



Title	Improved production of poly(lactic acid)-like polyester based on metabolite analysis to address the rate-limiting step
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Supplemental materials

"Improved production of poly(lactic acid)-like polyester based on metabolite analysis to address the rate-limiting step"

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Table S1. The list of plasmids used in this study

Plasmid name	Relevant genes	Reference
pPSPTG1	Transglutaminase gene with <i>cspB</i> promoter	Kikuchi et al. 2013
pPSDCP	pPSPTG1 derivative; multi-cloning sites	This study
pPS <i>ldhC1STQKpct</i>	pPSDCP derivative; <i>ldhA</i> gene from <i>E. coli</i> , <i>phaC1_{Ps}STQK</i> gene from <i>Pseudomonas</i> sp. 61-3, and <i>pct</i> gene from <i>M. elsdenii</i>	This study
pPS <i>ldheC1STQKpct</i>	pPSDCP derivative; <i>ldhA</i> gene from <i>E. coli</i> , codon-optimized <i>ephaC1_{Ps}STQK</i> gene from <i>Pseudomonas</i> sp. 61-3, and <i>pct</i> gene from <i>M. elsdenii</i>	This study

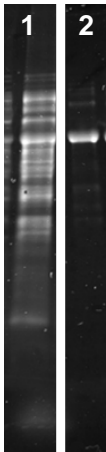


Figure S1. Protocol for purification of PCT. Recombinant *E. coli* JM109 harboring pQE30 (Qiagen), which includes His-tagged PCT at N-terminal, was grown on 1.5 mL LB medium containing 100 µg/l ampicillin at 37 °C for 15 h (preculture). The cells (1 mL) were transferred to 100 mL of the same medium and grown at 30 °C for 2 h. Then, the flasks were transferred to 25 °C incubator and 1 mM IPTG (final concentration) was added to the flask. The cells were further cultivated at 25 °C for 24 h. The cells were

harvested, and disrupted by sonication in lysis buffer [50 mM sodium phosphate (pH 8.0) containing 10 mM imidazole and 300 mM sodium chloride]. The supernatant was combined with 500 μ L of Ni-NTA His-Bind Resins (Novagen), and gently rotated at 4 °C for 1 h. The resin was applied to an empty column, and washed with 1 mL of lysis buffer, and subsequently, washed with 5 mL of lysis buffer but containing 20 mM imidazole. The bound protein was eluted with 4 mL of lysis buffer containing 250 mM imidazole, and immediately desalted using a PD-10 column (GE healthcare) with 50 mM sodium phosphate buffer (pH 7.0) containing 5% glycerol. The purified protein solution was frozen using liquid nitrogen and stored at -80 °C. Lane 1: crude extract. Lane 2: purified PCT.