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Metabolic engineering of 1,2-propanediol production from cellobiose using beta-glucosidase-expressing *E. coli*

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Abstract

Microbial 1,2-propanediol production using renewable feedstock is a promising method for the sustainable production of value-added fuels and chemicals. We demonstrated the metabolically engineered *Escherichia coli* for improvement of 1,2-propanediol production using glucose and cellobiose. The deletion of competing pathways improved 1,2-propanediol production. To reduce carbon flux toward downstream glycolysis, the phosphotransferase system (PTS) was inactivated by *ptsG* gene deletion. The resultant strain, GL3/PD, produced 1.48 ± 0.01 g/L of 1,2-propanediol from 20 g/L of glucose. A sugar supply was engineered by coexpression of β-glucosidase (BGL). The strain expressing BGL produced 1,2-propanediol from cellobiose at a concentration of 0.90 ± 0.11 g/L with a yield of 0.15 ± 0.01 g/g glucose (cellobiose 1g is equal to glucose 1.1 g). As cellobiose or cellooligosaccharides a carbon source, the feasibility of producing 1,2-propanediol using an *E. coli* strain engineered for β-glucosidase expression are demonstrated.

Keywords: metabolic engineering, 1,2-propanediol, *E. coli*, cellobiose

1. Introduction

Many commonly used chemicals are derived industrially from fossil fuels. Because of increasing concern regarding the environmental impact and depletion of fossil fuel stores, there has recently been increased demand for alternative routes of synthesis of these chemicals. Accordingly, interest in biorefinery processes has increased in recent decades. Owing to developments in synthetic biology and metabolic engineering, chemicals such as antibiotics and fuels are generated from biomass using microbes (Clomburg et al., 2017; Pontrelli et al., 2018).

Owing to its favorable characteristics, 1,2-propanediol is an important chemical commodity in the cosmetic industries, drug, and food and is widely used in daily life. This chemical is important to the medicine and food industries because it can dissolve organic compounds. It is used for unsaturated polyester resin preparation and biodegradable plastic production. Its low melting point and nontoxicity make 1,2-propanediol applicable as a de-icing agent and coolant. The global production of 1,2-propanediol was 1.5 million metric tons per year in 2007. Recently, the demand for 1,2-propanediol has been increasing yearly (Bennett and San, 2001; Saxena et al., 2010; Niu et al., 2018).

Microbial production of 1,2-propanediol using L-fucose or L-rhamnose as substrates was first reported in *Clostridium thermobutylicum* (Enebo, 1954), which was difficult to apply to industrial production because of high substrate cost (Saxena et al., 2010). Subsequently, the development of metabolic engineering for 1,2-propanediol production in *Escherichia coli* from glucose or glycerol has been achieved (Altaras and Cameron, 2000; Clomburg and Gonzalez, 2011). Figure 1a shows the metabolic pathway for 1,2-propanediol biosynthesis in *E. coli*. A central carbon metabolism

intermediate, dihydroxyacetone phosphate (DHAP), is metabolized to methylglyoxal catalyzed by methylglyoxal synthase. Then methylglyoxal is reduced by L-1,2-propanediol dehydrogenase and L-1,2-propanediol oxidoreductase, producing 1,2-propanediol. The highest titer of 1,2-propanediol achieved using engineered *E. coli* AG1 Δ*ldhA* has been 4.5 g/L (0.19 g 1,2-propanediol/g glucose) in a fermenter and 1.4 g/L (0.20 g/g glucose) in anaerobic flask cultivation (Altaras and Cameron, 2000). In general, anaerobic conditions are suitable for the production of chemicals, including 1,2-propanediol. The respiratory activity of *E. coli* under anaerobic conditions is weak because of the lack of the aerobic respiratory activity by electron transport chain (Gonzalez et al., 2017), and carbon flux to the TCA cycle is reduced, which causes poor cell growth. Microaerobic fermentation, having the advantages of both aerobic and anaerobic culture, has been used to produce reduced chemicals, such as (*R,R*)-2,3-butanediol and 1,3-propanediol. Jain et al. achieved 1,2-propanediol production under microaerobic conditions with an increased final 1,2-propanediol titer of up to 1.2 g/L (Jain et al., 2015a).

Lignocellulosic biomass has attracted attention as an inexpensive, abundant, and renewable feedstock (Bhatia et al., 2020; Zhao et al., 2020). Bio-based chemicals and fuels production from lignocellulose are a promising approach to sustainable economy and reduction of carbon dioxide emissions. However, most microorganisms are not able to metabolize lignocellulosic biomass directly. Processes for the degradation of lignocellulosic materials can be complex and expensive. For example, enzymatic saccharification process using endoglucanase, exoglucanase, and beta-glucosidase have been needed to hydrolyze cellulose to monomeric glucose. Endoglucanase cleaves the cellulose chain, and cellobiohydrolases then release cellobiose from cellulose polymers.

Finally, beta-glucosidases hydrolyze cellobiose and cellooligosaccharides to produce glucose (Khare et al., 2015). To avoid inhibition of beta-glucosidase activity caused by cellobiose and glucose, excess β-glucosidase is required to cellulase reactions (Van Dyk and Pletschke, 2012; Khare et al., 2015). Other inhibitors (gallic, hydroxy cinnamic, 4-hydroxybenzoic, tannic, and vanillin) have been shown to cause about 80% inhibition of β-glucosidase (Ximenes et al., 2011). Disaccharides, such as cellobiose, were found in the hydrolysates of these reactions (Li et al., 2010). Nevertheless, there has been sparse investigation of the use of cellobiose or cellooligosaccharides for 1,2-propanediol production.

Here, we constructed a 1,2-propanediol-producing *E. coli* strain using metabolic engineering. We evaluated genes related to the 1,2-propanediol metabolism pathway and glycolysis. The engineered GL3/PD strain produced 1.48 ± 0.01 g/L of 1,2-propanediol with a yield of 0.15 ± 0.00 g/g glucose. In addition, we attempted 1,2-propanediol production from cellobiose. Previously, we achieved production of various chemicals from cellobiose by expression of β-glucosidase (BGL) (Satowa et al., 2020; Sato et al., 2020; Matsuura et al., 2019). We applied this method to 1,2-propanediol production under microaerobic condition and achieved 0.90 ± 0.11 g/L of 1,2-propanediol production from cellobiose. These findings represent an important step toward efficient and sustainable production of this ubiquitously used industrial chemical.

2. Materials and Methods

2.1 Strains and medium

E. coli MG1655 was used as the host strain. Quick Taq HS (TOYOBO, Osaka, Japan) was used for polymerase chain reaction (PCR). For gene cloning, *E. coli* NovaBlue competent cells (Novagen, Cambridge, MA, USA) were utilized. DNA primers were purchased from the Invitrogen Custom DNA Oligos service (Thermo Fisher Scientific, Tokyo, Japan). Gene fragments (*mgsA* from *Bacillus subtilis*) were synthesized using the Invitrogen GeneArt Gene Synthesis service (Thermo Fisher Scientific, Tokyo, Japan). M9Y medium consisted of 20 g/L of glucose, 5 g/L of yeast extract, 0.1 mM $CaCl_2 \cdot 2H_2O$, 0.01 mM $FeSO_4 \cdot 7H_2O$, 1 mM $MgSO_4 \cdot 7H_2O$, 10 mg/L of thiamine hydrochloride, 0.5 g/L of NaCl, 17.1 g/L of $Na_2HPO_4 \cdot 12H_2O$, 3 g/L of KH_2PO_4, 2 g/L of NH_4Cl, 0.1 mM IPTG, 30 mg/L of chloramphenicol, and/or 100 mg/L of ampicillin.

2.2 Plasmids

We amplified insert fragment_1 using PCR with pHLA-*blc*-*Tfu0937* (Tanaka et al., 2011) as a template and pelB_Tfu0937_f1 and XhoI_Tfu0937_re primers. We then amplified insert fragment_2 using PCR with insert fragment_1 as a template and Bgl2_pelB_for and XhoI_Tfu0937_re primers. Insert fragment_2 was then cloned into the *Bgl*II and *Xho*I sites of pHLA, generating the pHLA-*pelB*-*Tfu0937* plasmid.

The pTΔgloAF plasmid was constructed by PCR amplification of the linearized fragment using pTargetF (Jiang et al., 2015) as a template and N20_del_gloA.Fw and N20_del_golA.Rv primers. The amplified fragment was self-ligated, and the resultant plasmid was named pTΔgloAF. The upstream and downstream DNA sequences of *gloA* were amplified using *E. coli* MG1655 and UP_del_gloA.Fw/UP_del_gloA.Rv and DOWN_del_gloA.Fw/DOWN_del_gloA.Rv primer pairs, respectively. The resultant

fragments were fused using overlap extension PCR with the UP_del_gloA.Fw/DOWN_del_gloA.Rv primer pair. The amplified donor was cloned into the *EcoR*I / *Hind*III sites of pTΔgloAF, resulting in the pTΔgloA plasmid. The other plasmids of the pTarget series, namely, pTΔgloAF, pTΔhchAF, pTΔghrAF, pTΔaldAF, pTΔzwfF, pTΔldhAF, pTΔackAF, pTΔpoxBF, pTΔptaF, and pTΔtpiAF, were constructed using the same procedures that were used to construct pTΔgloA.

The pTptsG::galP-glk plasmid was constructed by inverse PCR amplification of the linearized fragment using pTΔptsG as a template and ptargetptsg_for and ptargetptsG_re primers. Using *E. coli* CFT5 genomic DNA (Noda et al., 2016) as a template, the *galP-glk* gene fragment was amplified by PCR and XhoI_Plac_for and Avr2_term_re primers. Both fragments were ligated, and the resulting plasmid was named pTptsG::galP-glk.

Genetic deletions of *gloA*, *hchA*, *ghrA*, *aldA*, *zwf*, *ldhA*, *ackA*, *poxB*, *pta*, and *tpiA* were carried out using the CRISPR-Cas9-plasmid system (Jiang et al. 2015) in pTΔgloA, pTΔhchA, pTΔghrA, pTΔaldA, pTΔzwf, pTΔldhA, pTΔackA, pTΔpoxB, pTΔpta, pTΔtpiA, respectively.

2.3 Cultivation conditions

One colony from an LB plate was inoculated in LB medium and incubated 24h at 37°C while shaken at 220 rpm. This preculture was transferred into screw cap bottles containing 5 mL of M9 or M9Y medium until the OD_{600} was 0.05. These cultures were grown at 30°C and 150 rpm until collection of 300 µL at 24 and 48 h and 500 µL at 72 and 96 h for analysis. Experiments were carried out using three biological replicates.

2.4 Analytical methods

Cell growth was evaluated by the optical density at 600 nm on a UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A Prominence HPLC (Shimadzu) with a SUGAR KS-801 column (8.0 mmID × 300 mmL.; Shodex) was used for the analysis of oncentrations of sugars and 1,2-propanediol. The mobile phase was water with 0.8 mL/min flow rate at 50°C. HPLC profiles were detected using a refractive index detector. Lactate, acetate, and succinate were analyzed using HPLC with a SCR-102H column (7 μm, 8.0 mmID × 300 mmL.; Shimadzu) at 50°C. The mobile phase was 5 mM *p*-toluenesulfonic acid with a flow rate of 1.5 mL/min. A conductivity detector was used for monitoring of HPLC profile.

To measure NADH/NAD ratios in strains, we used an EnzyChrom™ NAD^+/NADH Assay Kit (E2ND-100, BioAssay Systems, Hayward, USA). Cells (5mL) after 24h cultivation was centrifuged at 3500*g* for 5 min. Extraction solution was added to collected cells then incubated at 4°C and 1000 rpm. After 24h, the supernatant was centrifuged at 13,000*g* for 5 min and evaporated. These samples were analyzed using an EnzyChrom NAD^+/NADH Assay Kit (E2ND-100) according to the manufacturer's instructions. BGL activity was measured according to previous report (Tanaka et al., 2011).

3. Results and Discussion

3.1 Reconstruction of the 1,2-propanediol production pathway in *E. coli* MG1655

The metabolic pathway of 1,2-propanediol synthesis is shown in Fig. 1a. Glyoxylase catalyzes the formation of lactate from methylglyoxal. It has been reported that the disruption of the *gloA* gene, which encodes glyoxylase, improves 1,2-

propanediol production (Altaras and Cameron, 2000). To eliminate this competing pathway, we deleted *gloA* from wild-type *E. coli* MG1655. To activate the 1,2-propanediol synthesis pathway, we used the pSAK-PD plasmid containing *Bacillus subtilis* *Bs_mgsA* and *E. coli gldA* and *fucO* encoding methylglyoxal synthase, glycerol dehydrogenase, and L-1,2-propanediol oxidoreductase, respectively (Fujiwara et al., 2020). The plasmid pSAK-PD was introduced into MG1655 and MG1655Δ*gloA*. The resulting strains were named MG1655/PD and MG1655Δ*gloA*/PD, respectively. Both strains were cultivated under microaerobic condition at 30 °C and 220 rpm for 24 h in M9 minimum medium with 20 g/L of glucose. MG1655Δ*gloA*/PD produced 0.14 ± 0.02 g/L of 1,2-propanediol under microaerobic conditions, and wild-type MG1655/PD did not produce 1,2-propanediol (data not shown).

3.2 Disruption of competing pathways in 1,2-propanediol production

Methylglyoxal and L-lactaldehyde, which are intermediates of the 1,2-propanediol synthesis pathway, are converted to other chemicals by various enzymes (Clomburg and Gonzalez, 2011). The deletion of enzymes in this pathway can lead to the redirection of the carbon flux into 1,2-propanediol production (Clomburg and Gonzalez, 2011). We first focused on two enzymes, namely, protein/nucleic acid deglycase (*hchA*), which converts methylglyoxal to lactate, and glyoxylate reductase (*ghrA*), which converts methylglyoxal to D-lactaldehyde. By disrupting *hchA* and/or *ghrA* in MG1655Δ*gloA*, we constructed three strains, namely, MG1655Δ*gloA*Δ*hchA*, MG1655Δ*gloA*Δ*ghrA*, and MG1655Δ*gloA*Δ*hchA*Δ*ghrA*. The pSAK-PD was introduced into these three strains, and 1,2-propanediol production was carried out for 24 h under microaerobic conditions at 30°C and 150 rpm in M9Y medium containing 20 g/L of

glucose. The MG1655Δ*gloA*/PD strain produced 0.57 ± 0.04 g/L of 1,2-propanediol, which was four-fold (0.14 vs 0.57) higher than that in the M9 medium. MG1655Δ*gloA*Δ*hchA*/PD, MG1655Δ*gloA*Δ*ghrA*/PD, and MG1655Δ*gloA*Δ*hchA*Δ*ghrA*/PD strains produced 0.57 ± 0.05, 0.58 ± 0.00, and 0.56 ± 0.03 g/L of 1,2-propanediol, respectively (Fig. 1b). Subsequently, aldehyde dehydrogenase encoded by *aldA*, which catalyzes the conversion of L-lactaldehyde to L-lactate, was disrupted. With the disruption of *aldA* in MG1655*ΔgloAΔhchA*, MG1655Δ*gloA*Δ*ghrA*, and MG1655Δ*gloA*Δ*hchA*Δ*ghrA*, we constructed MG1655Δ*gloA*Δ*hchA*Δ*aldA*/PD, MG1655Δ*gloA*Δ*ghrA*Δ*aldA*/PD, and MG1655Δ*gloA*Δ*hchA*Δ*ghrA*Δ*aldA*/PD strains. MG1655Δ*gloA*Δ*hchA*Δ*aldA*/PD achieved the highest production (0.60 ± 0.04 g/L) with a yield of 0.11 ± 0.01 g/g after 24 h of cultivation. This yield of 1,2-propanediol was also higher than in any other strains. Contrary to our expectation, the 1,2-propanediol titer produced by the MG1655Δ*gloA*Δ*hchA*Δ*ghrA*Δ*aldA*/PD strain was lower than those from other strains (Fig. 1b). To improve 1,2-propanediol production, it is also important to accumulate DHAP, which is an intermediate of glycolysis. Jain et al. reported that the deletion of *zwf*, which encodes glucose-6-dehydrogenase, prevented the leaking of the carbon flux into the pentose–phosphate pathway and improved 1,2-propanediol production (Jain et al., 2015a, 2015b). We constructed a *zwf*-deficient strain from MG1655Δ*gloA*Δ*hchA*Δ*ghrA*Δ*aldA* and introduced pSAK-PD, resulting in the MG1655Δ*gloA*Δ*hchA*Δ*ghrA*Δ*aldA*Δ*zwf*/PD strain. The production titer of 1,2-propanediol in MG1655Δ*gloA*Δ*hchA*Δ*ghrA*Δ*aldA*Δ*zwf*/PD was significantly decreased (0.08 ± 0.02 g/L). This result indicates that carbon is donated to the 1,2-propanediol synthetic pathway through PPP as well as glycolysis, through the reaction of converting

GAP to DHAP. On the basis of these results, we used MG1655Δ*gloA*Δ*hchA*Δ*aldA*, which achieved the highest production of 1,2-propanediol, for the following experiments, and this strain was renamed GL1.

3.3 Improvement in DHAP supply by *tpiA* disruption

Triose phosphate isomerase (TpiA, coded by *tpiA*) catalyzes a reversible reaction between GAP and DHAP. To prevent carbon leaks from DHAP to GAP, *tpiA*-deficient strains were used in previous studies of 1,2-propanediol production (Jain et al., 2015a, 2015b; Jung et al., 2008). We constructed a *tpiA*-deficient strain from GL1 (GL1-Δ*tpiA*), introduced pSAK-PD (GL1-Δ*tpiA*/PD), and carried out 1,2-propanediol production under microaerobic conditions. GL1-Δ*tpiA*/PD produced 0.19 ± 0.00 g/L of 1,2-propanediol with a yield of 0.08 ± 0.00 g/g after 24 h of cultivation, and both the titer and yield were lower than those of GL1/PD (Fig. 2a). The glucose consumption and cell growth of GL1-Δ*tpiA*/PD were also decreased compared to GL1/PD (Fig. 2a). These results suggested that TpiA led to carbon flux from DHAP to GAP in GL1/PD, consistent with the results for MG1655Δ*gloA*Δ*hchA*Δ*ghrA*Δ*aldA*Δ*zwf*/PD. Two studies from Yan's group were published about *zwf* deletion. They disrupted *tpiA* and *zwf* in *E. coli* BW25113 and could improve 1,2-propanediol titer (Jain et al., 2015a). However, additional disruption of *tpiA*, *zwf*, and *adhE* of BW25113Δ*gloA*Δ*ldhA* did not contribute toward improvement of 1,2-propanediol titer (Jain et al., 2015b). The disruptions of these genes may not have a positive effect on 1,2-propanediol production and also caused lower glucose consumption (Jain et al., 2015b).

3.4 Lactate dehydrogenase deletion to increase NADH availability

Because the biosynthesis of 1 mole of 1,2-propanediol from glucose requires 2 moles of NADH, NADH regeneration is an important factor for 1,2-propanediol production. Jain et al. reported that the disruption of lactate dehydrogenase encoded by *ldhA*, which converts pyruvate to lactate by consuming NADH, led to improved 1,2-propanediol production (Jain et al., 2015b). We constructed a *ldhA*-deficient strain derived from GL1 (GL2), introduced pSAK-PD (GL2/PD), and carried out 1,2-propanediol production under microaerobic conditions. Culture profiles of GL1/PD and GL2/PD are shown in Fig. 2a. GL1/PD produced 0.77 ± 0.08 g/L of 1,2-propanediol with a yield of 0.10 ± 0.01 g/g after 75 h of cultivation. Conversely, the 1,2-propanediol titer and yield from GL2/PD after 74 h of cultivation were 1.27 ± 0.16 g/L and 0.14 ± 0.02 g/g, respectively, which were 1.6-fold higher than those of GL1/PD. Although 0.98 ± 0.68 g/L of lactate accumulated during GL1/PD cultivation, no lactate was observed in GL2/PD cultures. This result was consistent with that of the previous study (Jain et al., 2015a). We measured the NADH/NAD ratio of MG1655Δ*gloA* and GL2 strains. The NADH/NAD ratio was similar among these strains (Fig. 2b). This result indicated that a shortage of NADH was not a rate-limiting factor. We speculated that the main factor of improvement of 1,2-propanediol was not NADH/NAD level but prevention of carbon flux from flowing into lactate.

3.5. The effect of acetate-producing pathways in GL2 on 1,2-propanediol production

Acetate is a major by-product of 1,2-propanediol production. Clomburg and Gonzalez reported that disruption of *ackA-pta* (acetate kinase and phosphate acetyltransferase) in *E. coli* led to improved 1,2-propanediol production (Clomburg and Gonzalez, 2011). It

was also reported that disruption of *poxB* (pyruvate oxidase) enhanced 1,2-propanediol production (Zhu et al., 2016). We constructed *ackA*, *poxB*, and *pta*-deficient strains derived from GL2 (GL2-Δ*ackA*, GL2-Δ*poxB*, and GL2-Δ*pta*, respectively), introduced pSAK-PD (GL2-Δ*ackA*/PD, GL2-Δ*poxB*/PD, and GL2-Δ*pta*/PD), and carried out fermentation for 72 h under microaerobic condition. GL2-Δ*ackA*/PD, GL2-Δ*poxB*/PD, and GL2-Δ*pta*/PD strains produced lower 1,2-propanediol concentrations (0.96 ± 0.07, 0.93 ± 0.12, and 0.18 ± 0.01 g/L, respectively) than the GL2/PD strain. Our results showed that disruptions of acetate biosynthesis pathways in GL2 did not improve 1,2-propanediol production. In the previous study, disruptions of acetate-synthetic pathway (*ackA* and *poxB*) in wild type did not improve the titer of 1,2-propanediol while the amount of acetate was decreased (Jain et al., 2015a). On the other hand, the combination of disrupting *tpiA*, *zwf*, and acetate-synthetic pathway might have a positive effect on 1,2-propanediol production (Jain et al., 2015a). In our study, disruptions of the acetate synthesis pathways of GL2, which remains intact *zwf* and *tpiA* genes, did not improve the titer of 1,2-propanediol. This result was consistent with that of the previous study (Jain et al., 2015a). In the case of alternative pathways for 1,2-propanediol production, such as lactate-derived pathway (Zhu et al., 2016) or glycerol-derived pathway (Clomburg and Gonzalez, 2011), disruption of acetate-synthetic pathway could improve its titer.

3.6. Alteration of glucose uptake systems for reducing pyruvate

From the results of our experiments, we speculated that low 1,2-propanediol production was due to the leakage of carbon to downstream glycolysis. To prevent these carbon leaks, we focused on the glucose uptake systems: the phosphotransferase system

(PTS) and the galactose permease/glucokinase system (GGS). In the PTS, 1 mole of phosphoenolpyruvate (PEP) is converted to 1 mole of pyruvate during the transport and phosphorylation of 1 mole of glucose into the cell. Alternatively, in the GGS, ATP is consumed as a phosphoryl group donor instead of PEP. To reduce PEP consumption and carbon flow to downstream glycolysis, we replaced the PTS with the GGS. A GGS operon containing the *galP* (galactose permease) and *glk* (glucokinase) genes under the control of a *lac* promoter was introduced into the *ptsG* locus in GL2, and the resultant strain was named GL3. Following the introduction of pSAK-PD (GL3/PD), production of 1,2-propanediol was carried out at 30°C and 150 rpm in M9Y medium. GL3/PD produced 1.48 ± 0.01 g/L of 1,2-propanediol titer with a yield of 0.15 ± 0.00 g/g at 72 h. This result showed that GGS was appropriate for microaerobic 1,2-propanediol production. With GL3/PD, succinate was detected as a major by-product (0.92 ± 0.00 g/L at 72 h) and was 1.57-fold more concentrated than that with GL2/PD (0.59 ± 0.13 g/L at 74 h). Under microaerobic conditions, the reverse citric acid cycle is slightly dominant (Thakker et al., 2012). In GL3/PD, PEP consumption via the PTS was diminished by *ptsG* disruption, and accumulated PEP flowed into oxaloacetate via phosphoenolpyruvate carboxylase, resulting in increased succinate accumulation. The important factors for producing 1,2-propanediol effectively were the repression of the carbon flux to TCA cycle and the improvement of glucose consumption. To repress the carbon flow to the TCA cycle, some previous studies have adopted cultures using anaerobic conditions and low phosphate medium. (Altaras and Cameron, 2000; Clomburg and Gonzales, 2011; Jain et al., 2015b). Yan's group also tried to improve the growth and glucose consumption by using optimized cell adaptation strategy (Jain et al., 2015b). This method triggered the adaptation of strains to low phosphate medium

gradually and enabled to increase cell growth. In our study, although the cell growth was better than those in the previous reports, the carbon flux to the TCA cycle under microaerobic condition was larger compared to anaerobic condition (Nikel et al.,2009; Gonzalez et al., 2017). To improve the 1,2-propanediol production further, the fine tuning of the carbon flux and/or optimization of culture condition might be required.

3.7. Production of 1,2-propanediol from cellobiose using a *pelB*-mediated BGL-expressing *E. coli* strain

To produce 1,2-propanediol from cellobiose, we adopted BGL Tfu0937 from *Thermobifida fusca* YX (Tanaka et al., 2011). Cell surface display is a technique for anchoring a target protein to a cell's outer membrane. The C-terminus of the anchor protein Blc (UniProt P0A901), a lipoprotein localized to the cell's outer membrane, was fused with the N-terminus of Tfu0937. The production of 1,2-propanediol from cellobiose was carried out using GL2/PD harboring pHLA-*blc-Tfu0937* (GL2/PDB) and GL3/PD harboring pHLA-*blc-Tfu0937* (GL3/PDB) at 30°C and 150 rpm in M9Y medium with 20 g/L of cellobiose. GL2/PDB produced 0.40 ± 0.19 g/L of 1,2-propanediol after 96 h of cultivation (Fig. 3a), and GL3/PDB exhibited no 1,2-propanediol production and little consumption of cellobiose under these conditions. The consumption of cellobiose in GL2/PDB remained low after 96 h (Fig. 3b), which may have been attributable to lower BGL activity at the cell surface. Therefore, we adopted signal sequence-mediated transport strategies for BGL expression on the cell surface. The PelB signal peptide, corresponding to the first 22 residues of pectate lyase B from *Erwinia carotovora* (UniProt P0C1C1), was fused to the N-terminus of Tfu0937, facilitating its transport into the periplasm without anchoring. As shown in Fig. 3c, the

BGL activity of GL2 harboring pHLA-*pelB-Tfu0937* (GL2/PelB) was 1.9-fold greater than that of the strain harboring pHLA-*blc-Tfu0937* (GL2/Blc), suggesting that a *pelB*-mediated expression system is useful for BGL expression under microaerobic conditions. Conversely, the BGL activity of GL3/PelB was lower than that of GL2/Blc. This result indicated that GL2 is better suited than GL3 for the production of 1,2-propanediol when using cellobiose as a carbon source.

We carried out 1,2-propanediol production from glucose or cellobiose as carbon sources using the GL2 strain harboring both pSAK-PD and pHLA-*pelB-Tfu0937* (GL2/PDP). As shown in Fig. 3a, GL2/PDP produced 0.90 ± 0.11 g/L of 1,2-propanediol from cellobiose and 1.07 ± 0.33 g/L of 1,2-propanediol from glucose. The 1,2-propanediol yield from cellobiose was 0.15 ± 0.01 g/g glucose (cellobiose 1 g is equal to glucose 1.1 g), which was similar to that with glucose as a carbon source (0.14 ± 0.02 g/g). Aso et al. also used cellobiose as a substrate for lactate production using *Escherichia coli* BW25113. This strain consumed 10 g/L cellobiose after 72 h fermentation under microaerobic condition (Aso et al., 2018). In our study, the consumed cellobiose by GL2/PDP was 9.55 ± 4.90 g/L after 72 h fermentation, which was similar to that observed in their study. Free glucose existed in the culture medium after 96 h cultivation. It indicates that the hydrolysis of cellobiose by BGL was enough and that enhancement of glucose uptake may contribute 1,2-propanediol production.

4. Conclusions

This study demonstrated direct 1,2-propanediol production from cellobiose. Metabolic engineering replacing PTS with GGS improved 1,2-propandiol production. PelB-mediated BGL expression resulted in higher BGL activity than Blc-mediated BGL

expression did, leading to the enhancement of 1,2-propanediol production from cellobiose. Using an engineered *E. coli* strain, our results demonstrate the effectiveness of producing 1,2-propanediol from cellobiose or cellooligosaccharides and represent an important step toward efficient and sustainable production of this ubiquitously used industrial chemical.

Supplementary materials

E-supplementary data for this work can be found in e-version of this paper online.

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CRediT authorship contribution statement

Daisuke Nonaka: Conceptualization, Investigation, Writing - original draft. Ryosuke Fujiwara: Conceptualization, Investigation, Writing - review & editing. Yuuki Hirata: Investigation. Tsutomu Tanaka: Conceptualization, Writing - review & editing, Funding acquisition. Akihiko Kondo: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure Captions

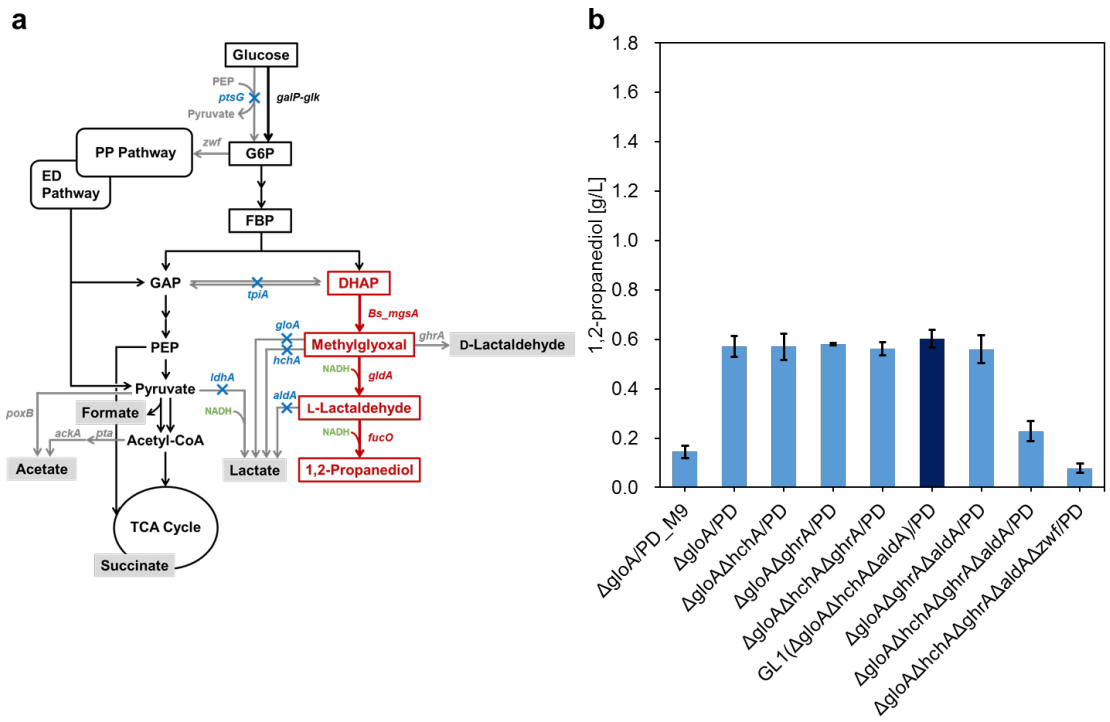
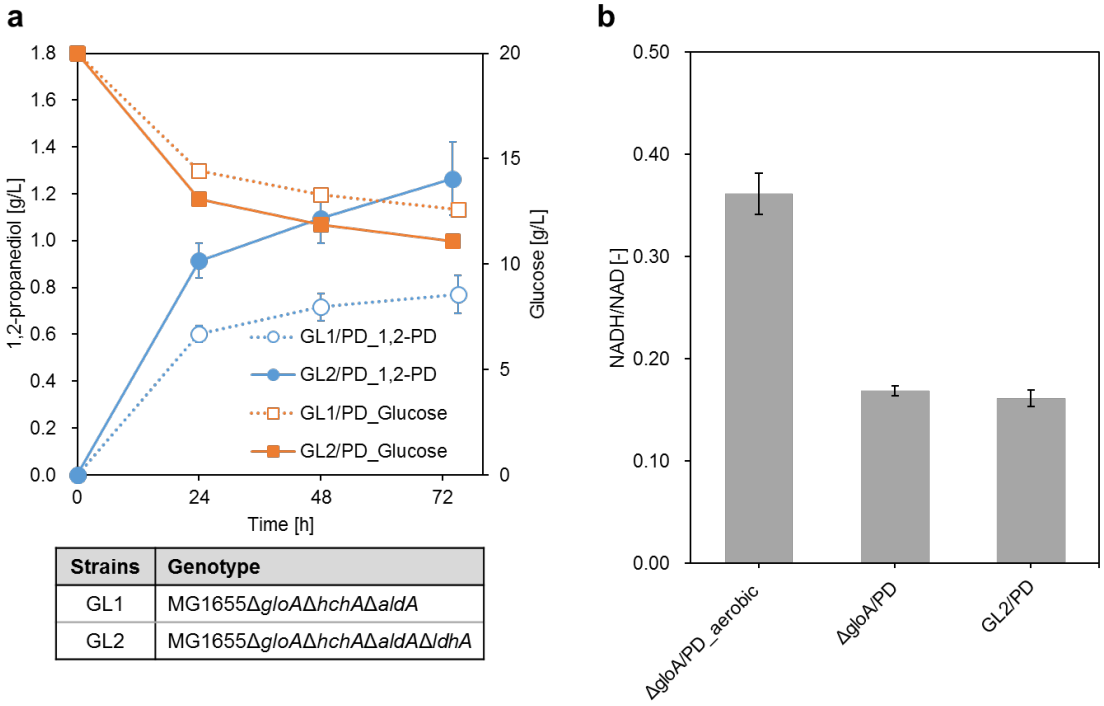


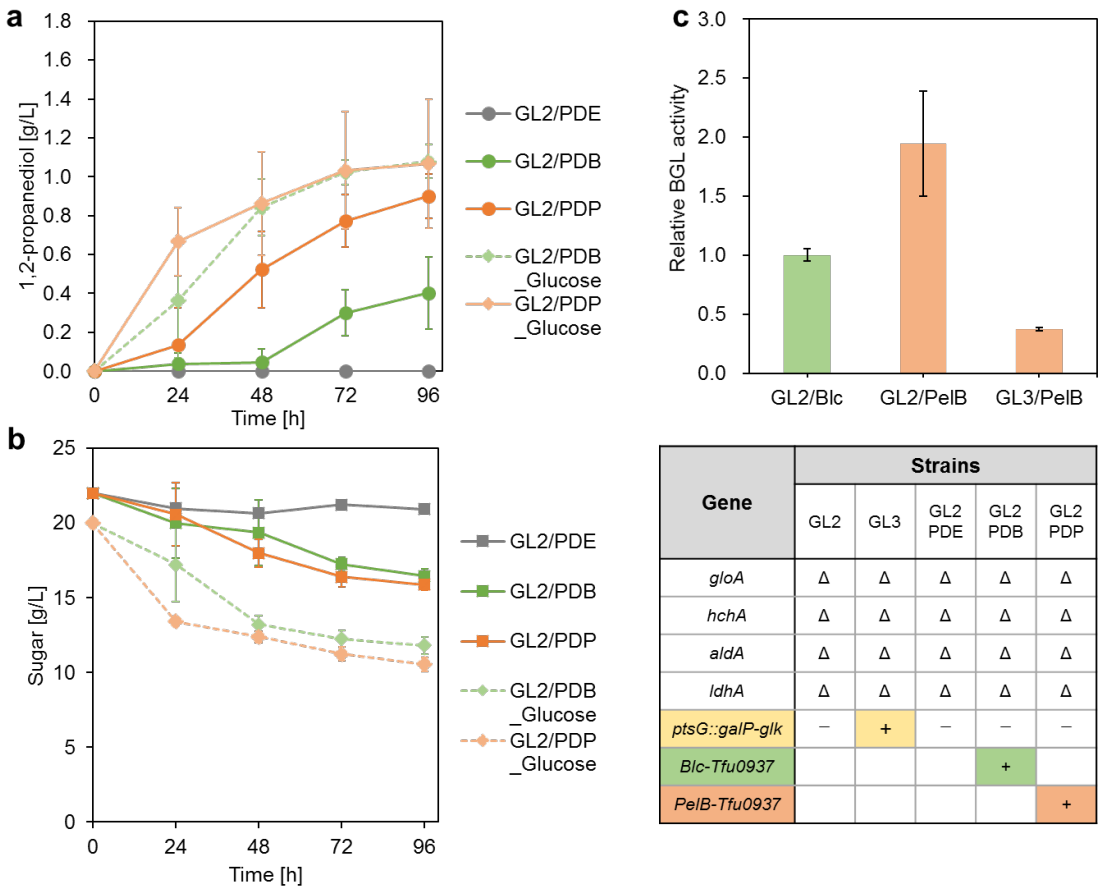
Fig. 1. (a) Metabolic engineering of 1,2-propanediol producing *E. coli*. The blue X indicates deletion of the *ptsG*, *tpiA*, *gloA*, *hchA*, *aldA*, and *ldhA* genes. Red shows genes involved in 1,2-propanediol synthesis. G6P, glucose 6-phosphate; FBP, fructose 1,6-bisphosphate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate; PP pathway, pentose–phosphate pathway; ED pathway, Entner–Doudoroff pathway; *mgsA*, methylglyoxal synthase; *gldA*, glycerol dehydrogenase; *fucO*, L-1,2-propanediol oxidoreductase; *gloA*, glyoxalase I; *hchA*, glyoxalase III and Hsp31 molecular chaperone; *ghrA*, glyoxylate/hydroxypyruvate reductase A; *aldA*, aldehyde dehydrogenase A; *zwf*, glucose-6-phosphate 1-dehydrogenase; *ackA*, acetate kinase A/propionate kinase 2; *pta*, phosphate acetyltransferase; *poxB*, pyruvate dehydrogenase; *ldhA*, D-lactate dehydrogenase; *ptsG*, fused glucose-specific PTS enzymes; *galP*, D-galactose transporter; *glk*, glucokinase. (b) 1,2-Propanediol production titer after 24 h of cultivation in each strain expressing

1,2-propanediol pathway genes. The data are shown as averages of three independent experiments with standard deviations.



Strains	Genotype
GL1	MG1655Δ*gloA*Δ*hchA*Δ*aldA*
GL2	MG1655Δ*gloA*Δ*hchA*Δ*aldA*Δ*ldhA*

Fig. 2. (a) Culture profiles of GL1 (MG1655Δ*gloA*Δ*hchA*Δ*aldA*)/PD and GL2 (MG1655Δ*gloA*Δ*hchA*Δ*aldA*Δ*ldhA*)/PD. (b) NADH/NAD ratio in strains. The data are shown as averages of three independent experiments with standard deviations.



Gene	Strains				
	GL2	GL3	GL2 PDE	GL2 PDB	GL2 PDP
gloA	Δ	Δ	Δ	Δ	Δ
hchA	Δ	Δ	Δ	Δ	Δ
aldA	Δ	Δ	Δ	Δ	Δ
ldhA	Δ	Δ	Δ	Δ	Δ
ptsG::galP-glk	–	+	–	–	–
Blc-Tfu0937				+	
PelB-Tfu0937					+

Fig. 3. (a) Time course of 1,2-propanediol production from cellobiose and (b) sugar consumption in GL2(MG1655Δ*gloA*Δ*hchA*Δ*aldA*Δ*ldhA*) harboring pSAK-PD and pHLA vector (gray), pSAK-PD and pHLA-*pelB-Tfu0937* (orange), or pSAK-PD and pHLA-*blc-Tfu0937* (green). Solid lines show profiles cultured with cellobiose, and dotted lines show those when glucose was used. (c) Relative BGL activity after 24 h of cultivation in GL2/Blc, GL2/PelB, and GL3 (MG1655*ptsG::galP-glk*Δ*gloA*Δ*hchA*Δ*aldA*Δ*ldhA*)/PelB under microaerobic condition. The data are shown as averages of three independent experiments with standard deviations.