Biosynthesis of 2-hydroxybutyrate containing polyhydroxyalkanoates in recombinant *Escherichia coli*

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Introduction. We have previously developed *in vivo* synthesis system of 2-hydroxyacid containing polyesters such as polylactic acid (PLA) and poly(3-hydroxybutyrate-*co*-lactate) [P(3HB-*co*-LA)] employing recombinant *Escherichia coli* strains by the expressions of evolved *Clostridium propionicum* propionyl-CoA transferase (Pct_{Cp}) and *Pseudomonas* sp. MBEL 6-19 polyhydroxyalkanoate (PHA) synthase 1 ($PhaC1_{Ps6\text{-}19}$) [1].

In this study, we have developed recombinant *E. coli* that could synthesize 2-hydroxybutyrate (2HB) containing PHAs from propionyl-CoA by the construction of metabolic pathways to generate 2HB-CoA and its polymerization. The precursor of 2HB, 2-ketobutyrate, was synthesized from propionyl-CoA and then 2-ketobutyrate was converted into 2HB by the 2-hydroxyacid dehydrogenase. Finally, 2HB-CoA generated by Pct_{Cp} was polymerized into 2HB-containing PHA by $PhaC1_{Ps6\text{-}19}$ in recombinant *E. coli*.

Methods. For the production of PHA copolymers containing 2HB, recombinant *E. coli* XL1-Blue was used as host strain. Plasmid p619C1437-pct540 has been previously described [1]. pKM22-PanE for the expression of the *L. lactis subsp. lactis Il1403 panE* gene and pKA32-PrpE for the expression of *R. eutropha prpE* gene were previously described [2]. For the synthesis of PHAs containing 2HB monomer, recombinant *E. coli* XL1-Blue was cultured in MR medium supplemented with 20 g/L of glucose and desired concentrations of sodium propionate and 3HB at 30 ^{o}C in a rotary shaker at 250 rpm for 96 h. Recombinant *E. coli* XL1-Blue was grown to the OD_{600} of 0.5 before induction with 1 mM of IPTG for the expression of the genes involved in PHA biosynthesis pathways. Ampicillin (Ap, 50 μg/mL), kanamycin (Km, 30 μg/mL) and chloramphenicol (Cm, 34 μg/mL) were added to the culture medium

Results. Metabolic pathway for the synthesis of 2HB from sodium propionate was constructed by the expressions of the *R. eutropha prpE* gene and the *panE* gene in recombinant *E. coli.* PHAs with different monomer fractions could be synthesized by varying 3HB concentrations. As 3HB concentration in the culture medium was increased, 3HB monomer fractions and PHA contents were increased up to 70 mol% and 66 wt%, respectively. But, 2HB and lactate monomer fractions in PHA copolymers were reduced.

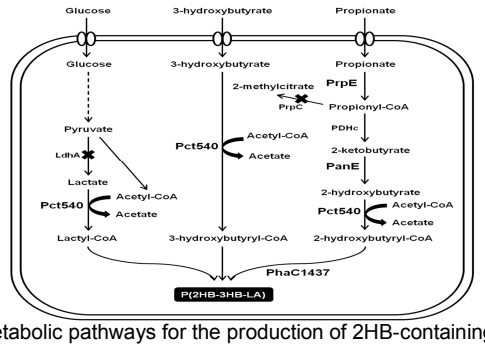

Fig.1 Metabolic pathways for the production of 2HB-containing PHA in recombinant *E. coli*

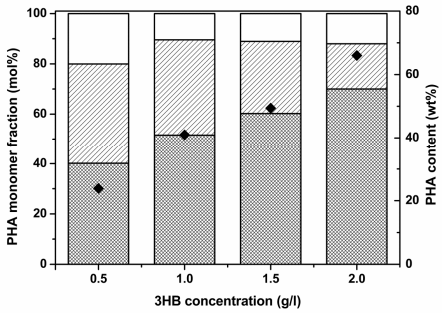

Fig.2 Biosynthesis of PHAs consisting of 2HB, 3HB, and lactate monomers in recombinant *E. coli* wild-type XL1-Blue harboring p619C1437-pct540, pKM22-PanE, and pKA32-PrpE by addition of different concentrations of 3HB and 1 g/L of sodium propionate into the culture medium (Filled diamonds are PHA contents. Dotted bar, dashed bar, and open bar are 3HB, 2HB, and lactate monomer fractions in PHA copolymer, respectively)

Conclusions. In this study, we report the metabolic engineering strategies for the biosynthesis of 2HB-containing PHA by using propionyl-CoA as precursor for 2HB monomer.

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References.

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