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The Role of the Pyruvate Acetyl-CoA Switch in the Production of 1,3-Propanediol by *Klebsiella pneumoniae*

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Abstract Pyruvate dehydrogenase-complex (AcoABCD) and pyruvate formate-lyase (PFL) are two pathways responsible for synthesis of acetyl-CoA from pyruvate (pyruvate acetyl-CoA switch). The two pathways were individually deleted in *Klebsiella pneumoniae*, and the role of the pyruvate acetyl-CoA switch in 1,3-propanediol production was investigated. Fermentation results showed that the two pathways were both active in the wild-type strain. Acetyl-CoA formation between the two pathways was equal in the wild-type strain. The pflB mutant produced high level of lactic acid, and deletion of ldhA eliminated lactic acid synthesis. The conversion ratio of glycerol to 1,3-propanediol in the pflB-ldhA mutant reached 0.541 g/g, which was 9.4 % higher than that of the wild-type strain. However, the productivity of 1,3-propanediol was decreased in the pflB-ldhA mutant. In contrast, the productivity of 1,3-

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propanediol was increased by 19 % in the acoABCD mutant, with the disadvantage of lower substrate conversion ratio. Regulating the pyruvate acetyl-CoA switch presents a novel way to improve the conversion ratio or productivity of 1,3-propanediol produced by *K. pneumoniae*.

Keywords *Klebsiella pneumoniae* · 1, 3-Propanediol · Pyruvate dehydrogenase complex · Pyruvate formate-lyase

Introduction

Klebsiella pneumoniae is an important resource for the biotechnology industry because it produces valuable chemicals such as 1,3-propanediol [22], 2,3-butanediol [3], acetoin [16], gluconic acid [15], 2-ketogluconic acid [19], and 3-hydroxypropionic acid [8]. 1,3-Propanediol is mainly used as a monomer to synthesize polytrimethylene terephthalate (PTT). PTT is a type of biodegradable polyester that exhibits better properties than those produced by 1,2-propanediol, butanediol, or ethylene glycol [20]. The researches of 1,3-propanediol production by *K. pneumoniae* were a hotspot in recent years, and this technology has been commercialized in China.

Pyruvate is a central intermediate metabolite, and many metabolic pathways diverge at this point. For example, two pathways synthesize acetyl-CoA from pyruvate. The former is synthesized by the pyruvate dehydrogenase complex (PDHc), and the latter is generated by pyruvate formate-lyase (PFL) (Fig. 1). The PDHc mediates the dissimilation of acetoin and is also called the acetoin dehydrogenase system (AcoABCD). This multienzyme complex comprises thiamine diphosphate-dependent acetoin dehydrogenase, dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase, which are encoded by *acoABCD* [16]. The AcoABCD pathway converts pyruvate to acetyl-CoA, which is accompanied by the release of CO_2 and the production of NADH. PFL encoded by *pflB* catalyzes the conversion of pyruvate to acetyl-CoA, which is accompanied by the formation of formic acid.

1,3-Propanediol is synthesized from glycerol (Fig. 1). In the reductive pathway, glycerol is first converted to a 3-hydroxypropionaldehyde, and reduced to form 1,3-propanediol. In a coupled oxidative pathway, glycerol is simultaneously oxidized to dihydroxyacetone, which is phosphorylated to dihydroxyacetone phosphate and channeled into glycolysis [17]. By-products of 1,3-propanediol production are succinic acid, lactic acid, acetic acid, and ethanol. 1,3-Propanediol synthesis from glycerol consumes one NADH, while lactic acid, acetic acid, and ethanol synthesis from glycerol are NADH-producing processes. NAD^+/NADH balance is a key factor for 1,3-propanediol production by *K. pneumoniae*. Previously, only PFL was considered responsible for the conversion of pyruvate to acetyl-CoA in 1,3-propanediol production. However, based on the NAD^+/NADH balance calculated from metabolites, Zeng et al. proposed the existence of further enzymes which participate in this reaction and produce NADH in this step, besides PFL pathway, such as pyruvate/ferredoxin oxidoreductase [23]. The AcoABCD was not recognized by Zeng et al. at that time.

Deletion of the gene encoding PFL from *K. pneumoniae* increases the yield of 2,3-butanediol [9]. We deleted the genes encoding the acetoin dehydrogenase system of

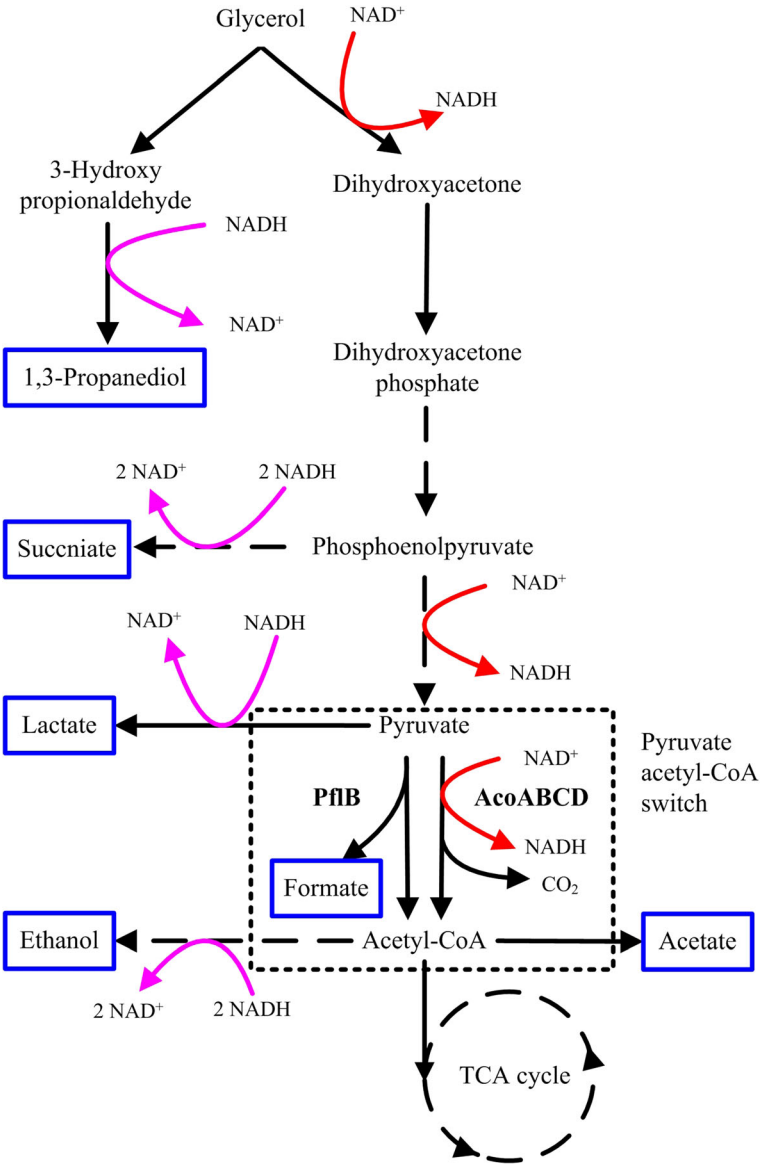

Fig. 1 Metabolic pathways of 1,3-propanediol synthesis and the pyruvate acetyl-CoA switch in *K. pneumoniae*

K. pneumoniae to eliminate the dissimilation of acetoin, which will prevent the cell to use acetoin as carbon source [16]. Because the two pathways that convert pyruvate to acetyl-CoA can be individually inactivated, we call them the pyruvate acetyl-CoA switch (Fig. 1). The pyruvate acetyl-CoA switch affects the net NADH production in acetic acid and ethanol synthesis processes and might affect the NAD^+/NADH balance of the cell. Therefore, the role of the pyruvate acetyl-CoA switch in 1,3-propanediol production was investigated here.

Materials and Methods

Strains, Plasmids, and Primers

Bacterial strains and plasmids are listed in Table 1. Primers used for PCR are listed in Table 2.

Construction of Mutants of *K. pneumoniae*

For mutant construction, *K. pneumoniae* and *E. coli* were grown in Luria–Bertani (LB) medium at 37 °C. The antibiotics used in selective medium were ampicillin (50 μg mL^{-1}), kanamycin (50 μg mL^{-1}), apramycin (50 μg mL^{-1}), and streptomycin (25 μg mL^{-1}).

K. pneumoniae ΔpflB was constructed according to a previously described method [18]. In brief, *pflB* was amplified from the genome of *K. pneumoniae* CGMCC 1.6366 using the primer pair pflB-s1/pflB-a1. The PCR product was then ligated into pMD18-T Simple to generate pMD18-T-*pflB*. Linear DNA with 39- and 40-bp homologous extensions flanking the apramycin resistance gene, *aac(3)IV*, were amplified from plasmid pIJ773 using the primer pair pflB-s2/pflB-a2. pMD18-T-*ΔpflB* was constructed by replacing part of *pflB* in plasmid pMD18-T-*pflB* with the *aac(3)IV* cassette using the Red recombinase system in *E. coli*. pMD18-T-*ΔpflB* was then used as a template for PCR preparation of linear DNA containing *aac(3)IV* with long homologous regions at either end. The primers used were pflB-s1/pflB-a1. Finally, the linear DNA was transformed into *K. pneumoniae* CGMCC 1.6366 which already host pDK6-red. Homologous recombination between linear DNA and the chromosome was mediated by Red recombinase. *K. pneumoniae ΔpflB* was isolated on apramycin plates, and the primer pair Test773 and pflB-s1 was used for PCR confirmation of the mutant.

pMD18-T-*ldhA* and pMD18-T-*ΔldhA* were constructed following the same method as pMD18-T-*pflB* and pMD18-T-*ΔpflB*. Excepted, pIJ773 was replaced by pIJ778, which hold the streptomycin resistance gene *aadA3*. *K. pneumoniae ΔpflB-ΔldhA* was constructed

Table 1 Strains and plasmids used in this work

Strain or plasmid	Relevant genotype and description	Reference or source
Strain		
E. coli DH5α	Host of plasmid	Lab stock
K. pneumoniae CGMCC 1.6366	Wild type	[6]
K. pneumoniae Δaco	*K. pneumoniae* CGMCC 1.6366, *ΔacoABCD*	[16]
K. pneumoniae ΔpflB	*K. pneumoniae* CGMCC 1.6366, *ΔpflB*	This work
K. pneumoniae ΔpflB-ldhA	*K. pneumoniae* CGMCC 1.6366, *ΔpflB*, *ΔldhA*	This work
Plasmid		
pDK6-red	Kan^r, carries λ-Red genes (gam, bet, exo)	[18]
pMD18-T-*ΔldhA*	Amp^r, carries *ΔldhA*	This work
pMD18-T-*ΔpflB*	Amp^r, carries *ΔpflB*	This work
pMD18-T-*ldhA*	Amp^r, carries *ldhA*	This work
pMD18-T-*pflB*	Amp^r, carries *pflB*	This work
pIJ773	$Apra^r$, *aac(3)IV* with FRT sites	[5]
pIJ778	Str^r, *aadA* with FRT sites	[5]

Table 2 Oligonucleotides used for PCR

Primer name	Sequence (5′-3′)
ldhA-s1	GTGTCGTTAATTATCTCGCTCATTGAGAGC
ldhA-a1	CTAATGCATCAGGTCGGCGAGCTTATAGAC
ldhA-s2	AACTTTGTCTTTCAAAGAGATTCCGCAAATATTCCGGGGATCCGTCGACC
ldhA-a2	AATCAGACGATAGCGTTCGGGCAGGTTTCATGTAGGCTGGAGCTGCTTC
pflB-s1	ATGTCCGAGCTTAATGAAAAGTTAGCCACA
pflB-a1	TTACATGGTCTGAGTGAAGGTACGGGTAAT
pflB-s2	CTGGTCTGCCGGATGCTTACGGCCGTGGTCATTCCGGGGATCCGTCGA CC
pflB-a2	GTGCAGTTTCTGAATTTTCTTCATGAAACGTGTAGGCTGGAGCTGCTTC
Test773	GCAAATACGGCATCAGTTACC

following the same method as *K. pneumoniae ΔpflB*, with *K. pneumoniae ΔpflB* replacing *K. pneumoniae* CGMCC 1.6366 as the target strain, and pMD18-T-*ΔldhA* replacing pMD18-T-*ΔpflB* used for linear DNA prepare. *K. pneumoniae ΔpflB-ΔldhA* was isolated on streptomycin plates.

Fermentation Media and Culture Conditions

Fermentation medium contained the following (g/L): glycerol, 30; $(NH_4)_2SO_4$, 4; $K_2HPO_4{\cdot}3H_2O$, 0.69; KH_2PO_4, 0.25; $MgSO_4$, 0.2; yeast extract, 1.5; $FeSO_4{\cdot}7H_2O$, 0.005; and 1 mL of trace element solution [17].

For the seed culture, 250-mL flasks containing 50 mL of LB medium were incubated in a rotary shaker at 37 °C and 200 rpm overnight. Seed culture was inoculated into a 5-L bioreactor (BIOSTAT-A plus Sartorius) with a working volume of 3 L. The culture pH was automatically controlled at 7. The air supplement and agitation were 2 L/min, 250 rpm. All the fermentation experiments were repeated in triplicate.

Analytical Methods

Biomass concentration was evaluated by determination of optical density (OD) at 600 nm with a NanoDrop-2000C spectrophotometer (Thermo-Scientific, Wilmington, DE). Chemical components in the broth were quantified by a Shimadzu 20AVP high performance liquid chromatograph system (Shimadzu Corp., Kyoto, Japan) with a RID-10A refractive index detector and a SPD-M20A photodiode array detector. The stationary and mobile phases were an Aminex HPX-87H column (300 mm × 7.8 mm) (Bio-Rad, USA) and a 0.005 mol/L H_2SO_4 solution at 0.8 mL/min flow rate.

Nucleotide extraction and assay were associated with the method described by Menzel et al. [11].

Calculations of Carbon Balance

Carbon balance was calculated based on the carbon and reducing equivalent recoveries that Zeng et al. reported [23], with some modifications. Consumed glycerol (molecular concentration) was set as 100, and all metabolites in the broth were transformed to a relative amount of

this substrate. CO_2 production was not measured in the process, and carbon conversion to CO_2, biomass, and other undetectable metabolites was set as lost mass. The lost mass was calculated as follows:

$$C_{lost} = C_{glycerol} - C_{1,3\text{-}propanediol} - C_{succinate} - C_{lactate} - C_{ethanol} - C_{acetate} - C_{TCA}$$

The amount of acetyl-CoA synthesized was defined as the sum of acetic acid and ethanol formed and the amount of acetyl-CoA transferred into the TCA cycle. We supposed that the ratio (K) of acetyl-CoA to TCA and to the sum of acetic acid and ethanol was stable in all the tested strains. Acetyl-CoA entering the TCA cycle was determined as the total amount of acetyl-CoA *minus* acetic acid and ethanol produced. Formic acid formed represents the acetyl-CoA synthesized through the PFL pathway. In the *acoABCD* mutant, acetyl-CoA synthesized through AcoABCD was eliminated, so the total acetyl-CoA synthesized equaled the formic acid produced. Acetyl-CoA synthesized from pyruvate through AcoABCD in other strains was determined from the acetic acid and ethanol and TCA amounts *minus* the amount of carbon pass through the PFL pathway (formic acid).

$$\begin{aligned} C_{Pfl} &= C_{formate} \\ C_{TCA} &= C_{Pfl} + C_{acoABCD} - C_{ethanol} - C_{acetate} \end{aligned}$$

In the AcoABCD mutant, $C_{acoABCD} = 0$

$$\begin{aligned} C_{TCA} &= C_{Pfl} - C_{ethanol} - C_{ethanol} \\ {C_{TCA}}/{(C_{ethanol} + C_{acetate})} &= K \end{aligned}$$

In other strains,

$$\begin{aligned} C_{acoABCD} &= C_{TCA} + C_{ethanol} + C_{acetate} - C_{Pfl} \\ C_{acoABCD} &= (1 + K) \times (C_{ethanol} + C_{acetate}) - C_{formate} \end{aligned}$$

Results

Effects of *pflB* Mutation on 1,3-Propanediol Production

The construction of the *K. pneumoniae* deletion mutants studied here was described in the "Materials and Methods" section. *K. pneumoniae ΔpflB* and the wild-type strain were cultured in a bioreactor to evaluate the effect of *pflB* mutation on 1,3-propanediol production. Fermentation processes are shown in Fig. 2.

Using glycerol as substrate, and cultured at micro-aerobic conditions, the main metabolite of *K. pneumoniae* is 1,3-propanediol. *K. pneumoniae* CGMCC 1.6366 consumed 28.9 g/L glycerol and produced 14.2 g/L 1,3-propanediol in 11.5 h. Beside 1,3-propanediol, 1.0, 2.2, 0.8, 4.4, and 0.3 g/L of lactic acid, formic acid, succinic acid, acetic acid, and ethanol were produced, respectively. The rate of glycerol consumption by *K. pneumoniae ΔpflB* was similar to that of the wild-type stain, and the former produced 14.6 g/L of 1,3-propanediol. The concentrations of formic acid, lactic acid, succinic acid, acetic acid, and ethanol produced were 1.2, 3.0, 0.7, 3.5, and 0.1 g/L, respectively. The productivities of 1,3-propanediol and all by-products (exclude lactic acid) of the *pflB* mutant were slower than that of the wild-type strain (Table 3, Fig. 2).

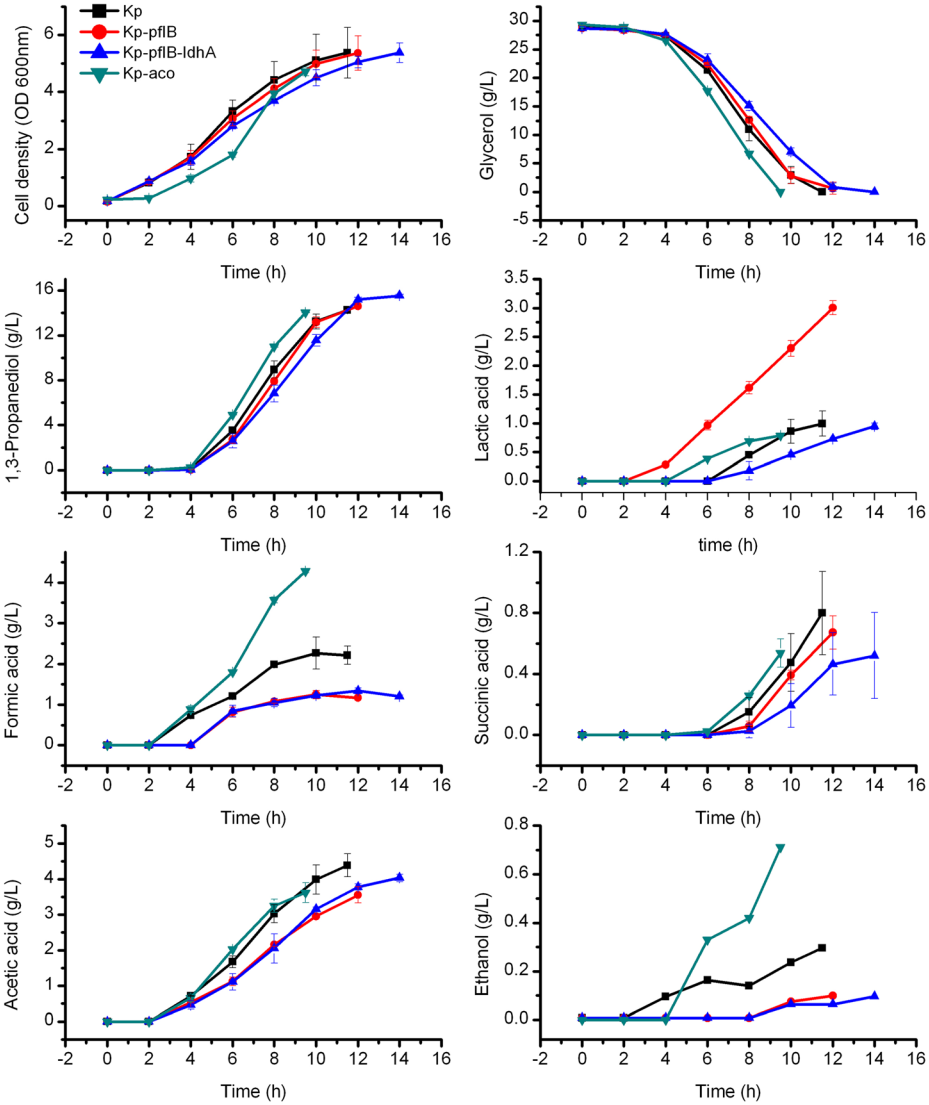

Fig. 2 Effects of the pyruvate acetyl-CoA switch on 1,3-propanediol production. 1,3-Propanediol production by *K. pneumoniae* CGMCC 1.6366 (*filled square*), *K. pneumoniae ΔpflB* (*filled circle*), *K. pneumoniae ΔpflB-ΔldhA* (*filled triangle*), and *K. pneumoniae Δaco* (*filled inverted triangle*)

Effects of *pflB* and *ldhA* Double Mutation on 1,3-Propanediol Production

Because lactic acid was the only by-product with elevated level at *K. pneumoniae ΔpflB*, the *pflB-ldhA* mutant was constructed to eliminate lactic acid synthesis. Fermentation results are shown in Fig. 2.

Lactic acid production decreased to 0.9 g/L in cultures of *K. pneumoniae ΔpflB-ΔldhA*, although there was no change in formic acid, acetic acid, and ethanol formation compared with *K. pneumoniae ΔpflB*. *K. pneumoniae ΔpflB-ΔldhA* produced a final concentration of 1,3-propanediol of 15.5 g/L, the conversion ratio of glycerol to 1,3-propanediol reached 0.541 g/g,

Table 3 Effects of different states of pyruvate acetyl-CoA switch on 1,3-propanediol-producing strain

	K. pneumoniae CGMCC 1.6366	*K. pneumoniae ΔpflB*	*K. pneumoniae ΔpflB-ΔldhA*	*K. pneumoniae Δaco*
Conversion ratio (g/g)	0.495 ± 0.008	0.509 ± 0.007	0.541 ± 0.004	0.478 ± 0.001
Productivity (g/Lh)	1.24 ± 0.11	1.22 ± 0.10	1.11 ± 0.08	1.47 ± 0.15

which is 9.4 % higher than the wild-type strain; however, 1,3-propanediol productivity decreased to 90 % of that in the wild-type strain (Table 3).

Effects of *acoABCD* Mutation on 1,3-Propanediol Production

AcoABCD encoding the pyruvate dehydrogenase complex, and it is another pathway responsible for conversion of pyruvate to acetyl-CoA. *K. pneumoniae Δaco* was cultured in a bioreactor to evaluate the effect of *acoABCD* mutation on 1,3-propanediol production. Fermentation processes are shown in Fig. 2.

Unlike the *pflB-ldhA* and *pflB* mutants, *K. pneumoniae Δaco* consumed glycerol at a high rate and produced 14.0 g/L of 1,3-propanediol. The productivity of 1,3-propanediol reached 1.47 g/Lh, which is 1.19 times of the wild-type strain; however, the conversion ratio of glycerol to 1,3-propanediol decreased to 0.478 g/g (Table 3). High levels of formic acid and ethanol were produced by *K. pneumoniae Δaco* compared with the wild-type strain.

Effects of Acetyl-CoA Switch on Redox Ratios in Cells

Nicotinamide nucleotides were measured in the fermentation process of the wild-type strain and the three mutants. Figure 3 shows the observed redox ratios.

The redox ratios were not stable during the culture process. The values for all four strains were around 1. Among the strains, the *pflB-ldhA* mutant had the highest redox ratio at 6 and 8 h of culture and the lowest at 10 h of culture.

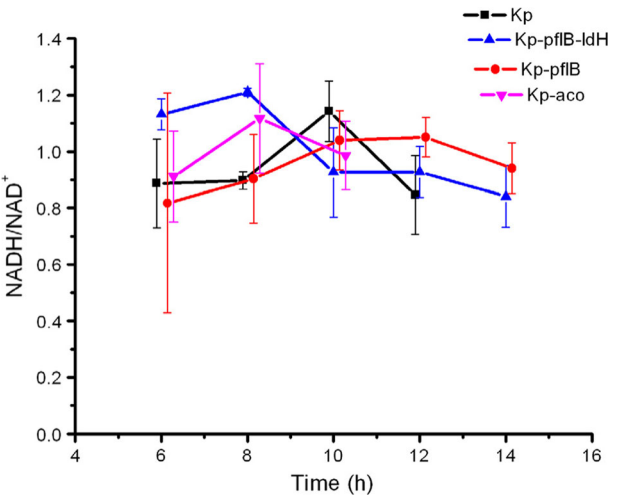

Fig. 3 Redox ratios in 1,3-propanediol production. *K. pneumoniae* CGMCC 1.6366 (*filled square*), *K. pneumoniae ΔpflB* (*filled circle*), *K. pneumoniae ΔpflB-ΔldhA* (*filled triangle*), and *K. pneumoniae Δaco* (*filled inverted triangle*)

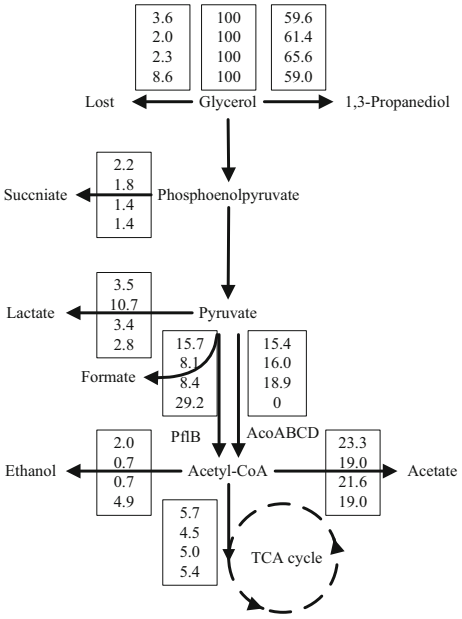

Fig. 4 Carbon balance at the pyruvate acetyl-CoA switch in 1,3-propanediol production. Values are for *K. pneumoniae* CGMCC 1.6366 (*the top line in each box*), *K. pneumoniae ΔpflB* (*the second line in each box*), *K. pneumoniae ΔpflB-ΔldhA* (*the third line in each box*), and *K. pneumoniae Δaco* (*the bottom line in each box*)

Carbon Distribution at the Pyruvate Acetyl-CoA Switch in 1,3-Propanediol Production

Based on the concentrations of metabolites in the fermentation broth, the carbon balance at the pyruvate acetyl-CoA switch is shown in Fig. 4.

Figure 4 shows that the carbon distribution between the PFL and AcoABCD pathways was equal in the wild-type strain. *pflB* and *acoABCD* mutation reduced the carbon distribution to the corresponding pathways. The reduced mass of lactic acid in the *ldhA* mutant coincided with an increased mass of 1,3-propanediol. The amount of 1,3-propanediol produced by the *pflB-ldhA* mutant was 87 % of the theoretical largest yield (based on the NAD^+/NADH balance, if all mass in the oxidation branch was transferred to acetic acid, the largest yield of 1,3-propanediol would be 0.75 mol/mol). The *acoABCD* mutant had the highest lost mass.

Discussion

In this study, *K. pneumoniae Δaco* and *K. pneumoniae ΔpflB* were engineered to use the PFL or the AcoABCD component of the acetyl-CoA switch, respectively.

The cleavage of pyruvate is a major branch point that determines in vivo fluxes of carbon in *E. coli.* Pyruvate entry into the fermentative pathway depends largely on the activity of PFL, whereas entry into the respiratory pathway is largely governed by the activity of PDHc (AcoABCD) [1]. Unlike in *E. coli*, carbon balance analysis results showed that the two pathways of the pyruvate acetyl-CoA switch were both active in wild-type *K. pneumoniae* in 1,3-propanediol production. *K. pneumoniae* was cultured in microaerobic conditions for 1,3-propanediol production; this choice of conditions might be one of the reasons that both pathways were active.

When acetyl-CoA formation was switched to the AcoABCD (*K. pneumoniae ΔpflB*), lactic acid accumulated to a high level. Other bacterial species employ a similar mechanism to regulate the distribution of pyruvate for its conversion to acetyl-CoA and lactate. A pyruvate formate-lyase mutant of *Klebsiella planticola* accumulates lactic acid at a level sixfold higher than that of the wild-type strain [4]. In *E. coli* MG1655, most pyruvate is converted to lactate by the *pflB* mutant, and the cells' growth of this mutant is decreased compared with the wild-type strain, the slower growth rate may be attributed to a lower ATP pool [14]. In another way, an increase in the activity of pyruvate formate-lyase in *Lactococcus lactis* decreases lactic acid production [10].

In the present study, we show that deletion of the gene encoding lactate dehydrogenase from *K. pneumoniae* (*pflB-ldhA* mutant) led to decreased production of lactic acid. A low level of lactic acid was still produced by the *ldhA* mutant because D-lactic acid can be synthesized through the methylglyoxal pathway, and L-lactic acid can be synthesized from pyruvate [2]. The reduced glycerol normally used for lactic acid production was all used for 1,3-propanediol synthesis in the *ldhA* mutant. Because, the *ldhA* mutation alone increased the conversion ratio of glycerol to 1,3-propanediol. *K. pneumoniae* HR526 is a high 1,3-propanediol-producing strain; the conversion was increased from 0.48 to 0.52 mol/mol by the mutation of *ldhA*. This change in the conversion ratio is the outcome of excessive NADH being available for 1,3-propanediol synthesis [21].

K. pneumoniae pflB-ldhA efficiently increased the substrate conversion ratios of glycerol to 1,3-propanediol; however, the productivity was decreased. It has been reported that the growth defect of *E. coli pflB-ldhA* is caused by ineffective regeneration of NAD^+, rather than acetyl-CoA formation [13]. Similarly, it has been reported that growth retardation of *K. pneumoniae ΔpflB-ΔldhA* in 2,3-butanediol production was caused by redox imbalance [9]. However, these two reports used glucose as substrate, and the redox ratios for 1,3-propanediol production in this study show different results. In the early stage of the exponential growth phase, the redox ratio of *K. pneumoniae ΔpflB-ΔldhA* was higher than that in the wild-type strain, but this was not the case during the whole growth. The *ldhA* mutant of *K. pneumoniae* HR526 has a lower NAD^+/NADH ratio compared with that of the wild-type strain, while this mutant showed a faster growth rate and higher 1,3-propanediol productivity than the wild-type strain [21]. The decrease in productivity on *pflB-ldhA* mutation might thus be caused by factors other than the redox ratio.

Acetyl-CoA formation switches to the PFL pathway in *K. pneumoniae Δaco*. Through this pathway, formic acid is produced along with acetyl-CoA. The distinct characteristic of the *acoABCD* mutant was that its productivity of 1,3-propanediol was increased. Cells seemed generally more metabolically active after *acoABCD* mutation; the productivities of all the by-products were also increased. Mass balance results show that the *acoABCD* mutant had the largest lost mass among the strains tested. In research into 2,3-butanediol production by *Klebsiella oxytoca*, the production rate of 2,3-butanediol was decreased in a *pflB–ldhA* mutant, but it was recovered by an increase in the air supplement. The fermentation results showed that the increased air supplement also resulted in an increase in the lost mass [12]. As the biomass produced was similar between the mutants and wild-type strain, the increased lost mass in the *acoABCD* mutant reflected an increase in CO_2 production. However, the carbon flux of acetyl-CoA into the TCA cycle was not increased in the *acoABCD* mutant. We suppose that more CO_2 was produced in the TCA cycle; however, glycerol went into the TCA cycle not through the pyruvate acetyl-CoA switch, but rather via pyruvate carboxylase or other pathways. In research into glutamate production by *Corynebacterium glutamicum*, a decrease in PDHc (AcoABCD) activity was accompanied with an increase in pyruvate carboxylase activity, which catalyzes the reaction of pyruvate to oxaloacetate [7]. There are many reports on the

mutation of *pflB* in different bacteria, but, few about *acoABCD* mutation. The mechanisms of the outcomes of *acoABCD* mutation require further investigation.

In summary, we show here that the carbon distribution at the points of pyruvate can be regulated by the pyruvate acetyl-CoA switch to a different state that affected the productivity and conversion ratio of glycerol to 1,3-propanediol. Introducing multiple mutations of *pflB* and *ldhA* may serve to increase the conversion ratio of glycerol to 1,3-propanediol, although the productivity is decreased. Alternatively, the *acoABCD* mutant increased the productivity of 1,3-propanediol with the disadvantage of a lower substrate conversion ratio. The two methods could be flexibly selected according to production goals.

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References

1. Alexeeva, S., de Kort, B., Sawers, G., Hellingwerf, K. J., & de Mattos, M. J. T. (2000). Effects of limited aeration and of the ArcAB system on intermediary pyruvate catabolism in *Escherichia coli*. *Journal of Bacteriology, 182*, 4934–4940.
2. Ashok, S., Raj, S. M., Rathnasingh, C., & Park, S. (2011). Development of recombinant *Klebsiella pneumoniae* ΔdhaT strain for the co-production of 3-hydroxypropionic acid and 1, 3-propanediol from glycerol. *Applied Microbiology and Biotechnology, 90*, 1253–1265.
3. Chen, C., Wei, D., Shi, J., Wang, M., & Hao, J. (2014). Mechanism of 2,3-butanediol stereoisomer formation in *Klebsiella pneumoniae*. *Applied Microbiology and Biotechnology, 98*, 4603–4613.
4. Feldmann, S., Sprenger, G. A., & Sahm, H. (1989). Ethanol production from xylose with a pyruvate-formate-lyase mutant of *Klebsiella planticola* carrying a pyruvate-decarboxylase gene from Zymomonas mobilis. *Applied Microbiology and Biotechnology, 31*, 152–157.
5. Gust, B., Challis, G. L., Fowler, K., Kieser, T., & Chater, K. F. (2003). PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proceedings of the National Academy of Sciences, 100*, 1541.
6. Hao, J., Lin, R., Zheng, Z., Liu, H., & Liu, D. (2008). Isolation and characterization of microorganisms able to produce 1, 3-propanediol under aerobic conditions. *World Journal of Microbiology and Biotechnology, 24*, 1731–1740.
7. Hasegawa, T., Hashimoto, K.-I., Kawasaki, H., & Nakamatsu, T. (2008). Changes in enzyme activities at the pyruvate node in glutamate-overproducing *Corynebacterium glutamicum*. *Journal of Bioscience and Bioengineering, 105*, 12–19.
8. Huang, Y., Li, Z., Shimizu, K., & Ye, Q. (2012). Simultaneous production of 3-hydroxypropionic acid and 1, 3-propanediol from glycerol by a recombinant strain of *Klebsiella pneumoniae*. *Bioresource Technology, 103*, 351–359.
9. Jung, M.-Y., Mazumdar, S., Shin, S. H., Yang, K.-S., Lee, J., & Oh, M.-K. (2014). Improvement of 2, 3-butanediol yield in *Klebsiella pneumoniae* by deletion of the pyruvate formate-lyase gene. *Applied and Environmental Microbiology, 80*, 6195–6203.
10. Melchiorsen, R. C., Jokumsen, V. K., Villadsen, J., Israelsen, H., & Arnau, J. (2002). The level of pyruvate-formate lyase controls the shift from homolactic to mixed-acid product formation in *Lactococcus lactis*. *Applied Microbiology and Biotechnology, 58*, 338–344.
11. Menzel, K., Ahrens, K., Zeng, A. P., & Deckwer, W. D. (1998). Kinetic, dynamic, and pathway studies of glycerol metabolism by *Klebsiella pneumoniae* in anaerobic continuous culture: IV. *Enzymes and fluxes of pyruvate metabolism. Biotechnol. Bioeng., 60*, 617–626.
12. Park, J. M., Song, H., Lee, H. J., & Seung, D. (2013). In silico aided metabolic engineering of *Klebsiella oxytoca* and fermentation optimization for enhanced 2, 3-butanediol production. *Journal of Industrial Microbiology & Biotechnology, 40*, 1057–1066.
13. Singh, A., Lynch, M. D., & Gill, R. T. (2009). Genes restoring redox balance in fermentation-deficient *E. coli* NZN111. *Metabolic Engineering, 11*, 347–354.
14. Utrilla, J., Gosset, G., & Martinez, A. (2009). ATP limitation in a pyruvate formate lyase mutant of *Escherichia coli* MG1655 increases glycolytic flux to D-lactate. *Journal of Industrial Microbiology & Biotechnology, 36*, 1057–1062.

15. Wang, D., Wang, C., Wei, D., Shi, J., Kim, C. H., Jiang, B., Han, Z., & Hao, J. (2016). Gluconic acid production by gad mutant of *Klebsiella pneumoniae*. *World Journal of Microbiology and Biotechnology, 32*, 132.
16. Wang, D., Zhou, J., Chen, C., Wei, D., Shi, J., Jiang, B., Liu, P., & Hao, J. (2015). R-acetoin accumulation and dissimilation in *Klebsiella pneumoniae*. *Journal of Industrial Microbiology & Biotechnology, 42*, 1–11.
17. Wei, D., Wang, M., Jiang, B., Shi, J., & Hao, J. (2014). Role of dihydroxyacetone kinases I and II in the dha regulon of *Klebsiella pneumoniae*. *Journal of Biotechnology, 177*, 13–19.
18. Wei, D., Wang, M., Shi, J., & Hao, J. (2012). Red recombinase assisted gene replacement in *Klebsiella pneumoniae*. *Journal of Industrial Microbiology & Biotechnology, 39*, 1219–1226.
19. Wei, D., Xu, J., Sun, J., Shi, J., & Hao, J. (2013). 2-Ketogluconic acid production by *Klebsiella pneumoniae* CGMCC 1.6366. *Journal of Industrial Microbiology & Biotechnology, 40*, 561–570.
20. Witt, U., Müller, R. J., Augusta, J., Widdecke, H., & Deckwer, W. D. (1994). Synthesis, properties and biodegradability of polyesters based on 1, 3-propanediol. *Macromolecular Chemistry and Physics, 195*, 793–802.
21. Xu, Y. Z., Guo, N. N., Zheng, Z. M., Ou, X. J., Liu, H. J., & Liu, D. H. (2009). Metabolism in 1, 3-propanediol fed-batch fermentation by a D-lactate deficient mutant of *Klebsiella pneumoniae*. *Biotechnology and Bioengineering, 104*, 965–972.
22. Zeng, A.-P., & Biebl, H. (2002). Bulk chemicals from biotechnology: the case of 1, 3-propanediol production and the new trends. *Advances in Biochemical Engineering/Biotechnology, 74*, 239–259.
23. Zeng, A. P., Menzel, K., & Deckwer, W. D. (1996). Kinetic, dynamic, and pathway studies of glycerol metabolism by *Klebsiella pneumoniae* in anaerobic continuous culture: II. *Analysis of metabolic rates and pathways under oscillation and steady-state conditions. Biotechnol. Bioeng., 52*, 561–571.