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Enhanced production of 3-hydroxypropionic acid from glycerol by modulation of glycerol metabolism in recombinant *Escherichia coli*

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HIGHLIGHTS

- 3-Hydroxypropionic acid (3-HP) is a valuable biochemical with high potential.
- The *E. coli* glycerol metabolism was modulated to enhance 3-HP production.
- Deletion of *glpK* and *yqhD* highly increased the glycerol flux to 3-HP production.
- A new semialdehyde dehydrogenase was expressed to reduce 3-HPA accumulation.
- The optimal *E. coli* strain produced 57 g/L 3-HP in a fed-batch culture.

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ABSTRACT

3-Hydroxypropionic acid (3-HP) is a valuable biochemical with high potential for bioplastic manufacturing. The endogenous glycerol metabolism and by-product formation pathway in *Escherichia coli* were modulated to enhance 3-HP production from glycerol. Double deletion of *glpK* and *yqhD* directed the glycerol flux to 3-HP biosynthesis and reduced the formation of 1,3-propanediol. Since 3-hydroxypropionaldehyde (3-HPA), a precursor of 3-HP, is toxic to cell growth, the gene encoding *Pseudomonas aeruginosa* semialdehyde dehydrogenase (PSALDH) highly active on 3-HPA was expressed in *E. coli*. Finally, fed-batch culture of recombinant *E. coli* BL21star(DE3) without *glpK* and *yqhD*, and expressing *Lactobacillus brevis* DhaB-DhaR, and *P. aeruginosa* PSALDH resulted in 57.3 g/L 3-HP concentration, 1.59 g/L-h productivity and 0.88 g/g yield. In conclusion, modulation of the glycerol metabolism in combination with enhanced activity of 3-HPA dehydrogenation improved the production of 3-HP from glycerol.

1. Introduction

3-Hydroxypropionic acid (3-HP) is a three-carbon carboxylic acid containing a β-hydroxyl group. 3-HP has been selected as one of the top value-added chemicals produced from biomass by US Department of Energy (Bozell and Petersen, 2010) and has a high potential to produce several chemicals such as acrylic acid, acrylonitrile, malonic acid, and 1,3-propanediol (1,3-PDO) (Kumar et al., 2013).

For microbial production of 3-HP, bacterial strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas denitrificans* were metabolically engineered to overexpress essential enzymes engaged in the 3-HP biosynthesis because natural 3-HP producers did not possess a high capacity for 3-HP production (Kumar et al., 2013). Glycerol, a by-product of biodiesel production using plant oils, is an attractive carbon source for biochemical production (Durnin et al., 2009). Currently, two metabolic pathways for 3-HP production from glycerol were developed: the CoA-dependent and CoA-independent pathways (Kumar et al., 2013). In the CoA-independent pathway (Fig. 1), glycerol dehydratase (DhaB) and aldehyde dehydrogenase (Aldh) are two essential enzymes for glycerol conversion to 3-HP. Glycerol dehydratase is a coenzyme B_{12}-dependent diol dehydratase (EC 4.2.1.30) catalyzing dehydration of glycerol to 3-hydroxypropionaldehyde (3-HPA). Since glycerol dehydratase undergoes irreversible inactivation when its substrate is glycerol (Toraya et al., 1976), the inactivated glycerol dehydratase should be reactivated by expression of reactivating proteins (Kwak et al., 2013; Mori et al., 1997). The second enzyme of aldehyde dehydrogenase converts 3-HPA to 3-HP and is specific for NAD^+ cofactor. To date, aldehyde dehydrogenases with 3-HPA oxidation activity have been identified in some microorganisms (Kumar et al., 2013; Valdehuesa et al., 2013). Since coenzyme B_{12}

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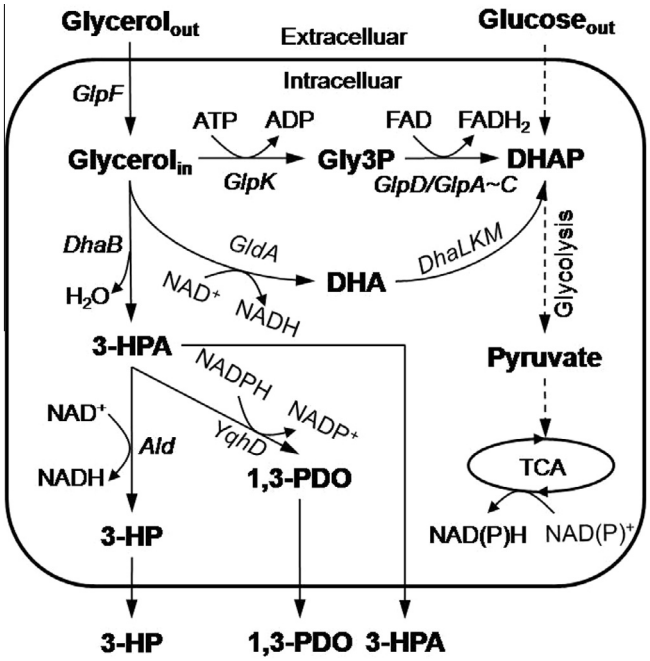

Fig. 1. Metabolic pathway for 3-HP production in recombinant *E. coli* BL21star(DE3) strain expressing glycerol dehydratase and aldehyde dehydrogenase. The name of metabolites are abbreviated as follows: 3-HP, 3-hydroxypropionic acid; 3-HPA, 3-hydroxypropionaldehyde; 1,3-PDO, 1,3-propanediol; Gly3P, glycerol-3-phosphate; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate. Italicized letters indicate metabolic enzymes: *DhaB*, glycerol dehydratase; *Ald*, aldehyde dehydrogenase; *YqhD*, 1,3-propandiol oxidoreductase; *GlpF*, glycerol symporter; *GlpK*, glycerol kinase; *GldA*, glycerol dehydrogenase; *GlpD*/*GlpA*~*C*, glycerol-3-phosphate dehydrogenases; *DhaLKM*, dihydroxyacetone kinase.

is required for dehydration of glycerol to 3-HPA, a natural coenzyme B_{12} producer of *K. pneumoniae* was engineered to produce 3-HP. Since *K. pneumoniae* produces 1,3-propanediol (1,3-PDO) as a main metabolite of glycerol, the endogenous *dhaT* and *yqhD* were disrupted and its aldehyde dehydrogenase genes of *puuC* and *dhaB* were overexpressed. A fed-batch culture with 5% dissolved oxygen content resulted in 28 g/L 3-HP concentration and 40% g/g yield (Ashok et al., 2013b). By-products of 3.3 g/L 1,3-PDO, 10.6 g/L acetate and 7.4 g/L 2,3-butanediol also accumulated in the culture broth. Recombinant *K. pneumoniae* expressing *E. coli* K12 *aldH* produced 48.9 g/L 3-HP and 25.3 g/L 1,3-PDO simultaneously in a microaerobic fed-batch fermentation while by-products of lactate, acetate and ethanol were also produced in the range of 3–25 g/L (Huang et al., 2013). Since *E. coli* does not have the 3-HP biosynthetic enzymes, genes for both glycerol dehydratase and aldehyde dehydrogenase should be introduced. Recombinant *E. coli* SH-BGK1 expressing *K. pneumoniae* DhaB123 and GdrAB, and *Azospirillum brasilense* KGSADH produced 38 g/L 3-HP in a 71 h fed-batch culture with 35% yield and 0.54 g/L-h productivity, in which by-products of 9.7 g/L 1,3-PDO and 7.2 g/L acetate were produced (Rathnasingh et al., 2009).

In our previous report (Kwak et al., 2013), new glycerol dehydratase and its reactivase from *Lactobacillus brevis* were identified. Their coding genes and the *E. coli* K12 *aldH* gene were overexpressed in *E. coli*. The two-step fed-batch process using dual substrates of glycerol and glucose resulted in 14.3 g/L 3-HP concentration, 0.26 g/L-h productivity and 0.14 g/g yield. Since the 3-HP yield based on glycerol consumed is only 15% relative to the theoretical yield, more metabolic engineering should be done to increase the glycerol flux to 3-HP production. As shown in Fig. 1, a wild type of *E. coli* has two glycerol metabolic pathways toward the central carbon metabolism, which are activated or deactivated by the presence of electron acceptors (Durnin et al., 2009). Recombinant *E. coli* stains expressing the 3-HP biosynthetic enzymes produced a by-product of 1,3-PDO by the action of endogenous oxidoreductase YqhD (Kwak et al., 2013; Zhang et al., 2006).

In this study, two genes (*glpK* and *gldA*) engaged in the central glycerol metabolism and the *yqhD* gene encoding an endogenous propanediol oxidoreductase were disrupted combinatorially in recombinant *E. coli* overexpressing the 3-HP biosynthetic enzymes in order to enhance 3-HP production. Since 3-HPA, an intermediate in the 3-HP biosynthesis, accumulated because of low activity of aldehyde dehydrogenase (Rathnasingh et al., 2009), new bacterial aldehyde dehydrogenases highly active on 3-HPA were identified and overexpressed in recombinant *E. coli*. Finally, a two-step fed-batch fermentation of the optimal *E. coli* strain was carried out to increase the performance of 3-HP production.

2. Methods

2.1. Strains and plasmids

E. coli DH5α and BL21star(DE3) (Invitrogen Co., Carlsbad, CA, USA) strains were used for plasmid construction and 3-HP production, respectively. Plasmids pELDRR expressing the *L. brevis* glycerol dehydratase complex ($DhaB_1B_2B_3$) and DhaB reactivase ($DhaR_1R_2$) genes and pCEa harboring the *E. coli aldH* gene (Kwak et al., 2013) were used for 3-HP production. Plasmids pKD46, pKD13 and pCP20 (Datsenko and Wanner, 2000) were used for deletion of the chromosomal *glpK*, *gldA* and *yqhD* genes, respectively. Each gene coding for putative semialdehyde dehydrogenases was cloned in plasmid pCDFDuet-1 (Novagen Co., Madison, WI, USA) with the *T7* promoter.

2.2. Genetic manipulation

Deletion of the chromosomal genes of *glpK*, *gldA* and *yqhD* genes followed the previous report with minor modification (Datsenko and Wanner, 2000). Briefly, each gene deletion cassette with the kanamycin resistance gene was amplified by a polymerase chain reaction (PCR) with the corresponding DNA oligomers (Table 1) and a template of plasmid pKD13: D1*glpK* and D4*glpK* for *glpK*, D1*gldA* and D4*gldA* for *gldA*, D1*yqhD* and D4*yqhD* for *yqhD*. Each PCR product was transformed into *E. coli* competent cells harboring plasmid pKD46 expressing λ recombinase. After selection of the transformants on LB medium with kanamycin and ampicilin, plasmid pCP20 expressing the yeast FLP recombinase was introduced into the transformants to remove the kanamycin resistance gene. The plasmids were removed by incubation of the transformants at 43 °C.

The genes encoding putative aldehyde dehydrogenases were PCR-amplified from the genomic DNA of *P. aeruginosa* ATCC10145 or *K. pneumoniae* 342 with the corresponding PCR primers (Table 1 and Supplement 2). For example, two PCR primers of F-PSPA3072 and R-PSPA3072 were used for amplification of the *P. aeruginosa* semialdehyde dehydrogenase (PSALDH) gene (PSPA7_3072, NCBI Gene ID: 5354745). After digestion of the PCR product with *Bam*HI and *Hind*III restriction enzymes, the nucleotides were ligated with plasmid pCDFDuet-1, resulting in the construction of plasmid pCPa72. Construction of other plasmids (pCKa19, pCKa47, pCKa57, pCKa99 and pCPa53) was described in Supplement 1.

Transformation of *E. coli* followed the $CaCl_2$ treatment or electroporation method and the sequences of DNA oligomers used in this study are listed in Table 1 and Supplement 2.

2.3. Culture conditions

E. coli was grown in LB medium (1% yeast extract, 2% bactotryptone and 1% NaCl) with appropriate antibiotics for genetic