

Deletion of Lactate Dehydrogenase in *Klebsiella Pneumoniae* to Enhance 1,3-Propanediol Production

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Abstract—1,3-Propanediol (1,3-PD) is an important chemical intermediate in manufacturing polyethers, polyurethanes and polyesters. Production of 1,3-PD by microbial fermentation has attracted increasingly attention of researchers. In this study, deletion mutant of *K.pneumoniae* HD-1 constructed by knocking out lactate dehydrogenase (LDH) gene in our previous work was cultivated in fermentation medium utilizing glucose as carbon source and original strain *K.pneumoniae* were simultaneously used as the control strain under absolutely the same conditions and their capability of 1,3-PD production were compared. The results showed that an increase of 19.98%, 20%, and 20.19% of 1,3-PD titer, productivity, and yield, respectively were obtained from *K.pneumoniae* HD-1. Obviously it can be inferred that the ability in the production of 1,3-PD of the *K.pneumoniae* HD-1 was better than that of the *K.pneumoniae*, therefore it could be a promising strain for 1,3-PD production in the further study of industrialized production.

Keywords—*K.pneumoniae*; lactate dehydrogenase; 1,3-propanediol; batch fermentation.

I. INTRODUCTION

1,3-Propanediol (1,3-PD) is an important chemical intermediate in manufacturing polyethers, polyurethanes and polyesters. It is widely applied in food, cosmetic and pharmaceutical industries^[1]. Besides, 1,3-PD can be used as intermediate to synthesize biocides and heterocyclic compounds and it is extensively used in the manufacture of polytrimethylene terephthalate (PTT) which demonstrates excellent potential of stretching and stretch-recovering in producing fiber for textiles and carpets that has been known as “new nylon” currently^[2-3]. In addition, compared with the commercial polyester from market, synthesis of polyester from 1,3-PD has a higher efficiency to reduce dyeing temperature, the higher the UV resistance and better fastness^[4].

Many microorganisms can be served as 1,3-PD producers when anaerobically cultivated in fermentation medium, while *K.pneumoniae* shows excellent ability to produce 1,3-PD with higher yield and productivity^[5]. There are primarily two branch pathways that are the reductive branch and the oxidative branch involved in glycerol metabolic pathway in *K.pneumoniae* which has

been comprehensive studied^[6-7]. In the reductive branch, 3-hydroxypropanal (3-HPA) is firstly synthesized by the conversion of glycerol at the catalysis of glycerol dehydratase which is then formed to 1,3-PD by 1,3-propanediol oxidoreductase^[8]. However, a series of by-products such as 2,3-butanediol (2,3-BD), acetate, lactate are produced due to the existence of oxidative branch, which lead to transforming of the carbon flux and the decreasing of 1,3-PD yield. It is mainly the accumulation of lactate in the stable period of cell growth that decreased the 1,3-PD productivity and thus will be costly in downstream purification process^[9]. It is of great significance to prevent the synthesis of lactate in 1,3-PD production^[10].

In this study, deletion mutant of *K.pneumoniae* HD-1 which was constructed by knocking out lactate dehydrogenase gene applying integrational mutagenesis to *K.pneumoniae* HD-1 in previously experiment in our laboratory and original strain *K.pneumoniae* were simultaneously used as strains in the fermentation medium utilizing glucose as carbon source under absolutely the same conditions, and 1,3-PD production capability were compared to verify whether the construction of *K.pneumoniae* HD-1 was successful and whether the gene expression of the deletion mutant was stable. The one with better ability to produce 1,3-PD will be chosen for further study on improving the yield of 1,3-PD.

II. MATERIALS AND METHODS

A. Microorganisms and medium

K.pneumoniae and *K.pneumoniae* HD-1 were used in the work. The seed cells were prepared in 250 ml flasks containing 100 ml pre-culture medium which contained (g/L): 6.0 (NH₄)₂HPO₄, 0.51 EDTA, 1.8 KCl, 0.29 sodium citrate, 0.6 MgSO₄·7H₂O, 0.21 citric acid, 1.5 yeast extract, 30 glucose, 1 ml microelement solution, 1 ml Fe³⁺ solution. The flasks were incubated at 37°C for 24 h and inoculated into the fermentation medium at 5% (v/v), the fermentation medium containing (g/L): 6.6 (NH₄)₂SO₄, 0.69 K₂HPO₄·3H₂O, 1.36 KH₂PO₄, 0.26 MgSO₄·7H₂O, 0.43 citric acid, 1.0 yeast extract, 1 ml microelement solution, 1 ml Fe³⁺ solution, 60 glucose.

B. Batch fermentation experiment

The batch fermentation process was performed in 500 ml flasks containing 150 ml fermentation medium with initial pH 6.8 and cultured at 37°C with a shaking speed of 150 rpm for 108 h. Samples were harvested every 12 hours and the pH was maintained using sterile CaCO₃ powder depending on the experiment.

C. Analytical methods

Cell growth was described by an optical density (OD) calibration curve determined at 650 nm. The concentrations of glucose, 1,3-PD, 2,3-BD, ethanol, acetate, succinate, and lactate obtained from the batch fermentation process were determined by a high-performance liquid chromatography (HPLC) System (Shimadzu Corp. Kyoto, Japan). Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) was used with 0.005 M H₂SO₄ as elution solvent at a flow rate of 0.8 ml/min, keeping column oven temperature at 65°C with RI detector.

D. Measurement of relative enzyme activities

For enzyme activities determination, the precipitate from 6 mL fermentation broth that was centrifuged at 5,000 rpm for 10 min at 4°C was washed twice with potassium phosphate buffer (100 mM, pH 7.4) and then was resuspended with the buffer. The supernatant from broken Cells which was performed by ultrasonic treatment in ice water bath after centrifugation (10,000 r/m, 10 min at 4°C) was used as crude enzymes liquor for the measurement of enzymes activities. The total protein concentration was determined by the Bradford method with bovine serum albumin as the standard. One unit of enzyme is defined as the amount of enzyme required to produce/consume 1mmol of product/substrate per minute^[11].

III. RESULTS AND DISCUSSION

A. LDH activity assays

The LDH activities of the parent strain and the mutant were determined. As shown in **Table.1**, the LDH activities were 0.232 and 0.128 of the mutant and the parent strain. The enzymatic analysis further proved that *ldhA* had been knocked out partially by integrational mutagenesis, resulting in the decrease of the LDH enzyme activity.

TABLE 1. LDH ACTIVITIES OF *K.PNEUMONIAE* AND *K.PNEUMONIAE* HD-1

| | strains | |
|----------------------------|---------------------|--------------------------|
| | <i>K.pneumoniae</i> | <i>K.pneumoniae</i> HD-1 |
| LDH activity(U/mg protein) | 0.232±0.02 | 0.128±0.03 |

B. The production of 1,3-PD in batch fermentation.

Growth curve of strains and pH value in the fermentation process were shown in **Figure.1**.

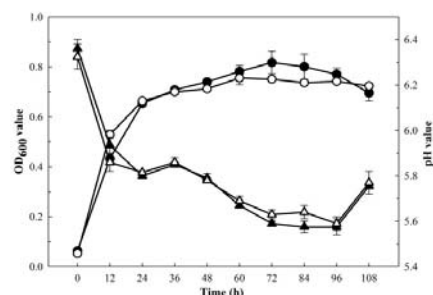


Figure 1. Batch fermentation of *K.pneumoniae* and *K.pneumoniae* HD-1. Closed circle, cell growth of *K.pneumoniae*; open circle, cell growth of *K.pneumoniae* HD-1; closed Triangle-up, pH value of *K.pneumoniae*; open Triangle-up, pH value of *K.pneumoniae* HD-1. The values were the mean of two independent samples.

The results of typical end-products in micro-aerobic batch fermentations were shown in **Fig.2** and the average values with 95% confidence intervals were listed in **Table.2**.

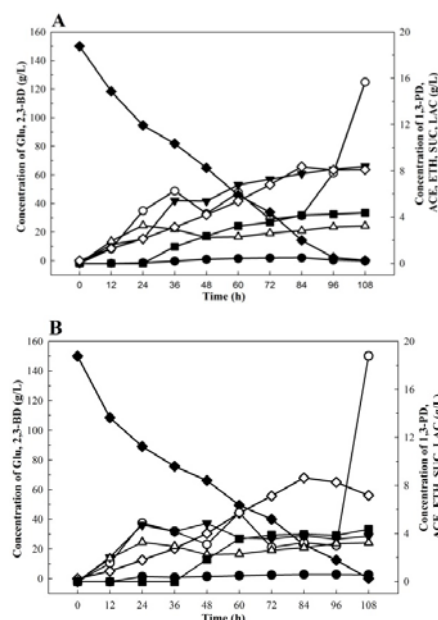


Figure 2. Time courses of *K.pneumoniae* (A) and *K.pneumoniae* HD-1 (B) batch fermentation under aerobic condition. Closed circle, succinate; open circle, 1,3-PD; square, acetate; closed diamonds, glucose; open diamonds, 2,3-BD; closed triangles down, lactate; open triangles up, ethanol.

TABLE 2. COMPARISON OF BATCH FERMENTATION BY *K.PNEUMONIAE* AND *K.PNEUMONIAE* HD-1

| | Strains | |
|----------------------------|---------------------|--------------------------|
| | <i>K.pneumoniae</i> | <i>K.pneumoniae</i> HD-1 |
| Glucose consumed (g/L) | 150±0.001 | 150±0.001 |
| 1,3-PD titer (g/L) | 15.66±0.015 | 18.79±0.026 |
| 2,3-BD titer (g/L) | 65.75±0.031 | 68.03±0.009 |
| Lactate titer (g/L) | 8.39±0.001 | 4.84±0.001 |
| succinate titer (g/L) | 0.486±0.002 | 0.591±0.005 |
| 1,3-PD yield (g/g) | 0.104±0.002 | 0.125±0.001 |
| 1,3-PD productivity(g/L·h) | 0.145±0.02 | 0.174±0.03 |

Theoretically the formation of lactate can be prevented by inactivating lactate dehydrogenase. The *K.pneumoniae* HD-1 was constructed by disrupting *ldhA* gene that encodes lactate dehydrogenase, which showed markedly lower lactate production (4.84 g/L) compared to the original strain (8.39 g/L). Concomitantly, the finally 1,3-PD titer and yield increased from 15.66 to 18.79 g/L and 0.104 to 0.125 g/g respectively. The increasing of other two important products that were 2,3-BD and succinate were 3.47% and 21.6%. In order to evaluate and compare the performance of 1,3-PD fermentation between *K.pneumoniae* and *K.pneumoniae* HD-1, the total glucose uptake concentration and 1,3-PD productivity were determined at the time the glucose was completely depleted in the fermentation broth (initially 150 g/L). And the total glucose uptake rate of the mutant was lower than the wild-type strain which led to the biomass of the mutant (**Fig.1**) and the 1,3-PD productivity was slightly higher from 0.145 to 0.174 g/L·h. Furthermore, the final concentrations of succinate increased to 0.105 g/L of the mutant while other by-products such as ethanol. and acetate were nearly similar to the wild-type strain.

Lactate was the main by-product in 1,3-PD production by *K.pneumoniae*, which competed for NADH against 1,3-PD pathway and certainly reduced the yield of 1,3-PD from glucose. In this study, *K.pneumoniae* HD-1 lacking fermentative lactate dehydrogenase was constructed and isolated to assess the physiological importance of lactate production and thus making the production of 1,3-PD more efficient. Synthesis of 1,3-PD plays significant role in regulating the balance of intracellular reducing equivalent by consuming NADH generated from the oxidation pathway (Zeng et al, 1993)^[12]. Furthermore, considerable quantity of pyruvate which is an important metabolize intermediate flowed to 2,3-BD pathway as the lactate synthesis pathway was cut off and part of the excessive NADH could be utilized. Thereby, 2,3-BD which is also known as a fuel additive with high value-added became the main by-product in *K.pneumoniae* HD-1 fermentation^[13].

IV. CONCLUSIONS

K.pneumoniae HD-1 lacking fermentative lactate dehydrogenase (LDH) exhibited higher 1,3-PD productivity and less lactate accumulation. In addition, 2,3-BD and succinate has also slightly increased. The

results showed that the ability in 1,3-PD production of genetic engineering strains was better than wild-type strain, which simultaneously was more suitable for further research of higher production of 1,3-PD.

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