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Genome-wide screening of transcription factor deletion targets in *Escherichia coli* for enhanced production of lactate-based polyesters

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Engineered *Escherichia coli* is a useful platform for production of lactate (LA)-based polyester poly[LA-*co*-3-hydroxybutyrate (3HB)] from renewable sugars. Here we screened all non-lethal transcription factor deletions of *E. coli* for efficient production of the polymer. This approach aimed at drawing out the latent potential of the host for efficient polymer production via indirect positive effects. Among 252 mutants from Keio Collection tested, eight mutants (Δ*pdhR*, Δ*cspG*, Δ*yneJ*, Δ*chbR*, Δ*yiaU*, Δ*creB*, Δ*ygfI* and Δ*nanK*) accumulated greater amount of polymer (6.2–10.1 g/L) compared to the parent strain *E. coli* BW25113 (5.1 g/L). The mutants increased polymer production per cell (1.1–1.5-fold) without significant change in cell density. The yield of the polymer from glucose was also higher for the selected mutants (0.34–0.38 g/g) than the parent strain (0.27 g/g). Therefore, the deletions of transcription factors should channel the carbon flux towards polymer production. It should be noted that the screening employed in this study identified beneficial mutants without analyzing causal relationship between the mutation and the enhanced polymer production. This approach, therefore, should be applicable to broad range of fermentation productions.

[**Key words:** Polyhydroxyalkanoate; Polyhydroxybutyrate; Poly(lactic acid); Biobased plastic; *Escherichia coli*; Transcription factor]

Microorganisms are useful platforms for synthesis of high-value-added compounds from renewable biomasses (1,2). An advantage of microorganisms is their tolerance to genetic modification that acquires high productivity and the ability to synthesize desired compounds (3). The rational approaches that overexpress/delete genes involved in the syntheses of target compounds are effective strategy to improve the productivity (4,5). In addition, genome-wide approaches that modify the expression levels of multiple genes are also able to gain indirect positive effects on the production of targets (6,7). For example, in the attempt of the minimum genome factories, physical deletion of the *Bacillus subtilis* genome by approximately 1 Mbp enhanced bacterial production of cellulases and proteases (6). In these approaches, the broad mutations in genome have no clear, direct causal relationship with the protein expression that accounts for the enhanced production.

Our group has been extensively working on the microbial production of lactate-based polyester P(lactate-*co*-3-hydroxybutyrate) using *Escherichia coli* as a platform (8). The polymer is processed into flexible and transparent plastic material, and potentially used in broad range of applications (9). In order to increase the productivity of the polymer, we previously reported that the deletion of a σ factor RpoN in *E. coli* increased P(LA-*co*-3HB) production with a 1.2-fold compared to the parent strain (10,11). The enhanced polymer production should be due to the changes in the expression levels of multiple genes caused by deletion of a sigma factor RpoN. This result suggests that the modulation of gene expressions, which are not directly involved in the P(LA-*co*-3HB) synthesis, could improve the polymer production.

This study, therefore, aimed at drawing out the latent potential of *E. coli* to further improve the polymer production. To meet this goal, we focused on transcription factors that contribute to the expression of multiple genes, but control less number of genes compared to the σ factors (12,13). Thus, the deletion of transcriptional factors allows us to observe the effect of changes in expression levels at narrower ranges on the polymer production. Furthermore, the collection of single-gene-deletion mutants of *E. coli* (Keio Collection) facilitates to conduct the systematic screening of transcription factor deletions (14). In this study, we screened these 252 mutant strains for the enhanced P(LA-*co*-3HB) production.

MATERIALS AND METHODS

Plasmids, strains, and growth conditions *E. coli* strains used in this study are listed in Table S1. *E. coli* BW25113 and the Keio Collection (purchased from National BioResource Project, Japan) were used as a host for polymer production (14). The expression vector pTV118N*pctphaC1*$_{Ps}$(ST/QK)*AB*, which harbors genes encoding propionyl-CoA transferase from *Megasphaera elsdenii* (*pct*), engineered PHA synthase with LA-polymerizing activity [*phaC1*$_{Ps}$(ST/QK)] from *Pseudomonas* sp. 61-3 and 3HB-CoA supplying enzymes β-ketothiolase and acetoacetyl-CoA reductase (*phaA*, and *phaB*) from *Ralstonia eutropha*, was used for P(LA-*co*-3HB) production (8,9). For polymer production, recombinant *E. coli* harboring pTV118N*pctphaC1*$_{Ps}$(ST/QK)*AB* were grown on 1.7 mL LB medium containing

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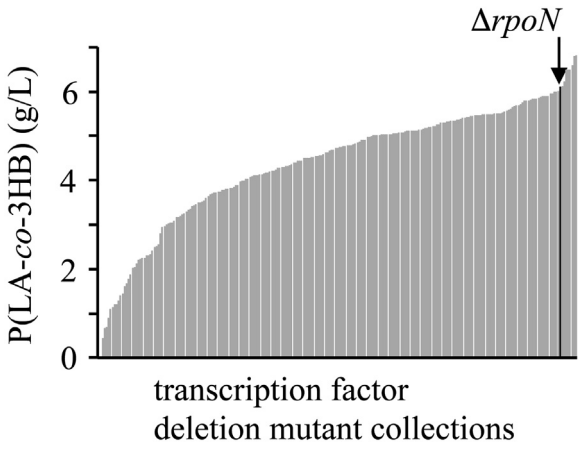

FIG. 1. The polymer production by the 252 mutant strains of *E. coli* carrying transcription factor deletions. The cells were grown on LB medium containing 20 g/L glucose. *E. coli* harboring pTV118N*pctphaC1*$_{Ps}$(ST/QK)*AB*. X axis is transcription factor deleted strains collection, and Y axis is polymer production (g/L).

several concentrations of glucose and 10 mM calcium pantothenate at 30°C for 48 h with reciprocal shaking at 180 rpm. Ampicillin (Amp; 100 μg/mL) and kanamycin (Km; 25 μg/mL) were added when needed.

Screening of the positive mutants from Keio Collection The *E. coli* was harvested by centrifugation at 1690 ×*g* for 15 min. The polymer content was determined as described previously (15). In brief, lyophilized cells were directly treated with concentrated sulfuric acid at 120°C to convert polyester into unsaturated carbonic acids, which were quantified using HPLC (JASCO engineering, Japan) equipped with UV detector at 210 nm and an aminex HPX-87H ion exclusion column (7.8 mm I.D × 300 mm, Bio-Rad laboratories, Hercules, CA, USA). The concentration of glucose in the supernatant was determined by HPLC equipped with a refractive index detector, as previously described (16).

Observation of cell morphology by microscopy For microscopic observation, cells were cultured on LB medium at 30°C for 48 h. The 1.7 mL of culture was harvested, concentrated to 10 μL, and a 5 μL aliquot was fixed on a glass slide. The cell shape was observed under a microscope (BZ-X700, Keyence Corporation) and controlled by BZ-X700 Analyzer software (Keyence Corporation).

Measurement of cell density using flow cytometry The volumetric cell density (cells/L) was measured by flow cytometry using a SH800 cell sorter (SONY). Cells grown under aforementioned conditions were harvested at 48 h (OD_{600} between 20 and 25) and 1000-fold diluted sample with water was used for cell counting. The flow rate was set to 37 μL/min (pressure 6). All FSC (forward scatter) and SSC (side scatter) images were recorded using SH800 software (SONY).

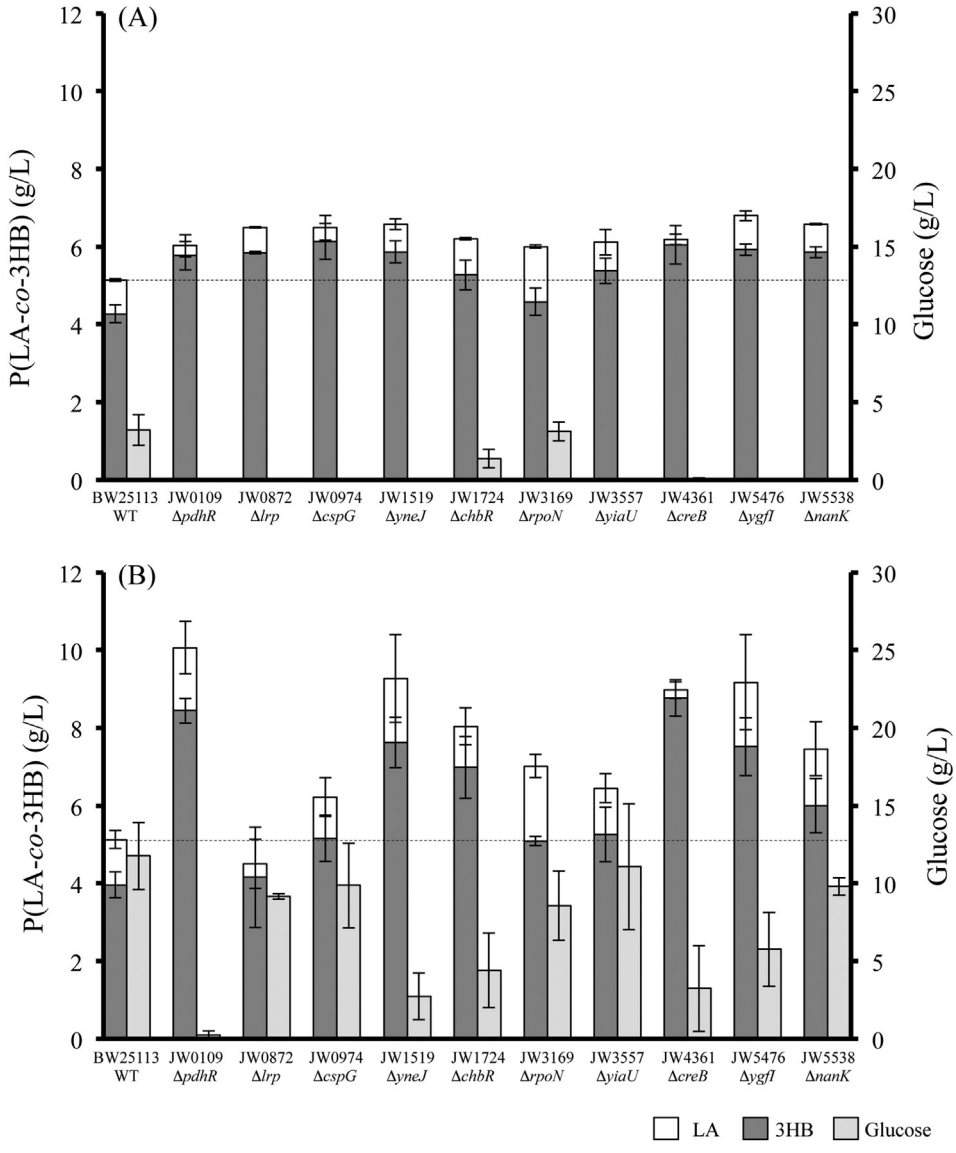

FIG. 2. P(LA-*co*-3HB) production and glucose consumption in the transcription factor deleted *E. coli*. *E. coli* BW25113 (parent strain) and transcription factor deleted strains harboring pTV118N*pctphaC1*$_{Ps}$(ST/QK)*AB* were grown on LB medium containing 20 g/L (A) and 30 g/L (B) glucose. Light shaded bars, amount of 3HB unit in the polymer. Open bars, amount of LA unit in the polymer. Dark shaded bars, glucose concentration in medium. The data represent the average ± standard deviation of three independent trials.

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