt containing plasmid pE39p-Pe^{EUP}, *Kana*^{R}	This work
ZMPt^{N1-EUP}	ZMPt containing plasmids pEZ-Pg^{N1} and pE39p-Pe^{EUP}, *Spe*^{R}, *Kana*^{R}	This work
ZMPt^{N2-EUP}	ZMPt containing plasmids ZM4Pg^{N2} and pE39p-Pe^{EUP}, *Spe*^{R}, *Kana*^{R}	This work
ZMPt-Flo	Deletion of a nucleotide thymine in *ZMO1082* resulting in the integration of *ZMO1082* with *ZMO1083*	This work
ZMPt-Flo^{N1}	ZMPt-Flo containing plasmid pEZ-Pg^{N1}, *Spe*^{R}	This work
ZMPt-Flo^{N2}	ZMPt-Flo containing plasmid pEZ-Pg^{N2}, *Spe*^{R}	This work
ZMPt-Flo^{N1-EUP}	ZMPt-Flo containing pEZ-Pg^{N1} and pE39p-Pe^{EUP}, *Spe*^{R}, *Kana*^{R}	This work
ZMPt-Flo^{N2-EUP}	ZMPt-Flo containing pEZ-Pg^{N2} and pE39p-Pe^{EUP}, *Spe*^{R}, *Kana*^{R}	This work

Table S2 Primers used in this study. Font underlined indicates homology arms.

Name	Sequence (5'—3')	Usage
Primers for construction of PHB		
P*tet*-CAB-F	GATCTCCCGGATCCATGGCCACCGGCAAAG	Clone and expression
P*gap*-CAB-F	CTTAATAAGTTAGGAGAATAAACATGGCCACCGGCAAAG	Clone and expression
phaC-R	CTTACTTTCTCTAGATTATGGTTAGGCTTTAGCTTTAACATAACGACCAG	Clone and expression
phaA-F	CCATAATCTAGAGAAAGTAAGCACATGACCGATGTTGTCATTGTCTCTG	Clone and expression
phaA-R	GTGCTTACTTTCTCTAGATTATGGTTATTTGCGTTCAACGGCCAAAG	Clone and expression
phaB-F	CTAGAGAAAGTAAGCACATGACCCAACGTATTGCCTATC	Clone and expression
phaB-R	GGCCGCTACTAGTTTAACCCATATGCAAGCCACCATTC	Clone and expression
ppnK-R	TCCTTTCTCCTCTTTTTAACATAAGCGAAATTGTTCGCG	Clone and expression
ppnK-phaC-F	GTTAAAAAGAGGAGAAAGGATCTCCCATGGCCACCGGCAAAG	Clone and expression
zwf-F	ATGACAAATACCGTTTCGACGATG	Clone and expression
zwf-R	GAGATCCTTTCTCCTCTTTTCAGTCATACCAAGTTACTCCATCAC	Clone and expression
zwf-phaC-F	AAAGAGGAGAAAGGATCTCCCATGGCCACCGGCAAAGG	Clone and expression
P*eno*-F	TGTCTATACTCCAGTTACTCAATACGTAACAATAATCAGTTTATCCTAAC	Clone and expression
P*eno*-R	ATCGAAACCTTTCTTAAAATCTTTTAGACGAG	Clone and expression
P*eno-ada*-F	AAAGATTTTAAGAAAGGTTTCGATATGGAACATAGCGTTATTGAACC	Clone and expression
ada-R	CTTCTAGACCCTGTGATTTAAGCAATACGAAACATATCAACCAAAAC	Clone and expression
adh2-F	ATCACAGGGTCTAGAAGGAGGTCGAAATGAGCATTCCGGAAACCC	Clone and expression
adh2-R	CGAGATCTATGGGACGTTATTTAGAGGTATCAACAACATAACGACC	Clone and expression
Primers for gene editing		
0038-gr1-F	GAAAGCGTCCAGCAAAATACGCCTTCTATTGATGAA	0038gRNA-F
0038-gr1-R	GAACTTCATCAATAGAAGGCGTATTTTGCTGGACGC	0038gRNA-R
0038-up-F	CACCAGCTCACCGTCTGCTTTTTGCCGACAAAGCG	Generate pL2R-*phaCAB-0038*
0038-up-R	TCACGCCCGACGCCAGACGGGATTAGAAATTTTGTCG	Generate pL2R-*phaCAB-0038*
0038-up-P*tet*-F	GGCGTCGGGCGTGATTAAGACCCACTTTCACATTTAAGTTGTTTTTCTAATC	Generate pL2R-*phaCAB-0038*
phaB-down-R	CGTCTATCTGAATATTTAACGATTAACCCATATGCAAGCCACC	Generate pL2R-*phaCAB-0038*
0038-down-F	TCGTTAAATATTCAGATAGACGGAGATAATAAACGGGAGAGAGGTCG	Generate pL2R-*phaCAB-0038*
0038-down-R	GCTCGAGATCTGATATCACTCAACAGATCAACC	Generate pL2R-*phaCAB-0038*
0038-up-P*gap*-F	GGCGTCGGGCGTGAGTTCGATCAACAACCCGAATCCTATC	Generate pL2R-*phaCAB-0038*
Flo-gr-F	GAAAGCTCTTATGGTGGTTGCTGTTCCGCTACCGCT	FlogRNA-F
Flo-gr-R	GAACAGCGGTAGCGGAACAGCAACCACCATAAGAGC	FlogRNA-R
Flo-up-F	ACCAGCTCACCGTCTTTAACTTTCATATCGGCGTACAAGAAGAAG	Generate pL2R-Flo-401
Flo-up-R	AGCAACCACCATAAGAGCTGCAACGGTAATCAAGCAAAGCAATG	Generate pL2R-Flo-401
Flo-down-F	GCTCTTATGGTGGTTGCTGTTCCG	Generate pL2R-Flo-401
Flo-down-R	GCTCGAGATCTGATATCACTGTCACAGTCAAAAATGCAGACTAATTCACC	Generate pL2R-Flo-401
Primers for gene detection		
15A-fwd	GGCAAAGCCACCCTATTTTTAG	Plasmid detection
15A-rev	CACTTCACTGACACCCTCAT	Plasmid detection

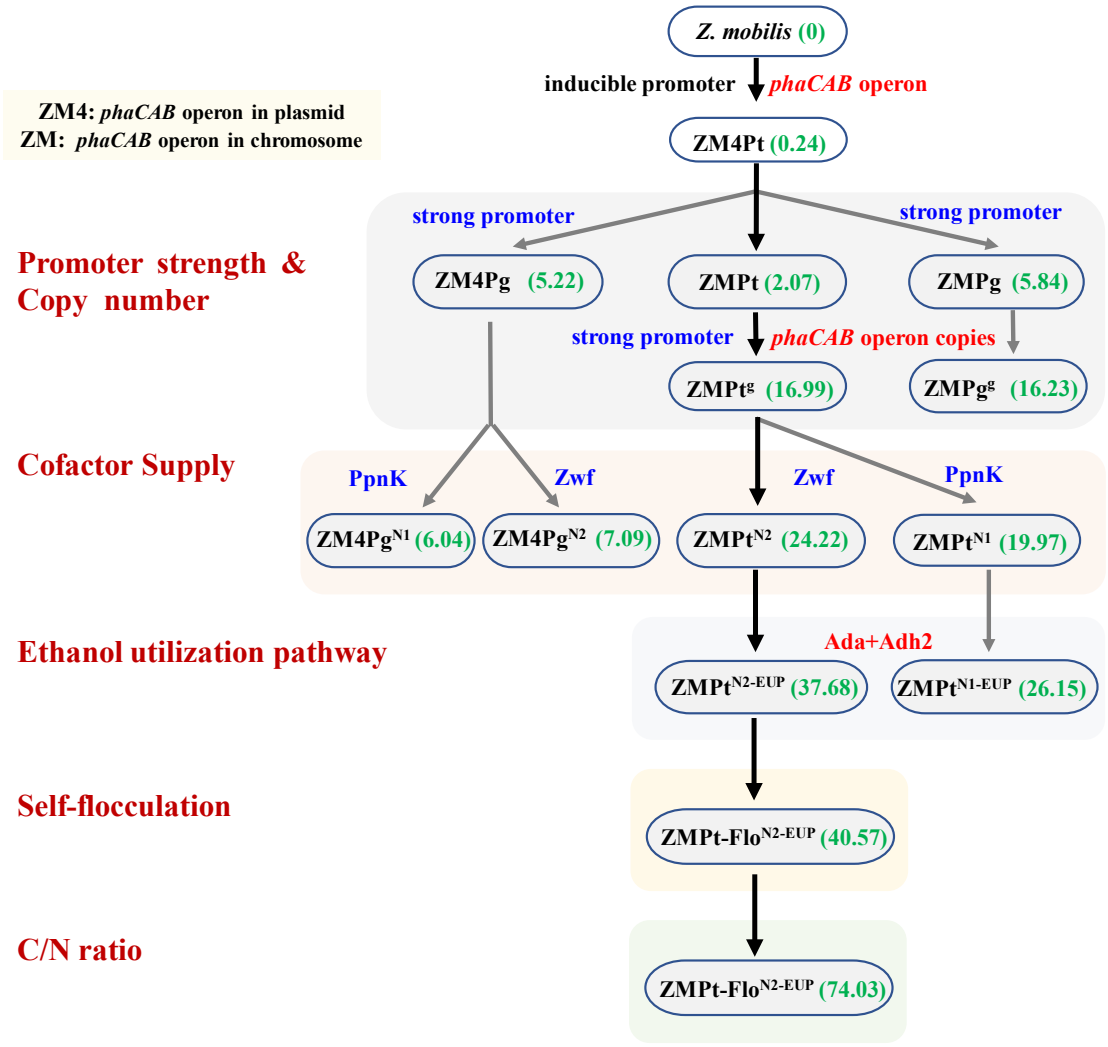

Fig S1. Overview of the strain constructed in *Z. mobilis* for PHB production. Native genes overexpressed or promoter replacement are shown in blue font, while heterologous genes are shown in red font. The PHB content of dry cell weight (%DCW) in recombinant *Z. mobilis* are shown in green font.