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Production of 1,2-Propanediol from Glycerol in *Saccharomyces cerevisiae*

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Glycerol has become an attractive carbon source in the biotechnology industry owing to its low price and reduced state. However, glycerol is rarely used as a carbon source in *Saccharomyces cerevisiae* because of its low utilization rate. In this study, we used glycerol as a main carbon source in *S. cerevisiae* to produce 1,2-propanediol. Metabolically engineered *S. cerevisiae* strains with overexpression of glycerol dissimilation pathway genes, including glycerol kinase (*GUT1*), glycerol 3-phosphate dehydrogenase (*GUT2*), glycerol dehydrogenase (*gdh*), and a glycerol transporter gene (*GUP1*), showed increased glycerol utilization and growth rate. More significant improvement of glycerol utilization and growth rate was accomplished by introducing 1,2-propanediol pathway genes, *mgs* (methylglyoxal synthase) and *gldA* (glycerol dehydrogenase) from *Escherichia coli*. By engineering both glycerol dissimilation and 1,2-propanediol pathways, the glycerol utilization and growth rate were improved 141% and 77%, respectively, and a 2.19 g 1,2-propanediol/l titer was achieved in 1% (v/v) glycerol-containing YEPD medium in engineered *S. cerevisiae*.

Keywords: *Saccharomyces cerevisiae*, glycerol, 1,2-propanediol, metabolic engineering

Owing to the limitation of fossil fuels [12], many researchers have made efforts to construct novel microbial systems to produce valuable chemicals and fuels from non-petroleum resources [4, 22, 23]. Glycerol, a by-product of biodiesel, occupies 10% of volume production of biodiesel [11]. As biodiesel production increased, glycerol has become an attractive and competitive carbon source because its price has decreased quickly. Moreover, its reduced state of carbon allows an easy conversion to other useful chemicals, such as 1,3-propanediol, dihydroxyacetone, ethanol, succinic acid, propionic acid, and citric acid [9].

The glycerol utilization pathway has been investigated in several enterobacteria species, such as *Klebsiella pneumonia* [1], *Enterobacter agglomerans* [5], and *Escherichia coli* [10]. Two different pathways were characterized for aerobic and anaerobic conditions, respectively [7, 21, 35]. Glycerol kinase and glycerol 3-phosphate dehydrogenase that convert glycerol to glycerol 3-phosphate to dihydroxyacetone phosphate are mainly expressed under aerobic condition. Meanwhile, glycerol dehydrogenase and glycerol 3-phosphate kinase that convert glycerol to dihydroxyacetone to dihydroxyacetone phosphate are expressed under anaerobic condition. With the pathway information, the production of ethanol and co-products from glycerol has been investigated in several microorganisms [17, 34, 38]. So far, the anaerobic pathway has been mainly utilized for this purpose. However, many valuable products can be produced at higher yields by microaerobic or aerobic culture conditions [11].

Glycerol overproduction has been widely investigated with *Saccharomyces cerevisiae* [24, 26, 29, 33], because *S. cerevisiae* can produce glycerol at the highest levels among microorganisms. Glycerol is also investigated as a stress-related metabolite in *S. cerevisiae* [30]. For example, osmotic stress seems to be intimately related to glycerol response, so that its signaling pathway, the HOG pathway, was well characterized [14]. However, metabolite overproduction using glycerol as a carbon source has been very limited with *S. cerevisiae*.

1,2-Propanediol, also called propylene glycol or propane-1,2-diol, is a major commodity chemical with global demand estimated around 3 billion lb/year for food, drug, and cosmetic industries. It is used as less-toxic antifreeze, solvent in the photographic industries, humectants in the food industries, or moisturizer and carrier of fragrance oils in the cosmetic and pharmaceutical fields [19]. The US Food and Drug Administration (FDA) has determined propylene

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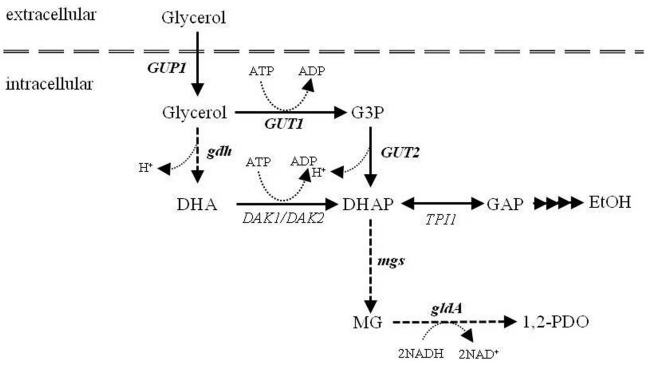

Fig. 1. Glycerol uptake pathway in *S. cerevisiae* and engineered 1,2-propanediol synthesis pathway in this study.
G3P, glycerol 3-phosphate; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; MG, methylglyoxal; 1,2-PDO, 1,2-propanediol; EtOH, ethanol; *GUT1* glycerol kinase gene; *GUT2*, glycerol 3-phosphate dehydrogenase gene; *DAK1/DAK2*, dihydroxyacetone kinase genes; *gdh*, glycerol dehydrogenase gene of *Pichia angusta*; *mgs*, methylglyoxal synthase gene of *E. coli*; *gldA*, glycerol dehydrogenase gene of *E. coli*. Bold italic letters indicate overexpressed genes in this study.

glycol to be "generally recognized as safe" for use in food, cosmetics, and medicines.

In this study, we metabolically engineered *S. cerevisiae* to produce 1,2-propanediol. The construction of a heterologous 1,2-propanediol pathway in *S. cerevisiae* increased the glycerol uptake rate. We further overexpressed three endogenous genes in the glycerol utilizing pathway, *GUT1* (glycerol kinase), *GUT2* (glycerol 3-phosphate dehydrogenase), and *GUP1* (aglycerol transport) (Fig. 1). We also introduced a glycerol dehydrogenase gene, which converts glycerol to dihydroxyacetone from *Pichia angusta* [27]. As a result, the growth rate, glycerol uptake rate, and 1,2-propanediol production were greatly enhanced in *S. cerevisiae*.

Materials and Methods

Strains and Plasmid Constructions

S. cerevisiae 499 strain (*MATa ura3-52 lys2-801amberade2-101orchetrp1-Δ63 his3-Δ200 leu2-Δ1*) was genetically modified for producing 1,2-propanediol (Table 1). *E. coli* strain DH5α was used for plasmid construction.

To construct expression vectors, pESC-URA, pESC-TRP, and pESC-LEU (Stratagene, San Diego, USA) were purchased. For producing 1,2-propanediol in *S. cerevisiae*, *mgs* and *gldA* genes were PCR-amplified using *E. coli* DH5α genomic DNA as a template and cloned into the pESC-URA vector as previously described [19]. For selection of the most strong glycerol dehydrogenase that converts from glycerol to dihydroxyacetone in *S. cerevisiae*, three different glycerol dehydrogenase genes, *gldA*, *dhaD*, and *gdh*, were PCR-amplified using *E. coli*, *C. freundii*, or *P. angusta* genomic DNAs as templates using the three sets of primers listed in Table 2, respectively. Each glycerol dehydrogenase gene was inserted into the pESC-LEU vector under a *GAL10* promoter using *Not*I/*Sac*I (Fig. 2A). For overexpression of the glycerol transporting-related *GUP1* gene, a pair of primers listed in Table 2 were used for PCR amplification using *S. cerevisiae* YPH499 genomic DNA as a template. The PCR product was cloned into the T-easy vector (Promega, Madison, USA), and then inserted into the pESC-LEU vector using *Bam*HI/*Sal*I under the *GAL1* promoter. We also constructed an expression vector for both the glycerol dehydrogenase (*gdh*) and *GUP1* genes into the pESC-LEU vector (Fig. 2B). For overexpression of the *GUT1* and *GUT2* genes, two sets of primers listed in Table 2 were used for PCR amplification using *S. cerevisiae* YPH499 genomic DNA as a template. Each PCR product was cloned into the pESC-TRP vector using *Bam*HI/*Sal*I under the *GAL1* promoter, and the *GUT1* gene was inserted under the *GAL10* promoter using *Eco*RI/*Sac*I (Fig. 2C). The results in expression vectors were transformed to *S. cerevisiae* by the lithium acetate method [16].

To develop a *dak1* deleted strain, a set of primers (dak1f and dak1r in Table 2) were used for PCR amplification using pFA6a-KanMX as a template [36]. The PCR product was transformed to *S.*

Table 1. *S. cerevisiae* strains used in this study.

Strain	Genotype	Reference
YPH499	*MATa ura3-52 lys2-801^{amber}ade2-101^{orche}trp1-Δ63 his3-Δ200 leu2-Δ1*	ATCC 204679
sJMG	YPH499 with pESC-URA-*mgs & gldA*	[19]
sJk1	YPH 499 with *dak1Δ :: KanMX6*	
sJD1k1	YPH499 with *dak1Δ :: KanMX6,* pESC-LEU-*gldA*	
sJD2k1	YPH499 with *dak1Δ :: KanMX6,* pESC-LEU-*dhaD*	
sJD3k1	YPH499 with *dak1Δ :: KanMX6,* pESC-LEU-*gdh*	
sJD	YPH499 with pESC-LEU-*gdh*	
sJP	YPH499 with pESC-LEU-*GUP1*	
sJT	YPH499 with pESC-TRP-*GUT1 & GUT2*	This study
sJDP	YPH499 with pESC-LEU-*gdh & GUP1*	
sJDPT	YPH499 with pESC-TRP-*GUT1 & GUT2*, pESC-LEU-*gdh & GUP1*	
sJDMG	YPH499 with pESC-URA-*mgs & gldA*, pESC-LEU-*gdh*	
sJPMG	YPH499 with pESC-URA-*mgs & gldA*, pESC-LEU-*GUP1*	
sJTMG	YPH499 with pESC-URA-*mgs & gldA*, pESC-TRP-*GUT1 & GUT2*	
sJDPMG	YPH499 with pESC-URA-*mgs & gldA*, pESC-LEU-*gdh & GUP1*	

Table 2. Primer sequences used in this study.

Primer	Sequence	Reference
GUP1f	5'-GGA TCC ATG TCG CTG ATC AGC ATC-3'	This study
GUP1r	5'-GTC GAC TCA GCA TTT TAG GTA AAT-3'	
GUT1f	5'-GAA TTC ATG TTT CCC TCT CTC TTC-3'	
GUT1r	5'-GAG CTC TTA TTG GAA GTT TTC TAG-3'	
GUT2f	5'-GGA TCC ATG TTT TCG GTA ACG AGA-3'	
GUT2r	5'-GTC GAC TTA GAC ACC AAA CGT CTT-3'	
gldAf	5'-GCG GCC GC ATG GAC CGC ATT ATT CAA-3'	[19]
gldAr	5'-GAG CTC TTA TTC CCA CTC TTG CAG-3'	
dhaDf	5'-GCG GCC GC ATG CTA AAA GTT ATT CAA TCT CCA-3'	[18]
dhaDr	5'-GAG CTC TTA ACG CGC CAG CCA CTG CTG-3'	
gdhf	5'-GCG GCC GC ATG AAA GGT TTA CTT TAT-3'	[27]
gdhr	5'-GAG CTC TTA GGA AAC CTC GTT CGG-3'	
dak1f	5'-CAA AGA ATA AGA TTA CAT TCT ATA TCT AAG ACT AAA TTT TAA ATG CGT ACG CTG CAG GTC GAC-3'	*Saccharomyces* Genome Deletion Project (http://www-sequence.stanford.edu/ group/ yeast_deletion_project)
dak1r	5'-ATA TAT CAT AAG TAT CTT GAT ATG TAT TCG TGA GCC AAG TAC TTA ATC GAT GAA TTC GAG CTC-3'	
dak1_CA	5'-TAT TTG TTA CTG TCA ATT GTC TGG C-3'	
dak1_CB	5'-ATC GGT CTT TCT GAA GAG AAT TTT T-3'	
dak1_CC	5'-GGT TAC ACT TTA GTG GCA GGA GTT A-3'	
dak1_CD	5'-TGT TAA CGG TAT TTC CTT CTT GTT C-3'	

The underlined sequences represent the introduced restriction enzyme sites for DNA recombination.

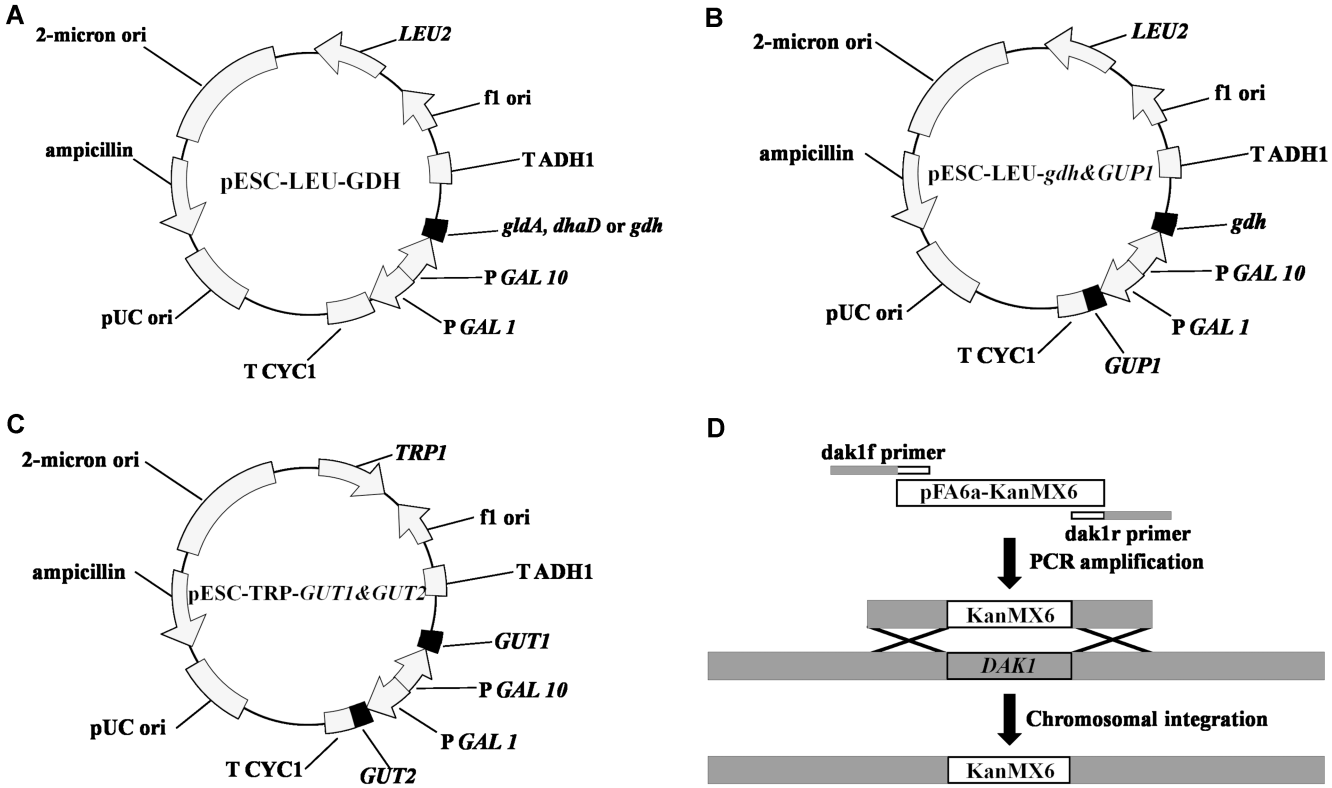

Fig. 2. Constructed plasmids and gene disruption method used in this study.
A. The map of pESC-LEU-GDH; *gldA*, *dhaD*, or *gdh* genes were cloned under *GAL10*. **B**. The map of pESC-LEU-*gdh1&GUP1*. **C**. The map of pESC-TRP-*GUT1&GUT2*. **D**. *dak1* gene disruption method using homologous recombination. dak1f and dakr primers have 45 base-pairs homologous to the DAK1 gene and 18 base-pairs homologous to the KanMX6 cassette, respectively.

cerevisiae by lithium acetate methods and the deletion mutant was selected by solid medium containing 200 μg/ml geneticin (Fig. 2D). The correct replacement of the *dak1* gene with KanMX6 was verified in the mutants by the appearance of PCR products of the expected sizes using primers that span the left and right junctions of the deletion module within the genome. Four ORF-specific confirmation primers (dak1_CA, dak1_CB, dak1_CC, and dak1_CD in Table 2) were used for verifying *dak1* gene deletion in the YPH499 strain ORF.

Media

LB medium was used for *E. coli*. Ampicillin 50 μg/ml was added for selection. Modified YEPD medium (yeast extracts 1%, peptone 2%, glycerol 1%, galactose 0.1%) and modified SD medium [glycerol 1%, yeast extract 0.2%, galactose 0.1%, yeast nitrogen base without amino acid 0.67%, CSM (amino acids supplement mixture) 0.074%] were used for production of 1,2-propanediol with *S. cerevisiae*. Different CSMs (amino acids supplement mixture; MP Biomedicals, Solon, USA) were used in cell cultivation depending on the auxotroph selection markers in the developed strains. For example, URA-medium was prepared with Yeast Nitrogen Base without amino acid 0.67%, glycerol 1.0%, yeast extract 0.2%, galactose 0.1%, and CSM-URA (amino acids supplement mixture) 0.074%. TRP, LEU, TRP-URA, and LEU-URA media were prepared in the same manner and used for *S. cerevisiae* selection. Solid media were made by adding 2% (w/v) agar.

Cultivation Condition

Growth rate was measured by detecting optical density at 660 nm using UV mini 1240 spectrometry (Shimadzu, Tokyo, Japan). Strains were inoculated at OD 0.04 and grown at 30°C in 250 ml flasks with shaking at 250 rpm. Galactose 0.1% (w/v) was added to the medium for overproducing *GUT1*, *GUT2*, *GUP1*, *gdh*, *dhaD*, *mgs*, and *gldA* gene products under GAL1 or GAL10 promoters.

Metabolite Assays

Glycerol, ethanol, and 1,2-propanediol were assayed with the HPLC LC-20H (Dong-il SHIMADZU Corp, Seoul, Korea) using the RI-detector RID-10A model and HPX-87X anion exchange column (Bio-Rad, Hercules, USA) at 40°C, 50°C, and 55°C, respectively, with 0.01 M sulfuric acid as the mobile phase at 0.5 ml/min velocity.

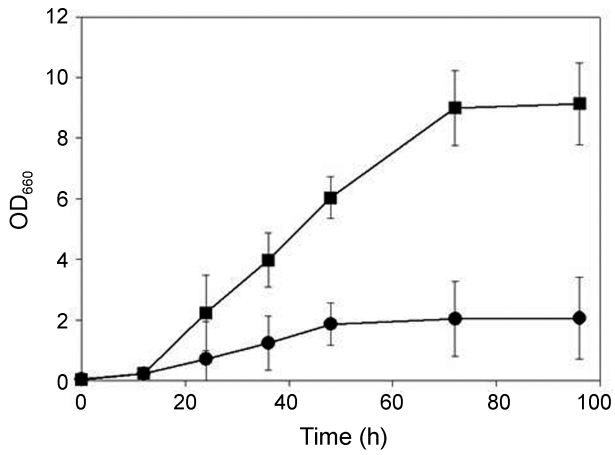

Fig. 3. Growth patterns of YPH499 wild-type strain in modified SD medium (■) and the same medium without glycerol (●).

Their peaks were detected at 14.5, 22.0, and 18.5 min, respectively. Extracellular media samples were prepared by taking supernatants after centrifugation of culture media on a UNION55R (Hanil Science Industrial Co., Ltd, Inchon, Korea) for 5 min at 3,000 rpm, followed by filtration with 0.20 μm nylon syringe filters (Albert, Barcelona, Spain).

Dihydroxyacetone was assayed with an enzymatic method [6]. Briefly, dihydroxyacetone was phosphorylated with ATP and glycerol kinase to dihydroxyacetone phosphate. Dihydroxyacetone phosphate was then reduced with NADH and glycerolphosphate dehydrogenase to L-(-)-glycerol 3-phosphate. The decrease of NADH concentration was measured by the change in absorbance at 339, 334, and 365 nm using spectrometry (UV mini 1240; Shimadzu, Tokyo, Japan), which was proportional to the amount of dihydroxyacetone.

RESULTS

Development of Strains

The production of 1,2-propanediol in *S. cerevisiae* as a host microorganism with glycerol has several hurdles. First, there is no 1,2-propanediol metabolic pathway in *S. cerevisiae*. This problem was solved in previous studies by introducing the methylglyoxal synthase (*mgs*) and glycerol dehydrogenase (*gldA*) genes from *E. coli* [19, 20]. Second, the low glycerol uptake rate and growth rate of *S. cerevisiae* make it difficult to reach the industrially feasible productivity. To solve the problem, we overexpressed glycerol dissimilation pathway genes as well as a glycerol transporting gene (Fig. 1). We combined two strategies to generate several metabolically engineered strains that showed increased cell growth rate, glycerol uptake ability, and 1,2-propanediol production. (Table 1).

Glycerol as a Carbon Source

To examine the carbon flux and utilization, *S. cerevisiae* must be cultivated in minimal media, such as SD medium. However, when the strain was grown with SD medium containing 1% (v/v) glycerol, the growth rate and glycerol consumption rate were extremely poor. To enhance the initial growth rate, modified SD medium was made by adding 0.2% (w/v) yeast extract, 1% (v/v) glycerol as a carbon source, and 0.1% galactose (w/v) to induce *GAL* promoters. *S. cerevisiae* YPH499 wild-type strain grew reasonably well in modified SD medium (Fig. 3). To prove that glycerol was used as a main carbon source, the strain was also grown in the modified SD medium without glycerol. The final OD in the medium without glycerol after 96 h was only a quarter compared with that in the modified SD medium, proving that glycerol was the main carbon source in the modified SD medium (Fig. 3).

Amplification of Glycerol Dissimilation Pathway Genes

To improve the growth rate of YPH499 in glycerol, sJT strain was made that overexpresses Gut1p, a glycerol kinase

converting glycerol to glycerol 3-phosphate, and Gut2p, a glycerol 3-phosphate dehydrogenase converting glycerol 3-phosphate to dihydroxyacetone phosphate [32]. We also developed sJP strain overexpressing Gup1p, a putative membrane-bound *O*-acyltransferase that was proposed to be involved in glycerol transport in *S. cerevisiae* [25]. Lastly, we constructed another glycerol dissimilation pathway *via* introducing heterologous glycerol dehydrogenase to *S. cerevisiae* (Fig. 1). For this purpose, we cloned three glycerol dehydrogenase genes, including *dhaD* of *Citrobacter freundii*, *gdh* of *Pichia angusta*, and *gldA* of *E. coli* [18, 27]. To find the most effective glycerol dehydrogenase, a *dak1*-deleted mutant (sJk1strain) that accumulates dihydroxyacetone was made as described in Fig. 2D. Three glycerol dehydrogenase genes were introduced to this strain, generating sJD1k1, sJD2k1, and sJD3k1, respectively. Three strains were cultivated in modified SD medium, and dihydroxyacetone was assayed as previously described. The strain overexpressing glycerol dehydrogenase (*gdh*) from *P. angusta* (sJD) produced the highest conversion rate from glycerol to dihydroxyacetone, which was 0.38 g/l dihydroxyacetone produced during 24 h cultivation. This titer was 3.16- and 2.11-fold higher than the glycerol dehydrogenases from *E. coli* and *C. freundii*, respectively (data not shown). This result was consistent with the reported data [27]. Together with these strains, the strains overexpressing both Gup1p and Gdh from *P. angusta* (sJDP) and all four genes (sJDPT) were constructed and grown in modified SD medium. All developed strains (sJT, sJP, sJD, sJDP, and sJDPT) that reinforced glycerol utilization pathway genes showed higher growth rates than the wild-type strain, meaning that glycerol uptake flux is a limiting step for *S. cerevisiae* to grow in glycerol (Fig. 4A).

We also compared the glycerol utilizing ability and ethanol production of these strains. The wild-type strain, YPH499, consumed only 4.24 g/l out of 12.62 g/l glycerol after 96 h cultivation. The sJT, sJP, and sJD stains consumed as much as 4.89 g/l, 5.11 g/l, and 5.18 g/l, glycerol respectively, showing improved glycerol uptake ability. Gup1p and gdh overexpression showed higher uptake rates than Gut1p and Gut2p. The sJDP and sJDPT consumed as much as 5.59 g/l and 6.24 g/l glycerol, respectively (Fig. 5). Manipulation of the glycerol dissimilation pathway improved glycerol consumption by as much as 1.15~1.47-fold. When ethanol production was monitored with four strains, ethanol concentrations were increased from 0.23 g/l to 0.35 g/l, 0.46 g/l, 0.44 g/l, and 0.64 g/l in sJT, sTP, sJD, and sJDP strains, respectively, which were 1.5~2.8-fold higher than the wild-type strain (Table 3). As we overexpressed four glycerol utilizing pathway genes in *S. cerevisiae*, cell growth increased 1.38-fold, glycerol was consumed 1.47-fold more, and ethanol was produced 3.3-fold higher than the wild-type strain in 96 h cultivation with glycerol as a major carbon source.

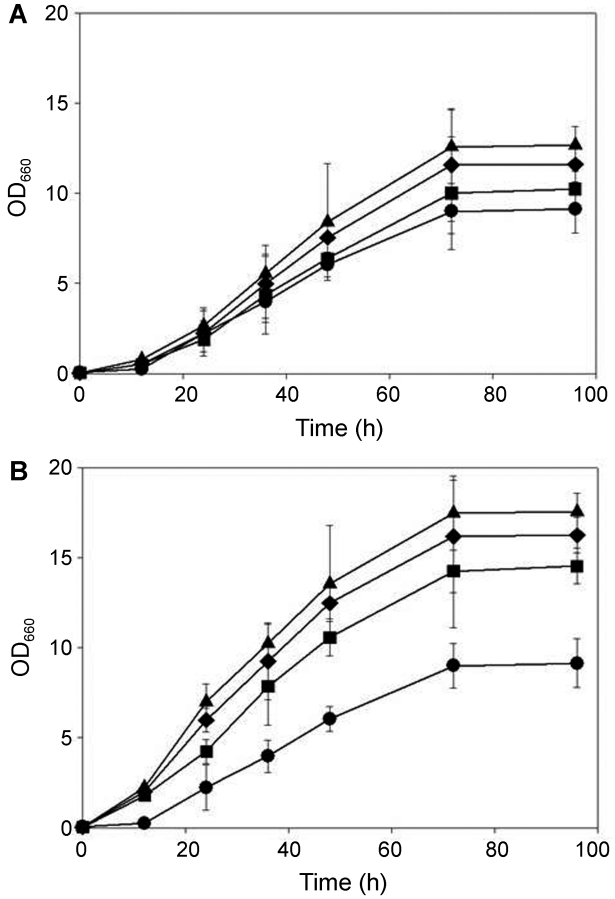

Fig. 4. Growth patterns of developed strains during 96 h flask cultivation.

A. The growth rates of wild-type (●), sJT, (■), sJDP, (◆), and sJDPT (▲) strains. **B**. The growth rates of wild-type (●), sJMG (■), sJTMG, (◆), and sJDPMG (▲) strains during 96 h flask cultivation.

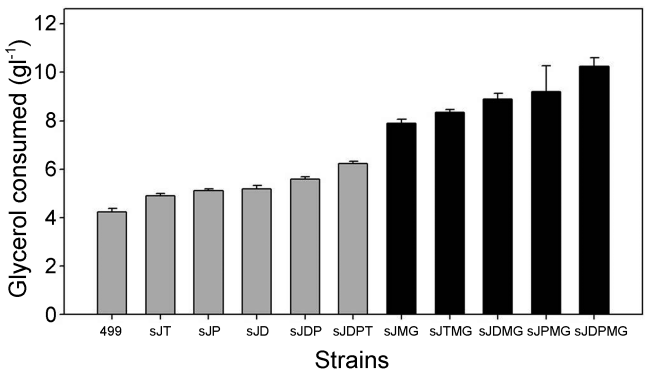

Fig. 5. Consumed glycerol during 96 h flask cultivation.

Gray bars represent consumed glycerol by the strains with the manipulated glycerol utilization pathway and black bars are that by the strains with manipulated glycerol utilization and 1,2-propanediol-producing pathway.

Table 3. Concentration of 1,2-propanediol and ethanol after 96 h flask cultivation in modified SD and YEPD media.

Strains	Overexpressed genes	Concentration of ethanol (g/l)	Concentration of 1,2-propanediol (g/l)	$Y_{X/S}$ (g/g)[b]	$Y_{1,2\text{-PDO}/S}$ (g/g)
499	None	0.232±0.13	N/D	0.496±0.10	N/A
sJT	*GUT1* and *GUT2*	0.354±0.10	N/D	0.481±0.05	N/A
sJD	gdh	0.435±0.08	N/D	0.515±0.18	N/A
sJDP	*gdh* and *GUP1*	0. 635±0.16	N/D	0.497±0.12	N/A
sJDPT	*GUT1*, *GUT2*, *gdh*, and *GUP1*	0.757±0.20	N/D	0.466±0.11	N/A
sJMG	*mgs* and *gldA*	0.287±0.10 [a]0.602±0.02	0.624±0.10 [a]0.987±0.05	0.308±0.06 [a]0.423±0.11	0.119±0.08 [a]0.125±0.04
sJTMG	*GUT1*, *GUT2*, *mgs*, and *gldA*	0.301±0.09 [a]0.558±0.04	0.745±0.15 [a]1.434±0.11	0.340±0.13 [a]0.448±0.14	0.134±0.21 [a]0.171±0.18
sJDMG	*gdh*, *mgs*, and *gldA*	0.298±0.16 [a]0.625±0.12	0.825±0.10 [a]1.728±0.21	0.337±0.10 [a]0.438±0.20	0.140±0.11 [a]0.194±0.13
sJPMG	*GUP1*, *mgs*, and *gldA*	0.255±0.05 [a]0.724±0.05	0.865±0.08 [a]1.865±0.08	0.324±0.02 [a]0.404±0.11	0.141±0.03 [a]0.202±0.11
sJDPMG	*gdh*, *mgs*, *gldA*, and *GUP1*	0.324±0.12 [a]0.895±0.31	0.982±0.16 [a]2.187±0.07	0.353±0.09 [a]0.394±0.18	0.147±0.14 [a]0.213±0.02

[a]Data measured in modified YEPD medium.
[b]Dry cell weight is calculated from OD_{660} value (1 OD_{660} = 0.23 dry cell weight g/l).

Introduction of 1,2-Propanediol Production Pathway Genes

We introduced 1,2-propanediol-producing pathway genes (*mgs* and *gldA* from *E. coli*) to *S. cerevisiae* (sJMG). Interestingly, the growth rate of this strain was increased significantly higher than that of the wild-type strain (Fig. 4B). The sJMG strain consumed 7.24 g/l glycerol within 96 h, which is almost 1.86-fold higher than the wild-type strain (Fig. 5). These results suggested that the introduction of the 1,2-propanediol pathway in *S. cerevisiae* had a positive effect on growth on glycerol and its consumption rate. The strain produced 1,2-propanediol (0.62 g/l) as well, which was 1.3-fold higher than the 1,2-propanediol produced form the same strain grown in SD medium with 2% (w/v) glucose.

We further overexpressed glycerol utilization pathway genes in this strain (sJMG). The sJTMG, sJDMG, sJPMG, and sJDPMG strains consumed glycerol by as much as 8.35 g/l, 8.88 g/l, 9.21 g/l, and 10.24 g/l, respectively, which were 1.96~2.41-fold higher than the wild-type strain (Fig. 5). Moreover, we assayed ethanol and 1,2-propanediol production from these strains. The ethanol concentration was almost similar, between 0.26 g/l and 0.32 g/l. However, the 1,2-propanediol concentrations was sharply increased when glycerol utilization pathway genes were overexpressed together. The concentration was increased from 0.62 g/l to 0.75, 0.83, and 0.87 g/l, when Gut1p/Gut2p, Gup1p, and Gdh from *P. angusta* were overexpressed, respectively. The sJDPMG strain produced 0.98 g/l 1,2-propanediol after 96 h flask cultivation. This is a 1.57-fold higher level of 1,2-propanediol concentration compared with the sJMG strain (Table 3). We also constructed the sJDPTMG strain overexpressing all six genes manipulated, but the growth rate of the strain was much poorer than sJDPMG (data not shown), possibly due to the nutrient problem. Lastly, we conducted flask cultivation in modified YEPD medium during 96 h. Our developed strains sJTMG, sJDMG, sJPMG, and sJDPMG produced as much as 1.43, 1.73, 1.87, and 2.19 g/l 1,2-propanediol, respectively (Table 3). This amount of 1,2-propanediol is the highest production reported in *S. cerevisiae*. Therefore, it was concluded that both introduction of the 1,2-propanediol production pathway and amplification of the glycerol utilization pathway contributed to improved growth rate and glycerol consumption rate, and the increase of the glycerol consumption contributed to the improved production of 1,2-propanediol.

Interestingly, $Y_{X/S}$ and $Y_{1,2\text{-PDO}/S}$ were increased in a similar way when the glycerol uptake pathway was amplified in modified SD medium. However, $Y_{X/S}$ was generally decreased whereas $Y_{1,2\text{-PDO}/S}$ was increased in YEPD medium by the same genetic modification. This showed that metabolic engineering may result in very different results in complex and minimal media. Further study is needed to characterize this phenomenon.

Discussion

Glycerol is recognized as an attractive carbon source and a platform chemical these days. The poor growth rate and glycerol utilization ability of *S. cerevisiae* made it difficult to use glycerol as a carbon source in its bioprocess, so that very few reports have been published in this aspect. In *E.*

coli, disruption of the 1,2-propanediol pathway reduced its growth rate by 29% compared with wild-type strain in glycerol and the reason suggested was that the 1,2-propanediol production pathway provides a means to consume reducing equivalents generated in the synthesis of cell mass, thus facilitating redox balance in the cytosol [13]. We expected a similar effect of the 1,2-propanediol pathway by introducing *mgs* and *gldA* genes in *S. cerevisiae*, which naturally does not produce 1,2-propanediol. Indeed, the growth rate and glycerol utilization ability were increased significantly by 1.59-fold and 1.86-fold over the wild-type strain, possibly due to the redox balance in the cytosol. This result is strongly supportive of the hypothesis that the 1,2-propanediol pathway makes strains grow more effectively in glycerol, although further investigation is needed to prove the mechanism.

Amplification of glycerol utilization pathway genes also improved the growth rate and glycerol uptake rate, suggesting that inefficient utilization of glycerol is another constraint of the *S. cerevisiae* grown in glycerol. By manipulating four genes, utilization of glycerol increased 1.47-fold, cell growth rate increased 1.39-fold, and ethanol production concentration increased 3.26-fold. Among the two glycerol utilization pathways, introduction of glycerol dehydrogenase has a bigger impact than the *GUT1* and *GUT2* pathway. *S. cerevisiae* has a *GCY1* gene encoding innate putative glycerol dehydrogenase, but its activity and function has not yet been clearly investigated [8, 28]. Therefore, we introduced the *P. angusta* glycerol dehydrogenase gene, which improved the glycerol utilization ability 1.22-fold.

Another gene that improved the glycerol utilization was *GUP1*. Gup1p is known as a plasma membrane protein involved in remodeling GPI anchors and its function is known to be that of an active glycerol uptake transporter in *S. cerevisiae* [15]. This gene had an effect on increasing of glycerol utilization 1.21-fold. *S. cerevisiae* has a *FPS1* gene that is also known to be involved in efflux of glycerol. However, when we overexpressed *FPS1* in the same manner, there was no increase of glycerol uptake ability (data not shown). This proved that Gup1p is more directly involved in glycerol uptake than Fps1p. More investigations are needed regarding the glycerol transporter, because several other putative glycerol transporters are found in *S. cerevisiae* genome annotations [31].

Recombinant *S. cerevisiae* and *E. coli* have been used for 1,2-propanediol production. Using glucose as a carbon source, 0.24 g/l [20] or 1.11 g/l [20] 1,2-propanediol was produced with *S. cerevisiae*. Meanwhile, 0.49 g/l 1,2-propanediol was produced with batch culture [2] and 4.5 g/l with fed-batch culture from glucose with recombinant *E. coli* [3]. Production of 2.19 g/l 1,2-propanediol from glycerol with recombinant *S. cerevisiae* in this paper is the highest concentration reported with *S. cerevisiae*.

In conclusion, metabolically engineered *S. cerevisiae* strains with overexpression of glycerol dissimilation pathway genes, including glycerol kinase (*GUT1*), glycerol 3-phosphate dehydrogenase (*GUT2*), glycerol dehydrogenase (*gdh*), and a glycerol transporter (*GUP1*), showed partially increased glycerol utilization and growth rate. Additionally, significant improvements of glycerol utilization and growth rate were accomplished by introducing 1,2-propanediol pathway genes in *S. cerevisiae.* The positive effect of the 1,2-propanediol pathway in *S. cerevisiae* on cell growth rate in glycerol medium was suspected to be the redox balance in the cytosol, but further investigation is required to reveal the exact mechanism. It would provide metabolic engineering tools to make *S. cerevisiae* grow more efficiently in glycerol as a carbon source and produce higher levels of 1,2-propanediol.

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