

TABLE S1. Sequences of primers used for saturation mutagenesis at position A479.

Primer name	Sequence (5' - 3')
A479X ^a	GGCCACAT <u>CGCC</u> GGCTCG
A479rev	GGATGCGCCCAGGACAAAGC

^a X indicates the 19 amino acid residues other than the original one. The substitution codon for Ala479 (GCC) is underlined. Codons used for substitution were: Cys (TGC), Asp (GAT), Glu (GAA), Phe (TTC), Gly (GGC), His (CAT), Ile (ATT), Lys (AAA), Leu (CTG), Met (ATG), Asn (AAC), Pro (CCG), Gln (CAG), Arg (CGC), Ser (AGC), Thr (ACC), Val (GTG), Trp (TGG), and Tyr (TAT).

TABLE S2. *In vivo* activities of wild-type and A479X mutants of PhaC_{CS}.

PHA synthase ^a	PHA content (wt%) ^b
Wild-type	35.6 ± 2.9
A479C	19.0 ± 6.9
A479D	27.4 ± 3.4
A479E	3.5 ± 1.8
A479F	0.3 ± 0.1
A479G	58.2 ± 2.8
A479H	39.2 ± 1.7
A479I	28.4 ± 2.8
A479K	0.4 ± 0.1
A479L	0.7 ± 0.3
A479M	46.5 ± 5.2
A479N	0.9 ± 0.1
A479P	27.4 ± 3.2
A479Q	36.2 ± 5.1
A479R	0.7 ± 0.1
A479S	32.4 ± 4.4
A479T	36.6 ± 5.4
A479V	44.6 ± 5.5
A479W	49.1 ± 3.2
A479Y	32.1 ± 3.1

^a Crude extract from *E. coli* LS5218 transformants harboring wild-type and individual A479 mutant PhaC_{CS} grown on LB medium containing 100 µg/mL of ampicillin and 50 µg/mL of kanamycin for 9 h at 30 °C

^b Determined via gas chromatography from freeze-dried cells

TABLE S3. Thermal properties of P(3HB) and P(3HB-*co*-3HHx) synthesized by *E. coli* LS5218 transformants harboring wild-type and various A479X mutant PhaC_S^a.

PHA synthase	Polymer	T_g (°C) ^b	T_m (°C) ^c	ΔH_m (J/g) ^d
Wild-type	P(3HB- <i>co</i> -1.6 mol% 3HHx)	8	162	65
A479E	P(3HB)	7	172	80
A479S	P(3HB- <i>co</i> -6.6 mol% 3HHx)	-4	154	40
A479T	P(3HB- <i>co</i> -4.4 mol% 3HHx)	-4	162	44
A479V	P(3HB- <i>co</i> -2.0 mol% 3HHx)	-2	163	52
A479W	P(3HB- <i>co</i> -0.8 mol% 3HHx)	3	165	68

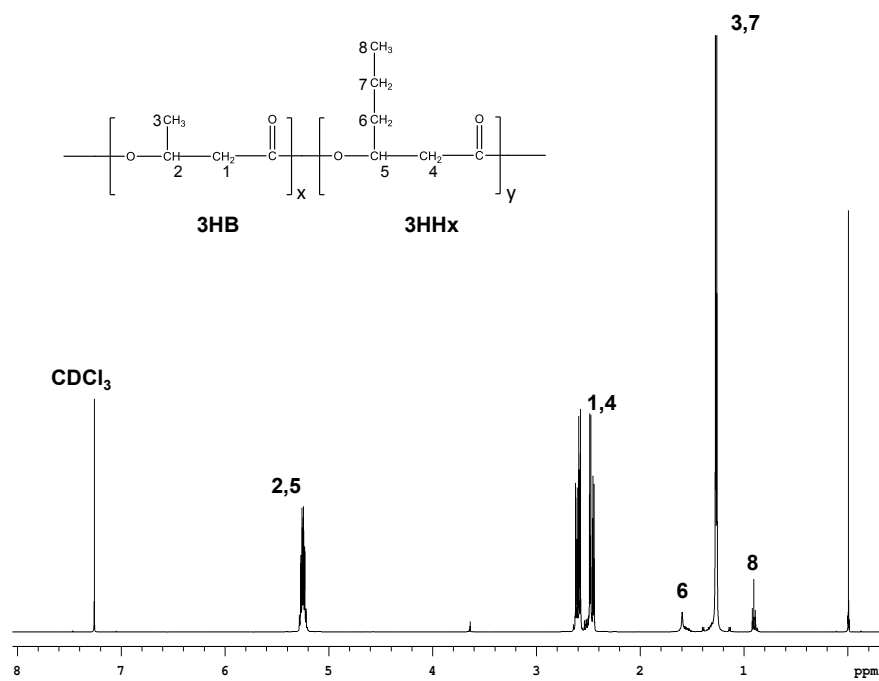
^a Cells were cultivated in 100 mL of M9 medium containing 0.25 % (w/v) dodecanoic acid, 0.4 % (v/v) Brij-35, 100 μ g/mL of ampicillin and 50 μ g/mL of kanamycin for 72 h at 30 °C

^b Glass transition temperature

^c Melting temperature

^d Enthalpy of fusion

A



B

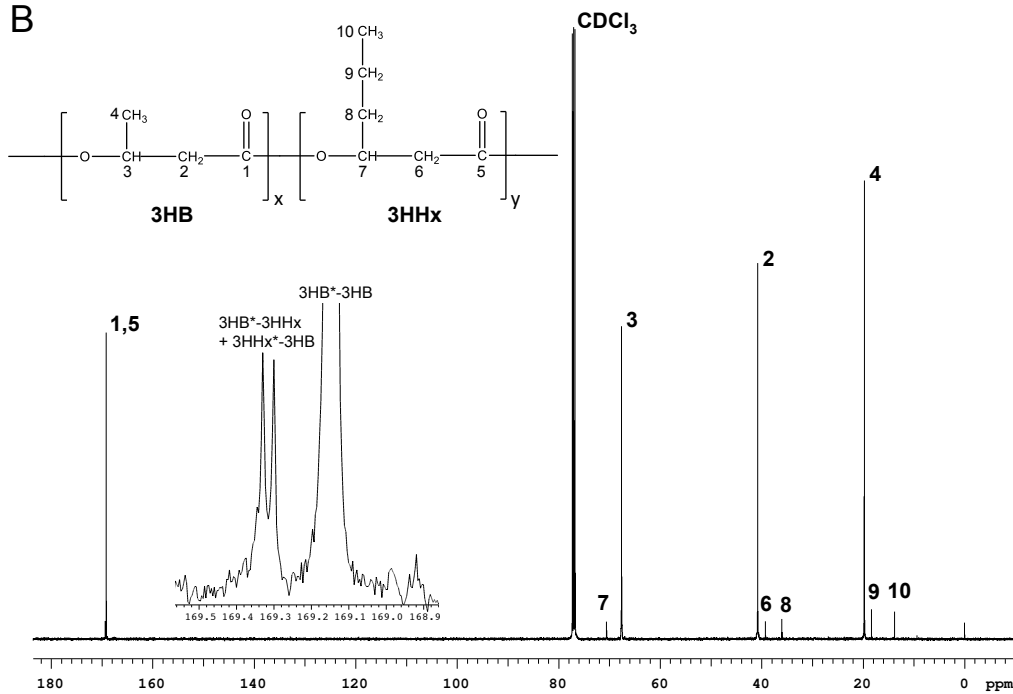


FIG S1. (A) $^1\text{H-NMR}$ and (B) $^{13}\text{C-NMR}$ spectra of P(3HB-co-6.6 mol% 3HHx) produced by *E. coli* LS5218 transformant harboring A479S mutant PhaC_S.