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Research Article

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Application of dissolved oxygen control and substrate feeding strategy to improve production of glutamate dehydrogenase protein of *Streptococcus suis* in *Escherichia coli*

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ABSTRACT

The glutamate dehydrogenase (GDH) protein of Streptococcus suis is used for protecting pigs against S. suis infection, and acetate is a primary inhibitory metabolite in expression of GDH by E. coli and the expression of GDH was increased by reduction of acetate accumulation with optimization of dissolved oxygen (DO) level and feeding strategy. In this study, the effect of different DO levels and DO stage control strategies on expression of GDH were investigated, and the results indicated that the DO level controlled at 50% (0-5 h) and 30% (5- 10 h) decreased accumulation of acetate and increased cell density and GDH production. Furthermore, the DO and pH feedback feeding were applied in production of GDH, and higher cell density and concentration of GDH were obtained with DO feedback feeding that were 1.57 (OD_{600}) and 38.24 mg/L and the accumulation of acetate decreased to 3.86 g/L. This study could provide theoretical foundation for industrial production of GDH and enhance the application market of GDH subunit vaccine.

Keywords glutamate dehydrogenase; *Escherichia coli*; *Streptococcus suis*; acetate; dissolved oxygen

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INTRODUCTION

Streptococcus suis is one of the most important swine pathogens worldwide and an important agent of zoonosis [1]. Because of a lack of effective means to control the infection of *S. suis*, the economic impact of the infection on the swine industry is substantial [2]. It has been reported that the glutamate dehydrogenase (GDH) protein of *S. suis* is antigenic and conserved in the strains tested, which reacts with serum from animals with *S. suis* type 2 infection, leading to making it a serological assay to detect *S. suis* infection [2, 3]. We previously constructed a recombinant *E. coli* designated as SS2-GDH that produces activity protein of GDH of *S. suis* serotype 2 [4], but the production of GDH was low because of high acetate accumulation. The GDH of *S. suis* used to distinguish *S. suis* infection will help to control the disease caused by *S. suis*, thus it is necessary to improve the production of GDH of *S. suis* type 2 and expand its application for the development of pig industry.

E. coli is used as the common host for production of recombinant protein, and acetate is a primary inhibitory metabolite in cultures of *E. coli*, since it causes inhibition for growth of strain and formation of desired product [5]. Many authors have reported that the high-yield production of recombinant protein was obtained by decreasing efficiency of acetate [6]. Acetate accumulated with the condition of aerobic was known as "overflow metabolism" that resulted from an imbalance between glycolysis and tricarboxylic acids (TCA) cycle [7]. The conversion of acetyl-CoA through the action of phosphotransacetylase (*pta*) and acetate kinase (*ackA)* and the conversion of pyruvate directly into acetate via pyruvate oxidase B (*poxB*) contribute to acetate formation [8]. The elimination of

Pta and AckA activities have resulted in a significant reduction in acetate accumulation [9].

The DO is an important factor in culture of *E. coli*, since it influences many physiological properties and a number of enzymes of the Embden-Meyerhof-Parnas (EMP), hexosemonophosphate (HMP) and TCA cycle pathways together with cytochrome measurements [10]. *E. coli* utilizes higher proportion of glucose via the HMP with high DO level than that of low DO level, which is caused by the low activities of phosphofructokinase [10, 11]. When the growth rate of strain and oxygen consumption became better balance, the formation of acetate could be overcome [12]. The acetate accumulation was found to be inversely correlated to the DO levels, and the expression level of key enzymes related to synthesis of acetate were affected by the DO levels, that is, at high oxygen only the Pta-AckA pathway genes are transcribed, while at low oxygen both the genes of the Pta-AckA pathway and the gene of PoxB seem to be transcribed [13]. Due to the high DO level maintained, the demand for bioreactor increases, and an efficient way to maintain aerobic growth conditions is to increase the oxygen concentration in the air supply by mixing the sparging air with pure oxygen [14], which will improve the cost of production. Increasing DO concentration during *E. coli* growth stimulates an increase in intracellular concentration of reactive oxygen species (ROS), which cause oxidative stress to *E. coli* cells associated with reduced or inhibited growth, including damage by inactivating proteins, breaking nucleic acid strands, and altering the lipids and fluidity of cell membranes [15]. Maintaining an appropriate DO level is important to reduce accumulation of acetate and improve production of desired product.

The accumulation of acetate can be reduced by optimizing the fermentation process and genetically modifying the cells [12]. In production of recombinant protein TRAIL, the excretion of acetate was prevented by using the combined feeding strategy of exponential feeding and pH-stat feeding, and the dry cell weight and active soluble TRAIL were increased by 58.54% and 47.37% compared with using a constant feeding rate strategy [16]. The natural glucose transport system was substituted by a constitutively expressed galactose permease in *E. coli* that allowed efficient growth by reducing the glucose uptake rate and consequently decreasing acetate production, leading to yielding the double of plasmid DNA per gram of cell than the parental strain [17].

In the present study, we investigated the effects of DO level on production of GDH by *E. coli*, and according to the requirement of cell growth and formation of GDH, a strategy of stage control of DO is used in production of GDH, which resulted in reduction of acetate accumulation and improvement of GDH production. Furthermore, the feeding strategy based on pH and DO were used in the GDH fermentation to increase the production of GDH.

EXPERIMENTAL SECTION

Microorganism and medium

The strain, *E. coli* SS2-GDH, was obtained from earlier work in our laboratory and stored at the Culture Collection of Shandong Binzhou Animal Science & Veterinary Medicine Academy.

The seed medium Luria Broth (LB) contained the components (in g/L): tryptone 10, yeast extract 5, and NaCl 5. The fermentation medium for producing GDH protein had the following composition (in g/L): glucose 5, yeast extract 10, $(NH_4)_2SO_4$ 2,, $MgSO_4{\cdot}7H_2O$ 2.5, and KH_2PO_4 1.5. The pH of both seed and fermentation media was adjusted to 7.0 with 4 mol/L NaOH.

Culture methods

The strain *E. coli* SS2-GDH was cultivated in a 10-L fermentor (GRJ-10D Fermentor System, Zhenjiang, China). A 500-mL baffled flask containing 100 mL seed medium was inoculated with a single colony of *E. coli* SS2-GDH and cultivated at 37℃ with shaking at 200 rpm for 12 h. The culture grown in the baffled flask was inoculated aseptically (2% v/v) into 5 L of production medium in a 10-L fermenter. The temperature was maintained at 37℃, and the pH was adjusted to 7.0 with 4 mol/L NaOH during the course of the cultivation period. The DO level was maintained at different values to meet specific experimental requirements. When the initial glucose was depleted, glucose solution (50 % w/v) was added to the fermentor. Once the cell density (OD_{600}) increased to between 0.7 and 0.8, the isopropyl thiogalactose (IPTG) as added into the fermentor to control the concentration of IPTG at 1 mmol/L to induce synthesis of GDH protein and the cultivation was continued for 8 h.

Analysis of fermentation products

The DO, pH, and temperature were measured automatically with electrodes attached to the fermentors. The cell density was determined as described previously [8]. The concentration of glucose was monitored using an SBA-40E Biosensor Analyzer (Biology Institute of Shandong Academy of Sciences, China). The concentration of acetate was determined using an Agilent 1206 (Agilent Technologies, Santa Clara, CA, USA) high-pressure liquid chromatography system. The concentration of GDH protein was determined as described previously [4].

Statistical analysis
All experiments were conducted in triplicate, and the data were averaged and presented as the mean±standard deviation. The significant differences was determined by using one-way analysis of variance followed as Dunnett's multiple comparison test, and statistical significance was defined as $P<0.05$.

RESULTS AND DISCUSSION

Effect of different DO levels on production of GDH
Cell density and concentration of GDH

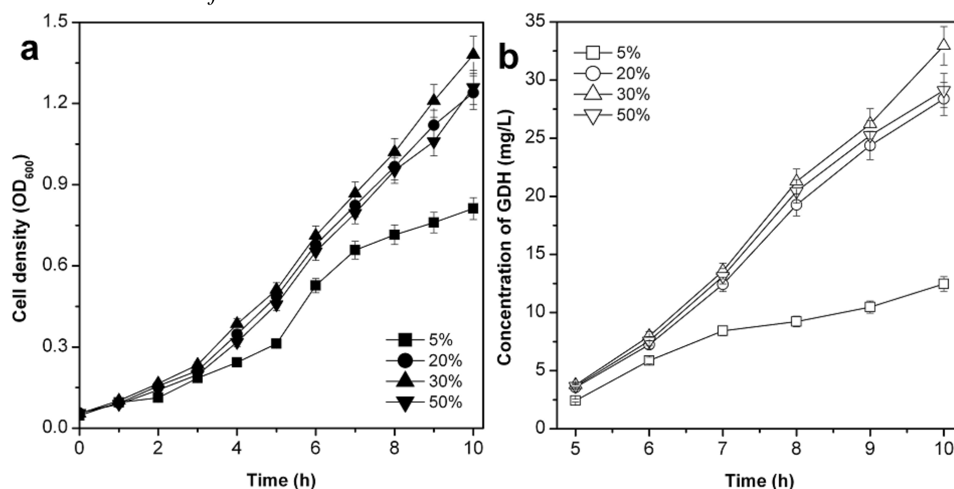

Figure 1 Effect of DO levels on cell density and concentration of GDH

The cell density and concentration of GDH with DO levels of 5%, 20%, 30% and 50% are displayed in Figure 1. The concentration of GDH increased with increasing cell density. The lowest cell density (0.81) and concentration of GDH (12.47 mg/L) were obtained with DO level of 5%, while the highest cell density and concentration of GDH were obtained with DO level at 30% that were 1.38 and 32.95 mg/L. The cell density and expression level of GDH at DO level of 50% were higher than those with DO level of 30%. High DO level was beneficial to increase cell density and expression level of GDH [13].

Accumulation of acetate and consumption rate of glucose
Figure 2 showed the accumulation of acetate and glucose consumption rate with different DO levels in GDH production. The accumulation of acetate increased with decreasing the DO level. The accumulations of acetate with DO levels of 5%, 20%, 30% and 50% were 8.78 g/L, 4.32 g/L, 4.12 g/L and 3.91 g/L, respectively. The enhanced acetate accumulation at lower DO was the result of lower TCA cycle activity and altered transcription levels of genes associated with glucose and acetate metabolism [13]. The glucose consumption rate with DO level of 5% during the whole fermentation period was lowest because of high accumulation of acetate [12]. With the DO level above 20%, the glucose consumption rate decreased with increasing DO level. Lower proportion of glucose via the EMP pathway with high DO level, leading to decreasing the consumption rate of glucose [11].

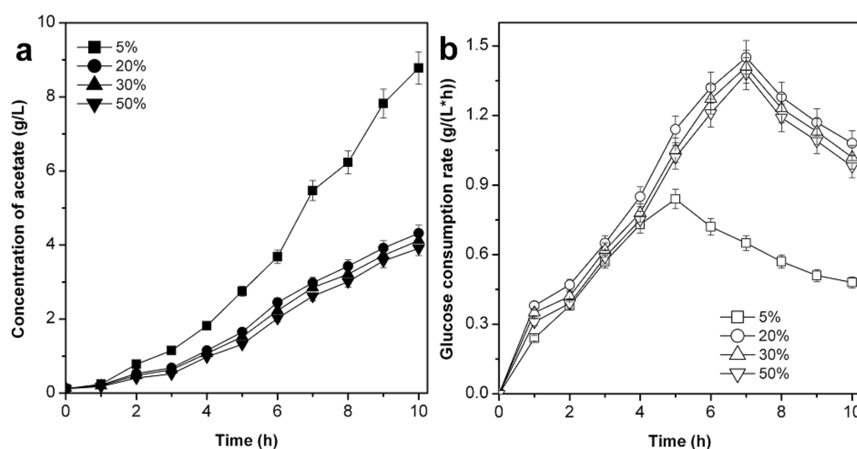

Figure 2 Effect of DO levels on acetate accumulation and glucose consumption rate

Effect of DO stage control on production of GDH

Based on the results with different DO levels, four strategies of DO stage control were developed in expression of GDH, as follows: strategy I 20% (0-5 h) and 30% (5- 10 h), strategy II 30% (0-5 h) and 20% (5-10 h), strategy III 50% (0-5 h) and 30% (5- 10 h), and strategy IV 50% (0-5 h) and 30% (5- 10 h).

Cell density and concentration of GDH

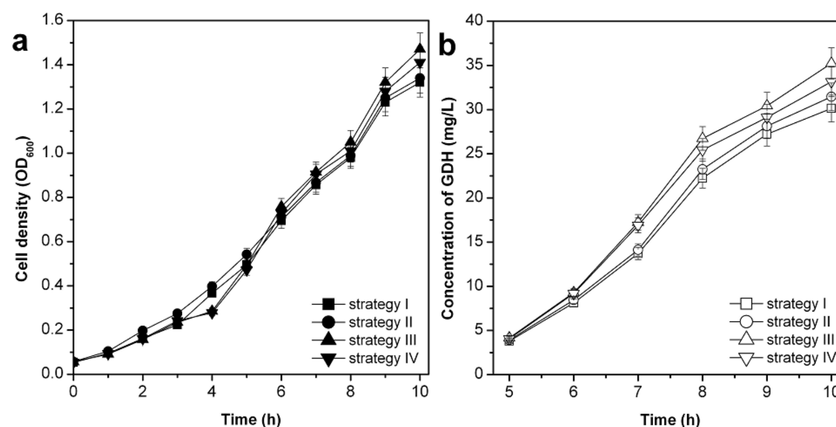

Figure 3 Effect of DO stage control on cell density and concentration of GDH

The cell density and concentration of GDH with DO stage control strategy are presented in Figure 3. Highest cell density and concentration of GDH were obtained with strategy III that were 1.45 and 35.24 mg/L, which were increased by 9.85% and 16.99% compared with those of strategy I, respectively. Cell density and concentration of GDH obtained with stragy III were higher than those with strategy II. Maintaining high DO level during the early fermentation phase increased the cell density and concentration of GDH [13].

Accumulation of acetate and consumption rate of glucose

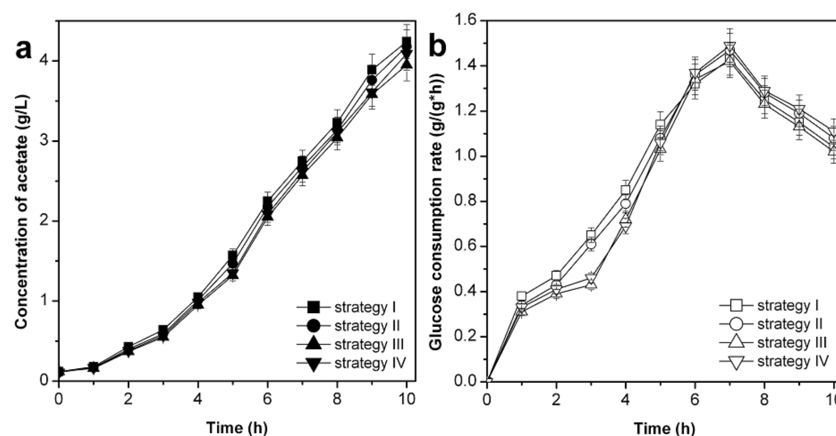

Figure 4 Effect of DO stage control on acetate accumulation and glucose consumption rate

The accumulation of acetate and glucose consumption rate with DO stage control strategy are displayed in Figure 4. Lower concentration of acetate was accumulated with higher DO level during the initial fermentation period. The accumulation of acetate (3.95 g/L) with strategy III was lowest, which was decreased by 6.84% compared with that of strategy I (4.24 g/L). The concentration of acetate accumulated with strategy II and IV were 4.18 g/L and 4.09 g/L. Due to the metabolic flux distribution with different DO levels, the glucose consumption rate was lower with higher DO level with the same fermentation period [10]

Application of pH and DO feedback feeding strategy in production of GDH

The cell density and concentration of GDH with pH and DO feedback feeding strategy are showed in Figure 5, along with accumulation of acetate and glucose consumption rate. The application of pH and DO feeding strategy decreased accumulation of acetate, leading to increasing cell density and concentration of GDH [12]. The cell density and concentration of GDH with DO feedback feeding strategy were 1.57 and 38.24 mg/L, which were 1.05- and 1.06-times higher than those with pH feedback feeding. However, the acelate accumulated (3.74 g/L) with pH

feedback feeding strategy was lower than that (3.86 g/L) of DO feedback feeding strategy. The variation of DO was more delicacy than that of pH, which led to higher feeding rate with DO feedback feeding strategy and resulted in higher glucose consumption rate and accumulation of acetate [8]. Due to the glucose limitation with pH feedback feeding strategy, the cell density and concentration of GDH were lower [17].

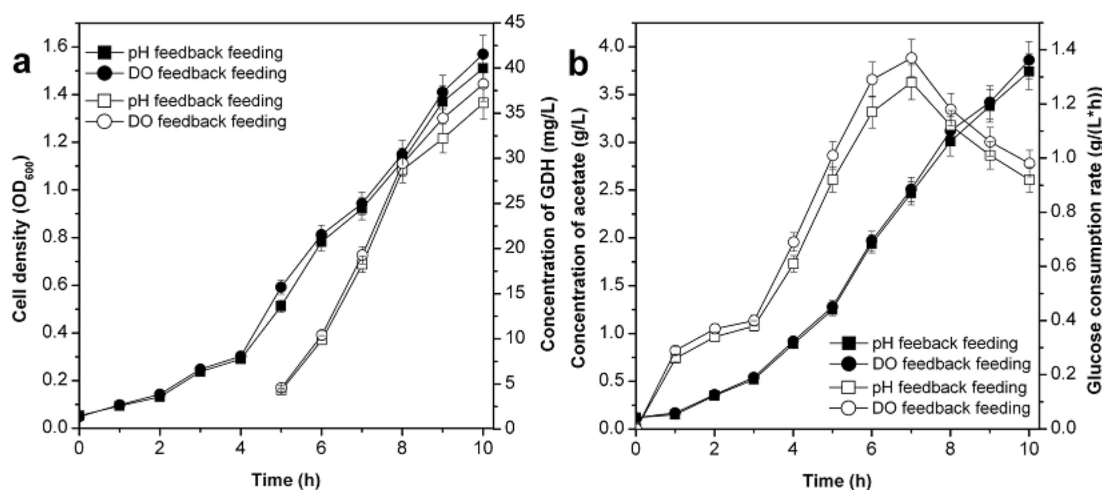

Figure 5 Effect of pH and DO feedback feeding strategy on expression of GDH

CONCLUSION

According to the mechanism of protein expression and acetate biosynthesis, the accumulation of acetate was decreased and the cell density and concentration of GDH were increased by using the DO stage control strategy and DO feedback feeding. The genetic modification of strain and optimization of culture conditions should be used to increase the cell density and expression of GDH. This study can provide theoretical foundation for industrial production of GDH and decrease its production cost, leading to enlarging the application market of GDH subunit vaccine and promoting the development of swine industry.

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