Microbial Conversion of Glycerol to 1,3-Propanediol by an Engineered Strain of Escherichia coli

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In an effort to improve industrial production of 1,3-propanediol (1,3-PD), we engineered a novel polycis-tronic operon under the control of the temperature-sensitive lambda phage P1Pl promoters regulated by the cls857 repressor and expressed it in Escherichia coli K-12 ER2925. The genes for the production of 1,3-PD in Clostridium butyricum, dhaB1 and dhaB2, which encode the vitamin B12-independent glycerol dehydratase DhaB1 and its activating factor, DhaB2, respectively, were tandemly arrayed with the E. coli yqhD gene, which encodes the 1,3-propanediol oxidoreductase isoenzyme YqhD, an NADP-dependent dehydrogenase that can directly convert glycerol to 1,3-PD. The microbial conversion of 1,3-PD from glycerol by this recombinant E. coli strain was studied in a two-stage fermentation process. During the first stage, a novel high-cell-density fermentation step, there was significant cell growth and the majority of the metabolites produced were organic acids, mainly acetate. During the second stage, glycerol from the fresh medium was rapidly converted to 1,3-PD following a temperature shift from 30°C to 42°C. The by-products were mainly pyruvate and acetate. During this two-stage process, the overall 1,3-PD yield and productivity reached 104.4 g/liter and 2.61 g/liter/h, respectively, and the conversion rate of glycerol to 1,3-PD reached 90.2% (g/g). To our knowledge, this is the highest reported yield and productivity efficiency of 1,3-PD with glycerol as the sole source of carbon. Furthermore, the overall fermentation time was only 40 h, shorter than that of any other reports.

In recent decades, the monomeric form of 1,3-propanediol (1,3-PD) has gained use in large-volume production of polyester fibers and polyurethanes (3, 10, 18). Over 107 tons of 1,3-PD are produced annually, primarily through chemical synthesis (26). However, these processes have low selectivity and produce toxic intermediates (29, 35), and the consequently high price hinders the utilization of 1,3-PD production. One potential method for improved 1,3-PD production is via the microbial fermentation of glycerol (6). The biological fermentation of glycerol to 1,3-PD is a two-step enzymatic reaction which primarily has been studied in the microorganisms Klebsiella pneumoniae (15, 16, 39), Citrobacter freundii (5, 9), and Clostridium butyricum (12, 13, 27). Specifically, vitamin B12-dependent glycerol dehydratase converts glycerol to an intermediate, 3-hydroxypropionaldehyde (3-HPA), which is then reduced to 1,3-PD by an NADH-dependent 1,3-propanediol oxidoreductase. The genes encoding glycerol dehydratase (EC 4.2.1.30) and NADH-dependent 1,3-propanediol oxidoreductase (EC 1.1.1. 202), the key enzymes in the production of 1,3-PD from glycerol, have been designated dhaB and dhaT, respectively (33).

Efforts to heterologously express these genes in a recombinant organism in an effort to improve 1,3-PD production have met with multiple challenges. LaFend and Nagarajan (19, 20) reported that recombinant Escherichia coli containing the genes dhaB and dhaT converts glycerol to 1,3-PD with low titers, possibly due to the failure of 1,3-propanediol oxidoreductase to completely catalyze the conversion of 3-HPA to 1,3-PD. Furthermore, 3-HPA levels above 30 mM have been shown to inhibit glycerol dehydratase (1). Recent studies have also shown that glycerol dehydratases undergo irreversible suicide inactivation by glycerol (9, 17, 31), despite glycerol being the substrate and the dehydratase being essential for glycerol breakdown. Finally, one of the key limitations of the application of this biological process to industry is that it requires the coenzyme B12-dependent glycerol dehydratase, necessitating the addition of large amounts of a high-cost molecule, vitamin B12, to the culture medium. Hence, to establish an ideal bioprocess for 1,3-PD production, at least three challenges need to addressed: (i) the effect of accumulated 3-HPA on glycerol dehydratase activity; (ii) the inactivation of glycerol dehydratase by glycerol (8); (iii) the expense of coenzyme vitamin B12 in fermentation.

Although several research groups reconstructed the 1,3-PD pathway in Escherichia coli by using the genes dhaB and dhaT from K. pneumoniae or C. freundii, the production levels of 1,3-PD by these recombinant strains were lower than with the wild type (25). Thus, we sought to engineer a novel bacterial strain with an improved 1,3-PD yield. Toward this end, we cloned the following three genes: (i) dhaB1, which encodes the vitamin B12-independent glycerol dehydratase, from C. butyricum; (ii) dhaB2, which encodes an activating factor for vitamin B12-independent glycerol dehydratase, from C. butyricum; (iii) yqhD, which encodes the 1,3-propanediol oxidoreductase isoenzyme, an NADP-dependent dehydrogenase, from wild-type E. coli. The product of yqhD is also called 1,3-propanediol

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NADP-dependent dehydrogenase and is sufficient to catalyze the interconversion of 3-HPA (25). In an effort to improve 1,3-PD production, we constructed a novel 1,3-PD operon of these three genes tandemly arrayed under the control of a constitutive, temperature-sensitive promoter in the vector pBV220 for heterologous expression in E. coli. Moreover, we also established a novel fermentation model of recombiant E. coli that was able to remarkably increase the conversion ratio of glycerol to 1,3-PD. This work promises to promote the development of an economical and eco-friendly biological process for the production of 1,3-PD from renewable resources.

MATERIALS AND METHODS

Bacterial strains and plasmids. Clostridium butyicum SYU 20108 was obtained from the Southern Yangtze University Microorganism Culture Center. We obtained a wild-type Escherichia coli strain (SYU 21132) from our laboratory mud culture collection. E. coli K-12 ER2925, obtained from New England Biolabs (Ipswich, MA) was used as the host for heterologous expression of the 1,3-PD operon. Temperature-sensitive plasmid pBV220 was kindly provided by Zhang Zhiqing (China Medicine Science Institute, Beijing, China).

DNA and RNA preparation and manipulation and Northern blotting. Total DNA from E. coli was prepared with Qiagen genomic tips (Valencia, CA). Plasmid DNA was prepared by a rapid alkaline lysis procedure (4) with a QIAprep spin miniprep kit (Qiagen). General DNA manipulations were carried out as described by Sambrook et al. (30). DNA sequencing of both strands was performed on an ABI 373 automated sequencer with dye-labeled terminators (Applied Biosystems Division, Perkin-Elmer, Foster City, CA). Oligonucleotides were synthesized by Bioneer (Chungweon, Korea). Genomic DNA from C. butyicum was isolated according to the method of Marmur (24). Total RNA was isolated by buffered phenol extraction. RNA integrity was checked by 1% formaldehyde agarose gel electrophoresis. DIG-labeled probes to each gene were generated by PCR amplification and purified with Probe Quant G5 (Amersham Biosciences, Buckinghamshire, England). Hybridization, washing conditions, and signal detection were performed according to the DIG kit manual (Roche, Germany).

PCR cloning of the dhaB1, dhaB2, and yqhD genes. Primer design was based on the published sequences of dhaB1 and dhaB2 (GenBank accession number AJ112989) and yqhD (GenBank accession number NC002655). The dhaB1 and dhaB2 sequences were tandemly arrayed (in the same direction of transcription) such that they could be amplified as a single dhaB fragment. The PCR primers used to amplify the dhaB fragment were 5′-CCGGCGATTCTAGCAGTACGGATTTAGTACCC-3′ (the introduced BamHI restriction site is underlined) and 5′-CCGGCGATTCTAGCAGTACGGATTTAGTACCC-3′ (the introduced SalI restriction site is underlined). The primers used to amplify yqhD were 5′-GGGCTGCCAAGCAGAAAAGGAAGCATTGAATGAC-3′ (the introduced SalI restriction site is underlined) and 5′-GGGCTGCCAAGCAGAAAAGGAAGCATTGAATGAC-3′ (the introduced SalI restriction site is underlined). All PCR products were cloned downstream of the temperature-sensitive PRLP promoter in plasmid pBV220. The resulting vector was designated pDY220. Sequence analysis was used to confirm the dhaB1, dhaB2, and yqhD genes in pDY220.

Media. Batch cultures of C. butyicum for genomic DNA preparation were grown anaerobically at 37°C in 2% YT medium (yeast extract, 10 g/liter; Bacto tryptone, 16 g/liter; NaCl, 4 g/liter) supplemented with 2% glucose. E. coli was routinely grown aerobically at 30°C in Luria-Bertani broth (30) supplemented with ampicillin (100 mg/ml)−1. E. coli inoculum for fermentation was cultivated overnight at 30°C on 240 rpm on a rotary shaker in Luria-Bertani broth at a concentration of 25 g/l. When the overnight culture reached an optical density at 600 nm (OD600) of 5.0, it was used to inoculate the fermentation cultures. The inoculum volume was 5% of the 15-liter working volume. The medium used for high-cell-density fermentation, as described by Bauer and Shiloach (2) with some modifications, and consisted of trisodium citrate (4 mM), (NH4)2SO4 (25 mM), KH2PO4 (25 mM), KH2PO4 (20 mM), and a trace metals solution (4 ml/liter). The pH was adjusted to 6.8 with NH4H2O and the medium was sterilized in place. Sterile MgSO4 (4 mM) and glucose (25 g/liter) were added to the cooled medium. Brexco FMT 30 (International Specialty Chemicals, Southampton, United Kingdom) was added at a concentration of 0.35 to 0.4 ml/liter to control foaming. Anaerobic fermentation medium consisted of the following: 30 g/liter glycerol, 9 g/liter yeast extract, KH2PO4 (25 mM), KH2PO4 (20 mM), MgSO4 (4 mM), and (NH4)2SO4 (28 mM).

First fermentation stage: aerobic fermentation. A standard 14-liter BioFlo 310 fermentor (New Brunswick Scientific Co., Inc., Edison, NJ) was used in these studies. The dissolved oxygen probe was calibrated at 0%, obtained by briefly disconnecting the cable, and at 100%, obtained by using 1,200-rpm agitation and 5 liters/min (1 volume per volume per minute [vvm]) airflow. After calibration, the fermentors were first equilibrated to a dissolved oxygen concentration of 100% air saturation. They were then inoculated to a cell concentration of 1 mg/l with cells from a freshly prepared high-cell-density fermentation medium which had been grown on a shaker for approximately 5 h at 30°C.

Initial fermentation conditions were 30°C, 2.0 standard liters of air per min, and agitation at 400 rpm. The dissolved oxygen concentration was maintained at 40% air saturation, first by increasing the agitation rate from 400 to 1,200 rpm, then by increasing the airflow rate from 2 to 3 standard liters of air per min, and later by increasing the oxygen flow rate from 0 to 3 standard liters of air per min. The pH was controlled at 6.8 with 7.4 M NH4OH, and glucose was fed into the system from a sterile 50% (wt/vol) solution by coupling its addition to the base titrant demand. Frequent measurements of the glucose concentration were made with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH), and the ratio of glucose feed to titrant flow was adjusted empirically to maintain a concentration of approximately 25 g/l throughout growth and induction. Repetition of selected fermentor experiments gave comparable results.

Second fermentation stage: perfusion experiments and anaerobic cultivation conditions. Perfusion experiments were conducted by using the apparatus illustrated in Fig. 1. The fermentations were run as described above until the cell density reached an OD600 of 60 (26 g [dry weight] per liter). A trial experiment indicated that a discontinuous procedure was the most effective in replacing spent culture medium with anaerobic fermentation medium. Perfusion was accomplished by first concentrating the culture in situ by using a cross-flow filtration apparatus (two HVLP-000-C5 Pellicon cassette filters; Millipore Corp., Billerica, MA) from approximately 13 to 8 liters while maintaining 0.4 vvm nitrogen after stopping aeration. The lost volume was then replaced by pumping in 5 liters of new anaerobic fermentation medium with salts, trace elements, glycerol concentrations, pH, and a temperature equivalent to those of fresh medium. This cycle was repeated so that approximately 30 liters of spent culture medium was replaced in 5-liter batches during each hour of medium exchange. This resulted in a medium replacement of approximately 99% in 2 h, after which the temperature was raised to 42°C for fermentation.

Preparation of cell extracts and enzyme assays. E. coli cell extracts were prepared by the anaerobic sonication procedure of Vasconcelos et al. (37). Glycerol dehydratase activity was assayed by an indirect method derived from that described by Toraya et al. (36), which is based on the measurement of NADH consumption when the aldehyde formed by the dehydratase is reduced to the corresponding alcohol by an excess of yeast alcohol dehydratase. The 1-mg glycerol dehydratase assay mixture contained 0.03 M (NH4)2SO4, 0.1 M 1,2-
RESULTS

Construction of a novel operon for the production of 1,3-propanediol. To construct an operon harboring the dhaB1, dhaB2, and yqhD genes for production of 1,3-PD, we PCR amplified the dhaB fragment containing the dhaB1 and dhaB2 open reading frames and a yqhD fragment including its Shine-Dalgalorno sequence. The yqhD gene was placed downstream of the dhaB fragment in plasmid pBY220, all of which were present in the fermentation broth were carried out with a Shimazu GC-14B gas chromatograph, which has a flame ionization detector, a 2-m by 0.5-mm stainless steel column packed with Chromosorb101, and is operated with N2 as the carrier gas at a flow rate of 40 ml min⁻¹ and with detector and column temperatures of 220°C and 210°C, respectively. The protein concentration was determined by use of Coomassie brilliant blue G250 (32).

Analytical methods. Biomass concentrations were measured as the OD₆₀₀. Determinations of the concentrations of 1,3-PD, glycerol, and other metabolites present in the fermentation broth were carried out with a Shimazu GC-14B gas chromatograph, which has a flame ionization detector, a 2-m by 0.5-mm stainless steel column packed with Chromosorb101, and is operated with N₂ as the carrier gas at a flow rate of 40 ml min⁻¹ and with detector and column temperatures of 220°C and 210°C, respectively. The protein concentration was determined by use of Coomassie brilliant blue G250 (32).

The tandem promoter P₃ P₅, obtained from λ phage. The cIts857 gene encodes a temperature-sensitive inhibitor from λ phage. rrnBT1T2 is a strong transcriptional stop sequence from the E. coli ribosomal protein gene rrsB. The gene yqhD harbors dhaB1 and dhaB2 from C. butyricum, and the gene yqhD is from E. coli.

Expression analysis. Northern blot analysis was used to confirm successful transcription of the 1,3-PD operon from the P₃ P₅ promoter in our novel engineered E. coli strain. Total RNA was hybridized with probes derived from the three genes in the operon, including dhaB1, dhaB2, and yqhD (Fig. 3). A single 4.5-kb hybridization signal, corresponding to the expected size of the 1,3-PD operon, was detected by probes internal to either dhaB1 or dhaB2 when cells were grown in glycerol at 42°C, but not when cells were grown in glucose at 30°C. A strong 4.5-kb hybridization signal and a weak 1.16-kb hybridization signal were detected by probes internal to the yqhD gene when cells were grown in glycerol at 42°C and a weak 1.16-kb yqhD hybridization signal was detected when cells were grown in glycerol at 30°C. Based on the detection of a 1.16-kb yqhD hybridization signal in RNA from the host strain alone grown in glucose at 30°C (data not shown), we concluded that this signal in our mutant must be due to basal transcription of the endogenous copy of yqhD in the E. coli K-12 ER2925 (data not shown). Thus, it appears that the P₃ P₅ promoter successfully transcribes our engineered 1,3-PD operon as a polycistronic message in a temperature-dependent manner when cells are shifted from 30°C in glucose to 42°C in glycerol.

Analysis of 1,3-PD production during high-cell-density fermentation. High-cell-density fermentation was divided into two stages, aerobic and anaerobic (Fig. 4). During the first stage (Fig. 4A), aerobic fermentation was used to obtain a high-density cell culture. Specifically, the recombinant strain was cultivated at 30°C in a high supply of glucose to an OD₆₀₀ of 60 after 10 h. During the second stage (Fig. 4B), anaerobic fermentation was used to promote the production of 1,3-PD from glycerol (Fig. 5). First, residual glucose medium from the first stage was completely replaced with glycerol medium over 1.16 kb

FIG. 2. Recombinant temperature-sensitive plasmid pDY220. Arrows indicate the direction of transcription. The tandem promoter P₃ P₅ was obtained from λ phage. The cIts857 gene encodes a temperature-sensitive inhibitor from λ phage. rrnBT1T2 is a strong transcriptional stop sequence from the E. coli ribosomal protein gene rrnB. The gene yqhD harbors dhaB1 and dhaB2 from C. butyricum, and the gene yqhD is from E. coli.
a period of 2 hours, after which anaerobic fermentation was stimulated by stopping aeration and maintaining 0.4 vvm nitrogen. Fresh glycerol fermentation medium was again added at 4, 11, 18, and 23 h by using a feed batch at a regular speed in order to maintain a glycerol concentration of approximately 35 g/liter. Although cell growth slowed with glycerol as the main carbon source, an OD_{600} of 106 was still reached at 25 h. We suspect this was due to the increase in temperature from 30°C to 42°C, as required to stimulate expression of the 1,3-PD polycistronic operon from the Pl_{PR} promoter for 1,3-PD production. During anaerobic fermentation, the recombinant strain successfully converted glycerol in the medium to 1,3-PD to a final concentration of 104.4 g/liter (Fig. 5).

Confirmation of enzymatic activities during fermentation. Glycerol dehydratase and 1,3-propanediol NADP-dependent dehydrogenase activities were not detected in fermentation cultures until the cultures underwent a temperature shift from 30°C to 42°C (Fig. 6). The highest activity of glycerol dehydratase detected was 0.4 U/mg of total protein after 25 h of anaerobic fermentation. The glycerol dehydratase activity eventually declined to 0.25 U/mg of total protein at the end point of fermentation. A similar phenomenon was observed for 1,3-propanediol NADP-dependent dehydrogenase activity. The highest 1,3-propanediol NADP-dependent dehydrogenase activity, 0.59 U/mg of total protein, was also detected after 25 h of anaerobic fermentation, after which activity declined to 0.45 units/mg. The activity patterns of the two enzymes (Fig. 6) were consistent with the stage of glycerol conversion to 1,3-PD by the recombinant E. coli strain (Fig. 5).

Analysis of by-product production during fermentation. Acetate, formate, lactate, and succinate, in addition to being by-products of glucose fermentation, are known by-products of conversion of glycerol to 1,3-PD (25). High by-product concentrations have been shown to slow or inhibit the growth rate.
of recombinant cells, thereby negatively impacting 1,3-PD production (10). Therefore, we assayed for by-product production during the fermentative conversion of glycerol to 1,3-PD (Fig. 7). By-product production followed the two phases of high-density cell fermentation (compare Fig. 7 and 4). In addition to a small amount of formate (Fig. 7A), acetate (Fig. 7A) was the main by-product produced as the culture density increased during aerobic fermentation (Fig. 4A). After the perfusion of glycerol to replace glucose in the fermentation medium (Fig. 4B), by-product concentrations in the fermentation broth decreased rapidly. Most notably, the concentration of acetate (Fig. 7B) decreased from 4.8 g/liter to 0.23 g/liter, and the concentration of formate (Fig. 7B) decreased from 0.5 g/liter to 0.03 g/liter. Furthermore, during anaerobic fermentation, as 1,3-PD rapidly accumulated (Fig. 5), steady increases in the concentrations of all but one of the measured by-products in the fermentation broth were observed (Fig. 7B). The main by-product was pyruvate (Fig. 7B), with a final concentration of 6.5 g/liter. The final concentrations of acetate, formate, and lactate were 5.5 g/liter, 2.2 g/liter, and 2.9 g/liter, respectively. In contrast, the concentration of succinate remained relatively constant, albeit at low levels (Fig. 7B).

**DISCUSSION**

Although many microorganisms are capable of the natural fermentation of glycerol to 1,3-PD, their application for large-scale 1,3-PD production has met with a myriad of difficulties. These include the following: (i) the continual and large accumulation of by-products in the culture broth, which have been shown to be cytotoxic and to strongly impair the formation of 1,3-PD; (ii) the requirement of the cost-ineffective cofactor vitamin B$_{12}$ for production; (iii) the role of microorganisms that naturally ferment glycerol into 1,3-PD as human pathogens (25). Thus, our focus, and that of others (25), has been to avoid these problems by developing an engineered *E. coli* strain capable of cost-effective 1,3-PD production.

To establish an improved bioprocess for large-yield 1,3-PD production via heterologous expression in recombinant *E. coli*, we combined the genes for two different mechanisms of glycerol conversion to 1,3-PD into one operon. First, in an effort to relieve the system from a dependence on the cost-ineffective cofactor vitamin B$_{12}$, we chose the genes *dhaB1* and *dhaB2* from *C. butyricum*, which encode the vitamin B$_{12}$-independent glycerol dehydratase and an activating factor for vitamin B$_{12}$-independent glycerol dehydratase, respectively. Second, in an effort to relieve the high accumulation of 3-HPA that often hampers efficient 1,3-PD production, we chose to use the 1,3-propanediol NADP-dependent dehydrogenase *YqhD*. Researchers from DuPont (Emptage and colleagues) first characterized the *yqhD* gene and constructed the *E. coli* engineered strain by utilizing *yqhD* and the *Klebsiella pneumoniae dha* regulon genes. They further found that *YqhD* had a higher efficiency for conversion of 3-HPA to 1,3-PD than that of the traditionally used NAD-dependent dehydrogenase DhaT (11).

Furthermore, we strove to maximize the effectiveness of our novel operon for 1,3-PD production by expressing it from a strong, regulatable promoter in a convenient host for heterologous expression. Temperature-sensitive gene induction provides a particularly attractive alternative to isopropyl-β-D-thiogalactopyranoside (IPTG) induction, an economically infeasible option for industrial applications currently in use by the majority of available laboratory heterologous expression systems. Thus, we chose the temperature-sensitive vector pBV220, which drives gene expression from the λ phage P$_{L}$P$_{R}$ promoter upon transfer of cells to 42°C. It also includes cIs875, a gene encoding a temperature-sensitive regulation protein, upstream of the promoter, and a strong downstream transcription stop sequence. Although pBV220 has been used to successfully express some medicinal proteins and enzymes on a laboratory scale (21, 34, 38, 41), this is its first documented use in an industrial process.

Our host of choice for expression of our novel operon was *E. coli* K-12, a common host cell for the construction of engineered strains for heterologous expression (25). Its benefits are several. First, it is not a human pathogen, in contrast to some of the naturally 1,3-PD-producing microorganisms. In fact, it is considered a food safety strain, which is eligible for favorable regulatory status in the United States and China. Second, its growth rate is very rapid (23), thereby showing promise for reduced fermentation times. Finally, the strain is a clean slate in which to produce heterologous proteins. Specifically, it has no capacity to produce 1,3-PD; thus, the engineered strain relies on a predominantly heterologous carbon pathway that diverts carbon from dihydroxyacetone phosphate, a major “pipeline” in central carbon metabolism, to 1,3-PD.

To test the efficacy of our novel 1,3-PD operon and its expression in our engineered *E. coli* strain, we first confirmed that our 1,3-PD polycistronic operon was expressed under temperature-regulated control of the P$_{L}$P$_{R}$ promoter in pBV220. Results of Northern analysis showed that the 1,3-PD operon was not transcribed at 30°C during aerobic fermentation in glucose; however, it was upregulated upon a shift to 42°C during fermentation in glycerol. Furthermore, we discovered that *E. coli* K-12 has one copy of the *yqhD* gene that is basally expressed at 30°C. Regardless of its presence, cells lacking the plasmid and vector-only controls did not show an increased rate of 1,3-PD accumulation, suggesting that the endogenous...
copy of yqhD alone does not provide this strain with the ability to produce 1,3-PD (data not shown). These results confirm that our 1,3-PD operon is expressed in a temperature-dependent manner by the recombinant E. coli strain, overcoming the need for IPTG and further increasing its potential for industrial applications.

In an effort to maximize 1,3-PD yield and at the same time minimize production time and by-product production, we established a two-stage two-substrate fermentation method for producing 1,3-PD with our engineered strain. During the first stage (0 to 10 h), dissolved oxygen was maintained well above the critical concentration, and glucose was added continuously to maintain a measured excess of 25 g/liter. The amounts of salts and trace elements in the medium were more than sufficient to support the accumulation of a cell mass equivalent to an OD<sub>600</sub> of 60, with a final cell density of 26 g (dry weight) per liter being reached. The second stage involved replacement of the glucose medium and by-products of the first stage with fresh glycerol fermentation medium over 2 hours. The temperature was then shifted to 42°C, after which the yield of 1,3-PD rapidly increased to a final concentration of 104.4 g/liter at the end point of fermentation.

Although we hoped to minimize by-product formation during 1,3-PD production by having it take place during anaerobic fermentation, the main by-products, pyruvate and acetate, were still produced. At the beginning of anaerobic fermentation, the by-product buildup did not limit 1,3-PD production; however, by 25 h it appeared that buildup of toxic products did start to limit 1,3-PD accumulation. In the catabolic pathway, glycerol not only can be catalyzed to 3-hydroxypropionaldehyde but also can be catalyzed to dihydroyacetone. The pyruvate in the broth is indirectly from dihydroyacetone, the acetate is from pyruvate, and the intermediate is acetyl-coenzyme A. Pyruvate formate lyase is extremely oxygen sensitive (28); therefore, during anaerobic cultivation, pyruvate formate lyase might show activity that leads to a further buildup of acetate. Thus, both the cell density and the 1,3-PD yield rate were evidently decreased in the anaephase of fermentation.

During anaerobic fermentation, the activities of DhaB1 and YqhD increased until 35 h, after which the activities of both declined. These reductions in enzyme activities were presumably due to cell death, as the cell density also decreased at the same time. Regardless, it appears that the reactivating factor DhaB2 effectively maintained stable DhaB1 activity for the duration of fermentation. Furthermore, it appears that YqhD effectively converted 3-HPA to 1,3-PD, resulting in a 1,3-PD titer far superior than with other engineered strains utilizing DhaT for this step (14) and unprecedented in a glycerol-fed fermentation using natural 1,3-propanediol-producing organisms. In contrast to DhaT, YqhD utilizes NADPH rather than NADH, and it is likely that the differences in the cofactor reduced/oxidized ratios contribute to the higher titer (25).

Thus far, with regard to the use of microbial conversion to produce 1,3-PD, our study reports the highest productive efficiency and yield of 1,3-PD using glycerol as the sole source of carbon. Specifically, we achieved a 1,3-PD yield of 104.4 g/liter, a productivity of 2.61 g/liter/h, and a conversion rate of glycerol to 1,3-PD of 90.2% (g/g). Other cases involving anaerobic fermentation with glycerol as the sole carbon source have reported lower 1,3-PD concentrations of 70.4 g/liter (for Clostridium butyricum) and 70 to 78 g/liter (for Klebsiella pneumoniae) (14, 22). Furthermore, the productivities and the conversion rates of glycerol to 1,3-PD in these systems were less than 1.57 g/liter/h and less than 75% (g/g), respectively (7, 25, 39). This work will be significant in exploring and finding a large-scale use for crude glycerol, which is the by-product of biodiesel, and will help lessen the requirements of growing supplies and give the biodiesel industry more revenue. Not only is it essential to find an economically viable refining process, but also it is important for the environment. In conclusion, we have developed a heterologous expression system that overcomes the common problems associated with microbial production of 1,3-PD from glycerol and is a large step ahead in the industrial production of 1,3-PD. Despite these improvements, we are still working to make the process more cost-efficient. Specifically, we are altering the system so that it instead utilizes glucose, which is a much cheaper substrate than glycerol, as the carbon source for direct conversion to 1,3-PD (40). Not only will the convenient and high-efficiency fermentation process developed in this study provide the opportunity to further improve the yield of 1,3-PD, it also promises to lend itself to the development of flexible methods to extend the substrate range of the 1,3-PD synthesis pathway to more-abundant renewable substrates, such as starch and sugar.

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