Bioprocess Biosyst Eng (2013) 36:757–763
DOI 10.1007/s00449-013-0901-y

ORIGINAL PAPER

Efficient production of 1,3-propanediol from glycerol upon constitutive expression of the 1,3-propanediol oxidoreductase gene in engineered *Klebsiella pneumoniae* with elimination of by-product formation

Baek-Rock Oh · Jeong-Woo Seo · Sun-Yeon Heo · Lian Hua Luo · Won-Kyung Hong · Don-Hee Park · Chul-Ho Kim

Received: 25 December 2012 / Accepted: 15 January 2013 / Published online: 30 January 2013

Abstract In the present study, we developed an efficient method of 1,3-propanediol (1,3-PD) production from glycerol by genetic engineering of *Klebsiella pneumoniae* AK mutant strains. The proposed approach eliminated by-product formation and IPTG induction resulted in maximal production of 1,3-PD. A series of recombinant strains was designed to constitutively express the *dhaB* and/or *dhaT* genes, using the bacteriophage T5 P_{DE20} promoter and the *rho*-independent transcription termination signal of the *Rahnella aquatilis* levansucrase gene. Among these strains, AK/pConT expressing *dhaT* alone gave the highest yield of 1,3-PD. Fed-batch fermentation resulted in efficient production of 1,3-PD from either pure or crude glycerol, without by-product formation.

Keywords 1,3-Propanediol · Glycerol · Constitutive expression · *Klebsiella pneumoniae* · By-product formation

B.-R. Oh · J.-W. Seo · S.-Y. Heo · L. H. Luo · W.-K. Hong · D.-H. Park · C.-H. Kim (✉)
Microbe-based Fusion Technology Research Center, Jeonbuk Branch Institute, KRIBB, Jeongeup, Jeonbuk 580-185, South Korea
e-mail: kim3641@kribb.re.kr

B.-R. Oh · D.-H. Park
Interdisciplinary Program of Graduate School for Bioenergy and Biomaterials, Chonnam National University, Gwangju 500-757, South Korea

L. H. Luo
Research Center of Bioactive Materials, Institute for Molecular Biology and Genetics, Chonbuk National University, Jeonju, Chonbuk 561-756, South Korea

Introduction

Currently, biodiesel synthesized from animal fat or plant oil is in great demand. However, a large amount of raw glycerol is formed as the main by-product of biodiesel production, which can be as much as 10 % (w/w) of the biodiesel generated [1]. This surplus raw glycerol has not only greatly disturbed the market for traditional glycerol in terms of both preparation and price, but is also a significant environmental problem because glycerol cannot be discharged directly into the environment [2]. Thus, a great deal of research effort has been devoted to the development of methods by which glycerol (a low-cost feedstock) can be refined into industrially valuable materials such as fuels, chemical building blocks, and bioactive substances.

1,3-Propanediol (1,3-PD) is a valuable chemical used mainly in the synthesis of polymethylene terephthalates by polymerization with terephthalates [3]. Applications for these polymers in the manufacture of materials such as textile fibers, films, and plastics are rapidly increasing. The 1,3-PD building block is currently produced by chemical processes such as hydroformylation of ethylene oxide or hydration of acrolein [4, 5]. Recently, a microbial fermentation process using a recombinant *Escherichia coli* strain containing genes from *Klebsiella pneumoniae* and *Saccharomyces cerevisiae* was developed, by which glucose is converted to 1,3-PD [6]. However, the original route to 1,3-PD production involved microbial fermentation using glycerol as substrate. Because glycerol is a major by-product of the biodiesel industry, biological conversion of this chemical is receiving considerable attention.

Klebsiella pneumoniae is typical of microorganisms that produce 1,3-PD, and the metabolic pathway responsible has been well studied [7]. Glycerol is first converted to 3-hydroxypropionaldehyde (3-HPA) by a coenzyme

B_{12}-dependent glycerol dehyratase (DhaB), and this material is next reduced to 1,3-PD in a reaction catalyzed by a reduced nicotinamide adenine dinucleotide (NADH)-dependent 1,3-PD oxidoreductase (DhaT). In addition to the reductive pathway, glycerol is metabolized by an oxidative pathway, through which glycerol is dehydrogenated to dihydroxyacetone (DHA) by an NAD^+-dependent glycerol dehydrogenase (DhaD) and DHA is phosphorylated to dihydroxyacetone phosphate (DHAP) by an ATP-dependent DHA kinase (DhaK). Glycerol assimilated via the oxidative branch results in an increase in biomass, resulting in the generation of many different metabolites such as acetate, ethanol, lactate, succinate, and 2,3-butanediol (2,3-BD).

The genes encoding the functionally linked proteins DhaB and DhaT of the reductive branch and DhaD and DhaK of the oxidative branch are clustered in a region of *K. pneumoniae* chromosomal DNA termed the *dha* regulon [8]. Many researchers have sought to enhance 1,3-PD production by metabolic engineering [9–12], either greatly increasing enzyme activities [13–15] or NADH availability [16]. However, minimization of by-product synthesis (which can be as much of 70 % [w/w] of 1,3-PD yield) must be considered when enhanced production of 1,3-PD is desired [17]. 2,3-BD is a major by-product formed during synthesis of 1,3-PD, making downstream purification of 1,3-PD difficult because the two materials have similar boiling points [18].

Recently, we engineered a mutant strain of *K. pneumoniae* (termed AK-VOT) to eliminate by-product formation during production of 1,3-PD from glycerol, by inactivation of the oxidative branch of the glycerol metabolic pathway [19]. In such recombinant strains, by-product formation, with the exception of acetate, was eliminated, resulting in a higher 1,3-PD yield relative to that of the wild-type strain. In the present study, we further improved 1,3-PD yield by constitutive expression of a *dha* gene to increase the economy of 1,3-PD production.

Materials and methods

Strains, plasmids, and media

The *K. pneumoniae* strains described in our previous study [18] were grown either in LB [yeast extract (Difco), 0.5 % (w/v); Bacto-trypton (Difco), 1.0 % (w/v); and NaCl, 1.0 % (w/v)] or in a defined glycerol-containing medium with 20 g/l of glycerol, 2 g/l $(NH_4)_2SO_4$, 3.4 g/l K_2HPO_4, 1.3 g/l KH_2PO_4, 0.2 g/l $MgSO_4$, 0.002 g/l $CaCl_2$ $2H_2O$, 1 g/l yeast extract, 1 ml Fe solution [5 g/l $FeSO_4$ $7H_2O$ and 4 ml/l HCl (37 %, w/v)], and 1 ml trace element solution [70 mg/l $ZnCl_2$, 100 mg/l $MnCl_2$ $4H_2O$, 60 mg/l H_3BO_3, 200 mg/l $CoCl_2$ $4H_2O$, 20 mg/l $CuCl_2$ $2H_2O$, 25 mg/l $NiCl_2$ $6H_2O$, 35 mg/l Na_2MoO_4 $2H_2O$, with 4 ml HCl (37 %, w/v)]. Tetracycline was added to a final concentration of 50 μg/ml.

Construction of recombinant plasmids

A schematic representation of the strategy used to construct pConT, pConB, pConBT, pConK, and pConTK is shown in Fig. 1. The primers used for amplification of DNA sequences by polymerase chain reaction (PCR) are shown in Table 1. The 75-bp promoter $P_{D/E20}$ of bacteriophage T5 (GenBank accession no. M11599) and the 80-bp sequence of the *rho*-independent transcription terminator (T_{lsrA}) from the levansucrase gene of *Rahnella aquatilis* (GenBank accession no. AAC36458) were synthesized by GenoTech (Daejeon, Korea). These DNA sequences were cloned into the pUC118 vector to generate pUC-$P_{D/E20}$-T_{lsrA}. The open reading frames (*orfs*) of the following genes were amplified from chromosomal DNA of *K. pneumoniae* MGH78578 using the primers shown in Table 1: $dhaB_\alpha$ (1.67 kb, GenBank accession no. ABR78884), $dhaB_\beta$ (0.59 kb, ABR78883), and $dhaB\gamma$ (0.43 kb, ABR78882) encoding glycerol dehydratase subunits (α, β and γ); $dhaB_{RA1}$ (1.82 kb, ABR78881) and $dhaB_{RA2}$ (0.35 kb, ABR78887) encoding glycerol dehydratase reactivators (1 and 2); and $dhaT$ (1.16 kb, ABR78886) and $yqhD_K$ (1.16 kb, ABR78827), encoding 1,3-PD oxidoreductase and an isozyme thereof [20], respectively. Amplified DNA fragments were cloned into the pGEM T-Easy (Promega, Madison, WI) vector followed by nucleotide sequencing to confirm the absence of any errors. The *Psh*BI and *Nde*I–*Sal*I fragments including $dhaB_{\beta\gamma}$ (pGEM-$dhaB_{\beta\gamma}$) and $dhaB_{RA1}$ were serially inserted into the *Nde*I and *Sac*I sites of pGEM-$dhaB_\alpha$ to create pGEM-*dhaB*. The pGEM-*dhaB*, -*dhaT*, -*yqhDk,* and -$dhaB_{RA2}$ plasmids were treated with *Eco*RI, *Nde*I–*Xho*I, *Bgl*II–*Xho*I, and *Nde*I–*Xho*I, respectively. After gap filling using the Klenow fragment, each DNA molecule was inserted into the *Eco*RV site of pUC-$P_{D/E20}$-T_{lsrA}. To generate pConBT, the *Not*I and *Spe*I-*Xba*I fragments, including *dhaB* (pUC-*dhaB*), $dhaB_{RA2}$ (pUC-$dhaB_{RA2}$), and *dhaT* (pUC-*dhaT*), were serially inserted into the *Not*I and *Xba*I sites of pBR322, respectively. Plasmids pConB, pConT, pConK, and pConTK were similarly constructed, as shown in Fig. 1. The resultant plasmids were transformed into *K. pneumoniae* AK by electroporation [19].

Fermentation by recombinant *K. pneumoniae* strains

Seed cells for fermentation were prepared in a 250-ml flask containing 50 ml of LB medium. Flasks were incubated at 37 °C for 12 h and the cultures subsequently inoculated into the fermentor at 2.5 % (v/v). Both batch and fed-batch fermentations were conducted at 37 °C, with stirring at

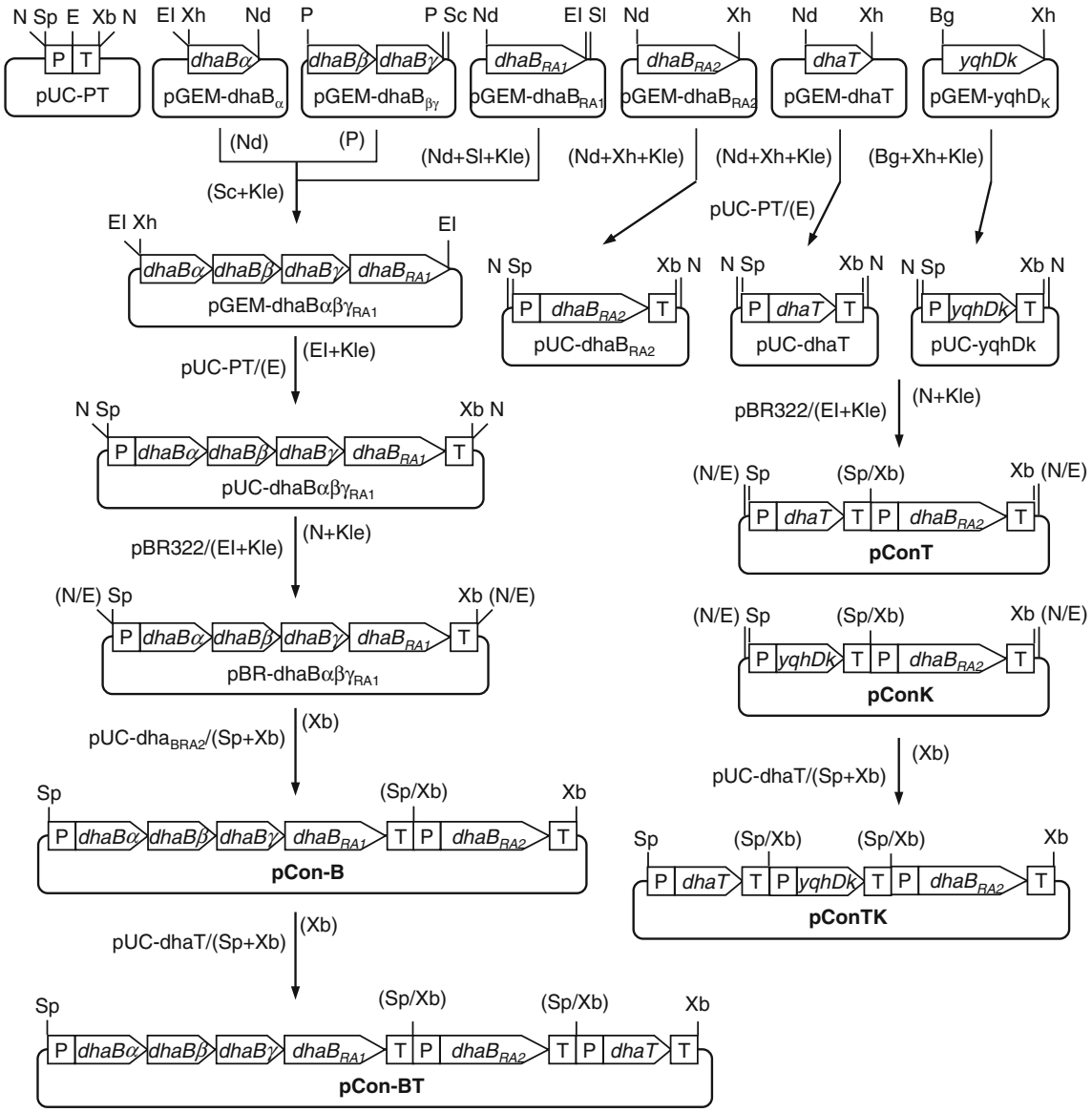

Fig. 1 Schematic representation of the strategies used for construction of pConT, pConB, pConBT, pConK, and pConTK. P and T indicate the 75 bp of promoter $P_{D/E20}$ of bacteriophage T5 and the 80 bp of the *rho*-independent transcription terminator (T_{lsrA}) of the levansucrase gene of *Rahnella aquatilis*, respectively. Abbreviations for restriction enzymes: Bg, *Bgl*II; E, *Eco*RV; EI, *Eco*RI; N, *Not*I; Nd, *Nde*I; P, *Psh*BI; Sc, *Sac*I; Sl, *Sal*I; Sp, *Spe*I; Xb, *Xba*I; Xh, *Xho*I. Kle, Klenow fragment

200 rpm and aeration at 0.5 vvm in a 5-l vessel (Kobiotech. Co., Ltd, Korea) containing 2 l of fermentation medium with tetracycline (10 μg/ml). pH was controlled by automatic addition of 28 % (v/v) NH_4OH. The carbon sources used were pure glycerol (purity 99 %, w/w) and crude glycerol (purity 70 %, w/w) derived from the biodiesel industry (ECO Solutions, Korea).

Enzyme activity assay

Cells were harvested by centrifugation at 6,000*g* for 15 min, and washed twice with 50 mM Tris buffer, pH 8.0. The cell pellet was resuspended in a small amount of buffer and cells were disrupted by ultrasonication for 5 min on ice. Cell debris was removed by brief centrifugation. Glycerol dehydratase (DhaB) activity was estimated using the 3-methyl-2-benzothiazolinon hydrazine (MBTH) method [16]. The activity of 1,3-PD oxidoreductase (DhaT or YqhD) was determined using the reverse reaction (conversion of 1,3-propanediol to 3-hydroxypropionaldehyde) according to the technique of Sulzenbacher et al. [17]. One unit of enzyme activity was defined as the amount of enzyme consuming 1 μmol substrate per minute. Protein levels were determined using a protein assay kit

Table 1 Oligonucleotides used in the present study

DNA (kb)	Primer	Oligonucleotide (5′ → 3′)	Description
dhaB$_{\alpha}$ (1.67)	F	CTCGAG*ATG*AAAAGATCAAAACGATTTGCAGTACTG	*Nde*I-*start codon*
	R	CATATG*TTA*TTCAATGGTGTCAGGCTGAACCACGCC	*Xho*I-*stop codon*
dhaB$_{\beta\gamma}$ (1.02)	F	ATTAAT*GTG*CAACAGACAACTCAAATTCAGCCCTCT	*Psh*BI-*start codon*
	R	ATTAAT*TTA*GCTTCCTTTACGCAGCTTATGCCGCTG	*Xho*I-*stop codon*
dhaB$_{RA1}$ (1.82)	F	CATA*TG*CCGTTAATAGCCGGGATTGATATCGGC	*Nde*I-*start codon*
	R	GTCGAC**GAATTC***TTA*ATTCGCCTGACCGGCCAGTAGCAGCCC	*Sal*I-***Eco*RI**-*stop codon*
dhaB$_{RA2}$ (0.35)	F	CATA*TG*TCGCTTTCACCGCCAGGCGTACGCCTG	*Nde*I-*start codon*
	R	CTCGAG*TCA*AGCGCAAGCATCAGG	*Sal*I-*stop codon*
dhaT (1.16)	F	CATA*TG*AGCTATCGTATGTTTGATTATCTGGTG	*Nde*I-*start codon*
	R	CTCGAG*TCA*GAATGCCTGGCGGAAAATCGCGGCAAT	*Xho*I-*stop codon*
yqhDk (1.16)	F	AGATCT*ATG*AATAATTTCGACCTGCA	*Nde*I-*start codon*
	R	CTCGAG*TTA*GCGTGCAGCCTCGTAAAT	*Xho*I-*stop codon*

(Bio-Rad), with bovine serum albumin (Sigma, St Louis, MO) as standard, according to the method of Bradford. Data are expressed as mean values ± SDs. Each experiment was performed three times, and the results showed no statistically significant difference, as determined using the Student's *t* test.

Metabolite analysis

Culture broth concentrations of metabolites including glycerol, acetate, and 1,3-PD were determined using a high-performance liquid chromatography system (Agilent 1200) equipped with a refractive index detector and an organic acid analysis column (300 × 78 mm; Aminex HPX-87H; Bio-Rad) [18]. The mobile phase was 0.005 mol/l H_2SO_4 and a flow rate of 0.8 ml per minute was maintained during elution. The column temperature was maintained at 65 °C.

Results and discussion

IPTG induction is essential for 1,3-PD production by *K. pneumoniae* AK/pVOT

In the recombinant *K. pneumoniae* AK/pVOT strain, the expression of both *dhaB* on chromosomal DNA and *dhaT* on plasmid DNA was under the control of the *E. coli lacZ* gene promoter [19], which allowed gene expression to be regulated by addition of an inducer such as isopropyl-D-thiogalactoside (IPTG). During cultivation of *K. pneumoniae* AK/pVOT, addition of IPTG (0.5 mM) induced 1,3-PD production from glycerol without by-product formation. Although IPTG is an efficient inducer of the *lacZ* promoter, development of an industrial 1,3-PD production process might be compromised by the high price of this chemical. We examined the effect of IPTG on glycerol metabolism by *K. pneumoniae* AK/pVOT upon cultivation in a 5-l bioreactor. The glycerol consumption rates by the recombinant strain were similar in both the absence and presence of IPTG (Fig. 2a), but cell growth was reduced in the presence of IPTG (Fig. 2b), probably because the chemical has some intrinsic toxicity. However, 1,3-PD production was enhanced in the presence of IPTG (Fig. 2c), indicating that IPTG induction is necessary for efficient production of 1,3-PD from glycerol by *K. pneumoniae* AK/pVOT. It is well known that lactose can usually substitute for IPTG as an inducer of the *lacZ* promoter. We thus examined whether lactose could replace IPTG during fermentative cultivation of *K. pneumoniae* AK/pVOT. However, as shown in Fig. 2, lactose negatively affected all of glycerol consumption rate, cell growth, and 1,3-PD production, to extents greater than those seen in the absence of IPTG.

Construction of recombinant *K. pneumoniae* AK strains constitutively expressing *dha* genes

We constructed a series of recombinant strains expressing *dha* genes in a constitutive manner, using the constitutive P_{DE20} gene promoter of bacteriophage T5 and an efficient *rho*-independent transcription termination signal from the *Rahnella aquatilis* levansucrase gene [21]. *K. pneumoniae* AK strains harboring pConB, pConT, or pConBT were prepared as described in Methods; these strains constitutively expressed *dhaB*, *dhaT*, or both the *dhaB* and *dhaT* genes, respectively. We also constructed *K. pneumoniae* AK strains harboring pConK or pConTK, thus with the *yqhD*$_k$ gene encoding an 1,3-PD oxidoreductase isozyme or with both *dhaT* and *yqhD*$_k$ (encoding two 1,3-PD

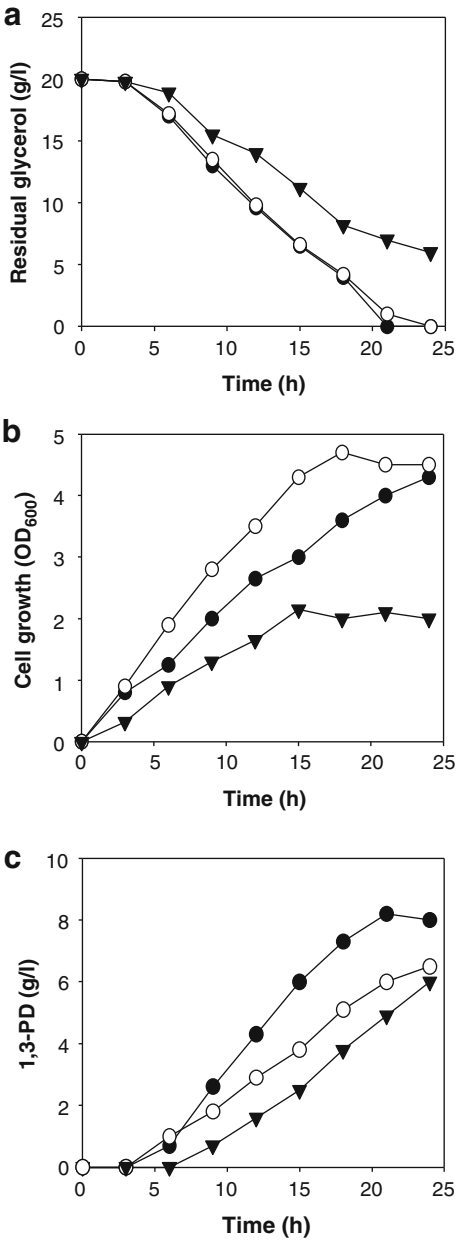

Fig. 2 Effects of IPTG induction on glycerol consumption rate (**a**), cell growth (**b**), and 1,3-PD production (**c**), of *K. pneumoniae* AK/pVOT under batch fermentation conditions. *Closed circles*, IPTG induction (0.5 mM); *open circles*, no IPTG induction; *closed triangles*, lactose induction (1 mM)

oxidoreductases), respectively. The activities of DhaB, DhaT, and YqhDk in the recombinant strains were as expected (Fig. 3).

Fermentation of glycerol by recombinant *K. pneumoniae* AK strains producing 1,3-PD

Upon batch fermentation of *K. pneumoniae* AK recombinant strains constitutively expressing *dhaB* (pConB), *dhaT*

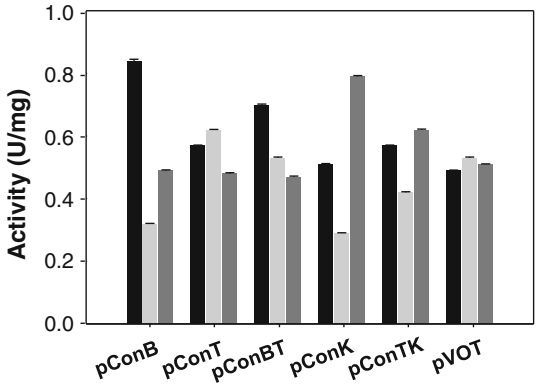

Fig. 3 Enzyme levels of DhaB (*closed bars*), DhaT (*gray bars*), and YqhD (*open bars*) in *K. pneumoniae* AK strains harboring recombinant plasmids pVOT (induced with IPTG at 0.5 mM), pConB, pConT, pConBT, pConK, or pConTK

(pConT), or both *dhaB* and *dhaT* (pConBT), in the absence of IPTG induction, the AK/pConT strain showed a faster glycerol consumption rate, a higher cell growth rate, and a greater 1,3-PD production level compared with the AK/pVOT strain with IPTG induction (Fig. 4). The AK/pConB and AK/pConBT strains showed the opposite effects, which may be attributable to an imbalance in metabolic flow, with accumulation of a toxic intermediate, 3-HPA, resulting from the increased activity of DhaB. Unexpectedly, high-level expression of the $yqhD_k$ gene encoding an 1,3-PD oxidoreductase isozyme negatively affected glycerol metabolism. This may be due to low DhaT activity, although it is unclear why DhaT activity should be reduced upon high-level expression of YqhDk. Glycerol metabolism was recovered by co-expression of *dhaT* and *yqhDk*. Thus, *K. pneumoniae* AK harboring pConT constitutively expressing *dhaT* alone produced the highest level of 1,3-PD from glycerol.

Fed-batch fermentation for 1,3-PD production by *K. pneumoniae* AK/pConT using pure or crude glycerol as carbon source

We examined 1,3-PD production from glycerol by *K. pneumoniae* AK/pConT, using fed-batch fermentation. The maximum levels of 1,3-PD production and productivity from pure glycerol were 26.6 g/l and 0.51 g/l/h in the absence of IPTG induction (Fig. 5a). Similar values (25.9 g/l and 0.54 g/l/h) were obtained using crude glycerol derived from the biodiesel industry as a carbon source (Fig. 5b). Although the maximum levels of 1,3-PD synthesized by AK/pConT were lower than those reported elsewhere [22, 23], the recombinant strain has one crucial advantage, namely, that no by-product, especially 2,3-PD, was formed by the AK/pConT strain, with the exception of acetate, thus rendering downstream 1,3-PD purification economical.

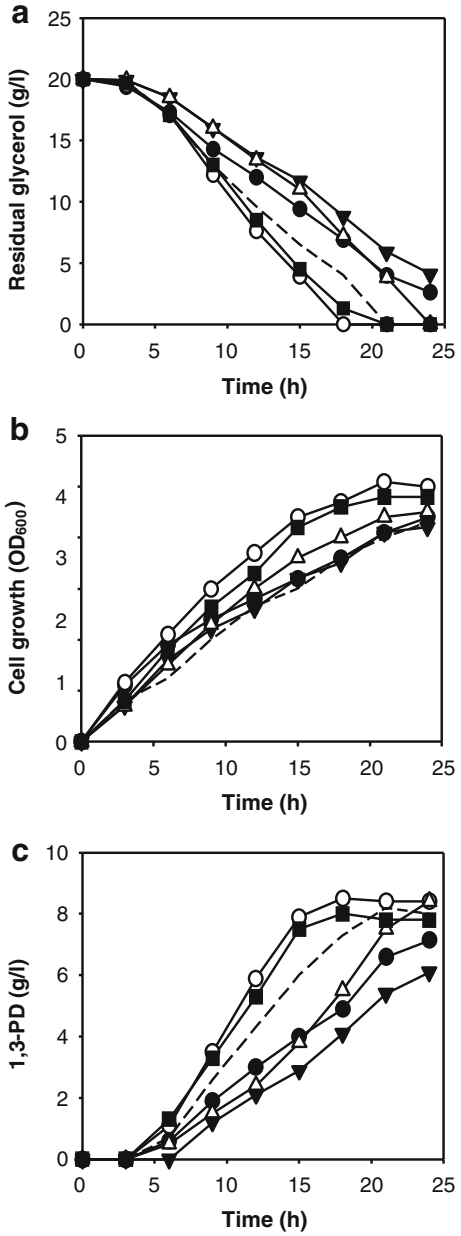

Fig. 4 Batch fermentation for 1,3-PD production by *K. pneumoniae* AK strains harboring recombinant plasmid pConB (*closed circles*), pConT (*open circles*), pConBT (*closed triangles*), pConK (*open triangles*), pConTK (*closed squares*), or pVOT (*dashed line*)

Genetic stability of the *K. pneumoniae* AK/pConT strain

Finally, we measured the stability of the plasmid expressing *dhaT* in *K. pneumoniae* AK/pConT in the absence of selective antibiotics. After culture for 56 h in the 5-l fermentor (thus, for about 150 generations), samples were collected and bacteria cultivated on LB plates. Next, 100 single colonies were replica plated to screen for tetracycline-resistant transformants. The plasmid was maintained in 93 % of cells of the recombinant strain, indicating that pConT is stable in *K. pneumoniae* AK.

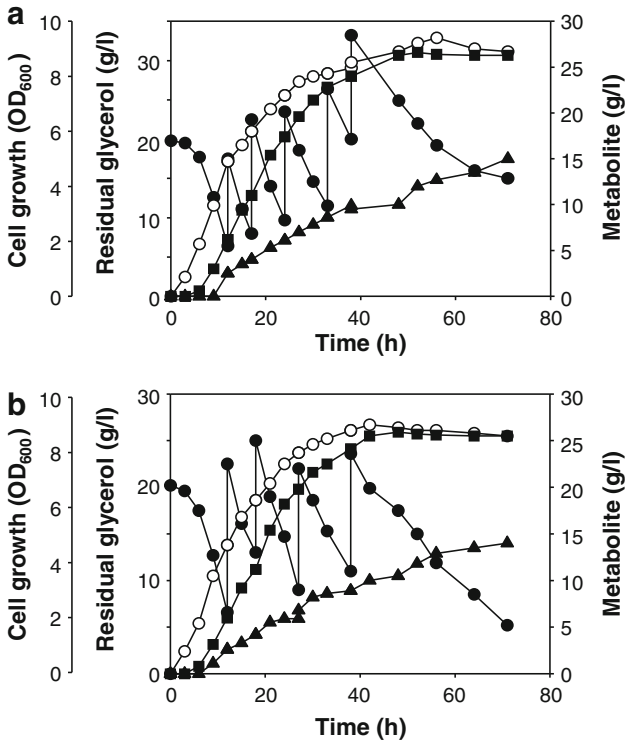

Fig. 5 Fed-batch fermentation for 1,3-PD production by *K. pneumoniae* AK strains harboring pConT, using pure (**a**) and crude (**b**) glycerol. Residual glycerol, *closed circles*; cell growth, *open circles*; 1,3-PD levels, *closed squares*; acetate levels, *closed triangles*

Conclusion

In the present study, we developed a series of recombinant *K. pneumoniae* AK strains engineered to eliminate by-product formation, in which the *dhaB* and/or *dhaT* genes encoding enzymes catalyzing the synthesis of 1,3-PD were constitutively expressed. Among these strains, AK/pConT expressing *dhaT* alone yielded the highest level of 1,3-PD. Fed-batch fermentation resulted in efficient production of 1,3-PD from either pure or crude glycerol, without by-product formation. Although the current level of 1,3-PD production by the recombinant strain is lower than those reported elsewhere, further engineering to enhance 1,3-PD yield will likely result in an economical biological process for production of this valuable material.

Acknowledgments This subject was supported by Korea Ministry of Environment as "Converging technology project" and by the New & Renewable Energy Technology Development Program of the Korea Institute of Energy Technology Evaluation and Panning (KETEP) Grant (2010T100100690) funded by the Korea government Ministry of Knowledge Economy.

References

1. Nakamura CE, Whited GM (2003) Metabolic engineering for the microbial production of 1,3-propanediol. Curr Opin Biotechnol 14:454–459
2. Biebl H, Menzel K, Zeng AP, Deckwer WD (1999) Microbial production of 1,3-propanediol. Appl Microbiol Biotechnol 52:289–297
3. Saxena RK, Anand P, Saran S, Isar J (2009) Microbial production of 1,3-propanediol: recent developments and emerging opportunities. Biotechnol Adv 27:895–913
4. Homann T, Tag C, Biebl H, Deckwer WD, Schink B (1990) Fermentation of glycerol to 1,3-propanediol by Klebsiella and Citrobacter strains. Appl Microbiol Biotechnol 33:121–126
5. Daniel R, Stuertz K, Gottschalk G (1995) Biochemical and molecular characterization of the oxidative branch of glycerol utilization by *Citrobacter freundii*. J Bacteriol 177:392–401
6. El-Ziney MG, Debevere JM (1998) The effect of reuterin on *Listeria monocytogenes* and *Escherichia coli* O157:h7 in milk and cottage cheese. J Food Prot 61:1275–1280
7. Marçal D, Rêgo AT, Carrondo MA, Enguita FJ (2009) 1,3-Propanediol dehydrogenase from *Klebsiella pneumoniae*: decameric quaternary structure and possible subunit cooperativity. J Bacteriol 191:1143–1151
8. Bhatia SK, Kurian JV (2008) Biological characterization of sorona polymer from corn-derived 1,3-propanediol. Biotechnol Lett 30:619–623
9. Ma J, Rao Z, Xu L, Liao X, Fang H, Zhuge B, Zhuge J (2009) Expression of dha operon required for 1,3-PD formation in *Escherichia coli* and *Saccharomyces cerevisiae*. Curr Microbiol 60:191–198
10. Gellissen G (2000) Heterologous protein production in methylotrophic yeasts. Appl Microbiol Biotechnol 54:741–750
11. Sohn JH (1997) Characterization of telomere-associated ARSs (autonomously replicating sequences) and their use for multiple gene integration in *Hansenula polymorpha*. PhD thesis. Korea Advanced Institute of Science and Technology, Taejon, Korea
12. Heo JH, Hong WK, Cho EY, Kim MW, Kim JY, Kim CH, Rhee SK, Kang HA (2003) Properties of the *Hansenula polymorpha*-derived constitutive *GAP* promoter, assessed using an HSA reporter gene. FEMS Yeast Res 4:175–184
13. Hill J, Donald KA, Griffiths DE (1991) DMSO-enhanced whole cell yeast transformation. Nucleic Acids Res 9:5791
14. Hong WK, Kim CH, Heo SY, Luo LH, Oh BR, Seo JW (2010) Enhanced production of ethanol from glycerol by engineered *Hansenula polymorpha* expressing pyruvate decarboxylase and aldehyde dehydrogenase genes from *Zymomonas mobilis*. Biotechnol Lett 32:1077–1082
15. Gonzalez-Pajuelo M, Meynial-Salles I, Mendes F, Soucaille P, Vasconcelos I (2006) Microbial conversion of glycerol to 1,3-propanediol: physiological comparison of a natural producer, *Clostridium butyricum* VPI 3266, and an engineered strain, *Clostridium acetobutylicum* DG1(pSPD5). Appl Environ Microbiol 72:96–101
16. Olivier C, Aline LF (2001) Primers and a specific DNA probe for detecting lactic acid bacteria producing 3-hydroxypropionaldehyde from glycerol in spoiled ciders. J Food Prot 64:833–837
17. Sulzenbacher G, Alvarez K, Van Den Heuvel RH, Versluis C, Spinelli S, Campanacci V, Valencia C, Cambillau C, Eklund H, Tegoni M (2004) Crystal structure of *E. coli* alcohol dehydrogenase YqhD: evidence of a covalently modified NADP coenzyme. J Mol Biol 342:489–502
18. Oh BR, Seo JW, Choi MH, Kim CH (2008) Optimization of culture conditions for 1,3-propanediol production from crude glycerol by *Klebsiella pneumoniae* using response surface methodology. Biotechnol Bioprocess Eng 13:524–532
19. Seo MY, Seo JW, Heo SY, Baek JO, Rairakhwada D, Oh BR, Seo PS, Choi MH, Kim CH (2009) Elimination of by-product formation during production of 1,3-propanediol in *Klebsiella pneumoniae* by inactivation of glycerol oxidative pathway. Appl Microbiol Biotechnol 84:527–534
20. Seo JW, Seo MY, Oh BR, Heo SY, Baek JO, Rairakhwada D, Luo LH, Hong WK, Kim CH (2010) Identification and utilization of a 1,3-propanediol oxidoreductase isoenzyme for production of 1,3-propanediol from glycerol in *Klebsiella pneumoniae*. Appl Microbiol Biotechnol 85:659–666
21. Seo JW, Hong WK, Rairakhwada R, Seo PS, Choi MH, Song KB, Rhee SK, Kim CH (2009) An efficient plasmid vector for constitutive high-level expression of foreign genes in *Escherichia coli*. Biotechnol Lett 31:877–881
22. Xu YZ, Guo NN, Zheng ZM, Ou XJ, Liu HJ, Liu DH (2009) Metabolism in 1,3-propanediol fed-batch fermentation by a D-lactate deficient mutant of *Klebsiella pneumoniae*. Biotechnol Bioeng 104:965–972
23. Zhuge B, Zhang C, Fang H, Zhuge J, Permaul K (2010) Expression of 1,3-propanediol oxidoreductase and its isoenzyme in *Klebsiella pneumoniae* for bioconversion of glycerol into 1,3-propanediol. Appl Microbiol Biotechnol 87:2177–2184