

Supplementary Materials

Table S1. Effect of coexpression of chaperones on 3HBO production in recombinant *E. coli* BW2511 Δ *adhE* with various ethanol concentrations. *E. coli* BW25113 Δ *adhE* harboring pGEM-*phaRC*_{YB4AB} or pGEM-*phaRC*_{YB4AB}+pG-Tf2 were cultivated in LB medium containing 20 g/L glucose at 30 °C for 48 h with various ethanol concentration.

Ethanol ^a (g/L)	Plasmid	Dry cell weight (g/L)	Extracellular 3HBO (g/L)	Extracellular 3HB (g/L)	Ethanol at 48 h ^b (g/L)
0	pGEM- <i>phaRC</i> _{YB4AB}	9.65	0.10	0.03	Trace
	pGEM- <i>phaRC</i> _{YB4AB} +pG-Tf2	9.76	0.39	0.03	Trace
0.5	pGEM- <i>phaRC</i> _{YB4AB}	9.70	0.21	0.03	0.20
	pGEM- <i>phaRC</i> _{YB4AB} +pG-Tf2	9.21	0.62	0.03	0.30
1	pGEM- <i>phaRC</i> _{YB4AB}	9.36	0.20	0.04	0.58
	pGEM- <i>phaRC</i> _{YB4AB} +pG-Tf2	9.12	0.85	0.04	0.63
3	pGEM- <i>phaRC</i> _{YB4AB}	8.92	0.65	0.04	2.23
	pGEM- <i>phaRC</i> _{YB4AB} +pG-Tf2	8.50	1.35	0.04	2.30
5	pGEM- <i>phaRC</i> _{YB4AB}	8.48	1.23	0.03	4.05
	pGEM- <i>phaRC</i> _{YB4AB} +pG-Tf2	8.22	1.60	0.05	3.82
10	pGEM- <i>phaRC</i> _{YB4AB}	7.53	1.90	0.04	8.38
	pGEM- <i>phaRC</i> _{YB4AB} +pG-Tf2	7.56	2.07	0.07	8.02

^a Ethanol was added at the beginning of cultivation. ^b Ethanol concentration in the culture supernatant after 48 h of cultivation.

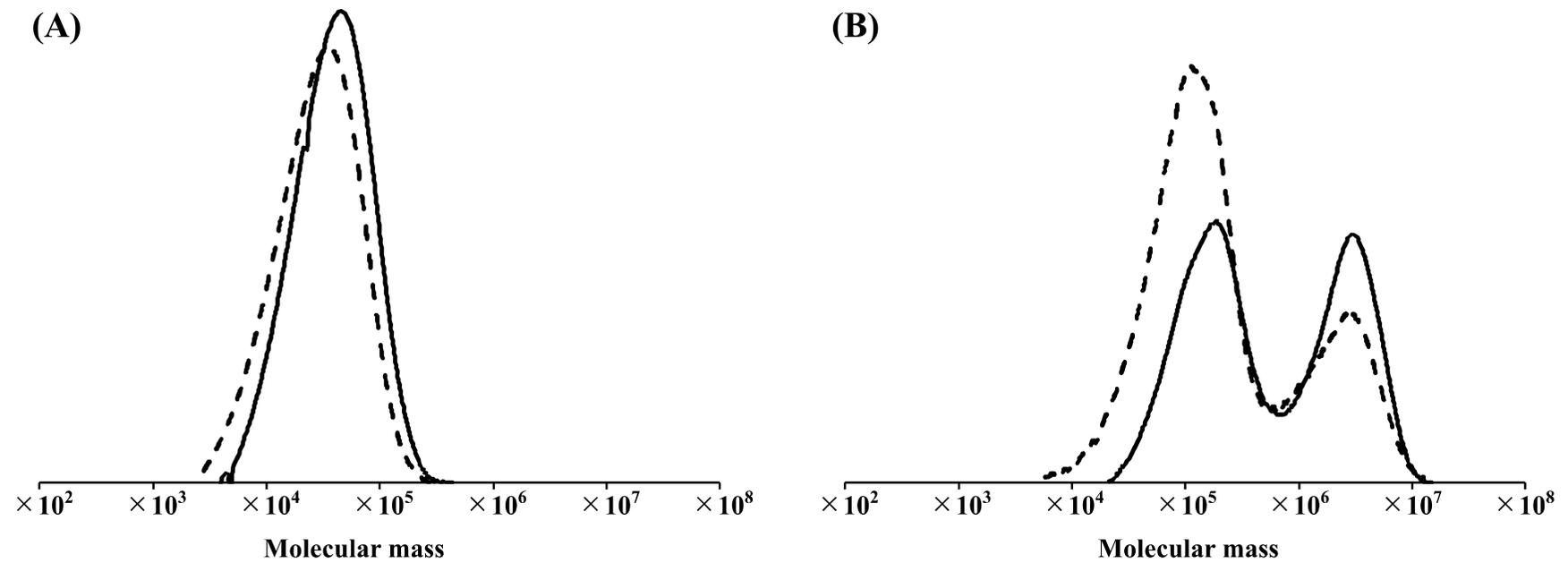


Figure S1. Molecular weight distribution of P(3HB) extracted from recombinant *E. coli* BW25113 WT (A) or $\Delta adhE$ (B) at 48 h of cultivation. Solid line, pGEM-*phaRC_{YB4AB}*; broken line, pGEM-*phaRC_{YB4AB}*+pG-Tf2.

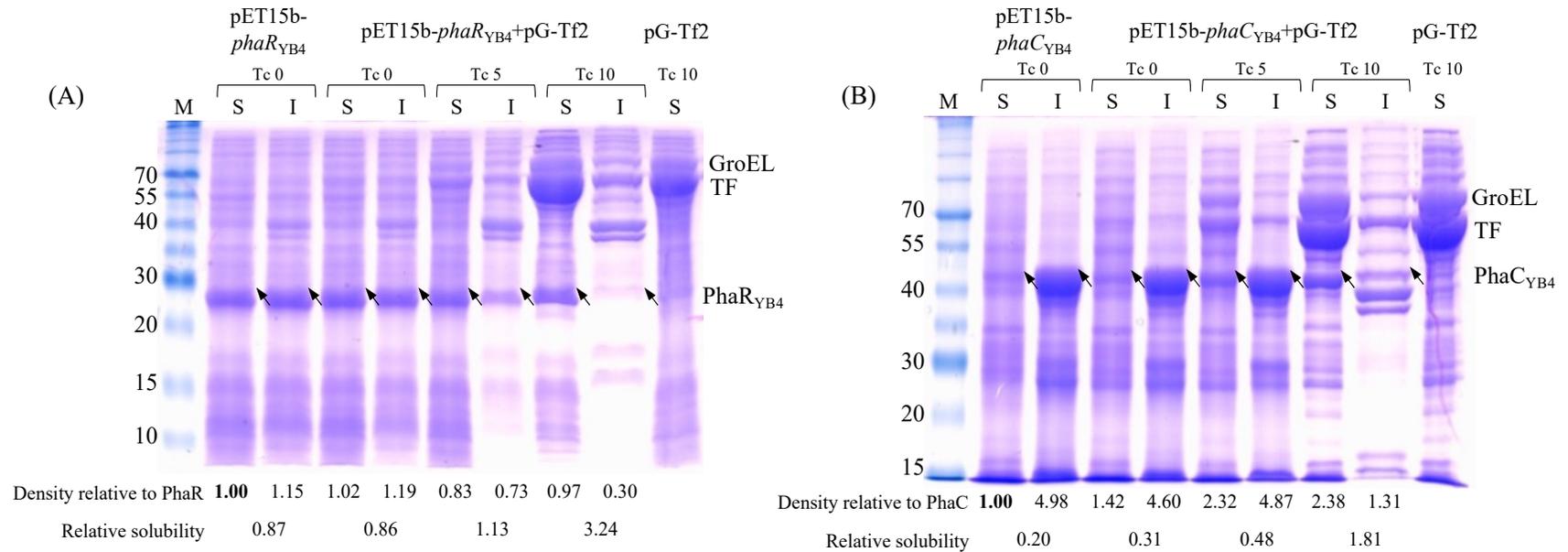


Figure S2. Effect of the chaperone plasmid pG-Tf2 on the solubilization of Pha_{YB4} and Pha_{CYB4}. The levels of solubilized Pha_{YB4} and Pha_{CYB4} were evaluated by SDS-PAGE. **(A)** *E. coli* BL21(DE3) harboring pET15b-*phaR*_{YB4}, pET15b-*phaR*_{YB4}+pG-Tf2 or pG-Tf2 and **(B)** *E. coli* BL21(DE3) harboring pET15b-*phaC*_{YB4}, pET15b-*phaC*_{YB4}+pG-Tf2 or pG-Tf2 were cultivated with 0-10 µg/L tetracycline (Tc). After sonication of each cell sample, soluble (S) and insoluble (I) fractions were analyzed by SDS-PAGE. The arrows indicate the bands corresponding to Pha_{YB4} and Pha_{CYB4}. The relative densities were calculated using ImageJ software. The density relative to the band of Pha_{YB4} or Pha_{CYB4} is the ratio of the density of each band to that of the band for the soluble fraction of Pha_{YB4} or Pha_{CYB4} alone (bold). The relative solubilities are the ratios of the S to I fractions within each sample.