Impact of gene modification of phosphotransferase system on expression of glutamate dehydrogenase protein of *Streptococcus suis* in *Escherichia coli*

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**ABSTRACT**

Glutamate dehydrogenase (GDH) protein of *Streptococcus suis* can be used for detection of *S. suis* infection and protection of pigs against *S. suis* infection. Acetate is a primary inhibitory metabolite for cell growth and formation of GDH protein. In this study, the *ptsG* gene, which encodes the integral membrane permease IICBGlcn in the phosphotransferase system, was deleted and the effect of this deletion on the expression of GDH protein was investigated. The plasmids containing *glf* (encoding glucose facilitator)–*glk* (encoding glucokinase) or *galP* (encoding galactose permease)–*glk* were transformed into *ptsG* mutant cells to recover the cell growth and glucose utilization of *ptsG* mutant. The mutants with deletion of *ptsG* decreased the accumulation of acetate; and higher cell density and GDH protein concentration were obtained with the *ptsG* mutants containing *glf–glk* or *galP–glk*. When the *ptsG* mutant containing *glf–glk* (SSGGFK) was used for expression of GDH protein, the cell density (optical density OD600 of 2.68) and the concentration of GDH protein (42.34 mg/L) were highest, with an increase by 12.61% and 14.84%, respectively, compared with the parental strain (SSG). The acetate accumulation was reduced to 2.35 g/L, i.e. a 37.33% decrease compared with the SSG strain. High concentration of GDH protein was obtained with reduction of acetate accumulation through gene modification of the phosphotransferase system. This can decrease the production cost and expand the applicability of the subunit vaccine of GDH protein and provide theoretical foundation for high-level expression of other recombinant proteins.

**INTRODUCTION**

The glutamate dehydrogenase (GDH) of *Streptococcus suis* is conserved and antigenic, as it reacts with serum from animals with *S. suis* type 2 infection, leading to it being utilized as a serological assay for diagnosis of *S. suis* infection [1,2]. It has been reported that the subunit vaccination based on GDH protein of *S. suis* is an effective strategy for protecting pigs against *S. suis* infection [3] but its market is limited because of the high cost. Improvement of the GDH protein by *Escherichia coli* can decrease the production cost and expand the application market of GDH subunit vaccine, leading to protection of the pig production industry and public health. The accumulation of acetate in the culture process is an important problem in the expression of recombinant proteins, since acetate inhibits cell growth and protein synthesis [4], and becomes more inhibitory to recombinant protein-producing cells than to wild-type cells [5]. There are examples where the expression of recombinant protein was significantly improved by decreasing the accumulation of acetate [4]. The accumulation of acetate is due to the combined high rates of glucose uptake and glucose catabolism by the Embden–Meyerhof–Parnas pathway, resulting in a rate of acetyl coenzyme A synthesis surpassing the capacity of the tricarboxylic acid (TCA) cycle to completely consume this metabolite [6,7]. In addition, modification of the glucose transport capacity is a successful approach for decreasing the excretion of acetate under aerobic conditions [7].

In *E. coli*, the phosphoenolpyruvate–sugar phosphotransferase system (PTS) participates in the transport and phosphorylation of several sugars [7]. The glucose-specific enzyme II*Glc* complex of the glucose-specific PTS containing the soluble IIA*Glc* enzyme (encoded by *crp*) and the integral membrane permease IICB*Glc* (encoded by *ptsG*) [8], and inactivation of *ptsG* are evaluated as...
effective strategies to reduce the glucose uptake capacity [7]. However, the PTS− strain is not suitable for industrial production because of its low growth and glucose utilization rate [8]. The expression of galactose permease (encoded by galP) of E. coli or glucose facilitator protein (encoded by glf) of Zymomonas mobilis, together with glucokinase (encoded by glk), is the most common approach for recovery of the cell growth and glucose utilization of a PTS− strain [9]. When the PTS− Glc+ strain, where glucose is transported and phosphorylated by an alternative transport system (GalP and Glk), is used for L-phenylalanine production, the yield from glucose in the synthesis of phenylalanine can increase by 57% as compared to the isogenic PTS+ strain [10]. In addition, the effect of genetic modification of the PTS system on the expression of GDH protein by recombinant E. coli was studied to select the strain for higher expression of GDH protein.

In the present study, the ptsG mutant was constructed and the plasmids containing glf−glk or galP−glk were transformed to the ptsG mutant. In addition, the effect of genetic modification of the PTS system on the expression of GDH protein by recombinant E. coli was studied to select the strain for higher expression of GDH protein.

### Materials and methods

**Bacterial strains, plasmids and primers**

All bacterial strains, plasmids and primers used here are listed in Table 1.

**Media**

The media used for generating and propagating recombinant strains and plasmids were prepared according to published procedures [12]. The seed medium Luria–Broth contained the following components: 10 g/L tryptone, 5 g/L yeast extract and 5 g/L NaCl. The fermentation medium for producing GDH protein had the following composition: 5 g/L glucose, 10 g/L yeast extract, 2 g/L (NH4)2SO4, 2.5 g/L MgSO4, 7H2O and 1.5 g/L KH2PO4. The pH of both seed and fermentation media was adjusted to 7.0 with 4 mol/L NaOH.

**Culture methods**

The culture conditions used for the construction of recombinant strains and plasmids were controlled according to published procedures [12]. A 500-mL baffled flask containing 100 mL seed medium was inoculated with a single colony of E. coli SS2-GDH and

| Table 1. Strains, plasmids and primers used in this study. |
|----------------------------------------------------------|
| **Name** | **Characteristics** | **Source** |
| **Strains** | | |
| Z. mobilis | ATCC3 I 821 | Lab collection |
| E. coli MG1655 (wild type) | Lab collection |
| PARG | Derived from PAR, but ΔptsG | This study |
| PARGFK | Derived from PARG, and containing pSTV-FK (glf and glk) | This study |
| PARGPK | Derived from PARG, and containing pSTV-PK (galP and glk) | This study |
| SSG | E. coli SS2-GDH (E. coli BL21 containing gdhA of S. suis type 2) | [3] |
| SSGG | Derived from SSG, but ΔptsG | This study |
| SSGGPK | Derived from SSGG, and containing pSTV-FK (glf and glk) | This study |
| SSGGPK | Derived from SSGG, and containing pSTV-PK (galP and glk) | This study |
| **Plasmids** | | TaKaRa |
| pSTV28 | CmR, cloning vector | |
| pSTV-FK | pSTV28 inserted with glfZmobilis and glkE. coli | This study |
| pSTV-PK | pSTV28 inserted with gaPE. coli and glkE. coli | This study |
| pKD46 | AmR, red-expressing vector | [11] |
| pK3 | CmR, template vector | [11] |
| pCP20 | AmR, CmR, FLP-expressing vector | [11] |
| Primers | | |
| ptsG-P1 | 5’-ATGTATAGAATGATCAGTTGTAAGACTCCGAAAAGGTGCGGTACTCGGAATCCTGGAATCGATGGTGCAGCTGTTCCGAGGTTGCTGTTGTTGTTCTGTCGTAGATACGAGATAGAGACT-3’ | This study |
| ptsG-P2 | 5’-TTAGTTGTTAGGATGTTAGCTATCATATCGGTTTTCTCACGATTGATTGCTGTTGTTGTTGTTGTTCTGTCGTAGATACGAGATAGAGACT-3’ | This study |
| ptsG-P3 | 5’-ATGTGAAAGCGTGATAGCCGTCC-3’ | This study |
| ptsG-P4 | 5’-AACGTTGGAAGGTCTATCGTTAC-3’ | This study |
| glf-P1 | 5’-TGCACTGCAACCCGTCATCTCGGATTGTAAG-3’ (PstI) | This study |
| glf-P2 | 5’-ACATGCCATACAGGATTCACGCAACGCTGTAAG-3’ (SphI) | This study |
| gal-P1 | 5’-GCACTGCAAGGATTCACGCAACGCTGTAAG-3’ (PstI) | This study |
| gal-P2 | 5’-ACATGCAAGGATTCACGCAACGCTGTAAG-3’ (SphI) | This study |
| glf-P1 | 5’-GGACGTCGATTTAGCGGAGGATTG3’ (SacI) | This study |
| glf-P2 | 5’-TGGACGTCGATTTAGCGGAGGATTG3’ (PstI) | This study |

*The underlines indicate 56-nt homology extensions of a target knockout gene.*

*The underlines indicate the restriction enzyme cutting site.*
cultivated at 37 °C with shaking at 200 r/min 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 (GRJ-10D Fermentor System, Zhenjiang, China). The temperature was maintained at 37 °C, and the pH was adjusted to 7.0 with 4 mol/L NaOH during the course of the cultivation period. The dissolved oxygen (DO) level was maintained at 20%. When the initial glucose was depleted, glucose solution (50% w/v) was added to the fermentor according to the DO feedback feeding strategy [13]. Once the cell density (optical density at 600 nm, OD600) increased to 0.5–0.6, isopropyl thiogalactose (IPTG) was added into the fermentor to control the concentration of IPTG at 0.5 mmol/L to induce synthesis of the GDH protein.

**Construction of plasmids pSTV-FK and pSTV-PK**

Genes \(\text{glf}_{\text{Z.mobilis}}\), \(\text{galP}_{\text{E.coli}}\) and \(\text{glk}_{\text{E.coli}}\) were amplified by polymerase chain reaction (PCR) with the primers \(\text{glf-P1-glF-P2}\), \(\text{galP-P1-galP-P2}\) and \(\text{glk-p1-glk-p2}\), respectively. The PCR products were purified using a purification kit. The purified \(\text{glk}_{\text{E.coli}}\) fragment digested with Sacl and PstI was inserted into pSTV28 that was digested with Sacl and PstI to construct pSTV-K. The purified \(\text{glf}_{\text{Z.mobilis}}\) and \(\text{galP}_{\text{E.coli}}\) fragments digested with PstI and SphI were inserted into pSTV-K that was digested with PstI and SphI to construct the plasmids pSTV-FK and pSTV-PK, respectively [12].

**Assays of plasmids pSTV-FK and pSTV-PK**

The expression of genes \(\text{glf}\) and \(\text{galP}\) in pSTV-FK and pSTV-PK was determined by comparing the glucose consumption rate of PAR, PARG, PARGFK and PARGPK. The expression of \(\text{glk}_{\text{E.coli}}\) was monitored by assay of the glucokinase activity of PAR, PARG, PARGFK and PARGPK. Glucokinase activity was determined by monitoring the Glucokinase activity of PAR, PARG, PARGFK and PARGPK. The deletion of \(\text{ptsG}\) mutants with deletion of \(\text{ptsG}\) were constructed [16].

**Analysis of substrates and by-products**

The DO, pH and temperature were measured automatically with electrodes attached to the fermenters. The cell density was determined as described previously [14]. The glucose concentration was monitored 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 [15].

**Results and discussion**

**Construction of ptsG mutants**

The deletion of \(\text{ptsG}\) in \(\text{E.coli}\) was confirmed by colony PCR using primers \(\text{ptsG-P3-ptsG-P4}\), and the PCR results are presented in Figure 1. The lengths of the fragments agreed with their theoretical lengths, indicating that mutants with deletion of \(\text{ptsG}\) were constructed [16].

**Construction of plasmids pSTV-FK and pSTV-PK**

The glucokinase activity of PARGFK and PARGPK was 2.17- and 2.15-fold higher than that of PAR and PARG, which suggested that \(\text{glk}_{\text{E.coli}}\) was actively expressed in plasmids pSTV-FK and pSTV-PK [8], and there was no difference in the glucokinase activity of PAR and PARG. The cell density (OD600), cell growth rate and glucose consumption rate with the strains PRA, PARG, PARGFK and PARGPK are presented in Figure 2. The mutant with lesion in \(\text{ptsG}\) showed the lowest growth rate, cell density and glucose consumption rate. The deletion of \(\text{ptsG}\) impaired the
capacity for cell growth and glucose consumption, which resulted in PARG not being used as a host strain for GDH protein expression [9]. The capacity for cell growth and glucose consumption of the ptsG mutant containing the plasmids pSTV-FK or pSTV-PK were higher than those of the ptsG mutant, and the plasmids pSTV-FK or pSTV-PK could recover the cell growth rate and glucose uptake of the ptsG mutant. This indicated that the plasmids pSTV-FK and pSTV-PK expressed the active glkZ.mobilis and galPE.coli, respectively. The glucose consumption rate of PARGFK was higher than that of PARGPK, which was due to the higher glucose transport velocity and lower energy cost of Glf [17].

**GDH expression with the parental strain and mutants with modification of PTS**

**Cell density and growth rate**

Figure 3 shows the cell density and cell growth rate of SSG and the mutants. The cell density and growth rate of SSSG were lowest because of the deletion of ptsG, and the cell density of SSSG was 1.22 (OD_{600}), which was a decrease by 48.74% compared with that of SSG (OD_{600} of 2.38) [7]. The cell density of SSSGFK and SSSGPK were 1.13- and 1.09-times higher than that of SSG. The cell growth rate of SSSGFK and SSSGPK were also higher than that of SSG during the later fermentation phase. The highest cell density and growth rate were obtained with SSSGFK. Overexpression of glf is more feasible for increasing the glucose uptake rate of E. coli than galP, which resulted in obtaining higher cell density and growth rate [17].
Concentration and production rate of GDH protein

The concentration and production rates of GDH protein with different strains are presented in Figure 4. Due to the low cell density and growth rate, the lowest concentration and production rate of GDH protein was obtained with SSGG, and the GDH protein concentration of SSGG was 12.12 mg/L. The GDH protein concentrations of SSGGFK and SSGGPK were 42.34 and 39.87 mg/L, which were increased by 14.84% and 8.14% compared with SSG (36.87 mg/L), respectively. A study indicated that cultures of a ptsG-strain growing in complex medium supplemented with 20 g/L of glucose resulted in significant reduction of acetate excretion and more than 50% increase in recombinant protein synthesis compared

Figure 3. Effect of modification of the PTS system on the cell density (a) and cell growth rate (b) in expression of GDH protein by E. coli ($P < 0.05$).

Figure 4. Effect of modification of the PTS system on the concentration (a) and production rate (b) of GDH protein in expression of GDH protein by E. coli ($P < 0.05$).
with the wild-type strain cultures [18]. The production rate of GDH protein of SSGGFK and SSGGPK were higher than that of SSG during the whole induction period.

**Concentration of acetate and glucose consumption rate**

The acetate concentration and glucose consumption rate of different strains are displayed in Figure 5. The deletion of *ptsG* decreased the accumulation of acetate, and the mutants with lesion in *ptsG* accumulated lower concentration of acetate [4,7]. The acetate accumulation of SSGG (1.85 g/L) was lowest, and the acetate accumulation of SSGGFK and SSGGPK were 2.35 and 2.17 g/L, which marked a decrease by 37.33% and 42.13%, respectively. Due to the lower accumulation of acetate, higher cell density and GDH protein concentration were obtained with SSGGFK and SSGGPK [4,18]. The glucose consumption rate of SSGG was lowest, which resulted in the lowest cell density and GDH protein concentration when SSGG was used [8]. The expression of pSTV-FK or pSTV-PK increased the glucose consumption rate of the *ptsG* mutant, and the glucose consumption rates of SSGGFK and SSGGPK were higher than that of SSG. The glucose transport rate of Glf is near five times higher than that of GalP, and Glf does not require metabolic energy in the form of a proton potential, while GalP operates by a sugar-proton symport mechanism [7,9]. Thus, application of Glf is more energetically favourable than GalP, and the glucose utilization rate of SSGGFK was higher than that of SSGGPK. The concentration of GDH protein was increased with reduction of acetate accumulation through genetic modification of the PTS. Coordinated expression of *glf* and *glk* of *E. coli* was required for improving glucose utilization and the expression of the GDH protein, which can provide theoretical foundation for genetic modification of the PTS in *E. coli* and high-level expression of other recombinant proteins.

**Conclusions**

In the present study, the accumulation of acetate was decreased by the deletion of *ptsG*. Higher cell density and concentration of GDH protein were obtained by the *ptsG* mutants containing the plasmids pSTV-FK or pSTV-PK, and the cell density and GDH protein concentration of SSGGFK were highest. The enhancement of the expression of GDH protein can decrease the production cost and expand the application market for the subunit vaccine of the GDH protein, which may aid in the control and prevention of diseases caused by *S. suis*, and thus promote the development of the pig industry. In addition, the construction strategy of the strain in this study can provide the theoretical basis for expression of other recombinant proteins.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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