Title

Fermentative production of 1-propanol from D-glucose, L-rhamnose and glycerol using recombinant *Escherichia coli*

Running title

1-PROPANOL PRODUCTION USING RECOMBINANT *E. COLI*

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Abstract

Fermentative production of 1-propanol, which is one of the promising precursors of polypropylene production, from D-glucose, L-rhamnose and glycerol using metabolically engineered *Escherichia coli* was examined. To confer the ability to produce 1-propanol from 1,2-propanediol (1,2-PD) in recombinant *E. coli*, a part of the *pdu* regulon including the diol dehydratase and the propanol dehydrogenase genes together with the adenosylcobalamin (AdoCbl) regeneration enzyme genes of *Klebsiella pneumoniae* was cloned, and an expression vector for these genes (pRSF_pduCDEGHOQS) was constructed. Recombinant *E. coli* harboring pRSF_pduCDEGHOQS with 1,2-PD synthetic pathway (pKK_mde) genes, which was constructed in our previous report (Urano et al., Appl. Microbiol. Biotechnol., 99, 2001-2008 (2015)), produced 16.1 mM of 1-propanol from D-glucose with a molar yield of 0.36 mol/mol after 72 h cultivation. 29.9 mM of 1-propanol was formed from L-rhamnose with a molar yield of 0.81 mol/mol using *E. coli* carrying only pRSF_pduCDEGHOQS. In addition, 1-propanol production from glycerol was achieved by addition of the ATP-dependent dihydroxyacetone kinase gene to *E. coli* harboring pKK_mde and pRSF_pduCDEGOQS. In all cases, 1-propanol production was achieved by adding only a small amount of AdoCbl.

Introduction

Due to widespread concerns about global increases in greenhouse gas levels and the depletion of petroleum reserves, various attempts have recently been made to extract, from biomass and other renewables, fuels and chemicals that were previously obtained from fossil resources (1). Propylene is one such compound and used as a monomer material for synthesis of polypropylene, which is a general-use and petroleum-derived polymer. There are several reported methods for the production of propylene from biomass, most of them dependent on the chemical conversion of ethanol or propanol (isopropanol and 1-propanol) fermentatively obtained from biomass into propylene. Commercial production of ethanol using *Saccharomyces cerevisiae* has already been developed and the resulting ethanol has been used as a biofuel (2). Moreover, isopropanol has been efficiently produced by recombinant microorganisms into which the isopropanol biosynthetic pathway of acetone-butanol fermenting bacteria, *Clostridium*, was introduced (3,4). Meanwhile, 1-propanol production methods have been reported by several groups (5–7), but their productivities were not sufficient. However, when the production efficiency of these alcohols is compared from the viewpoint of carbon yield in the fermentation process, 1-propanol has advantages over ethanol and isopropanol. 1-Propanol can be produced from D-glucose without CO₂ release, while the biosynthetic pathway of ethanol or isopropanol includes CO₂ emission steps. Theoretically, therefore, 1 mol of D-glucose (C₆ unit) would be converted to 2 mol of ethanol (C₂ unit), 1 mol of isopropanol (C₃ unit) or 2 mol of 1-propanol (C₃ unit); that is, the carbon yields in the respective fermentation processes would be 67%, 50% and 100%.

The designed 1-propanol biosynthetic pathway is shown in Fig. 1. In previous studies, 1-propanol production methods from D-glucose using recombinant *Shimwellia blatte*

carrying the 1,2-propanediol (1,2-PD) synthetic pathway (8) or recombinant *Escherichia coli* carrying both the 1,2-PD synthetic and 1-propanol synthetic pathways have been reported (5,6). In this paper, in addition to the 1,2-PD synthetic pathway that was constructed in our previous report (8), we also constructed the 1-propanol synthetic pathway. The newly constructed 1-propanol synthetic pathway also included the regeneration system of adenosylcobalamin (AdoCbl), which was required as a cofactor in the 1,2-PD dehydration reaction. We then examined the 1-propanol production from D-glucose using recombinant *E. coli* harboring the designed 1-propanol biosynthetic pathway.

In addition, we attempted to produce 1-propanol from L-rhamnose and glycerol. L-Rhamnose and glycerol are underutilized biomasses, and both are expected to be useful as substrates in fermentative production. When L-rhamnose is used as a substrate, an endogenous L-rhamnose utilization pathway is used in combination with the engineered 1-propanol synthetic pathway. In the case that glycerol is used as a starting material, an engineered glycerol utilization pathway is further introduced into the 1-propanol-producing *E. coli*.

MATERIALS AND METHODS

Bacterial strains and culture conditions All strains used are listed in Table S1. *E. coli* JM109 was used as a host strain for plasmid construction, and *Klebsiella pneumoniae* AKU30 (NBRC12019) was used as the source of *pduCDEGH*, *pduO* and *pduQS* (GenBank accession no. LC073314). *S. blattae* ATCC33430 was used as the source of *dhaK* (GenBank accession no. LC073756). All strains were cultivated in Luria-Bertani (LB) medium at 37°C (9,10). *E. coli* BW38029 was used as a host for

1-propanol production. For the fermentative experiments, preculture in test tubes containing 5 ml of LB medium was performed at 37°C overnight on a reciprocal shaker (300 rpm). An aliquot of overnight culture (0.25 ml) was added to 25 ml of enriched M9 medium (EM9 medium) in 28 ml screw-capped tubes. EM9 medium was comprised of 6 g/l Na₂HPO₄, 3 g/l KH₂PO₄, 0.5 g/l NaCl, 1 g/l NH₄Cl, 2 mM MgSO₄, 100 µM CaCl₂, 5 g/l yeast extract, 5 g/l CaCO₃ and 200 µM IPTG. When necessary, various carbon sources (D-glucose, L-rhamnose or glycerol), 1.3 or 10 µM AdoCbl and 50 µg/ml appropriate antibiotics (kanamycin sulfate, ampicillin sodium salt and/or chloramphenicol) were added to the medium. Cultivation was carried out at 37°C without shaking.

Construction of pRSFAK30, pACYCAK30, pRSF_pduCDE, pRSF_pduCDEGHOQS, pAC_sbdhaK and plasmid introduction into *E. coli*

All basic recombinant DNA procedures were followed as standard protocols (11). All plasmids are listed in Table S1 and all primers used for the construction of plasmids are listed in Table S2. PCR was performed using a PTC-200 thermal cycler (MJ Research Inc., Watertown, MA) and PrimeSTAR DNA polymerase (Takara Bio Inc., Shiga, Japan). Thermal cycling consisted of 98°C for 1 min followed by 30 cycles of 98°C for 10 s, 55°C for 5 s and 72°C for 1 min/kb. pRSFAK30 was constructed as follows. DNA fragment A containing the *tac* promoter, multiple cloning site and transcriptional terminator of pKK223-3 (GE-Healthcare UK, Ltd., Little Chalfont, UK) was amplified using primers A-F and A-R with pKK223-3 as template. DNA fragment B containing RSF ori and the kanamycin-resistance gene was amplified using primers B-F and B-R with pRSFDuet-1 (Merck Millipore, Billerica, MA) as template. After these two DNA fragments were digested with *Pvu*II and *Xba*I, fragment A was ligated with fragment B. The resulting plasmid was named pRSFAK30. pACYCAK30 was

constructed in almost the same way as pRSFAK30. DNA fragment C containing P15A ori and the chloramphenicol-resistance gene was amplified using primers C-F and C-R with pACYCDuet-1 (Merck Millipore) as template. After DNA fragment C was digested with *EcoRV* and *SpeI*, this fragment was ligated with *PvuII/XbaI*-treated DNA fragment A. The resulting plasmid was named pACYCAK30.

pRSF_pduCDE and pRSF_pduCDEGHOQS for the conversion of 1,2-PD to 1-propanol was constructed as follows. The coding regions of *pduCDE*, *pduCDEGH*, *pduO* and *pduQS* were PCR-amplified with various flanking restriction enzyme sites using the appropriate primer sets (**CDE-F & CDE-R, CDE-F & CDEGH-R, O-F & O-R and QS-F & QS-R, respectively**) listed in Table S2 and the genomic DNA of *K. pneumoniae* AKU30 as template. **The *pduCDE* gene amplified with the flanking *EcoRI* and *HindIII* sites was inserted into pRSFAK30 doubly digested with *EcoRI* and *HindIII*. The resulting plasmid was named pRSF_pduCDE.** The *pduCDEGH* gene amplified with the flanking *EcoRI* and *HindIII* sites was inserted into pRSFAK30 doubly digested with *EcoRI* and *HindIII*. The resulting plasmid was digested with *NotI* and *NheI*, and ligated with *pduO* amplified with the flanking *NotI* and *NheI* sites. Then, the resulting plasmid was digested with *NheI* and *HindIII*, and ligated with *pduQS* amplified with the flanking *NheI* and *HindIII* sites. As for the ribosome binding sequence (RBS), synthesized RBS (12) was used for *pduO* and *pduQS* (Table S2, underlined). The resulting plasmid was named pRSF_pduCDEGHOQS.

pAC_sbdhaK for the conversion of dihydroxyacetone (DHA) to dihydroxyacetone phosphate (DHAP) was constructed as follows. The coding region of *dhaK* was amplified with the flanking *EcoRI* and *HindIII* sites by PCR using primers dhaK-F and dhaK-R listed in Table S2 and the genomic DNA of *S. blattae* ATCC33430 as template. The *dhaK* gene amplified with the flanking *EcoRI* and *HindIII* sites was inserted into

pACYCAK30 doubly digested with *EcoRI* and *HindIII*. The resulting plasmid was named pAC_sbdhaK.

These plasmids were introduced into *E. coli* BW38029 by the heat-shock method (13). After incubation for 30 min on ice, a mixture of chemically competent *E. coli* cells and plasmid DNA was placed at 42°C for 45 sec (heat-shock) and then placed back on ice for 5 min. Heat-shocked cells were diluted with 1 ml SOC medium and incubated for 1 h at 37°C. They were then spread on an LB agar plate containing 50 µg/ml of the appropriate antibiotics.

1-Propanol-producing 1,2-PD conversion activity assay Recombinant *E. coli* strains, BW38029 [pRSFAK30], BW38029 [pRSF_pduCDE] and BW38029 [pRSF_pduCDEGHOQS] were used for the 1-propanol-producing activity assay with 1,2-PD as a substrate. Preculture in test tubes containing 5 ml of LB medium was performed at 37°C overnight on a reciprocal shaker (300 rpm). An aliquot of overnight culture (0.25 ml) was added to 25 ml of EM9 medium containing 22.7 mM 1,2-PD, 54.5 mM D-glucose and 50 µg/ml kanamycin sulfate in 28 ml screw-capped tubes. When necessary, 1.3 or 10 µM AdoCbl was added to the medium. Cultivation was carried out at 37°C without shaking.

ATP-dependent dihydroxyacetone kinase (DHAK) activity assay

Recombinant *E. coli* cells bearing an expression plasmid, pAC_sbdhaK, were cultured in LB medium at 37°C. When the OD₆₆₀ reached 0.8, 200 µM IPTG was added to the medium and further cultivation was performed for 3 h at 37°C. The cells were harvested and washed by centrifugation, suspended in 50 mM triethanolamine hydrochloride buffer (pH 7.0), and then disrupted with an ultrasonic oscillator (Insonator 201M; Kubota Corp., Tokyo, Japan). After centrifugation, the resulting supernatant (cell-free extract: CFE) was used for enzyme assays. Protein concentrations

were determined with a protein assay CBB solution (Nacalai Tesque Inc., Kyoto, Japan) using bovine serum albumin as a standard.

ATP-dependent DHAK activity was determined as described previously (14). The reaction mixture contained 50 mM triethanolamine hydrochloride buffer (pH 7.0), 10 mM DHA, 15 mM NADH, 15 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 2.0 mM dithiothreitol, 10 mM α, α' -dipyridyl, 25 mM ATP, 3 units glycerol-3-phosphate dehydrogenase (Takara Bio) and an appropriate amount of CFE. Enzyme activity was determined by monitoring the decrease of absorbance at 340 nm, corresponding to the oxidation of NADH ($\epsilon_{340 \text{ nm}} = 6300 \text{ M}^{-1} \cdot \text{cm}^{-1}$) to NAD^+ at 37°C. One unit of ATP-dependent DHAK activity was defined as the amount of enzyme catalyzing the consumption of 1 μmol of NADH—that is, the formation of 1 μmol of DHAP—per min.

Analytical methods Sugars (D-glucose and L-rhamnose), glycerol, alcohols (1-propanol, ethanol and 1,2-PD) and organic acids (lactate, formate and acetate) in the medium were determined by HPLC as described previously (8). The optical density at 660 nm (OD_{660}) of the culture broth was also determined as described previously (8).

RESULTS

Conversion of 1,2-PD to 1-propanol by *E. coli* carrying the 1-propanol synthetic pathway To examine the conversion ability of 1,2-PD to 1-propanol, *E. coli* harboring **pRSF_pduCDE** or pRSF_pduCDEGHOQS was cultured in a medium containing 1,2-PD. When recombinant *E. coli* BW38029 [pRSFAK30] was cultured anaerobically in EM9 medium containing 1,2-PD, D-glucose and **1.3 or 10 μM AdoCbl** for 72 h, the formation of 1-propanol were not observed (Table 1). **Similarly, when recombinant *E. coli* BW38029 [pRSF_pduCDE] was cultured under the same**

conditions, small amount of 1-propanol was formed from 1,2-PD (Table 1). On the other hand, recombinant *E. coli* BW38029 [pRSF_pduCDEGHOQS] converted almost all of 1,2-PD in the culture medium to 1-propanol under the same conditions (Table 1). However, recombinant *E. coli* BW38029 [pRSF_pduCDE] or *E. coli* BW38029 [pRSF_pduCDEGHOQS] cultured without AdoCbl did not convert 1,2-PD to 1-propanol (Table 1). Thus, the introduction of pRSF_pduCDEGHOQS enabled *E. coli* to convert 1,2-PD to 1-propanol efficiently.

1-Propanol production from D-glucose For 1-propanol production from D-glucose, the 1,2-PD synthetic pathway (pKK_mde) was introduced along with the 1-propanol synthetic pathway (pRSF_pduCDEGHOQS). First, recombinant *E. coli* BW38029 [pKK_mde, pRSFAK30] carrying only the 1,2-PD synthetic pathway was cultured anaerobically in EM9 medium containing D-glucose and AdoCbl for 72 h. As a result, 11.1 mM of 1,2-PD was accumulated, but no 1-propanol was detected in the culture medium (Fig. 2A). However, when recombinant *E. coli* BW38029 [pKK_mde, pRSF_pduCDEGHOQS] was cultured under the same conditions, 44.5 mM of D-glucose was consumed, and 16.1 mM (= 0.97 g/l) of 1-propanol was formed with a molar yield of 0.36 mol/mol D-glucose in the culture medium (Fig. 2B). Then, 45.0 mM of lactate, 31.4 mM of acetate and 7.69 mM of ethanol were formed as by-products in the culture medium.

1-Propanol production from L-rhamnose Since wild-type *E. coli* already has an endogenous L-rhamnose utilization pathway, introduction of only the 1-propanol synthetic pathway into *E. coli* BW38029 was attempted. Recombinant *E. coli* BW38029 [pRSF_pduCDEGHOQS] was cultured anaerobically in EM9 medium containing

L-rhamnose and AdoCbl for 72 h. As a result, 36.8 mM of L-rhamnose was consumed and 29.9 mM (= 1.80 g/l) of 1-propanol was formed with a molar yield of 0.81 mol/mol L-rhamnose (Fig. 3).

1-Propanol production from glycerol

In order to use glycerol for the production of 1-propanol, the introduction of an engineered glycerol utilization pathway consisting of glycerol dehydrogenase (GLDH) and ATP-dependent DHAK was required. Since the GLDH gene was introduced by pKK_mde, pAC_sbdhaK possessing only the ATP-dependent DHAK gene of *S. blattae* was constructed and introduced into *E. coli* BW38029. Gene expression of pAC_sbdhaK was examined by enzyme activity assays. The pAC_sbdhaK-introduced *E. coli* showed significantly higher ATP-dependent DHAK activity than the pACYCAK30-introduced one (Table 2). Thus, pAC_sbdhaK was introduced into recombinant *E. coli* BW38029 [pKK_mde, pRSF_pduCDEGHOQS].

Recombinant *E. coli* BW38029 [pACYCAK30, pKK_mde, pRSF_pduCDEGHOQS] not carrying an engineered glycerol utilization pathway was cultured anaerobically in EM9 medium containing glycerol and AdoCbl for 72 h. As a result, a small amount of glycerol (13.8 mM) was consumed with the accumulations of acetate (6.43 mM) and ethanol (10.1 mM), but no accumulation of 1,2-PD or 1-propanol was detected in the culture medium (Fig. 4A). When recombinant *E. coli* BW38029 [pAC_sbdhaK, pKK_mde, pRSF_pduCDEGHOQS] was cultured under the same conditions, 46.0 mM of glycerol was consumed and 12.6 mM (= 0.75 g/l) of 1-propanol was formed with a molar yield of 0.27 mol/mol glycerol in the culture medium (Fig. 4B). Then, 8.10 mM of lactate, 7.48 mM of acetate and 22.6 mM of ethanol were formed as by-products in the culture medium.

DISCUSSION

In order to realize the production of propylene from biomass and other renewable resources, we examined the fermentative production of 1-propanol, which is one of the key intermediates of propylene production and is expected to give a higher carbon yield. Two main pathways have been reported for the synthesis 1-propanol from D-glucose: a pathway via 2-ketobutyrate as an intermediate (15–17), and the pathway via 1,2-PD presented here. However, the 1-propanol synthetic pathway via 2-ketobutyrate also has a CO₂ emission step, and thus, in theory, the carbon yield of this pathway should be lower than that via 1,2-PD.

In our previous report (8) we constructed the 1,2-PD synthetic pathway (pKK_mde), while in this study we constructed the 1-propanol synthetic pathway converting 1,2-PD to 1-propanol. The 1,3-propanediol (1,3-PD)-forming glycerol metabolizing enzyme system has been shown to catalyze the conversion of 1,2-PD to 1-propanol (18). Several enteric bacteria, such as *S. blattae* and *K. pneumoniae*, carry the *dha* regulon, which codes for the enzyme system to convert glycerol to 1,3-PD under anaerobic conditions (19,20). Glycerol dehydratase (GLDHt) and 1,3-PD oxidoreductase (PDOR) were respectively encoded by *dhaBCE* and *dhaT* on the *dha* regulon. GLDHt converts glycerol or 1,2-PD to 3-hydroxypropionaldehyde (3-HPA) or propionaldehyde (PA), and PDOR converts 3-HPA or PA to 1,3-PD or 1-propanol, respectively (21). In addition, *Salmonella enterica* and *K. pneumoniae* carry the *pdu* regulon, which has functions similar to the *dha* regulon (GenBank accession no. CP000647). In *S. enterica*, diol dehydratase (DDHt) encoded by *pduCDE* in combination with propanol dehydrogenase (PRDH) encoded by *pduQ* converts 1,2-PD to 1-propanol (22). Since the whole cells of

K. pneumoniae AKU30 cultivated with glycerol as a sole carbon source could convert 1,2-PD to 1-propanol (data not shown), the genes coding for the 1-propanol synthetic pathway were cloned from *K. pneumoniae* AKU30 based on the *pdu* regulon sequences.

In addition to *pduCDE* (DDHt gene) and *pduQ* (PRDH gene), the *pdu* regulon includes other enzyme genes, such as *pduGH* (ATP-dependent reactivation factor gene), *pduO* (adenosyl transferase gene) and *pduS* (cobalamin reductase gene). These 3 enzymes are required for regeneration of AdoCbl in DDHt inactivated during dehydration reaction.

In previous reports (5,6), the 1-propanol synthetic pathway in *E. coli* cells was constructed by introducing the DDHt gene encoded by *ppdABC* of *Klebsiella oxytoca* or GDHt gene encoded by *gldABC* of *K. pneumoniae* or *dhaB12* of *Clostridium butyricum*. The endogenous alcohol dehydrogenase activity of *E. coli* was used as a PA reductase instead of PDOR or PRDH. In this study, *pduCDE* was introduced together with the AdoCbl regeneration enzyme genes, *pduGH*, *pduO* and *pduS*, using a newly constructed plasmid, pRSF_pduCDEGHOQS, for the conversion of 1,2-PD to 1-propanol. Moreover, to improve the conversion of PA to 1-propanol, *pduQ* coding PRDH, which has specific affinity with PA (23), was also introduced by pRSF_pduCDEGHOQS. As compared to 1-propanol-producing recombinant *E. coli* not carrying the regeneration system of AdoCbl and PRDH in previous reports (5,6), the recombinant *E. coli* BW38029 [pRSF_pduCDEGHOQS] could efficiently convert 1,2-PD to 1-propanol using only about 13% much AdoCbl (1.3 μ M).

1-Propanol production from D-glucose was previously performed using recombinant *S. blattae* [pKK_mde] (8). In this case, since an endogenous 1-propanol synthetic pathway of *S. blattae* was used in combination with the 1,2-PD synthetic pathway, glycerol must have been added to induce the gene expression of the endogenous

1-propanol synthetic pathway. Recombinant *S. blattae* [pKK_mde] was able to begin using glycerol after the complete consumption of D-glucose due to catabolite repression, and thus 1,2-PD was accumulated until the consumption of glycerol commenced. In contrast, when using recombinant *E. coli* BW38029 [pKK_mde, pRSF_pduCDEGHOQS] carrying the 1,2-PD synthetic and 1-propanol synthetic pathways, 1-propanol production from D-glucose proceeded without the accumulation of 1,2-PD and the addition of glycerol (Fig. 2B). Moreover, in previous reports (5,6), using 1-propanol-producing recombinant *E. coli* not carrying the AdoCbl regeneration system and PRDH, 0.62 g/L of 1-propanol was produced at the maximum with 1.51 g/L of 1,2-PD remaining unconverted, from D-glucose, however, in this study, 0.97 g/L of 1-propanol was produced without the accumulation of 1,2-PD, which is the largest titer in 1-propanol production using single recombinant *E. coli* and the same pathways.

1-Propanol production from L-rhamnose has also been performed using wild-type *S. blattae* (8). However, as when using D-glucose as a substrate, glycerol was required as an inducer for this synthetic scheme. On the other hand, by using recombinant *E. coli* BW38029 [pRSF_pduCDEGHOQS], 1-propanol production from L-rhamnose proceeded without the addition of glycerol (Fig. 3). In the endogenous L-rhamnose utilization pathway, 1 mol of L-rhamnose was converted into 1 mol of DHAP and 1 mol of 1,2-PD (24–26). Since the number of conversion steps, especially for the NAD(P)H-dependent reactions to 1-propanol from L-rhamnose, was less than that from D-glucose, the carbon yield of 1-propanol toward L-rhamnose is expected to be higher than that toward D-glucose. At present, L-rhamnose is not suitable material for the fermentative production, because it is a rare and expensive sugar. However, L-rhamnose is the major component (2.2 mol%) of pectin, which is an underutilized biomass, next to D-galacturonic acid (27). If a saccharification technology of pectin could be developed,

L-rhamnose would have value as a reasonable substrate.

Glycerol, which has been produced in large amounts as a by-product of biodiesel production in recent years, is expected to be used as a substrate for fermentative production because of its availability and low cost (28,29). To utilize glycerol for 1-propanol production, an additional two-step conversion of glycerol to DHAP is required. Wild-type *E. coli* holds two types of endogenous glycerol utilization pathways (30,31). In one, glycerol is converted to DHAP via glycerol-3-phosphate by phosphorylation and the subsequent dehydrogenation reactions. In the other pathway, glycerol is converted to DHA by GLDH, and then DHA is converted to DHAP by phosphoenolpyruvate (PEP)-dependent DHAK (32). In the present study, since GLDH was introduced as one of the enzymes in the 1,2-PD synthetic pathway (pKK_mde), it was considered that glycerol should be taken by *E. coli* via DHA. However, PEP-dependent DHAK consumes 1 mol of PEP to phosphorylate 1 mol of DHA, and hence a large amount of glycerol, which should be used as a starting material for production of 1-propanol, would be lost for pyruvate formation (33). Actually, *E. coli* BW38029 [pACYCAK30, pKK_mde, pRSF_pduCDEGHOQS] using an endogenous PEP-dependent DHAK could not produce 1-propanol (Fig. 4A), despite consuming glycerol and producing acetate and ethanol derived from pyruvate. Thus, we considered that introduction of ATP-dependent DHAK would be reasonable. The construction of an engineered glycerol utilization pathway using ATP-dependent DHAK of *S. blattae* was examined, and consequently 1-propanol production from glycerol was achieved (Fig. 4B).

1-Propanol could be produced using D-glucose, L-rhamnose or glycerol as a substrate, but the carbon yields of 1-propanol toward each substrate were 18.1%, 40.7% and 27.3%, respectively, and were not sufficient as compared to theoretical one. To improve

the carbon yield, limiting the flux to by-product-producing pathways would be effective, because when D-glucose was used as a substrate, large amounts of fermentative by-products were observed in the culture medium. On the other hand, substrate consumption rate and 1-propanol production rate decreased during the latter period of 1-propanol fermentation (Figs. 2B, 3 and 4B). This might be due to damage of *E. coli* cells caused by 1-propanol accumulation. Therefore, optimization of the culture conditions, such as the temperature, pH, aeration rate and substrate concentration, would lead to improvements in 1-propanol productivity.

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Figure legends

FIG. 1. Biosynthetic pathway of 1-propanol from D-glucose, glycerol or L-rhamnose.

FIG. 2. Time course of a batch fermentation of recombinant *E. coli* BW38029 [pKK_mde, pRSFAK30] (A) and recombinant *E. coli* BW38029 [pKK_mde, pRSF_pduCDEGHOQS] (B) in EM9 medium containing 53.0 mM D-glucose and 1.3 μ M AdoCbl. D-Glucose (gray triangles), 1,2-PD (open circles), 1-propanol (closed circles), and OD₆₆₀ (crosses with dotted lines).

FIG. 3. Time course of a batch fermentation of recombinant *E. coli* BW38029 [pRSF_pduCDEGHOQS] in EM9 medium containing 46.0 mM L-rhamnose and 1.3 μ M AdoCbl. L-Rhamnose (closed triangles), 1,2-PD (open circles), 1-propanol (closed circles), and OD₆₆₀ (crosses with dotted lines).

FIG. 4. Time course of a batch fermentation of recombinant *E. coli* BW38029 [pACYCAK30, pKK_mde, pRSF_pduCDEGHOQS] (A) and recombinant *E. coli* BW38029 [pAC_sbdhaK, pKK_mde, pRSF_pduCDEGHOQS] in EM9 medium containing 50.0 mM glycerol and 1.3 μ M AdoCbl. Glycerol (open triangles), 1,2-PD (open circles), 1-propanol (closed circles), and OD₆₆₀ (crosses with dotted lines).

Figure(s)

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

TABLE 1. Conversion of 1,2-PD to 1-propanol by recombinant *E. coli* BW38029 [pRSFAK30], BW38029 [pRSF_pduCDE] or BW38029 [pRSF_pduCDEGHOQS].

Plasmid	AdoCbl (μ M)	OD ₆₆₀	Concentration (mM)	
			1,2-PD	1-Propanol
pRSFAK30	1.3	1.13 $\pm$ 0.05	21.2 $\pm$ 0.46	n.d. ¹
pRSFAK30	10	1.10 $\pm$ 0.02	21.4 $\pm$ 0.86	n.d.
pRSF_pduCDE	0	1.06 $\pm$ 0.05	21.4 $\pm$ 0.37	n.d.
pRSF_pduCDE	1.3	1.11 $\pm$ 0.10	21.0 $\pm$ 0.27	0.10 $\pm$ 0.01
pRSF_pduCDE	10	1.09 $\pm$ 0.07	21.4 $\pm$ 0.48	0.09 $\pm$ 0.01
pRSF_pduCDEGHOQS	0	1.08 $\pm$ 0.01	21.2 $\pm$ 0.05	n.d.
pRSF_pduCDEGHOQS	1.3	1.10 $\pm$ 0.04	0.18 $\pm$ 0.16	22.4 $\pm$ 0.19
pRSF_pduCDEGHOQS	10	1.07 $\pm$ 0.03	0.08 $\pm$ 0.09	22.4 $\pm$ 0.16

¹n.d., not detected.

TABLE 2. ATP-dependent DHAK activities of recombinant *E. coli*.

Plasmid	ATP-dependent
	DHAK activity (unit/mg)
pACYCAK30	0.02 $\pm$ 0.02
pAC_sbdhaK	0.34 $\pm$ 0.02