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RESEARCH PAPER

Production of 1,3-Propanediol from Glucose by Recombinant *Escherichia coli* BL21(DE3)

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Abstract A range of recombinant strains of *Escherichia coli* were developed to produce 1,3-propanediol (1,3-PDO), an important C3 diol, from glucose. Two modules, the glycerol-producing pathway converting dihydroxyacetone phosphate to glycerol and the 1,3-PDO-producing pathway converting glycerol to 1,3-PDO, were introduced into *E. coli*. In addition, to avoid oxidative assimilation of the produced glycerol, glycerol oxidative pathway was deleted. Furthermore, to enhance the carbon flow to the Embden-Meyerhof-Parnas pathway, the Entner-Doudoroff pathway was disrupted by deleting 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase. Finally, the acetate production pathway was removed to minimize the production of acetate, a major and toxic by-product. Flask experiments were carried out to examine the performance of the developed recombinant *E. coli.* The best strain could produce 1,3-PDO with a yield of 0.47 mol/mol glucose. Along with 1,3-PDO, glycerol was produced with a yield of 0.33 mol/mol glucose.

Keywords: *Escherichia coli* BL21(DE3), glycerol, 1,3-PDO, pathway engineering

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1. Introduction

The production of bio-based metabolites has attracted considerable attention recently due to unstable oil price and serious environmental concerns. 1,3-Propanediol (1,3-PDO), a C3 diol, can be produced from glycerol and/or glucose and has a wide range of industrial applications, such as the production of adsorbents, adhesives, paints, fibers, pharmaceuticals, andcosmetics. In addition, it can be used as a monomer for the production of the polyester, polytrimethylene terephthalate (PTT) [1,2].

Many strains, including *E. coli*, *Klebsiella pneumoniae*, and *Clostridium* were developed for the biological production of 1,3-PDO [3-5]. *K. pneumonia* can naturally produce 1,3-PDO from glycerol, and ithas the ability to synthesize coenzyme B12, which is an essential cofactor for glycerol dehydratase [6]. On the other hand, *K. pneumonia* is pathogenic, and produces many by-products, such as ethanol, lactate, and acetate, at high concentrations under limited oxygen supply conditions. *Clostridium* is strictly anaerobic and a genetic toolbox for metabolic engineering has not been well developed [7-10]. *E. coli* cannot naturally produce 1,3-PDO, but has several advantages in the production of 1,3-PDO over *K. pneumonia* or *Clostridium*. *E. coli* can be engineered to produce 1,3-PDO and less by-products are generated, especially compared to *K. pneumoniae*. In addition, for *E. coli*, a fully-developed genetic toolbox is available, and genomic, proteomic, and physiological information required for metabolic engineering are well documented [11].

1,3-PDO can be produced from glycerol or glucose. Glycerol is more reduced and can be readily converted to 1,3-PDO. However, its availability is limited because the production is strictly dependent on biodiesel industry. The only crude glycerol at present, which is economically-

feasible for the use in fermentation industry, is produced as byproduct during biodiesel production. In comparison, glucose is the most abundant and most popular carbon source which can be derived from starch. Furthermore, it can be produced from cellulosic biomass. Using glucose as carbon source, DuPont and Genencor (USA) successfully commercialized their benchmark process for 1,3-PDO production [12]. Their *E. coli* strains have two heterologous modules for the production of 1,3-PDO from glucose; conversion of dihydroxyacetone phosphate (DHAP) to glycerol and subsequently to 1,3-PDO. The first module, glycerol synthesis pathway, is composed of the NADH-dependent glycerol-3-phosphate dehydrogenase (GPD) and glycerol-3-phosphate phosphatase (GPP), both from *Saccharomyces cerevisiae*. The second module, the 1,3-PDO pathway to produce 1,3-PDO from glycerol, is composed of the coenzyme B12-dependent glycerol dehydratase (DhaB) and NAD(P)H-dependent 1,3-PDO oxidoreductase (DhaT), both from *K. pneumonia* [13,14]. Despite the successful commercialization, details of the strain development have not been disclosed. In addition, owing to its commercialization, in-depth science behind the 1,3-PDO production from glucose has not attracted proper attention from scientific community.

The purpose of this study is to develop recombinant *E. coli* for the efficient production of 1,3-PDO from glucose. First, the pathways to convert glucose to 1,3-PDO were introduced to *E. coli* and the production of 1,3-PDO from glucose was confirmed. Then, the recombinant strain was extensively modified to improve the production of 1,3-PDO, which include deletion of glycerol degradation pathways (*glpK, glpABC* and *glpD*), glycerol-3-phosphate regulon repressor (*glpR*), the ED pathway genes (*edd_eda*), the acetate production pathway (*pta_ackA*), *etc* (Fig. 1). For the developed strains, flask cultures were performed to examine the effect of gene modification on 1,3-PDO production.

2. Materials and Methods

2.1. Strains and materials

E. coli BL21 (DE3) was purchased from Novagen (Merck Millipore Corporation, Darnstadt, Germany). Restriction enzymes were obtained from New England Biolabs (Beverly, Mass.) and DNA polymerase was from SolGent Co. Ltd (Seoul, Korea). Plasmid DNA isolation and Gel DNA extraction kit were acquired from Cosmo Gentech Co.Ltd. (Seoul, Korea). Primers were synthesized and sequenced

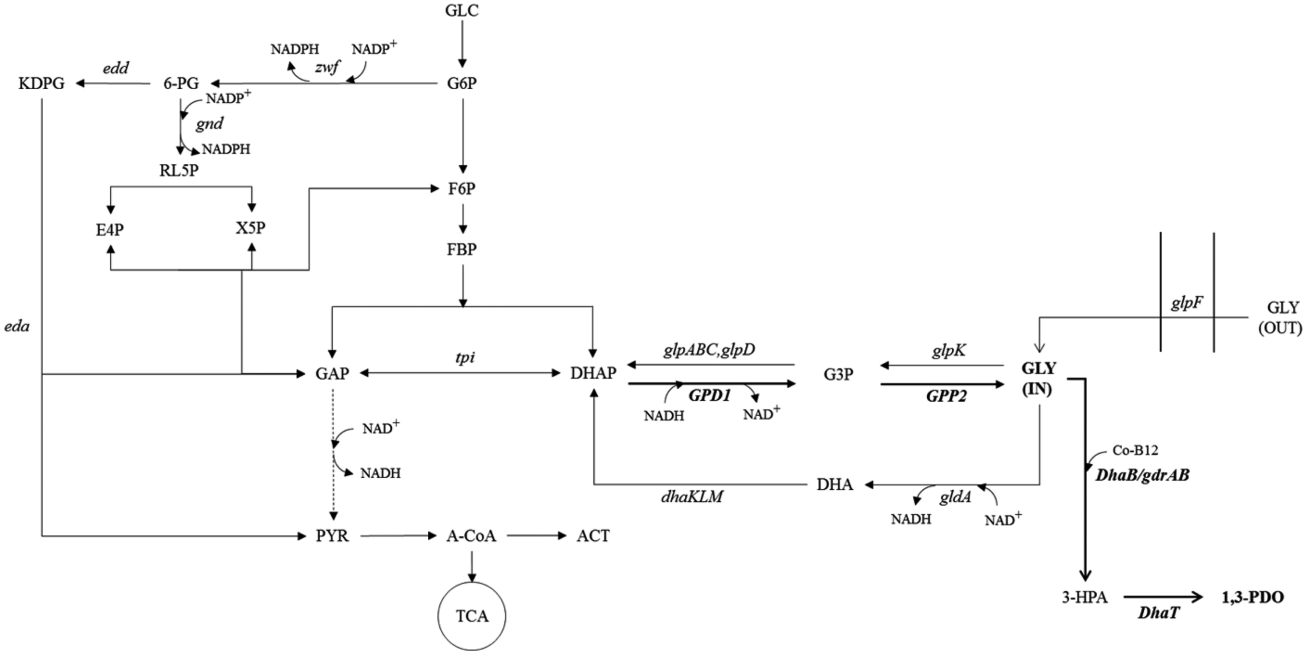

Fig. 1. Metabolic pathway related to1,3-PDO production in recombinant *Escherichia coli* BL21(DE3). Abbreviations for metabolites: GLC, glucose; G6P, glusoe-6-phosphate; 6PG, 6-phosphogluconate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; RL5P, ribulose 5-phosphate; E4P, erythrose 4-phosphate; X5P, xylulose 5-phosphate; GAP, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; G3P, glycerol-3-phosphate; GLY, glycerol; DHA, dihydroxyacetone; 3-HPA, 3-hydroxypropionaldehyde; 1,3-PDO, 1,3-propanediol; PYR, pyruvate; Co-B12, coenzyme B12. Italicized letters indicate genes/enzymes: *GPD1,* glycerol- 3-phosphate dehydrogenase; *GPP2*, glycerol-3-phosphate phosphatase; *glpD/glpABC*, glycerol-3-phosphate dehydrogenase; *glpK*, glycerol kinase; *gldA*, glycerol dehydrogenase; *dhaKLM*, dihydroxyacetone kinase; *glpF*, glycerol transporter; *DhaB*, glycerol dehydratase; *gdrAB*, glycerol dehydratase reactivase; *DhaT*, 1,3-propanediol oxidoreductase.

by Macrogen Co. Ltd. (Seoul, Korea). Bacto TM tryptone (Cat.211705), yeast extract (Cat.212750), and LB broth (Cat.244610) were procured from Difco (Becton Dickinson; Franklin Lakes, NJ, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, Mo., USA).

2.2. Culture conditions

For routine gene manipulation and development of the recombinant strains, the Luria_Bertani (LB) medium was used. Cells were cultured aerobically at 37°C in a shaking incubator with an agitation speed of 200 rpm. For 1,3-PDO production, the M9 minimal medium was used; 100 mM potassium phosphate buffer (pH 7.5), 1.0 g/L yeast extract, 1.0 g/L NaCl, 1.0 g/L NH_4Cl, 0.25 g/L $MgSO_4$ $7H_2O_2$. As carbon source, glucose was added at 10 g/L. For the maintenance of the recombinant plasmids, kanamycin (at 50 μg/mL) and chloramphenicol (at 25 μg/mL) were added to the culture medium. The inoculum culture was prepared by incubating the cells for 15 h in the M9 medium. Main culture was conducted at 30°C in 250 mL flasks under varying aeration conditions. The cultivation was started by inoculating the inoculum culture to the M9 medium to be 0.1 OD at 600 nm. To induce the 1,3-PDO synthesis pathway, isopropyl-β-D-thiogalactopyranoside (IPTG) was added at 0.1 mM when OD_{600} reached at ~0.5. Coenzyme B12 (10 μM) was also supplemented to the culture medium when IPTG was added. To vary the aeration intensity, different culture volume (25, 50, or 100 mL) and/or different agitation speed (100 or 200 rpm) were adopted.

2.3. Construction of plasmids and mutant strains

Table 1 lists the strains and plasmids used in this study. The genetic deletion strains were developed using pKOV plasmids [15]. The ~500 bp upstream and ~500 bp downstream regions of the target gene were amplified using the appropriate primers by PCR. These two fragments were

Table 1. Strains and plasmids used in this study

Strains and plasmids	Description	Source
Strains		
E. coli DH5α	Cloning host	Takara
EB	*Escherichia coli* BL21(DE3)	Novagen
EB3	*Escherichia coli* BL21(DE3) Δ*glpK*Δ*glpABC*Δ*glpD*	This study
EB6	*Escherichia coli* BL21(DE3) Δ*glpK*Δ*glpABC*Δ*glpD* Δ*glpR*	This study
EB7	*Escherichia coli* BL21(DE3) Δ*glpK*Δ*glpABC*Δ*glpD* Δ*glpR*Δ*eda_edd*	This study
EB9	*Escherichia coli* BL21(DE3) Δ*glpK*Δ*glpABC*Δ*glpD* Δ*glpR*Δ*ptsG*	This study
EB13	*Escherichia coli* BL21(DE3) Δ*glpK*Δ*glpABC*Δ*glpD* Δ*glpR*Δ*eda_edd*Δ*pta_ackA*	This study
EB-0	EB Harboring pDP	This study
EB-1	EB Harboring pDPT and pABG	This study
EB3-1	EB3 Harboring pDPT and pABG	This study
EB6-1	EB6 Harboring pDPT and pABG	This study
EB7-1	EB7 Harboring pDPT and pABG	This study
EB9-1	EB9 Harboring pDPT and pABG	This study
EB13-1	EB13 Harboring pDPT and pABG	This study
Plasmids		
pKOV	Temperature sensitive pSC101; SacB; Cm^R	Harvard University
pQE80L	lacIq; ColE1-ori; His_6-N; Amp^R	Qiagen
pDK7(p15A)	p15A-ori; P_{tac}; Cm^R	[16]
pDP	pQE80L_*GPD1_GPP2*	[17]
pDPT	pQE80L_*GPD1_GPP2_DhaT*	[17]
pABG	pDK7(p15A)_*DhaB_gdrAB*	[16]
pKOV-*glpK*	pKOV-*glpK*-plasmid used to develop *E. coli* BL21(DE3) mutant strain	This study
pKOV-*glpABC*	pKOV-*glpABC*-plasmid used to develop *E. coli* BL21(DE3) mutant strain	This study
pKOV-*glpD*	pKOV-*glpD*-plasmid used to develop *E. coli* BL21(DE3) mutant strain	This study
pKOV-*glpR*	pKOV-*glpR*-plasmid used to develop *E. coli* BL21(DE3) mutant strain	This study
pKOV-*eda-edd*	pKOV-*eda-edd*-plasmid used to develop *E. coli* BL21(DE3) mutant strain	This study

ligated through overlapping PCR and cloned into the pKOV plasmid. These plasmids were used to develop the gene deletion strains through homologous recombination. The gene deletion strains were finally identified by PCR and sequence analysis. For overexpression, the GPD1 and GPP2 genes derived from *S. cerevisiae* and DhaT derived from *Klebsiella pneumoniae* were cloned into the pQE80LK vector, pDPT recombinant plasmid [16], and pABG recombinant plasmid [17], respectively. The DhaB and GdrAB genes (*dhaB1*, *dhaB2*, *dhaB3*,*gdrA*, and *gdrB*) derived from *K. pneumoniae* were cloned into the pDK7(p15A) vector. The recombinant plasmids were isolated using a mini prep kit and transformed into the recombinant strains by electroporation.

2.4. Protein expression and gel electrophoresis

For protein expression, cells were cultured at 30°C and 100 rpm in LB medium and induced at 0.1 OD_{600} with 0.1 mM IPTG. At 12 h after induction, the cells were harvested by centrifugation, washed twice with potassium phosphate buffer (50 mM, pH 7.0), and resuspended in the same buffer. The resuspended cells were disrupted using a French press (FA-078A, Thermo Electron Corp.; Waltham, Mass., USA) at 1,250 psi and the resulting cell lysate was centrifuged at 10,000 g for 20 min at 4°C and collected supernatants. The supernatants were used for SDS-PAGE analysis. Protein bands were stained with Coomassie brilliant blue and confirmed using an image analyzer, BIO-RAD Gel Doc 2000.

2.5. Analytical methods

Cell density was determined in a 10-mm-path-length cuvette using a double-beam spectrophotometer (Lambda 20, Perkin Elmer, USA) at 600 nm (OD_{600}). Glycerol, 1,3-PDO, and various metabolites were analyzed by high-performance liquid chromatography (HPLC, Agilent Technologies, HP, 1200 series) using 2.5 mM H_2SO_4 as the mobile phase. For HPLC analyses, culture broths were centrifuged at 13,000 rpm for 20 min, and the supernatant was passed through a 0.2 μm nylon filter. The 300 mm × 7.8 mm Aminex HPX-87H (Bio-Rad, USA) column was used.

3. Results and Discussion

3.1. Expression of the glycerol biosynthetic pathway

To produce glycerol from glucose, the first module derived from *S. cerevisiae* and composed of GPD1 and GPP2, was constructed in the pQE80LK plasmid [12–18]. The resulting pDP recombinant plasmid was introduced into *E. coli* to generate EB-0 strain. As confirmed by SDS-PAGE (Fig. 2A), the recombinant EB-0 well expressed both GPD1 (42 kDa) and GPP2 (29 kDa) enzymes in the soluble fraction.

The recombinant EB-0 strain could produce glycerol from glucose successfully (Fig. 2C). The effect of aeration on glycerol production was examined by varying the culture volume and agitation speed. EB-0 was cultured in 250 mL flasks under the following conditions (working volume/agitation speed); (i) fully-aerobic, 25 mL/200 rpm; (ii) micro-aerobic I, 50 mL/200 rpm; (iii) micro-aerobic II, 50 mL/100 rpm; and (iv) Micro-aerobic III, 100 mL/100 rpm.The cell density was the highest under the fully-aerobic conditions and it decreased gradually as aeration was reduced (Fig. 2B). On the other hand, glycerol production increased as aeration decreased. Acetate formation also increased as aeration decreased (Fig. 2D). Under high aeration conditions, it is expected that, it is expected that, at the DHAP node, the glycolysis rate is high and relatively less carbon is diverted for glycerol production (Fig. 1). The highest glycerol yields and production were obtained under the micro-aerobic II conditions were ~ 0.83 mol/mol and 50.19 mM, respectively (Fig. 2C).

The effect of GPD and GPP expression on glycerol production was investigated by changing the concentration of IPTG at 0.00, 0.02, 0.05, 0.1, and 0.2 mM under the micro-aerobic II condition. Cell growth was lowered slightly at the high IPTG concentrations above 0.05 mM (Fig. 3A). The production of glycerol increased with increasing IPTG up to 0.1 mM and remained constant at 0.2 mM (Fig. 3B.). Therefore, the optimal IPTG concentration for GPD and GPP expression in the current recombinant *E. coli* was considered to be 0.1 mM. Interestingly, the IPTG concentration affected the formation of byproducts, such as ethanol and acetate (Fig. 3C). Ethanol appeared when IPTG was not added only, where glycerol production was not evident. Although glycerol production was almost the same at 0.1 and 0.2 mM, acetate production was higher at 0.2 mM. It is not clear why more acetate is produced at higher IPTG. Higher production of heterologous enzymes in the recombinant strain could require more ATP which is produced when acetate is formed.

3.2. Expression of the 1.3-PDO biosynthetic pathway

To convert the produced glycerol to 1,3-PDO, the 1,3-PDO production module was established in EB-0. Two recombinant plasmids, the pABG plasmid [16] harboring the genes encoding DhaB and GdrAB (*dhaB1, dhaB2, dhaB3, gdrA,* and *gdrB*), and the pDPT plasmid [17] containing the *dhaT* gene (along with *gpd1* and *gpp2* constituting for the glycerol production module), were introduced into *E. coli* BL2 (DE3) and the recombinant *E. coli* was designated EB-1. From SDS-PAGE analysis, the expression of DhaB1 (64 kDa), DhaB2 (22 kDa), DhaB3

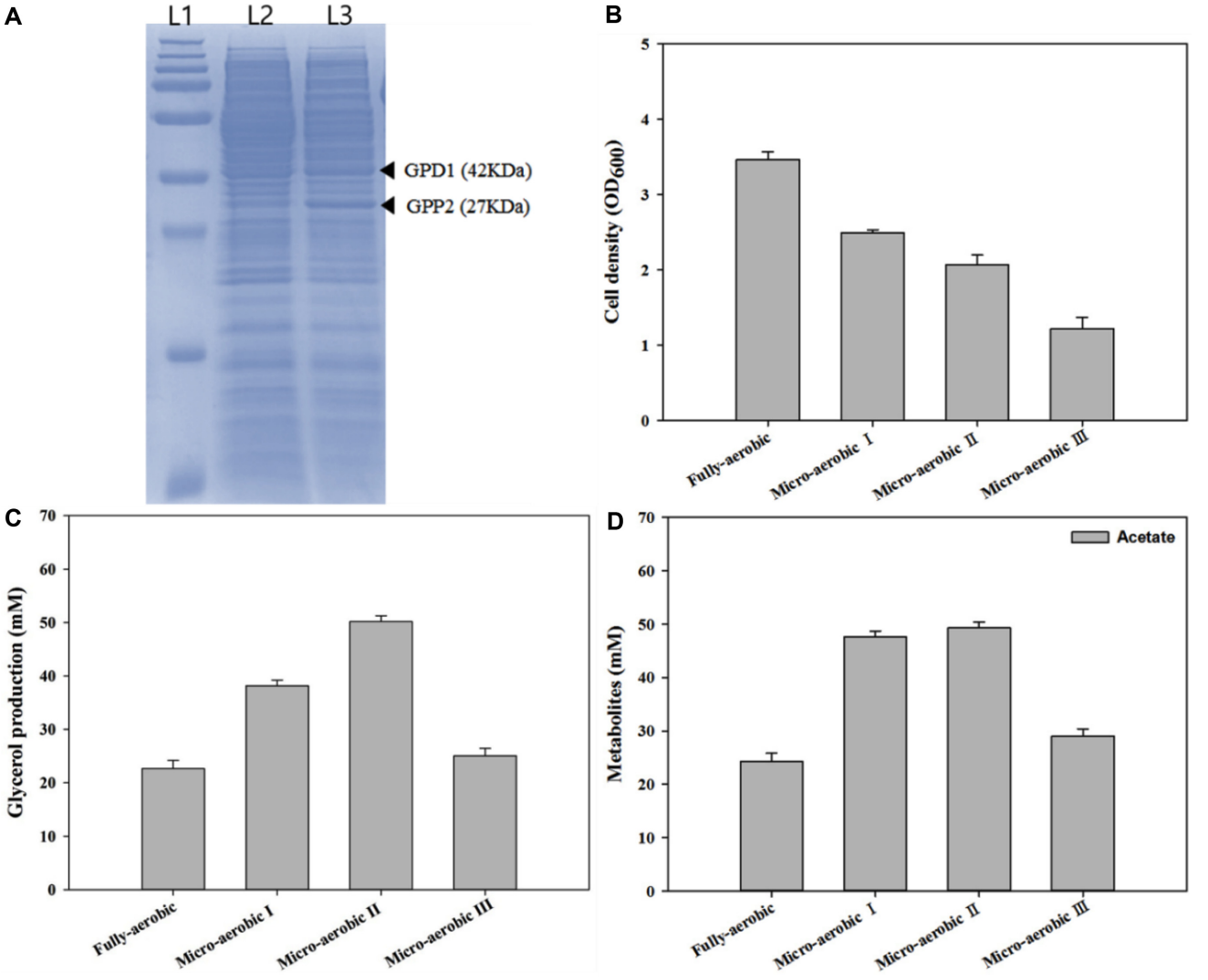

Fig. 2. Characterization and performance of the EB-0 strain expressing *gpd1* and *gpp2*. EB-0 was cultured in 250 mL flasks for 24 h under different aeration conditions by varying the liquid working volume (mL) and agitation speed (rpm). The conditions adopted were as follows; Fully-aerobic, 25 mL/200 rpm; Micro-aerobic I, 50 mL/200 rpm; Micro-aerobic II, 50 mL/100 rpm; Micro-aerobic III, 100 mL/100 rpm. The data are the average of three independent experiments. (A) SDS-PAGE analysis. The heterologous proteins are marked with arrows. Lane 1, marker; Lane 2, cell-free extract of host *E. coli* BL21(DE3); Lane 3, cell-free extract of EB-0. (B) Cell density. (C) Glycerol production. (D) Acetate accumulation.

(16 kDa), GdrA (61 kDa), and GdrB (12 kDa) in the EB-1 strain were confirmed (Fig. 4A). Fig. 4B shows the time course profiles of 1,3-PDO production, glycerol production, cell growth and by-products (acetate) formation during flask culture of EB-1. EB-1 showed similar cell growth to EB-0, but its glucose consumption rate was lower than that of EB-0 (Fig. 4B). As desired, the recombinant strain, EB-1, harboring both the glycerol and 1,3-PDO production pathways, showed 1,3-PDO production although the yield and production of 1,3-PDO were low 0.24 mol/mol glucose and 14.34 mM, respectively. A significant amount of glycerol accumulated (0.50 mol/mol glucose and 30.35 mM, respectively), and the ratio of 1,3-PDO to glycerol (1,3-PDO/glycerol) was 0.48 (Fig. 4C). The high level of glycerol accumulation was attributed to the insufficient NADH supply. NADH is the essential cofactor for the conversion of glycerol to 1,3-PDO and this conversion cannot proceed without NADH.

3.3. Improvement of 1,3-PDO production by pathway engineering

Although EB-1 could produce 1,3-PDO using glucose as a substrate, the 1,3-PDO yield was far from the theoretical maximum, 1.5 mol/mol glucose [19]. The following genetic modifications were conducted to improve the yield: removal of the glycerol degradation pathway, enhancement of the

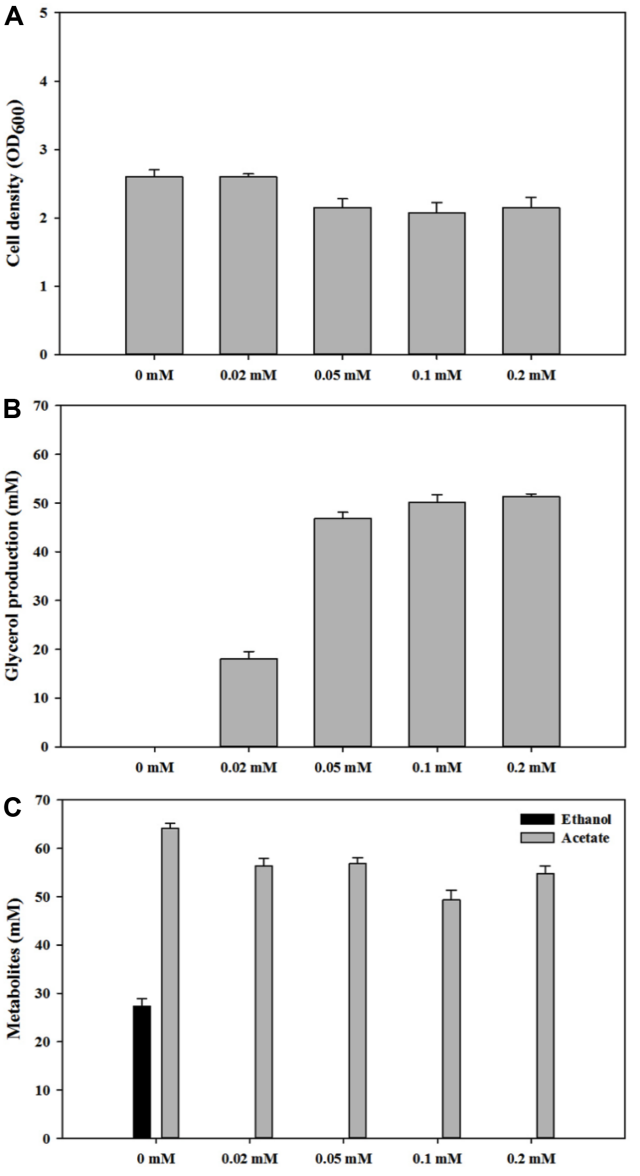

Fig. 3. The performance of EB-0 under varying the IPTG concentrations of 0, 0.02, 0.05, 0.1, and 0.2 mM. (A) Cell density. (B) Glycerol production. (C) Accumulation of by-products, ethanol and acetate. The cells were cultured under micro-aerobic II conditions (50 mL/100 rpm) for 35 h and the data are the average of three independent experiments.

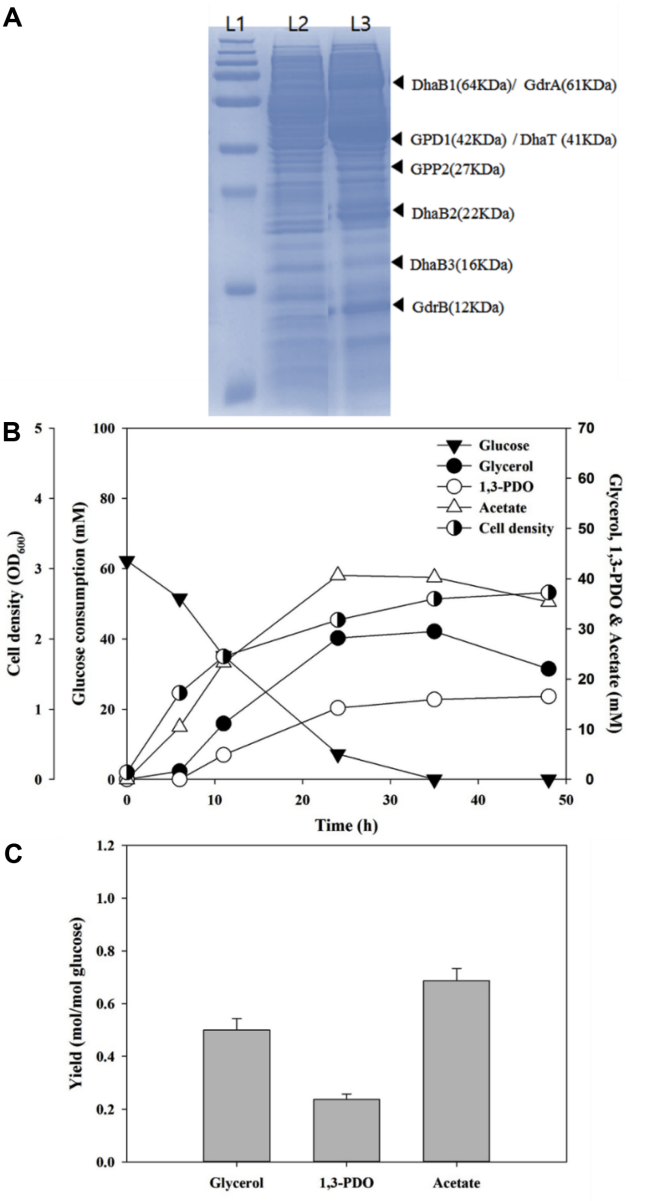

Fig. 4. Characterization of the strain EB-1 expressing both glycerol module (GPD and GPP) and 1,3-PDO module (DhaB and DhaT). (A) SDS-PAGE analysis. The heterologous proteins are marked with arrows. Lane 1, marker; Lane 2, cell-free extract of host *E. coli* BL21(DE3); Lane 3, cell-free extract of induced EB-1. (B) Performance of EB-1. (C) Yield of glycerol, 1,3-PDO, and acetate. The cells were cultured under micro-aerobic II (50 mL/100 rpm) condition for 35 h and induced by 0.1 mM IPTG induction. In (C), the data are the average of three independent experiments.

expression of the glycerol transport facilitator, elimination of the ED pathway, removal of the *ptsG* gene associated with glucose transport, and removal of the *pta-ackA* genes in the acetate production (Fig. 1).

3.4. Deletion glycerol degradation pathways

Glycerol produced from glucose can be assimilated through the glycerol oxidation pathway before it is channeled to the 1,3-PDO (Fig. 1). To prevent the oxidative asimilation, the *glpK, glpABC* and *glpD* genes were deleted and the resulting strain was designated EB3-1. EB3-1 showed similar cell growth and glucose consumption as EB-1. However, the yield of 1,3-PDO increased to 0.32 mol/mol glucose, which is 39% higher than that of EB-1. Sum of glycerol and 1,3-PDO of EB3-1 was 0.76 mol/mol glucose, which is comparable to that of EB-1 (Fig. 5B). On the other hand,

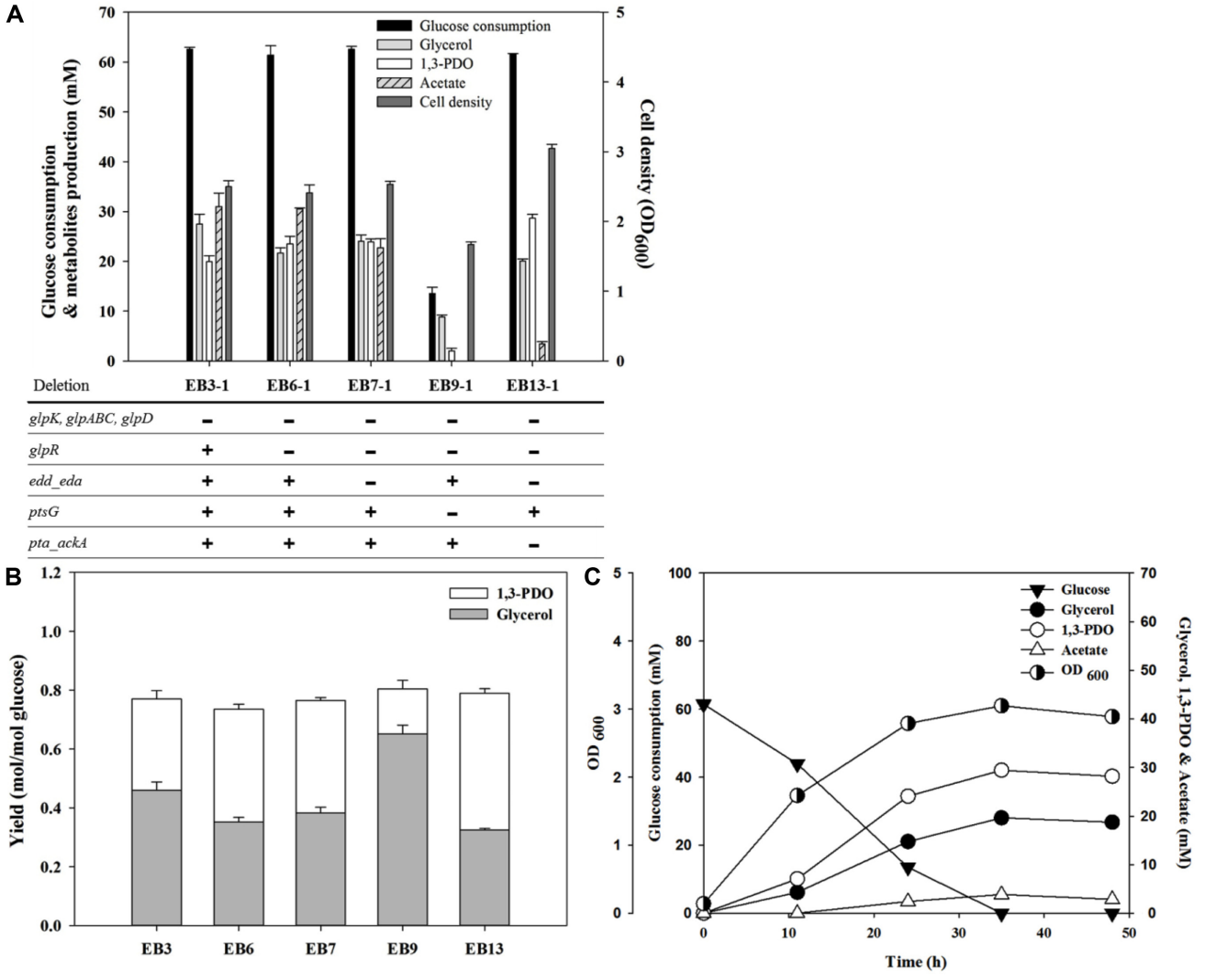

Fig. 5. Performance of various recombinants developed from the hosts, EB3, EB6, EB7, EB9, and EB13. (A) Growth, glucose consumption and metabolites production of various recombinants. (B) Yield of glycerol and 1,3-PDO at 35 h (C) Time course profile of cell growth and production of glycerol, 1,3-PDO, and acetate of EB13-1. The data are the average of three independent experiments. Cells were cultured under the micro-aerobic II (50 mL/100 rpm) condition, and induced by 0.1 mM IPTG.

the only byproduct, acetate, was reduced to 0.50 mol/mol glucose, which is approximately 25% lower. This suggests that a deletion of the glycerol oxidation pathway increased the availability of glycerol for 1,3-PDO production. The decrease in acetate production was attributed to a reduction of the overflow metabolism, which is caused by decrease in the glucose consumption rate and/or the reduced flux to pyruvate at the DHAP node by the prevention of glycerol re-assimilation.

3.5. Deletion of glycerol-3-phosphate regulon repressor (*glpR*)

The presence of a large amount of glycerol in the medium indicates that the glycerol produced from glucose is excreted extracellularly. The 1,3-PDO production can decreased if the glycerol does not moved back to the cell. The transport of glycerol in *E. coli* is known to be promoted by the glycerol uptake facilitator GlpF protein, and its expression is suppressed by the negative regulatory protein, GlpR. According to Jung *et al*., elimination of GlpR changed glycerol uptake rate and dramatically improved glycerol assimilation in *E. coli* [20-22]. To increase the glycerol transport rate, the EB6 strain was developed by removing the *glpR* gene from EB3 and pDPT and pABG plasmids were introduced to construct EB6-1. EB6-1 produced 23.51 mM 1,3-PDO and 21.66 mM glycerol at 36 h cultivation. The 1,3-PDO production yield was 0.38 mol/mol glucose and the glycerol production yield was 0.36 mol/mol glucose, respectively, which are approximately18% higher than those of EB3-1. In addition, the 1,3-PDO/glycerol

production ratio increased to 1.08, which is approximately 50% higher than that of EB3-1. These results indicate that the availability of the intracellular glycerol is increased through the enhanced GlpF expression, which in turn improves 1,3-PDO production.

3.6. Deletion of the ED pathway genes (*edd_eda*)

To improve the production yield of 1,3-PDO, it is necessary to increase carbon flux to glycerol from DHAP which is the immediate precursor of glycerol. Furthermore, the rate of NADH regeneration must be increased. In *E. coli*, glucose is converted to pyruvate through three pathways: Embden-Meyerhof-Parnas (EMP), Pentose-Phosphate (PP), and Entner-Doudoroff (ED). Among these pathways, the ED pathway does not contribute to the production of 1,3-PDO in the aspects of the NADH regeneration and carbon flux to DHAP. The EB7 strain was constructed from the EB6 strain by a deletion of *edd_eda*, the key genes of the ED pathway, and the pDPT and pABG plasmids were introduced to construct EB7-1. No significant change in cell growth was observed, but the production of 1,3-PDO and glycerol were improved; 0.38 mol 1,3-PDO/mol glucose and 0.38 mol glycerol/mol glucose, respectively, were obtained. As a result, sum of glycerol and 1,3-PDO also increased to 0.77 mol/mol glucose, which is comparable to that of EB6-1 (Fig. 5B). The acetate was also reduced by 25%, probably due to reduced overflow metabolism (Fig. 5A). This suggests that deletion of the ED pathway is beneficial for 1,3-PDO production by improving the carbon flux to the 1,3-PDO production pathway and reducing overflow metabolism.

3.7. Reduction of acetate formation

Acetate production is caused by an overflow metabolism, *i.e*., the imbalance between the production and consumption of pyruvate. Acetate production is accompanied by and thus contributes to the supply of ATP but significantly reduces the 1,3-PDO yield. One method to reduce acetate production is to decrease the glucose transport rate through the cell membrane. In *E. coli*, there are five glucose transport systems. Among them, the phosphoenolpyruvate-dependent phosphotransferase system (PTS system) plays a key role [22,23]. The PTS system consists of EI, HPr, EII, *etc*., and the EII consists of the cytosolic IIAGlc protein (encoded by crr gene) and membranous IICBGlc protein (encoded by *ptsG* gene). In this PTS system, conversion of PEP to pyruvate is coupled with glucose transport and this coupling also contributes to the overflow metabolism. The *ptsG* gene was disrupted in EB6 strain, and the EB9 and EB9-1 strains were constructed. As expected, in the newly developed *ptsG*-deficient strains, the glucose consumption rate and cell growth decreased significantly. Only 70% of the initial glucose was consumed even after 48 h. Also, cell growth reached only 1.6 OD_{600}, 60% of EB-7. In addition, the co-production of 1,3-PDO and glycerol was reduced significantly to 30.79 mM. On the other hand, the production yields on glucose were not significantly different; sum of 1,3-PDO and glycerol was 0.72 mol/mol glucose, and 1,3-PDO production was 0.40 mol/mol glucose. Notably, acetate production was reduced by 25% (Supplementary Fig. 1), indicating the overflow metabolism was reduced. However, the overall performance of the *ptsG* deletion mutant was unsatisfactory.

To reduce the production of acetate, a deletion of the acetate production pathway (*pta_ackA*) was also attempted. In this case, the EB7 strain, where *ptsG* is intact, was used. The production of acetate in the *pta_ackA*-deficient EB13-1 strain was reduced greatly to 3.43 mM. In contrast to EB9-1, cell growth and 1,3-PDO production improved slightly (Figs. 5A and 5C). The yield of 1,3-PDO was 0.47mol/mol glucose, which is 19% higher than that by EB7-1. In addition, the yield of glycerol decreased to 0.33 mol/mol glucose from the 0.38 mol/mol glucose of EB7-1 (Fig. 5B). It is speculated that deletion of *pta_ackA* diverts more carbon to the TCA cycle, which should have increased the NADH supply.

4. Conclusion

In this study, recombinant *E. coli* strains were developed and examined for the production of 1,3-PDO from glucose. Glycerol production was confirmed by the overexpression of the GPD1 and GPP2 genes, both derived from *S. cerevisiae*. 1,3-PDO could be produced from glucose through the additional overexpression of DhaB, GdrAB, and DhaT, all derived from *K. pneumoniae*. To improve 1,3-PDO production, the glycerol oxidative pathway (*glpK, glpABC* and *glpD*), glycerol-3-phospate regulon repressor (*glpR*), ED pathway (*edd_eda*), and acetate production pathway (*pta_ackA*) were deleted. The best strain (EB13-1) showed a 1,3-PDO production yield of 0.47 mol/mol glucose and the co-production yield (1,3-PDO and glycerol) of 0.80 mol/mol glucose (Fig. 5B). The production of 1,3-PDO can be improved further by increasing the NADH regeneration rate and controlling the carbon flux distribution at the DHAP node.

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