Inactivation of hydrogenase-3 leads to enhancement of 1,3-propanediol and 2,3-butanediol production by *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae can use glucose or glycerol as carbon sources to produce 1,3-propanediol or 2,3-butanediol, respectively. In the metabolism of *Klebsiella pneumoniae*, hydrogenase-3 is responsible for H_2 production from formic acid, but it is not directly related to the synthesis pathways for 1,3-propanediol and 2,3-butanediol. In the first part of this research, *hycEFG*, which encodes subunits of the enzyme hydrogenase-3, was knocked out, so *K. pneumoniae* Δ*hycEFG* lost the ability to produce H_2 during cultivation using glycerol as a carbon source. As a consequence, the concentration of 1,3-propanediol increased and the substrate (glycerol) conversion ratio reached 0.587 mol/mol. Then, *K. pneumoniae* Δ*ldhA*Δ*hycEFG* was constructed to erase lactic acid synthesis which led to the further increase of 1,3-propanediol concentration. A substrate (glycerol) conversion ratio of 0.628 mol/mol in batch conditions was achieved, which was higher compared to the wild type strain (0.545 mol/mol). Furthermore, since *adhE* encodes an alcohol dehydrogenase that catalyzes ethanol production from acetaldehyde, *K. pneumoniae* Δ*ldhA*Δ*adhE*Δ*hycEFG* was constructed to prevent ethanol production. Contrary to expectations, this did not lead to a further increase, but to a decrease in 1,3-propanediol production. In the second part of this research, glucose was used as the carbon source to produce 2,3-butanediol. Knocking out *hycEFG* had distinct positive effect on 2,3-butanediol production. Especially in *K. pneumoniae ΔldhAΔadhEΔhycEFG*, a substrate (glucose) conversion ratio of 0.730 mol/mol was reached, which is higher compared to wild type strain (0.504 mol/mol). This work suggests that the inactivation of hydrogenase-3 may have a global effect on the metabolic regulation of *K. pneumoniae*, leading to the improvement of the production of two industrially important bulk chemicals, 1,3-propanediol and 2,3-butanediol.

Key words: Hydrogen, 1,3-propanediol, 2,3-butanediol, *Klebsiella pneumoniae*, *hycEFG*

Introduction

1,3-Propanediol is a type of bulk chemical widely used as a solvent in the cosmetics industry, and particularly in polymer industries as a building block for the synthesis of polytrimethylene terephthalate (PTT) [1]. PTT has many advantages over its polyester cousin polyethylene terephthalate (PET) and polybutylene terephthalate (PBT). PTT has high resiliency, superior elastic recovery, high bulk, and soft handle, which makes it attractive for making carpets, textile fibers, and nonwovens [2]. PTT can replace PET in all applications, but the high price of 1,3-propanediol limits the competitiveness of PTT in the market. Therefore, producing 1,3-propanediol with high efficiency is a hot topic worldwide. 1,3-propanediol is a metabolite of many microorganisms cultured with glycerol as the feedstock, such as *Citrobacter freundii* [3], *Clostridium butyricum* [4], *Lactobacillus reuteri* [5], and *Klebsiella pneumoniae* [6]. Among them, *K. pneumoniae* is the best one, having the highest conversion ratios and highest titers. Since glycerol is a by-product of biodiesel production, it is available at low prices. In general, during biodiesel production 10% (w/w) of glycerol is produced [7]. The technology of 1,3-propanediol production using glycerol as the feedstock and *K. pneumoniae* as the producer has been already industrialized in China.

The pathway of 1,3-propanediol synthesis from glycerol is a dismutation process, which contains two branches [8]. In the oxidization branch, glycerol is converted to dihydroxyacetone which is catalyzed by glycerol dehydrogenase. Dihydroxyacetone is phosphorylated to phosphate dihydroxyacetone and flows into the glycolysis pathway. This reaction is catalyzed by two kinases [9]. In the reduction branch, glycerol is converted to 3-hydroxypropionaldehyde which is catalyzed by glycerol dehydratase. This enzyme contains three subunits and is a vitamin B12-dependent enzyme. 3-Hydroxypropionaldehyde is further reduced to 1,3-propanedioland catalyzed by 1,3-propanediol dehydrogenase [10]. During the conversion of glycerol to 1,3-propanediol, one molecule of NADH is consumed. NADH, on the other hand, is produced in the oxidation branch and in other catabolic pathways to maintain the balance of NAD^+ and NADH [6]. Glycerol dehydratases, 1,3-propanediol dehydrogenase, glycerol dehydrogenase, dihydroxyacetone kinase I and II are encoded by *dhaBCE*, *dhaT*, *dhaD*, *dhaK,* and *dhaK123*, respectively. These genes and a regulatory protein DhaR form a *dha* operon. This operon is induced by dihydroxyacetone (Figure 1) [8]. Besides the *dha* operon, the *pdu* regulon which is responsible for 1,2-propanediol catabolism was also involved in 1,3-propanediol synthesis from glycerol in *K. pneumoniae*. *pduCDE* encodes a diol dehydratase, which is an isoenzyme of the *dhaBCE* encoded glycerol dehydratase. Diol dehydratase and other enzymes of the *pdu* regulon are located in the Pdu microcompartment, a

bacteria organelle, rather than in the cytoplasm of the cell [11].

Many studies in metabolic engineering have focused on the production of 1,3-propanediol by *K. pneumoniae*. Lactic acid is a by-product of 1,3-propanediol production and is synthesized from pyruvate. *ldhA* encoded lactate dehydrogenase catalyzes this reaction. It has been proven that *ldhA* disrupted *K. pneumoniae* strain can lead to the increase of 1,3-propanediol concentration [12] 2,3-butanediol is also a by-product in the synthesis of 1,3-propanediol from glycerol, and the key enzyme in this process is decarboxylase encoded by *budA*. However, a *budA* disrupted *K. pneumoniae* was unable to synthesize 2,3-butanediol, and at the same time the concentration of 1,3-propanediol was also reduced [13].

2,3-butanediol is a main catabolite of *K. pneumoniae* during cultivation on glucose as the substrate. 2,3-butanediol is synthesized from pyruvate, in a way that two molecules of pyruvate form one molecule of acetolactate which is catalyzed by a *budB* encoded acetolactate synthetase. Acetolactate is further converted to acetoin, catalyzed by a *budA* encoded decarboxylase, while the acetoin is converted to 2,3-butanediol, catalyzed by butanediol dehydrogenase (Figure 1).

The conversion of pyruvate to acetyl-CoA is a central reaction of living organisms' metabolic pathways. *AcoABCD* concedes a pyruvate dehydrogenase. This enzyme catalyzes the conversion of pyruvate to acetyl-CoA and CO_2, with one molecule of NAD^+ converted to NADH. At the same time, a *pflB* encoded pyruvate formatelyase catalyzes pyruvate conversion to acetyl-CoA and formic acid, but in this reaction NADH is not generated. The two enzymic reactions are both active in the wild type strain of *K. pneumonia* (Figure1). In our previous research, a *pflB* knock out *K. pneumonia* strain was constructed, and the formation of acetyl-CoA from pyruvate must undergo the reaction catalyzed by pyruvate dehydrogenase. Thus, more NADH was generated in this strain than that of the wild type strain, and the 1,3-propanediol production of this strain was improved. However, small amounts of formic acid were still produced in the broth of this strain [14].

Hydrogen is an anaerobic metabolic product of many *Enterobacteria*. The mechanism of hydrogen production by bacteria is triggered by anaerobic conditions. Under aerobic conditions, the carbon sources are completely oxidized to CO_2. Electrons formed in the process are finally transferred to O_2 through the respiratory chain to form H_2O. Under anaerobic conditions, some catabolites accept electrons to form incomplete oxidative compounds, such as lactic acid, acetic acid, formic acid, ethanol, etc. However, the production of organic acids leads to a decrease in the pH of the environment, and too low a pH is lethal to bacteria. H_2 production is a mechanism used by some bacteria to reduce the drop in pH of the environment under anaerobic conditions. Normally, formic acid is produced by bacteria and released into

the environment. When the pH of the environment decreases, the formic acid is reabsorbed by the cell and converted to H_2 and CO_2 by catalysis of the formate hydrogenlyase complex [15]. There are four hydrogenases encoding genes in the genome of *E. coli*, and formate hydrogenlyase complex (hydrogenase-3) is responsible for nearly all H_2 production. The structure of this complex has been well-resolved. It is membrane-bounded and contains a formate dehydrogenase and a [NiFe] hydrogenase. Formate dehydrogenase is encoded by *fdhF*. Hydrogenase contains 6 subunits and they are encoded by *hycBCDEFG,* respectively. Formate dehydrogenase catalyzes the oxidation of formic acid to form CO_2 and proton. Two electrons are transferred to *hycE* and driving proton reduction to H_2 [16].

As a kind of clean energy, H_2 production by microorganisms especially by *Enterobacteria* has been a hotspot in recent years [17]. As the Thauer limit, scientists generally assume that a maximum of 4 moles of H_2 can be produced from one molecule of glucose. However, there are some reports that H_2 production has exceeded the Thauer limit [18]. These results are dependent on external electro-power input. The reaction of H_2 production from formic acid is reversible, and this reaction has been used for CO_2 fixation [19]. Although the efficiency of CO_2 fixation is low, this has been investigated a lot in recent years.

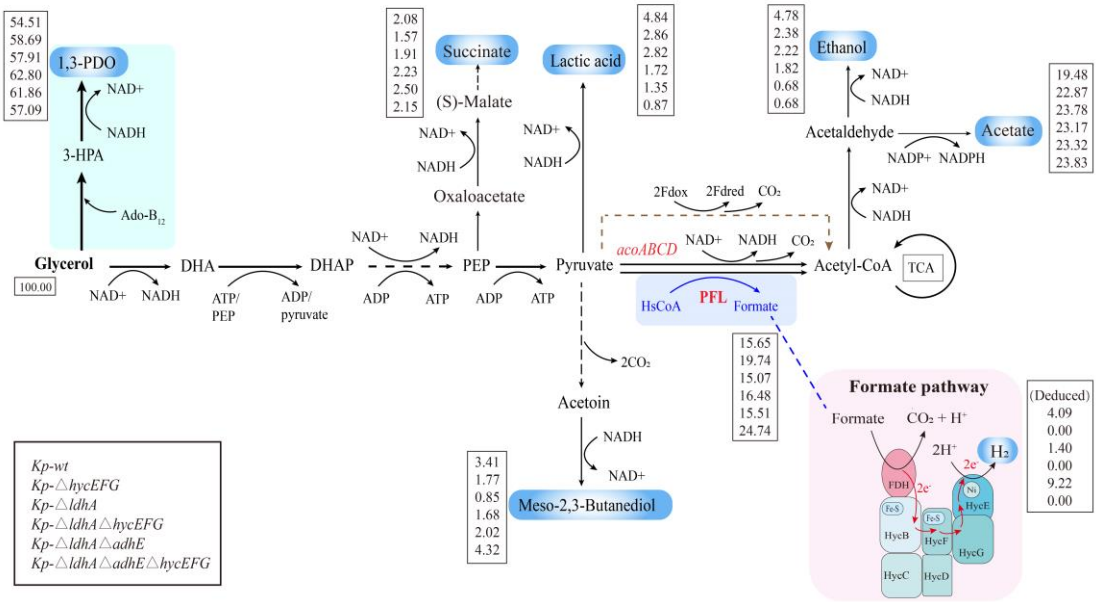

Figure 1. Metabolic pathways related to 1,3-propanediol synthesis and H_2 synthesis of *K. pneumoniae.* Values shown in the boxes are mole conversion ratios of glycerol to metabolites for wild type *K. pneumoniae* (the top line), *K. pneumoniae* Δ*hycEFG* (the second line), *K. pneumoniae* Δ*ldhA* (the third line), *K. pneumoniae* Δ*ldhA*Δ*hycEFG* (the fourth line), *K. pneumoniae* Δ*ldhA*Δ*adhE* (the fifth line), and *K. pneumoniae* Δ*ldhA*Δ*adhE*Δ*hycEFG* (the bottom line) under microaerobic condition.

Although there are some reports on H_2 production by *K. pneumonia* [20-23], there are no reports on

K. pneumonia losing its ability to synthesize H_2. In this work, the encoding genes of hydrogenase-3 in *K. pneumoniae* were knocked out, and their effects on the production of 1,3-propanediol and 2,3-butanediol, have been studied in detail. Results show that the inactivation of hydrogenase-3 has a distinct enhancement on 1,3-propanediol and 2,3-butanediol production by *K. pneumoniae*, even at microaerobic cultivation conditions.

Materials and methods

Strains, Plasmid and Primers

Bacterial strains and plasmids are listed in Table 1, while the primers used in this study are presented in Table 2.

Table 1 Bacterial strains and plasmids

Strains	Relevant genotype and description	Reference or source
K. pneumoniae strains		
CGMCC 1.6366	wild type	[6]
Δ*hycEFG*	Δ*hycEFG*, Apr^r	This work
Δ*ldhA*	Δ*ldhA*, Str^r	[13]
Δ*ldhA*Δ*hycEFG*	Δ*ldhA*, Δ*hycEFG*, Str^r, Apr^r	This work
Δ*ldhA*Δ*adhE*	Δ*ldhA*, Δ*adhE*	This work
Δ*ldhA*Δ*adhE*Δ*hycEFG*	Δ*ldhA*, Δ*adhE*, Δ*hycEFG* Apr^r	This work
E. coli DH5α	Host of plasmid	Lab stock
Plasmid		
pDK6	Kan^r, 5118 bp	[24]
pDK6-red	Kan^r, carries λ-Red genes (gam, bet, exo), 7003 bp	[25]
pDK6-flp	Kan^r, carries the yeast FLP recombinase gene, 6390 bp	[25]
pMD18-T	Amp^r, TA cloning vector, 2692 bp	Takara®
pMD18-T-Δ*hycEFG*	Apr^r, Amp^r, carries part of hycEFG, 5477 bp	This work
pIJ773	Apr^r, aac(3)IV with FRT sites	[26]
pIJ778	Str^r, aadA with FRT sites	[26]

Table 2 Primers

Primer name	Sequence (5′-3′)
hycEFG-up-F	TCATGGTGGGCGTGATGCTGA

hycEFG-up-R	GGTCGACGGATCCCCGGAATGCTCAAACTCTCTTAATCACGCCACC
hycEFG-down-F	CGAAGCAGCTCCAGCCTACAGCGAGACGGTGGTGTTCAGTC
hycEFG-down-R	GTTGAGCGGCATGTTATGGGT
adhE-up-F	CTTATTGCGCACTGCTGCCG
adhE -up-R	GGTCGACGGATCCCCGGAATCAAGCGCGTTCAGTTCAGCG
adhE -down-F	CGAAGCAGCTCCAGCCTACATCGACAAGCATAAACGTTCTGCCAG
adhE -down-R	GGGATGCGTACCGTCGTT
apr-F	ATTCCGGGGATCCGTCGAC
apr-R	TGTAGGCTGGAGCTGCTTCG

Construction of Mutants of *K. pneumoniae*

hycEFG disrupted strains were constructed using the Red recombinase associated gene replacement method. The detailed protocol was described previously [25]. Briefly, the upstream sequence of hycEFG was amplified from the genome of *K. pneumoniae* by PCR using a primer pair hycEFG-up-F and hycEFG-up-R. Similarly, the downstream sequence was amplified by hycEFG-down-F and hycEFG-down-R. Apramycin resistance gene was amplified from plasmid pIJ773 with a primer pair apr-F and apr-R. Upstream and downstream homologous sequences and resistance genes were linked together to obtain a linear DNA fragment using the ClonExpress Ultra One Step Cloning Kit (Vazyme, Nanjing, China). The linear DNA was transformed into *K. pneumoniae* containing pDK6-red. Red recombinase promotes homologous recombination between the linear DNA and chromosomal DNA, resulting in the deletion of hycEFG and obtaining *K. pneumoniae* △*hycEFG*.

K. pneumoniae △*ldhA* was stored in the laboratory. *K. pneumoniae* △*ldhA*△*adhE* was constructed from *K. pneumoniae* △*ldhA* following the same method as *K. pneumoniae* △*hycEFG* construction. adhE-up-F, adhE-up-R, adhE-down-F, and adhE-down-R were used as primers. *K. pneumoniae* △*ldhA*△*hycEFG* and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* were constructed from *K. pneumoniae* △*ldhA*, and *K. pneumoniae* △*ldhA*△*adhE*, respectively.

Fermentation conditions

Composition of nutrient medium

The nutrient medium contained 25 g/L glucose or 30 g/L glycerol, 1.5 g/L yeast extract, 4 g/L $(NH_4)_2SO_4$, 0.69 g/L $K_2HPO_4{\cdot}3H_2O$, 0.25 g/L KH_2PO_4, 0.2 g/L $MgSO_4$, 0.005 g/L $FeSO_4{\cdot}7H_2O$ and 1 mL of trace element solution (200 mg/L $CoCl_2{\cdot}6H_2O$, 100 mg/L $MnSO_4{\cdot}4H_2O$, 70 mg/L $ZnCl_2$, 60 mg/L H_3BO_3, 35 mg/L $Na_2MoO_4{\cdot}2H_2O$, 29.28 mg/L $CuSO_4{\cdot}5H_2O$, 25 mg/L $NiCl_2{\cdot}6H_2O$ and 0.9 mL/L 37% HCl).

Anaerobic fermentation

Anaerobic fermentation was performed in 250 mL Erlenmayer flasks containing 50 mL nutrient medium in a rotary shaker (Zhicheng, Shanghai, China) at 37 °C and 150 rpm. Nitrogen sparging was performed to purge the residual air for 0.5 min to create anaerobic conditions after autoclaving the nutrient medium. A biogas sampling bag (E-switch, Shanghai, China) was used to collect gases generated in the process.

Microaerobic fermentation

Microaerobic batch fermentation was carried out in the 5 L bioreactor (BIOSTAT-A plus Sartorius) with a working volume of 3 L at the following process conditions: pH = 6.8, T = 37 °C, air flow rate 2 L/min, n = 250 rpm.

For the seed culture, 50 mL of nutrient medium in a 250 mL flask was incubated overnight at 37 °C and 200 rpm. Seed culture was inoculated into a 5-L bioreactor.

All the fermentation experiments were done in triplicate.

Analytical Methods

Biomass concentration was determined by measuring the absorbance at 600 nm using a 722G spectrophotometer (Jingke Co., Shanghai, China). Measurements of the concentrations of glycerol, glucose, succinic acid, lactic acid, formic acid, acetic acid, 1,3-propanediol, 2,3-butanediol, and ethanol in the broth were done by a Shimadzu 20AVP high performance liquid chromatograph (HPLC) system (Shimadzu Corp., Kyoto, Japan) with a RID-20A refractive index detector and an 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.

The volumes of gas generated in the flasks were measured with a 100 mL syringe (Cofoe, Changsha, China) The concentration of H_2 and CO_2 in the collected gas were analyzed by a gas chromatograph (GC) (2010 Plus, Shimadzu, Japan) equipped with TDX-01 stainless steel column (2 m × 2 mm, Shimadzu, Japan) and a thermal conductivity detector (TCD). The injector and detector temperature were all set at 150 ℃, and the oven temperature was held at 120 ℃ for 10 min. The sample injection volume was 1.0 mL. Argon was used as a carrier gas at a flow rate of 40 mL/min.

The intracellular concentrations of NADH and NAD+ were measured by colorimetric method, according to the manual of the NAD(H) Content Assay Kit (Sangon, Shanghai). Briefly, cells were harvested and resuspend in basic or acidic solutions to extract NADH or NAD^+, respectively. The sample was ultrasonic disrupted for 2 min (intensity 20% or 200W, disrupt for 2 s, stop for 1 s), and boiled for 5 min. NADH reduces oxidized Thiazolyl Blue Tetrazolium Bromide (MTT) to formazan by proton

transfer of PMS. NAD^+ can be reduced to NADH by alcohol dehydrogenase, and further detected by MTT reduction method. The luminescence from samples in a 96-well plate was measured using a microplate reader (BioTek Synergy H1, Agilent, America).

Results

The effect of inactivation of hydrogenase-3 on glycerol catabolism of *K. pneumoniae*

In this work, *hycEFG*, which encodes the hydrogenase-3, was knocked out in the genome of wild type strain to obtain *K. pneumoniae* △*hycEFG*. Since the production of 1,3-propanediol is mainly attributed to the *dha* operon under anaerobic conditions, *K. pneumoniae* △*hycEFG* and wild type strain were cultivated in 50 mL shake flasks under anaerobic conditions with glycerol as a carbon source to produce 1,3-propanediol. The fermentation results are shown in Figure 2.

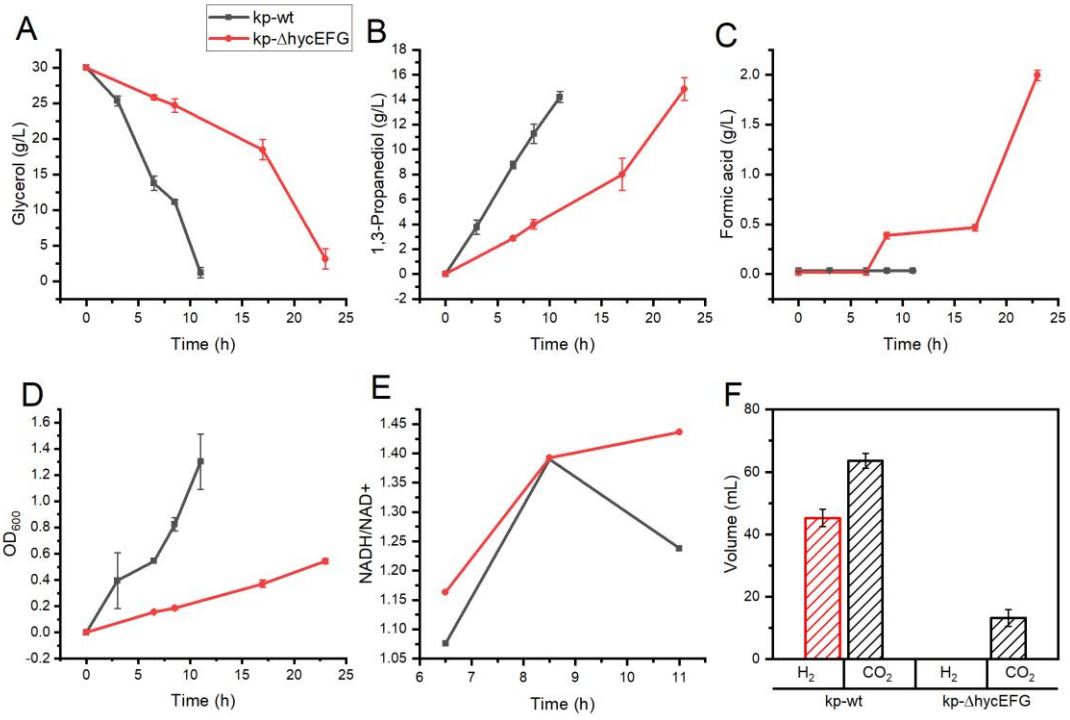

Figure 2. Cell growth and catabolite production of wild type *K. pneumoniae* and *K. pneumoniae* △*hycEFG* cultured with glycerol as the carbon source in shake flasks at anaerobic conditions. A) Glycerol concentration, B) 1,3-Propanediol concentration, C) Formic acid concentration, D) Optical density, E) $NADH/NAD^+$, F) H_2 and CO_2 volumes. The data indicated here is from the replicates of three independent duplicates; error bars represent stdev.

As already emphasized, when glycerol is used as a carbon source, the main product of *K. pneumoniae* is 1,3-propanediol. In the experiment performed with the wild type strain, 28.30 g/L glycerol was consumed and 13.91 g/L of 1,3-propanediol was produced after 11 hours of fermentation. The conversion

ratio of glycerol to 1,3-propanediol was 0.59 mol/mol. In this experiment, 52.83 mL of H_2 and 73.06 mL of CO_2 were produced. The conversion ratios of glycerol to H_2 and CO_2 were 0.15 and 0.21 mol/mol, respectively. No formic acid was detected in the broth.

In the experiment performed with *K. pneumoniae* △*hycEFG,* the growth rate was slower and 25.86 g/L glycerol was consumed and 14.21 g/L of 1,3-propanediol was produced after 24 hours of fermentation. The conversion ratio of glycerol to 1,3-propanediol was 0.66 mol/mol. In this experiment, no H_2 was produced, only 14.84 mL of CO_2 with the conversion ratio of CO_2 from glycerol of 0.047 mol/mol. This means that the hydrogen synthesis pathway was completely blocked in *K. pneumoniae* △*hycEFG*. 1.96 g/L of formic acid was detected in the broth, with a substrate conversion ratio of 0.15 mol/mol. This conversion ratio was equal to that of H_2 production from glycerol in the wild type strains. This suggested the hydrogen produced by the wild type strain all came from formic acid.

The intracellular concentrations of NADH and NAD^+ were also detected, and the results are shown in Figure 2E. The value of NADH/NAD^+ of *K. pneumoniae* △*hycEFG* was higher than those of the wild type strain at 6.5 and 11 hours of cultivation. The NADH/NAD^+ values of the two strains were equal at 8.5 hours of cultivation.

The effects of inactivation of hydrogenase-3 on 1,3-propanediol production at microaerobic conditions

3-hydroxypropanal is a toxic intermediate in the production of 1,3-propanediol. During anaerobic fermentation large amounts of 3-hydroxypropanal (cca.10 mmol/L) can be accumulated, which leads to cell death and the cease of fermentation [27]. In microaerobic fermentation, NADH content can be increased by strengthening the oxidative branch flow, which promotes the conversion of 3-HPA to 1,3-propanediol [28]. For this reason, additional experiments were performed in 5 L bioreactor under microaerobic conditions to reveal the effects of knocking out *hycEFG* on 1,3-propanediol production. The results are shown in Figure 3.

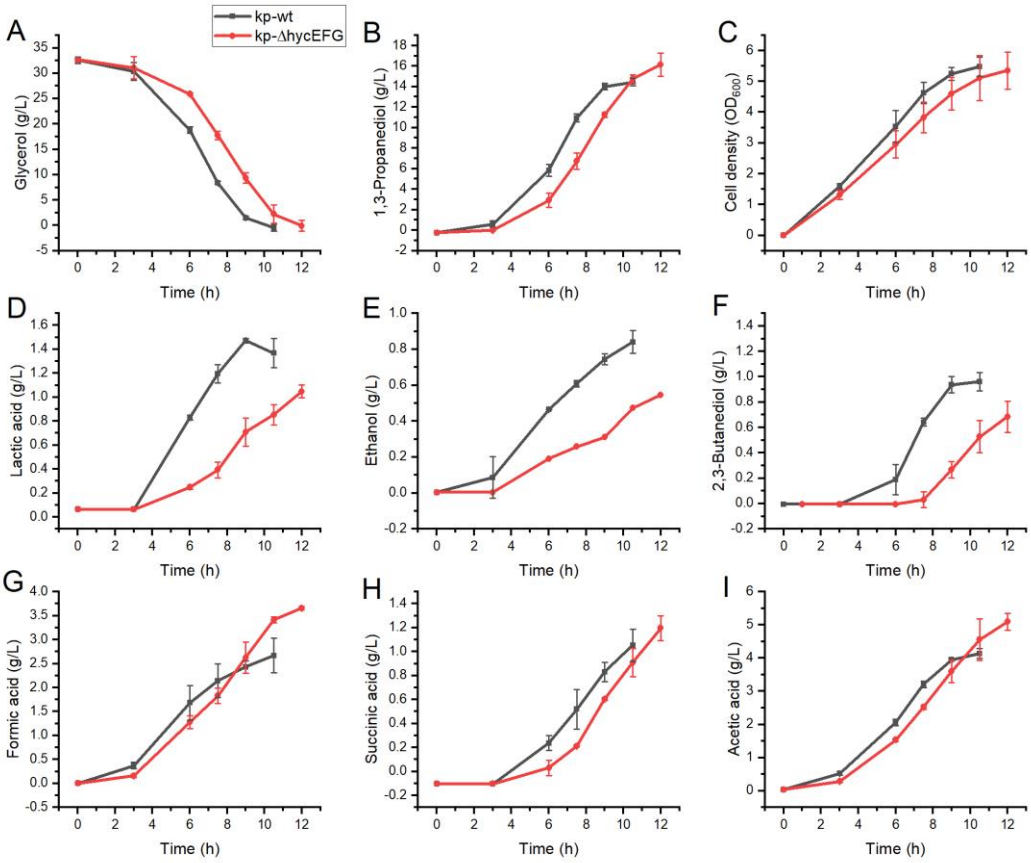

Figure 3. Cell growth and catabolite production of wild type *K. pneumoniae* and *K. pneumoniae* △*hycEFG* for 1,3-propanediol production in 5L bioreactor batch culture. A) Glycerol concentration, B) 1,3-Propanediol concentration, C) Optical density, D) Lactic acid concentration E) Ethanol concentration, F) 2,3-Butanediol concentration, G) Formic acid concentration, H) Succinic acid concentration, I) Acetic acid concentration. The data indicated here is from the replicates of three independent duplicates; error bars represent stdev.

After 10 hours of cultivation, 31.08 g/L of glycerol was consumed by the wild type *K. pneumoniae*. 14.00 g/L of 1,3-propanediol was produced, and the substrate conversion ratio was 0.55 mol/mol. Besides 1,3-propanediol, 1.05 g/L of succinic acid,1.37 g/L of lactic acid, 2.67 g/L of formic acid, 0.96 g/L 2,3-butanediol, 4.14 g/L of acetic acid and 0.84 g/L of ethanol were produced, respectively. The glycerol consumption rate of *K. pneumoniae* △*hycEFG* was slower than that of the wild type strain, it took a further 2 hours to deplete the glycerol. The final cell densities of the wild type and *K. pneumoniae* △*hycEFG* were similar, and both reached 5.40 OD_{600}. The concentration of 1,3-propanediol produced by *hycEFG* mutant strain was 16.14 g/L, and the substrate conversion ratio was 0.59 mol/mol. The concentration of produced formic acid was 3.66 g/L, which was 37% higher than that obtained with wild type strain. The concentrations of lactic acid, ethanol, and 2,3-butanediol produced by *K. pneumoniae*

△*hycEFG* were 1.05 g/L, 0.54 g/L, and 0.68 g/L, respectively. They were all lower than those produced by the wild type strain. 1.20 g/L of succinic acid and 5.10 g/L of acetic acid were produced by *K. pneumoniae* △*hycEFG*, which was also higher compared to the concentrations of succinic acid and acetic acids produced by the wild type strain.

The effects of inactive hydrogenase-3 and lactate dehydrogenase on 1,3-propanediol production

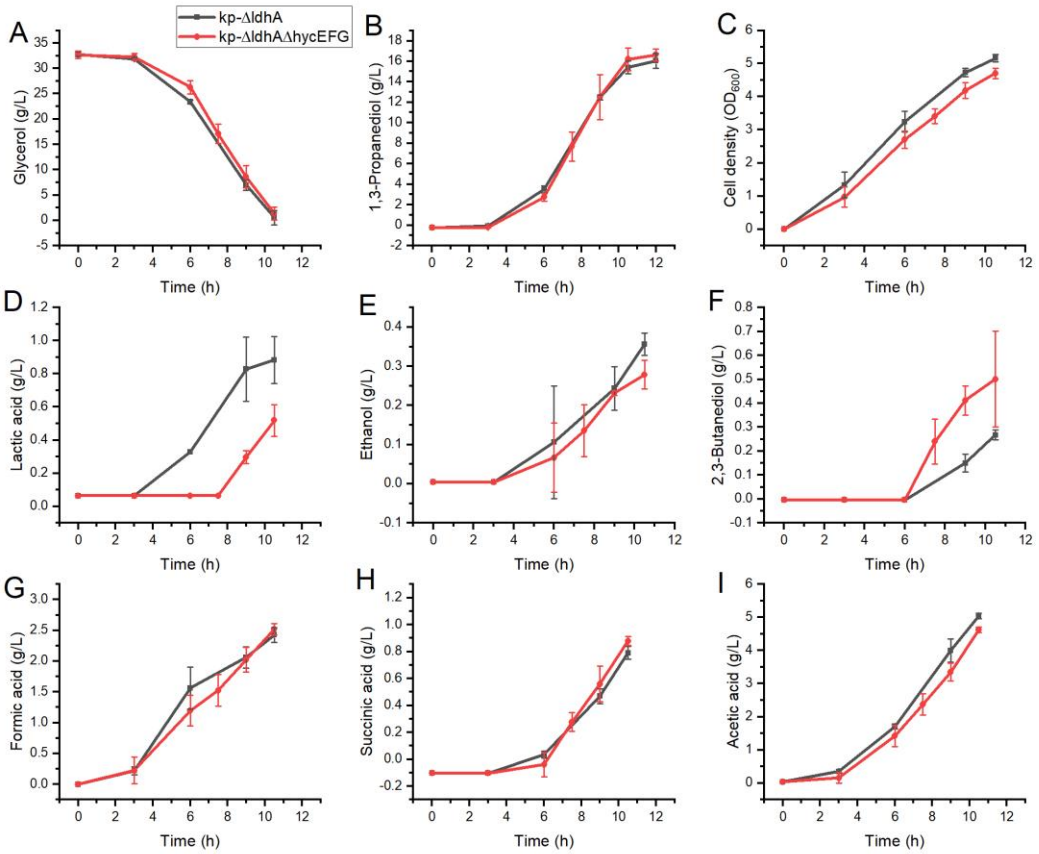

Figure 4. Cell growth and catabolite production of *K. pneumoniae* △*ldhA* and *K. pneumoniae* △*ldhA*△*hycEFG* for 1,3-propanediol production in 5L bioreactor batch culture. A) Glycerol concentration, B) 1,3-Propanediol concentration, C) Optical density, D) Lactic acid concentration E) Ethanol concentration, F) 2,3-Butanediol concentration, G) Formic acid concentration, H) Succinic acid concentration, I) Acetic acid concentration. The data indicated here is from the replicates of three independent duplicates; error bars represent stdev.

Lactic acid is also one of the by-products of 1,3-propanediol production. The synthesis of lactic acid competes for the substrate and NADH with 1,3-propanediol synthesis. In addition, lactic acid and other organic acids produced in the broth increase the burden of the downstream process. Lactic acid is synthesized from pyruvate, and lactate dehydrogenase (*ldhA*) is responsible for this reaction. *K. pneumoniae* △*ldhA* had shown an increase in 1,3-propanediol production efficiency. Here, *K.*

pneumoniae △*ldhA*△*hycEFG* was constructed to reveal the combined effects of inactive hydrogenase-3 and lactate dehydrogenase on the 1,3-propanediol production. *K. pneumoniae* △*ldhA*△*hycEFG* and *K. pneumoniae* △*ldhA* were cultured in 5L bioreactors under microaerobic conditions, and the fermentation results are shown in Figure 4.

Lower concentration of lactic acid was produced by *K. pneumoniae* △*ldhA* (0.88 g/L) and *K. pneumoniae* △*ldhA*△*hycEFG* (0.52 g/L), compared to wild type strains (1.37 g/L). Those values were 64.23 % and 37.96 % lower than that of the wild type strain. Glycerol consumption rates of *K. pneumoniae* △*ldhA* and *K. pneumoniae* △*ldhA*△*hycEFG* were similar, and all close to that of *K. pneumoniae* △*hycEFG* (shown in Figure 3). Compared with *K. pneumoniae* △*hycEFG*, 1,3-propanediol produced by *K. pneumoniae* △*ldhA*△*hycEFG* was further improved to 16.61 g/L, with a substrate conversion ratio of 0.63 mol/mol. 16.03 g/L of 1,3-propanediol was produced by *K. pneumoniae* △*ldhA*. The 1,3-propanediol level of *K. pneumoniae* △*ldhA* was higher than that of the wild type *K. pneumoniae* (14.42 g/L shown in Figure 3), but slightly lower than that of the *K. pneumoniae* △*hycEFG* (16.14 g/L shown in Figure 3) and *K. pneumoniae* △*ldhA*△*hycEFG*.

Cell growth of *K. pneumoniae* △*ldhA*△*hycEFG* was slightly slower than that of *K. pneumoniae* △*ldhA*. The concentrations of ethanol, succinic acid, formic acid, and acetic acid produced by *K. pneumoniae* △*ldhA* were 0.36 g/L, 0.79 g/L, 2.43 g/L, and 5.04 g/L, respectively. These by-product concentrations were similar to those of *K. pneumoniae* △*ldhA*△*hycEFG*, which were 0.28 g/L, 0.88 g/L, 2.52 g/L, and 4.63 g/L, respectively. 0.50 g/L of 2,3-butanediol was produced by *K. pneumoniae* △*ldhA*△*hycEFG*, which was higher than those of *K. pneumoniae* △*ldhA* (0.27 g/L).

The effects of inactive hydrogenase-3, lactate dehydrogenase, and alcohol dehydrogenase on 1,3-propanediol production

Ethanol is another by-product of the 1,3-propanediol production. Ethanol is synthesized from acetaldehyde and an *adhE* encoded alcohol dehydrogenase catalyzes this reaction. To further investigate the role of hydrogenase-3 on 1,3-propanediol synthesis, *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG was* constructed based on *K. pneumoniae* △*ldhA*△*hycEFG. K. pneumoniae* △*ldhA*△*adhE* was constructed as a control. The two strains were cultured in bioreactors for 1,3-propanediol production under microaerobic condition, and the results are shown in Figure 5.

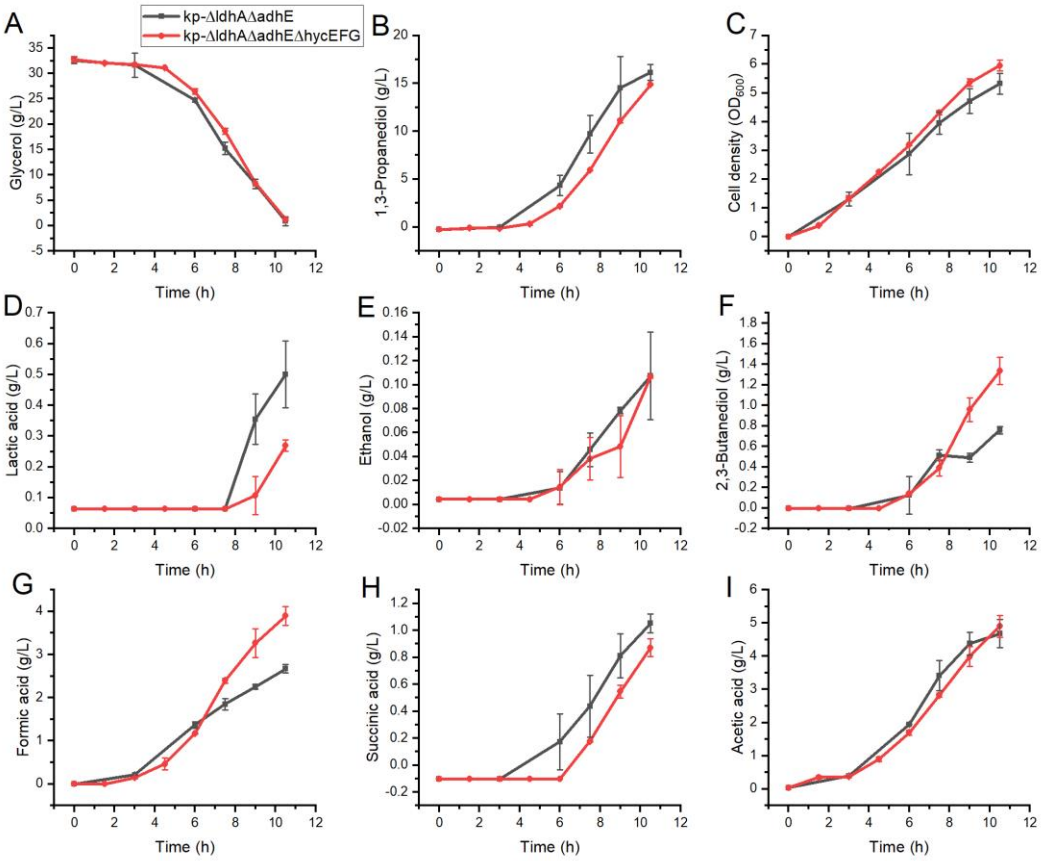

Figure 5. Cell growth and catabolite production of *K. pneumoniae*△*ldhA*△*adhE*, and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* for 1,3-propanediol production in 5L bioreactor batch culture. A) Glycerol concentration, B) 1,3-Propanediol concentration, C) Optical density, D) Lactic acid concentration E) Ethanol concentration, F) 2,3-Butanediol concentration, G) formic acid concentration, H) Succinic acid concentration, I) Acetic acid concentration. The data indicated here is from the replicates of three independent duplicates; error bars represent stdev.

The ethanol synthesis pathway was blocked in *K. pneumoniae*△*ldhA*△*adhE* and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG*. The ethanol produced by the two strains was decreased to 0.10 g/L. Glycerol consumption rates of *K. pneumoniae*△*ldhA*△*adhE* and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* were similar, and glycerol was all exhausted after 10.5 hours of cultivation. A cell density of 5.95 OD_{600} was reached by *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG*, and it was higher than that of *K. pneumoniae*△*ldhA*△*adhE* (final cell density 5.32 OD_{600}). 14.87 g/L of 1,3-propanediol was produced by *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG*, and the substrate conversion ratio was 0.57 mol/mol. This value was lower than that of *K. pneumoniae* △*ldhA*△*hycEFG* (0.62 mol/mol). *K. pneumoniae* △*ldhA*△*adhE* consumed 31.69 g/L glycerol and produced 16.20 g/L of 1,3-propanediol, and the substrate conversion ratio was 0.62 mol/mol, which was the same as that of *K. pneumoniae* △*ldhA*△*hycEFG*.

Formic acid and 2,3-butanediol production both showed a distinct difference between *K. pneumoniae* △*ldhA*△*hycEFG* and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG*. Formic acid concentrations were 2.67 g/L and 3.90 g/L for the two strains respectively. Furthermore, 2,3-butanediol concentrations produced by the two strains were 0.76 g/L and 1.33 g/L, respectively.

H_2 generated at 1,3-propanediol production

H_2 is generated from formic acid with the catalysis of hydrogenase-3. Under anaerobic flasks culture (Figure 2), H_2 was generated by the wild type strain, but not by *K. pneumoniae* △*hycEFG*. At the same time, no formic acid was produced by the wild type strain but by *K. pneumoniae* △*hycEFG*. It indicates that all the formic acid synthesized by the wild type strain was used by the cell to generate H_2. At microaerobic cultures (Figure 3, 4, 5), 2 L/min of air flowed into the bioreactor, H_2 generated was diluted and the total volume of H_2 could not be precisely measured. The exhaust gas of *K. pneumoniae* △*ldhA*△*adhE* at 11 hours of cultivation was detected, and the concentrations of H_2 and CO_2 in the sample were in the ratio of 5.38:93.79. We supposed that the difference in formic acid formed between *hycEFG* wild type strain and *hycEFG* knock out strain was due to the formation of H_2 and CO_2. H_2 generated in the processes was deduced from the difference in concentration of formic acid. The mole conversion ratios of 1,3-propanediol, formic acid, and other by-products from glycerol (set as 100 mol) were calculated. These data are listed in Table 3 and shown in Figure 1.

Table 3. Conversion ratios of catabolites synthesis from glycerol of different *K. pneumoniae* strains (mol/100 mol glycerol)

	Wild type	△*hycEFG*	△*ldhA*	△*ldhA*△*hycEFG*	△*ldhA*△*adhE*	△*ldhA*△*adhE*△*hycEFG*
Succinic acid	2.08±0.18	2.32±0.18	1.91±0.01	2.24±0.17	2.51±0.43	2.15±0.18
Lactic acid	4.84±0.11	2.86±0.14	2.83±0.62	1.73±0.25	1.35±0.09	0.87±0.05
Formic acid	15.65±0.67	19.74±3.46	15.08±0.11	16.48±1.22	15.51±2.15	24.74±1.19
H_2 (deduced)	4..09	0	1.40	0	9.22	0
Acetic acid	19.48±0.01	22.87±1.87	23.78±1.39	23.17±1.30	23.32±2.39	23.83±1.42
1,3-Propanediol	54.51±0.69	58.69±2.37	57.92±1.07	62.81±1.78	61.86±0.39	57.09±0.14
2,3-butanediol	3.41±0.64	1.77±0.42	0.85±0.12	1.68±0.73	2.02±0.84	4.33±1.38
Ethanol	4.78±0.24	2.38±1.13	2.22±0.30	1.82±0.31	0.68±0.27	0.68±0.01

Formic acid formed by the wild type strain and *K. pneumoniae* △*hycEFG* were 15.65 and 19.74 mol/100 mol glycerol, respectively. The formic acid difference between the two strains was 4.09 mol/100

mol glycerol, and this part of formic acid was deduced to be converted to H_2 and CO_2 in the wild type strain. Using the same assumption, H_2 generated by *K. pneumoniae* △*ldhA*, and *K. pneumoniae* △*ldhA*△*adhE* were deduced to be 1.40 and 9.22 mol/100 mol glycerol, respectively. These data showed that only a small part of formic acid was converted to H_2 and CO_2 for *hycEFG* containing strains (including *K. pneumoniae*, *K. pneumoniae* △*ldhA*, and *K. pneumoniae* △*ldhA*△*adhE*), and most formic acid remained in the fermentation broth in these microaerobic fermentations.

The effect of inactive hydrogenase-3 on glucose catabolism of *K. pneumoniae*

In this experiment, glucose was used as a substrate for *K. pneumoniae* cultivation. The wild type strain and *K. pneumoniae* △*hycEFG* were cultured in anaerobic flasks, and fermentation results are shown in Figure 6.

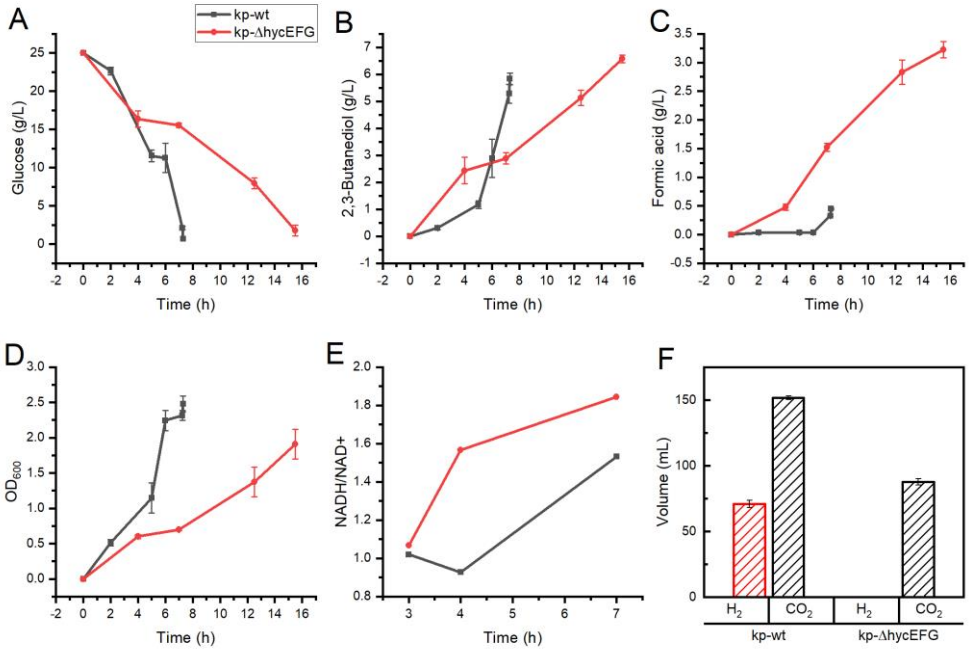

Figure 6. Cell growth and catabolite production of wild type *K. pneumoniae* and *K. pneumoniae* △*hycEFG* cultured with glucose as the carbon source in shake flasks under anaerobic conditions. A) Glucose concentration, B) 2,3-Butanediol concentration, C) Formic acid concentration, D) Optical density, E) NADH/NAD+ ratio, F) H_2 and CO_2 volumes. The data indicated here is from the replicates of three independent duplicates; error bars represent stdev.

Compared with the wild type strain, the cell growth and glucose consumption rate of *K. pneumoniae* △*hycEFG* were slow. This result is similar to the experiment using glycerol as the carbon source (shown in Fig 2). Instead of 1,3-propanediol, 2,3-butanediol became the main catabolite of *K. pneumoniae*, when

glucose was used as the carbon source. 5.68 g/L of 2,3-butanediol was produced by the wild type strain, while the 2,3-butanediol titer was increased to 6.47 g/L in *K. pneumoniae* △*hycEFG*. Conversion ratios of 2,3-butanediol synthesis from glucose were 0.47 mol/mol and 0.57 mol/mol for the two trains, respectively. Formic acid produced by the two strains was 0.40 g/L and 3.12 g/L, with conversion ratios of 0.06 mol/mol and 0.54 mol/mol.

77.32 ml of H_2 and 168.85 ml of CO_2 were produced by the wild type *K. pneumoniae*. The conversion ratios of glucose to H_2 and CO_2 were 0.51 mol/mol and 1.12 mol/mol, respectively. Like the cultivation with glycerol as the carbon source, *K. pneumoniae* △*hycEFG* did not produce H_2. 96.11 ml of CO_2 was produced by *K. pneumoniae* △*hycEFG*, with a conversion ratio of 0.64 mol/mol. CO_2 produced by *K. pneumoniae* △*hycEFG* was almost equal to the value of CO_2 subtracted by the value of H_2 in the wild type strain.

The NADH and NAD^+ concentrations in the cells were detected, and the results are shown in Figure 6. In the whole fermentation process, the ratios of NADH/NAD^+ of *K. pneumoniae* △*hycEFG* were higher than those of *K. pneumoniae* △*hycEFG*.

The effects of inactive hydrogenase-3 on 2,3-butanediol production

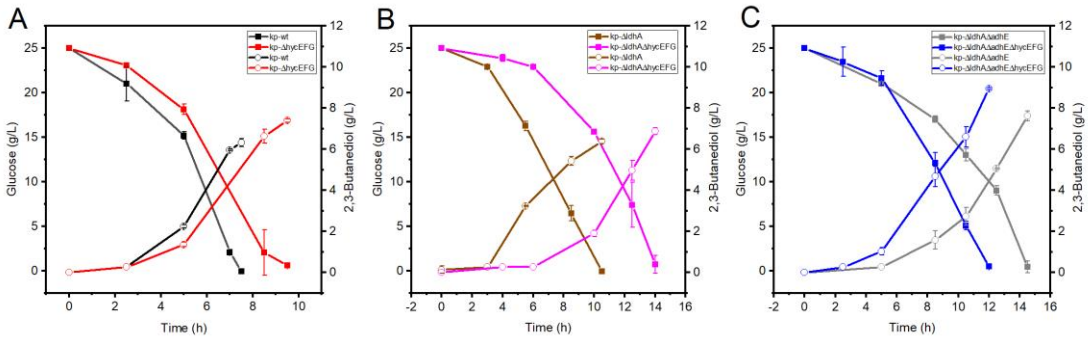

Figure 7. Glucose consumption and 2,3-butanediol production of *K. pneumonia* with glucose as the carbon source in shake flasks at microaerobic conditions. A) wild type *K. pneumoniae* and *K. pneumoniae* △*hycEFG*, b) *K. pneumoniae* △*ldhA* and *K. pneumoniae* △*ldhA*△*hycEFG*, c) *K. pneumoniae* △*ldhA*△*adhE* and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG*. The data indicated here is from the replicates of three independent duplicates; error bars represent stdev.

Figure 6 shows the inactivation of hydrogenase-3 affected 2,3-butanediol production in *K. pneumoniae* strains under anaerobic conditions. Next, wild type strain, *K. pneumoniae* △*hycEFG*, *K. pneumoniae* △*ldhA*, *K. pneumoniae* △*ldhA*△*hycEFG*, *K. pneumoniae* △*ldhA*△*adhE*, and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* were cultured in 250 mL shake flasks and with glucose as the carbon source for 2,3-butanediol production under microaerobic conditions, and fermentation results are shown in Figure

7. The substrate conversion ratios of metabolites of these strains are listed in Table 4.

Similar to the anaerobic shake flask cultures, the glucose consumption rate of *K. pneumoniae* △*hycEFG* was slower than that of the wild type strain. *K. pneumoniae* △*hycEFG* took 10 hours to exhaust the 25 g/L of glucose, while the wild type strain exhausted the glucose after 8 hours of cultivation. The glucose consumption rate of *K. pneumoniae* △*ldhA*△*hycEFG* was further decreased. It took 14 hours to consume all the glucose. *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* was different from other *hycEFG* disrupted strains. Though, the glucose consumption rate of this strain was slower than the wild type strain and *K. pneumoniae*△*ldhA*, it was faster than *K. pneumoniae* △*ldhA*△*adhE*.

The 2,3-butanediol produced by wild type *K. pneumoniae*, *K. pneumoniae* △*ldhA*, and *K. pneumoniae* △*ldhA*△*adhE*, were 6.32 g/L, 6.39 g/L, and 7.63 g/L respectively. While 7.40, 6.88, and 8.95 g/L of 2,3-butanediol were produced by *K. pneumoniae* △*hycEFG. pneumoniae* △*ldhA*△*hycEFG* and *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG*, respectively. The levels of 2,3-butanediol increased significantly after the disruption of *hycEFG*. *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* had the highest 2,3-butanediol levels. The substrate conversion ratio of this strain was 0.73 mol/mol, which was 44.74% higher than that of the wild type strain.

No formic acid was synthesized by *K. pneumoniae* △*ldhA* or *K. pneumoniae* △*ldhA*△*adhE*. Whereas formic acid produced by the wild type strain was 0.4 g/L. These results indicated most formic acid formed in the process of 2,3-butanediol production was converted to H_2 and CO_2 with the catalysis of hydrogenase-3. Thus, the hydrogenase-3 in the cell was kept active during this microaerobic cultivation.

Table 4, Conversion ratios of catabolites synthesis from glucose of different *K. pneumonia* strains (mol/100 mol glucose)

	Wild type	*△hycEFG*	*△ldhA*	*△ldhA△hycEFG*	*△ldhA△adhE*	*△ldhA△adhE△hycEFG*
Succinic acid	2.86±0.02	2.97±0.30	3.19±0.07	2.39±0.74	1.83±0.25	4.95±0.03
Lactic acid	4.22±0.52	5.36±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Formic acid	4.34±0.50	14.05±0.82	0.0±0.0	13.05±2.39	0.00±0.00	6.95±0.21
Acetic acid	6.58±1.17	7.36±0.15	7.56±3.52	9.34 ±0.39	20.93±2.79	10.33±0.67
2,3-butanediol	50.44±1.59	61.06±1.83	50.97±0.89	56.73±3.72	62.99±2.47	73.01±0.51
Ethanol	36.12±1.84	29.37±4.85	50.46±0.22	28.21±1.97	0.00±0.00	0.00±0.00

Discussion

Hydrogenase 3 encoded by *hycEFG* is responsible for H_2 production in *K. pneumoniae*

Although *K. pneumonia* and *E. coli* belong to the same family of *Enterobacteria*, *E. coli* has 4 hydrogenases, namely hydrogenase 1, hydrogenase 2, hydrogenase 3, and hydrogenase 4 [29], while the hydrogenase-3 encoding genes were only found in the genome of *K. pneumoniae* CGMCC 1.6366.

In catabolism process of *E. coli*, hydrogenase-1, and hydrogenase-2 have hydrogen uptake activity [30], while hydrogenase-3 has hydrogen evolution activity [31]. The function of hydrogenase 4 is still unclear, and there are inconsistent reports. For example, hydrogenase-4 is an isoenzyme of hydrogenase-3 [32], whereas hydrogenase-4 is silent with a high concentration of glucose [33]. Through genome-wide sequence alignment, only the *hyc* operon was found in *K. pneumoniae* CGMCC 1.6366, and this operon encodes hydrogenase-3. In formate hydrogen production pathway, hydrogenase-3 along with formate dehydrogenase (*fdh*) form a formate hydrogen lyase complex, which catalyzes the oxidation of endogenous formic acid to CO_2 and transfers electrons to generate gaseous hydrogen [34].

In the present study, formic acid (0.15 mol/mol) produced by *K. pneumoniae* △*hycEFG* is equivalent to the H_2 (0.15 mol/mol) generated by the wild type with glycerol as a carbon source (Figure 2). Accordingly, similar results of 0.51 mol/mol of H_2 or formic acid were obtained with glucose as the carbon source (Figure 6). These results suggested the H_2 produced in the wild type strain all came from formic acid.

Some researchers believe that there is a NADH dependent H_2 formation pathway in *E. aerogenes* [35]. A membrane-bound NADH oxidase catalyzes NADH to generate NAD^+ and electrons. In *Clostridial sp.*, NADH:Fd oxidoreductase catalyzes the conversion of NADH and oxidized ferredoxin to NAD+ and reduced ferredoxin [36]. Then, the electrons are transported to protons through membrane-bound hydrogenase to form hydrogen. If this NADH dependent H_2 pathway was present in the *K. pneumoniae*, the production of hydrogen in the wild type strain should be more than the accumulation of formic acid in *K. pneumoniae* △*hycEFG*. On the other hand, H_2 production from NADH dependent pathway is limited by thermodynamics. The standard electrode potential of $NADH/NAD^+$ is -320 mV [37], which is energetically unfavorable to reducing ferredoxin (E_0' -420 mV) [38, 39]. In theory, if the partial pressure of hydrogen is low enough (<60 Pa), H_2 can be produced through NADH dependent pathway. However, most of the NADH may be oxidized through other fermentation pathways, such as the synthesis of 1,3-propanediol [40]. Therefore, the NADH pathway is probably not present in *K. pneumoniae*, and the formate pathway mediated by hydrogenase-3 is the only hydrogen production pathway.

Hydrogenase-3 is not very sensitive to oxygen

Based on the structure of the active site, hydrogenases can be divided into [NiFe]-hydrogenases (active site: binuclear Ni-Fe center), [FeFe]-hydrogenases (active site: binuclear Fe-Fe center) and [Fe]-hydrogenases (active site: a mononuclear Fe center) [41]. [FeFe]-hydrogenase is extremely sensitive to oxygen. Even traces of oxygen can bind to di-iron domain (2FeH) and attack the [4Fe-4S] domain [42], causing [FeFe]-hydrogenase inactivation. In earlier studies, [NiFe]-hydrogenases isolated from anaerobic bacteria were also highly sensitive to oxygen [43]. Later, the [NiFe]-hydrogenases isolated from facultative anaerobic bacteria showed some tolerance to oxygen [44, 45]. In this study, we performed fermentation experiments under both anaerobic and microaerobic conditions. H_2 was detected in the exhaust gas of both processes. Thus, the hydrogenases-3 of *K. pneumoniae* retain some levels of activity at microaerobic culture conditions.

In the experiments of 2,3-butanediol production, there are no formic acid synthesized by *K. pneumoniae* △*ldhA* or *K. pneumoniae* △*ldhA*△*adhE* (Table 4), also indicated the tolerance of hydrogenase-3 to oxygen at microaerobic conditions.

Inactivation of hydrogenase-3 promoted 1,3-propanediol production

Here, the deletion of *hycEFG* leads to an increase in 1,3-propanediol production in *K. pneumoniae* △*hycEFG* (Figure 2, 3). With the exception of acetic acid, the concentration of all other by-products (succinic acid, lactic acid, 2,3-butanediol, ethanol) decreased in *K. pneumoniae* △*hycEFG*. When acetic acid was synthesized from glycerol, 4 or 5 molecules of NADH were generated, which is the highest amount of NADH among all these by-products. The NADH and NAD^+ concentrations were measured in *K. pneumoniae* △*hycEFG*, and an increase in the ratio of NADH/NAD^+ was observed compared to the wild type strain (Figure 2). The increase in NADH concentration is beneficial for 1,3-propanediol synthesis, as one molecule of NADH is consumed during the conversion of glycerol to 1,3-propanediol. In previous research of our group, the *K. pneumonia* △*pflB* strain showed an increase in the ratio of NADH/NAD^+[14]. The increase in the ratio of NADH/NAD^+ in *K. pneumoniae* △*hycEFG* might have a relation to that of *K. pneumonia* △*pflB*.

Knocking out *ldhA*, which blocked the lactic acid synthesis, led to an improvement in 1,3-propanediol production (Figure 3). This is in agreement with a previous study by Xu et.al [12], which claimed that the conversion of total 1,3-propanediol and 2,3-butanediol increased from 0.55 mol/mol to a maximum of 0.65 mol/mol after *ldhA* knockout. In this research, *K. pneumoniae* △*ldhA*△*hycEFG* showed a further improvement of 1,3-propanediol production compared to *K. pneumoniae* △*ldhA*. Ethanol produced by *K. pneumoniae* △*ldhA*△*hycEFG* was reduced compared with *K. pneumoniae* △*ldhA*. However, the

concentrations of succinic acid and 2,3-butanediol produced by *K. pneumoniae* △*ldhA*△*hycEFG* increased.

K. pneumoniae △*ldhA*△*adhE* showed an improvement of 1,3-propanediol production compared to *K. pneumoniae* △*ldhA* and wild type strain. Thus, the removal of lactic acid and ethanol production was beneficial to 1,3-propanediol production. This is consistent with a previous study. Zhou has indicated the formation of lactic acid and ethanol competes with the synthesis of 1,3-propanediol in carbon flux. Meanwhile, the accumulation of lactic acid inhibits cell growth [46]. However, the 1,3-propanediol produced by *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* was lower than either *K. pneumoniae* △*hycEFG, K. pneumoniae* △*ldhA, K. pneumoniae* △*ldhA*△*hycEFG,* or *K. pneumoniae* △*ldhA*△*adhE*. A main characteristic of *K. pneumoniae* △*ldhA*△*adhE*△*hycEFG* was that the 2,3-butanediol concentrations were improved. The inactivation of hydrogenase-3 in the cell blocked the H_2 production from formic acid. As a result, the concentration of formic acid in the broth was increased. 1,3-propanediol, lactic acid, ethanol, succinic acid, acetic acid, and 2,3-butanediol concentrations in the fermentation broth were all affected by the inactivation of hydrogenase-3. However, these catabolites' synthesis pathways have no direct relation with hydrogenase-3. Thus, the inactivation of hydrogenase-3 might have a global regulation of the cell. *K. pneumoniae* △*ldhA*△*hycEFG* exhibited the highest efficiency of 1,3-propanediol production from glycerol. But, the detailed mechanism of this improvement needs further investigation.

Inactivation of hydrogenase-3 promoted 2,3-butanediol production

2,3-butanediol is a by-product of the production of 1,3-propanediol from glycerol. This chemical has been used as a solvent in the cosmetic industry, and can also be used in biofuel production and other applications [47]. Bacteria of the *Enterobacteria* spp. and *Bacillus* spp. are commonly used strains for 2,3-butanediol production. Knocking out *ldhA* to prevent lactic acid formation has been shown to improve the 2,3-butanediol production by *Klebsiella oxytoca* [48]. A *pflB* knock out *K. pneumoniae* strain showed an improvement in the 2,3-butanediol production [49]. The mechanism of 2,3-butanediol synthesis by *Enterobacteria* is similar to that of H_2 synthesis. Under anaerobic conditions, acetic acid and other organic acids are synthesized, leading to a decrease in the pH of the environment. The 2,3-butanediol synthesis operon is induced by acetic acid. Cell synthesis of 2,3-butanediol instead of acetic acid prevents the toxicity to the cell caused by the lower pH [50, 51]. As mentioned above, the 2,3-butanediol synthesis pathway is not directly linked to hydrogenase-3. The positive effect of inactivating hydrogenase-3 on 2,3-butanediol synthesis may be due to the regulation of the expression of the 2,3-

475 butanediol synthesis operon. Therefore, this study could provide a new strategy to improve the efficiency
476 of 2,3-butanediol production by *Klebsiella* spp.

477 **Conclusion**

478 *K. pneumonia* is an important industrial bacterium that can be used for the production of a variety of
479 chemicals. In this work, it has been shown that the inactivation of hydrogenase-3 blocks H_2 generation
480 and enhances 1,3-propanediol and 2,3-butanediol production with glycerol or glucose as carbon sources
481 respectively. The inactivation of hydrogenase-3 might affect the global regulation of the cell, and this
482 provides a novel strategy for constructing *K. pneumonia* strains for the production of industrially relevant
483 chemicals.

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The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

JH designed this study. WJ, YC, SS, and WW conducted the research. WJ and JH analyzed the data. WJ and JH wrote the manuscript. MT and FB review and editing the manuscript. All authors reviewed and approved the final manuscript.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.