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Enhancement of fructose utilization from sucrose in the cell for improved L-serine production in engineered *Corynebacterium glutamicum*

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ABSTRACT

Microbial fermentative production of L-serine has attracted increasing attention, and in our previous study, *Corynebacterium glutamicum*-SYPS-062ΔSSA has been successfully screened and engineered to produce L-serine from sucrose. It was noticed that there was significant fructose accumulation during L-serine fermentation process using sucrose as substrates, which is the preferred carbon resource for this strain. Lack of fructokinase may be responsible for poor fructose utilization. In the present study, a shortcut pathway for fructose utilization has been constructed via heterologous expression of *scrK* (fructokinase). Furthermore, *pfkA* (phosphofructokinase) was over-expressed to avoid over accumulation of fructose-6-phosphate, the resulting strain showed significantly improved cell growth and L-serine accumulation. In a 5-L bioreactor, the engineered *C. glutamicum* strain produced 30.6 g/L L-serine (0.99 mol/mol sucrose yield, with a theoretical maximal yield of 4 mol/mol sucrose), whereas the reference strain produced 20.54 g/L L-serine (with a yield of 0.67 mol/mol sucrose) at 120 h. After two feedings of sucrose, the L-serine production increased up to 37.0 g/L at 96 h.

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1. Introduction

As a central cellular intermediate, L-serine plays an important role in metabolism as well as formation of activated C1 units [1]. Currently, L-serine is widely used in modern industries, ranging from pharmaceutical to food and cosmetics, and is also utilized as a building block chemical. The main processes employed for L-serine production include extraction of L-serine from proteins or enzymatic conversion of glycine plus C1 compounds such as methanol to L-serine. However, application of these two methods at an industrial scale is limited owing to their low yield and high cost, making them less attractive. Thus, production of L-serine through microbial fermentation has attracted increasing attention.

Corynebacterium glutamicum is regarded as a moderate candidate for producing amino acids, and has been successfully used to produce L-serine with metabolic engineering. Peters-Wendisch et al. identified the key enzymes in the L-serine metabolic pathway in *C. glutamicum* ATCC 13032 [2]. Subsequently, by overexpressing or deleting the key enzymes, the engineered *C. glutamicum* strain could produce up to 9.1 g/L L-serine from glucose [3]. Furthermore, 36.3 g/L L-serine was achieved by reducing folate supply in *C. glutamicum* [4]. In our previous study, *Corynebacterium glutamicum*-SYPS-062ΔSSA has been successfully screened and engineered to produce L-serine from sugar, and sucrose is the preferred carbon resource for this strain, when compared with other sugars (such as glucose and fructose) [5].

Sugar utilization may be also a key in microbial fermentation of L-serine. It has been established that *C. glutamicum* uses phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) to take up and phosphorylate glucose, fructose, and sucrose, the major sugars from agricultural crops that are used as primary feedstocks in industrial amino acid production [6]. Moreover, the representative *C. glutamicum* ATCC 13032 has been reported to have four sets of PTS, each specific for glucose, fructose, sucrose, and an unknown substrate [7,8]. In addition, sucrose uptake in wild-type *C. glutamicum* has been noted to depend exclu-

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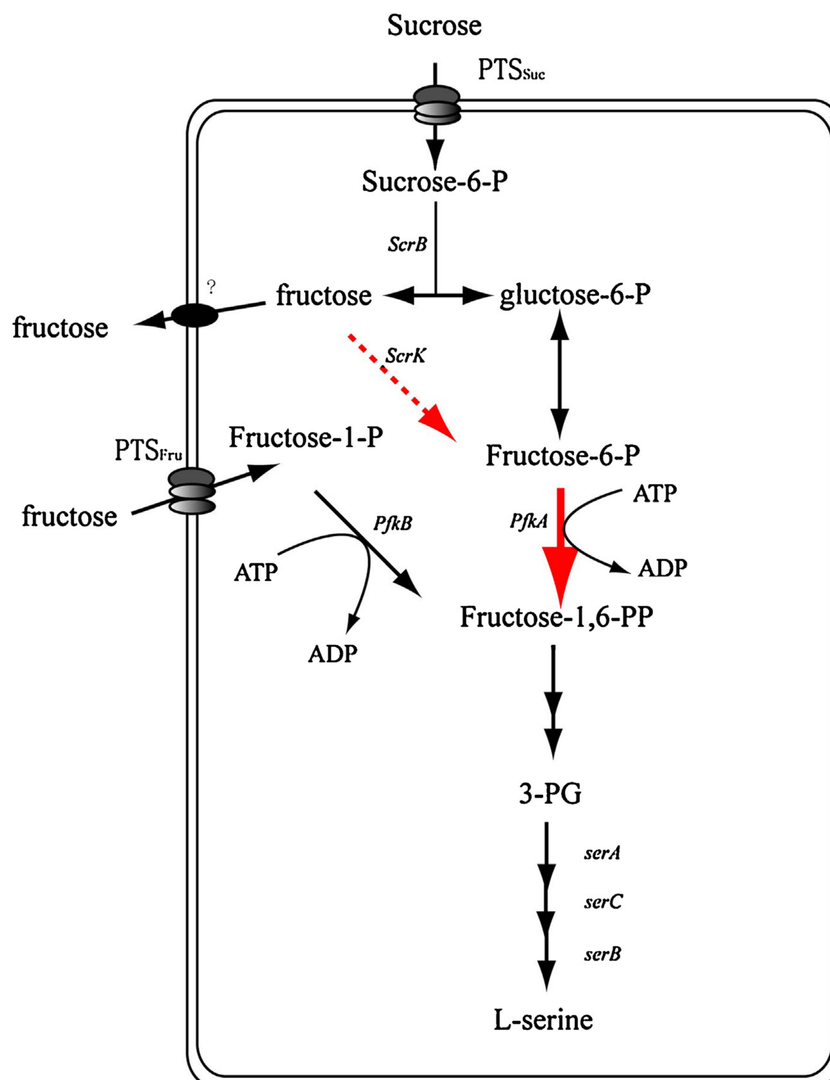

Fig. 1. L-Serine biosynthesis pathways from sucrose in *C. glutamicum*. *ScrB*, encodes sucrose 6-phosphate hydrolase; *ScrK*, encodes fructokinase; *PfkA*, encodes phosphofructokinase; *PfkB*, encodes phosphofructokinase; *serA*, encodes 3-phosphoglycerate dehydrogenase; *serC*, encodes phosphoserine aminotransferase; *serB*, encodes phosphoserine phosphatase. Solid arrows indicate existing metabolic pathways and dashed arrows denote constructed pathways.

sively on sucrose PTS, in which intracellular sucrose-6-phosphate is hydrolyzed to glucose-6-phosphate and fructose by sucrose-6-phosphate hydrolase. Intracellular fructose cannot be further metabolized owing to the lack of fructokinase activity in *C. glutamicum* [9]. The free fructose generated in the cell is exported outside by an unidentified carrier and re-imported by the fructose PTS to generate fructose-1-phosphate [9,10] (Fig. 1). In order to phosphorylate directly the fructose without any fructose efflux, heterologous expression of the *ScrK* gene (fructokinase) from *Clostridium acetobutylicum* in *C. glutamicum* is proposed [9]. Our studies showed that there was significant fructose accumulation during L-serine fermentation process using sucrose as substrates. Therefore, *Scr*K expression may be a potential strategy for solving fructose accumulation and enhancing L-serine production during growth on sucrose.

In the present study, a shortcut pathway for fructose utilization has been constructed via heterologous expression of *scrK* (fructokinase). Furthermore, *pfkA* (phosphofructokinase) was overexpressed to avoid over accumulation of fructose-6-phosphate, and effect of these modification on cell growth, sugar consumption and L-serine production have been investigated.

2. Materials and methods

2.1. Strains and plasmids

The strains and plasmids used in this study are listed in Table 1. *Escherichia coli* JM109 was used as the host for plasmid construction. *C. glutamicum* SYPS-062-33a, derived from *C. glutamicum* SYPS-062 by random mutagenesis, has been deposited in China General Microbiological Culture Collection Center (CGMCC) under the accession no. 8667. The engineered *C. glutamicum* SYPS-062-33aΔSSA, derived from *C. glutamicum* SYPS-062-33a with the deletion of 591 bp from the C-terminal domain of *serA* and deletion of *sdaA* and *alaT,* has also been deposited in CGMCC under the accession no. 8668, and was stored in our laboratory.

2.2. Media formulation

For plasmid construction, Luria-Bertani (LB) medium was used, which contained (per liter) 5.0 g of yeast extract, 10.0 g of tryptone, and 10.0 g of NaCl. The brain-heart infusion medium (BHI; Difco) was employed as the complex medium for *C. glutamicum* cultivation at 30 °C on a rotary shaker at 120 rpm. Ampicillin or

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