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Metabolic engineering and flux analysis of *Corynebacterium glutamicum* for L-serine production

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L-Serine plays a critical role as a building block for cell growth, and thus it is difficult to achieve the direct fermentation of L-serine from glucose. In this study, *Corynebacterium glutamicum* ATCC 13032 was engineered *de novo* by blocking and attenuating the conversion of L-serine to pyruvate and glycine, releasing the feedback inhibition by L-serine to 3-phosphoglycerate dehydrogenase (PGDH), in combination with the co-expression of 3-phosphoglycerate kinase (PGK) and feedback-resistant PGDH ($PGDH^r$). The resulting strain, SER-8, exhibited a lower specific growth rate and significant differences in L-serine levels from Phase I to Phase V as determined for fed-batch fermentation. The intracellular L-serine pool reached (14.22±1.41) μmol g_{CDM}^{-1}, which was higher than glycine pool, contrary to fermentation with the wild-type strain. Furthermore, metabolic flux analysis demonstrated that the over-expression of PGK directed the flux of the pentose phosphate pathway (PPP) towards the glycolysis pathway (EMP), and the expression of $PGDH^r$ improved the L-serine biosynthesis pathway. In addition, the flux from L-serine to glycine dropped by 24%, indicating that the deletion of the activator GlyR resulted in down-regulation of serine hydroxymethyltransferase (SHMT) expression. Taken together, our findings imply that L-serine pool management is fundamental for sustaining the viability of *C. glutamicum*, and improvement of C_1 units generation by introducing the glycine cleavage system (GCV) to degrade the excessive glycine is a promising target for L-serine production in *C. glutamicum*.

***Corynebacterium glutamicum*, L-serine, intracellular metabolites, metabolic engineering, elementary mode analysis**

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The amino acid L-serine is one of the major components for nutritional growth and development of the central nervous system of mammals [1,2], but is also a predominant source of one-carbon (C_1) units in *de novo* biosynthesis of purines, amino acids and thymidylate [3–5] as well as an intermediate for phospholipid biosynthesis [6] in a variety of organisms. Given its critical role as a building block for cell growth, the bacterium *Corynebacterium glutamicum*, for example, has as much as 7.5% of its total glucose-originating carbon flux distributed to L-serine biosynthesis [7]. In *C. glutamicum*, L-serine biosynthesis is initiated from the glycolytic intermediate 3-phosphoglycerare and carried through three sequential reactions catalyzed by 3-phosphoglycerate dehydrogenase (PGDH), 3-phosphoserine aminotransferase (PSAT) and 3-phosphoserine phosphatase (PSP), respectively. PGDH is the gene product of *serA* and catalyzes the oxidation of 3-phosphoglycerare to generate 3-phosphohydroxypyruvate. PSAT is encoded by *serC* and catalyzes the transamination of 3-phosphohydroxypyruvate to yield 3-phosphoserine. 3-Phophoserine is then dephosp-

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horylated to yield L-serine catalyzed by PSP, a gene product of *serB* (Figure 1). Among the three enzymes, PDGH is found to be sensitive to feedback inhibition by L-serine and only remains 60% activity in the presence of 10 mmol L^{-1} L-serine [8]. It has also been identified that the carboxy-terminal 197 amino acids of PGDH are responsible for the feedback inhibition by L-serine and its deletion has only a minimal effect on the enzyme activity [8].

Catabolism of L-serine in *C. glutamicum* proceeds via pyruvate in the presence of glucose. This conversion reaction is catalyzed by L-serine dehydratase (L-SerDH) encoded by the gene *sdaA* [9]. L-Serine is also used to provide building blocks for amino acid biosynthesis including glycine, cysteine and tryptophan [9–11]. Isotope tracer analysis demonstrated that the carbon skeleton of ^{13}C-labeled L-serine is mainly converted to glycine [9]. This reaction is catalyzed by the gene product of *glyA*, a serine hydroxymethyltransferase (SHMT) [12]. SHMT catalyzes the reversible, simultaneous conversion of L-serine to glycine and tetrahydrofolate to 5,10-methylenetetrahydrofolate. Since this reaction provides the majority of the C_1 units needed for cell growth, disruption of the *glyA* gene is lethal to host cells [12]. A recent study identified that, in the stationary growth phase, *glyA* gene expression is tightly controlled by an activator GlyR at the transcriptional level [13]. This finding provides a possible regulatory tool to control the flux from L-serine to glycine by manipulating GlyR expression.

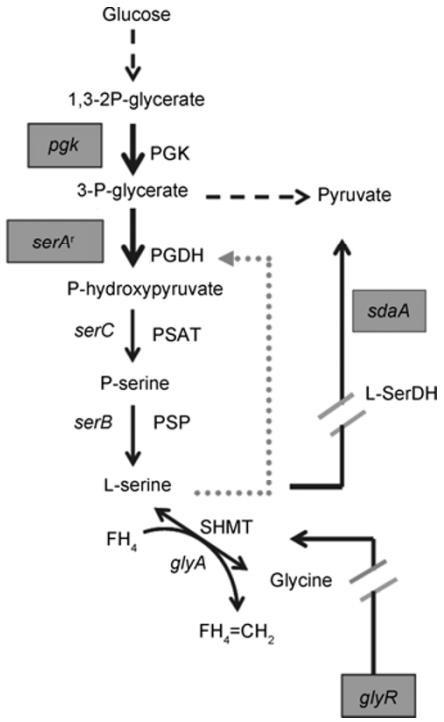

Figure 1 Strategy to engineer *C. glutamicum* for L-serine production. Genetic manipulation includes the inactivation of the catabolic genes *sdaA* and *glyR* (double short lines), the release of the feedback inhibition of 3-phosphoglycerate dehydrogenase (PGDH) by L-serine (dotted long arrow) and the over-expression of the biosynthesis genes *pgk* and feedback-resistant *serA*^{r} (boldfaced arrows). 1,3-2P-glycerate: 1,3-diphosphoglycerate; 3P-glycerate: 3-phosphoglycerate; P-hydroxypyruvate: phosphohydroxypyruvate; P-serine: phosphoserine; FH_4: tetrahydrofolate and FH_4=CH_2: 5,10-methylenetetrahydrofolate.

At present, the industrial production of L-serine mainly depends on the enzymatic or cellular conversion of the substrate glycine and a C_1 compound in the presence of an enzyme system composed of SHMT [14]. Many methanol-utilizing bacteria, such as *Hyphomicrobium methylovorum*, have been applied to produce L-serine by using glycine and methanol [15]. These systems either use expensive substrates or exhibit low productivity that extremely restricts their application for L-serine production. In addition, using these industrial strains, large-scale fermentation for L-serine production is not achieved and unlike the production of other amino acids, the conversion of cheap sugar to L-serine is very difficult.

C. glutamicum is amenable to genetic modification and shows robust fermentation characteristics using glucose as a carbon source, hence making it a good candidate to produce amino acids [16,17]. Subjected to systems metabolic engineering in recent years, *C. glutamicum* has been successfully applied to produce amino acids including L-lysine, L-valine and L-methionine [17–19]. However, the L-serine production from this bacterium is not yet suitable for industrial application [20], mainly due to the fact that the metabolic regulatory systems for intracellular L-serine accumulation remain largely unknown. In this study, we constructed an engineered strain SER-8, quantified its extra- and intra-cellular products and analyzed its carbon flux redistributions by constructing elementary modes to obtain functional insights into the performance of metabolic networks.

1 Materials and methods

1.1 Bacterial strains, plasmids, and cell growth

All bacterial strains and plasmids used in this study are listed in Table 1. Luria-Bertani medium was used for *E. coli* at 37°C. Brain heart infusion medium was used as a complex medium for *C. glutamicum* at 30°C. As a minimal medium, CGX medium was used with 40 g L^{-1} glucose [24]. If necessary, antibiotics were added at the following concentrations: 100 μg mL^{-1} ampicillin, 50 μg mL^{-1} kanamycin or 20 μg mL^{-1} chloramphenicol for *E. coli* and 25 μg mL^{-1} kanamycin or 10 μg mL^{-1} chloramphenicol for *C. glutamicum*. The strains harboring the plasmid with the P_{tac} promoter were cultivated with the addition of 1 mmol L^{-1} isopropyl-β-D-thiogalactoside (IPTG).

1.2 Construction of plasmids and strains

All genetic modifications were introduced into the *C. glutamicum* genome using the homologous *sacB* recombination system [25,26]. Gene disruption was performed as described previously [27]. Correct integration and in-frame

Table 1 Strains and plasmids used in this study

Strains and plasmids	Relevant characteristics	Reference or source
E. coli		
DH5α	*sup*E44, *φ*80 *lacZ*ΔM15, *hsdR*17, *recA*1, *endA*1, *gyrA*96,*thi*-1,*relA*1	[21]
C. glutamicum		
WT	Wild-type, ATCC 13032	ATCC[a)]
SER-0	WT/pXMJ19	This work
SER-1	WT-Δ*sdaA*	This work
SER-2	WT-Δ*sdaA* Δ*glyR*	This work
SER-3	WT-Δ*sdaA* Δ*glyR serA*^{r}	This work
SER-4	SER-3/pXMJ19	This work
SER-5	SER-3/pWYE1115	This work
SER-6	SER-3/pWYE1116	This work
SER-7	SER-3/pWYE1117	This work
SER-8	SER-3/pWYE1118	This work
Plasmids		
pK18mob*sacB*	Km^r [b)], containing *lacI*^{q}	[22]
pXMJ19	Shuttle vector of *E. coli* and *C. glutamicum.* Cm^r [b)]	[23]
pWYE260	pK18mob*sacB*-Δ*sdaA*	This work
pWYE297	pK18mob*sacB*-Δ*glyR*	This work
pWYE1101	pK18mob*sacB*-*serA*^{r}	This work
pWYE1115	pXMJ19-*pgk*	This work
pWYE1116	pXMJ19-*serA*^{r}	This work
pWYE1117	pXMJ19-*pgk*-*serA*^{r}	This work
pWYE1118	pXMJ19-*serA*^{r}-*pgk*	This work

a) ATCC, American Type Culture Collection. b) Km^r, kanamycin resistance; Cm^r, chloramphenicol resistance.

gene deletion were verified by PCR and sequencing. The genes were amplified with the primers listed in Appendix Table 1 in the electronic version. The PCR product was sub-cloned into the pMD19-T vector (Takara, Japan). The resultant plasmid was digested with *Xba* I/*Kpn* I, and the *pgk* or *serA*^{r}-containing insert was ligated into *Xba* I/*Kpn* I-treated pXMJ19 to construct pWYE1115 (pXMJ19-*pgk*) and pWYE1116 (pXMJ19-*serA*^{r}), respectively. Considering the polar effect, two other plasmids harboring *pgk* and *serA*^{r} in different tandem orders were constructed as follows: the pMD19-T-*serA*^{r}-R was digested with *Kpn* I/*Eco*R I and ligated into pWYE1115 to construct pWYE1117 (pXMJ19-*pgk*-*serA*^{r}). The pMD19-T-*pgk*-R was digested with *Kpn* I/*Eco*R I and ligated into pWYE1116 to construct pWYE1118 (pXMJ19-*serA*^{r}-*pgk*).

1.3 Fermentation in shake flasks and a bioreactor

In the shake-flask growth experiment, *C. glutamicum* strains were precultured in the seed medium CGIII at 30°C and 200 r min^{-1} until the A_{600} reached 12. 1 mL of seed culture was inoculated in a 500-mL baffled shake flask with 30 mL CGX medium [24]. The cells were grown in triplicate at 30°C and shaken at 120 r min^{-1}. The pH was maintained at 7.0–7.2 by supplementation with ammonia.

Fed-batch fermentation was carried out in a 7.5-L bioreactor (BioFlo®/CelliGen®115, New Brunswick, USA) with a working volume of 2 L CGX medium containing 20 g L^{-1} initial glucose. After 4 h of growth, from an A_{600} of 0.2 to an A_{600} of 2.0, 0.8 mmol L^{-1} IPTG was added for induction of P_{tac}. The feed started after the residual sugar concentration was <10 g L^{-1} with feed medium containing 400 g L^{-1} glucose. Temperature was maintained at 30°C using cooling water circulation. The pH was maintained at 7.2 by automated addition of ammonia and 100 mmol L^{-1} H_3PO_4. Dissolved oxygen was determined using a pO_2 electrode and maintained above 30% saturation level by variation of the stirrer speed.

1.4 Determination of intracellular metabolites—sampling, quenching, and extraction

Bioreactor-grown cells were harvested at mid-exponential growth phase. 5 mL of culture was injected into the quenching solutions (60% aqueous methanol+70 mmol L^{-1} Hepes) and directly centrifuged at 5200×*g* at –20°C. After the removal of the supernatant, cell pellets were immediately frozen at –80°C [28,29]. Subsequently, cell pellets were extracted three times by boiling at 90°C for 5 min as described previously [30]. The obtained extracts were evaporated to dryness in a pump under vacuum at room temperature. After suspension of the extracts in 500 μL of water, cell debris was removed by centrifugation for 10 min at 5500×*g*. After decanting, the supernatant was stored at –80°C until further analysis [29]. To reduce variations in feeding and maintain accuracy, SER-0 and SER-8 were grown in CGX minimal medium with 40 g L^{-1} glucose in a batch-fermentation process.

1.5 Analytical methods

The glucose concentration was measured using an SBA-40D biosensor analyzer (Institute of Biology of Shandong Province Academy of Sciences, Shandong, China). Cell dry mass (CDM) was estimated with a spectrophotometer (1.0 A_{600} is equivalent to 0.27 g L^{-1} CDM). Amino acids in the culture supernatant were determined using high performance liquid chromatography with a Zorbax Eclipse XDB-C_{18} column (4.6 mm×250 mm, 5 μm; Agilent) at 40°C after derivatization with 2,4-dinitrofluorobenzene. Mobile phase A was 55% (v/v) acetonitrile whereas mobile phase D consisted of 40 mmol L^{-1} KH_2PO_4 at pH 7.0–7.2. The flow rate of the mobile phase was 1 mL min^{-1}.

1.6 Elementary mode and metabolic flux analysis

The *C. glutamicum* metabolic network was constructed as shown in Figure 3. The model was based on utilizing glucose as the carbon source. The complete set of reactions used for the elementary mode analysis is listed in Appendix Table 2 in the electronic version. The pathway details were collected from the KEGG database (http://www.kegg.com), comprising reactions involved in the glycolysis pathway (EMP), the pentose phosphate pathway (PPP), the tricarboxylic acid cycle (TCA), anaplerotic reaction converting phosphoenol pyruvate (PEP) into oxaloacetic acid (OAA) and synthetic reactions for serine, glycine, pyruvate, alanine, valine, isoleucine, leucine, glutamine, lysine, succinic acid and histidine. Intracellular fluxes were estimated from measurements of metabolite uptake and output rates (exchange fluxes) by a pseudo-steady-state approximation [31,32]. Elementary mode analysis was constructed using MATLAB software version 7.0 (MathWorks). Stoichiometric coefficients of the external metabolites present in the modes were written in the form of a matrix equation (eq. (1)):

$$Ax(t)=r(t), \quad (1)$$

where A is an $m\times n$ matrix of stoichiometric coefficients, the number of rows was equal to the number of external metabolites and the number of columns was equal to the number of modes [32].

The stoichiometric model in this study (Appendix Tables 2 and 3 in the electronic version) consists of 29 reactions and 17 compounds; the degree of freedom (DF) is 29–17=12, which means that changes in the rate of twelve compounds should be measured to determine all of the reaction rates in the metabolic network. External metabolites considered to generate flux distribution maps during the course of the fermentation were measured for quantification, including glucose, serine, glycine, alanine, valine, isoleucine, leucine, glutamine, lysine, succinic acid, histidine and biomass.

2 Results

2.1 Metabolic engineering of *C. glutamicum* for serine production

Our overall strategy to optimize *C. glutamicum* for L-serine production was to simultaneously enhance its biosynthesis and reduce its degradation (Figure 1; also see introduction section). For the latter, an *sdaA*-null strain, SER-1, was first created to block the degradation of L-serine to yield pyruvate. The *glyR* gene was deleted subsequently in SER-1 to create the *sdaA*- and *glyR*-double-null mutant, SER-2. SER-2 lacks the transcriptional activator GlyR to up-regulate the SHMT expression in the stationary phase and is supposed to reduce the conversion of L-serine to glycine. In order to enhance the L-serine biosynthesis, strain SER-3 was created from SER-2 to have the endogenous PGDH replaced with a C-terminal 197 amino acid truncated one. Based on SER-3, three further steps were applied to increase the supply of the key precursor substrate for L-serine biosynthesis, 3-phosphoglycerate, by over-expressing either PGK (SER-5) or the feedback-resistant PGDH variant ($PGDH^r$) (SER-6). Following this strategy, a series of mutant strains were generated to facilitate the metabolic dissection of the contribution of these individual enzymes to L-serine accumulation. This design also made the analysis of the effects of these gene modifications on the metabolism of *C. glutamicum* possible (Figure 1).

2.2 Metabolic analysis of the mutant strains for L-serine production

Use of both the SER-1 and SER-2 strains did not improve the L-serine production, indicating that blocking the degradation of L-serine to pyruvate and reducing the conversion of L-serine to glycine hardly contributed to L-serine accumulation. Strain SER-3 can accumulate L-serine at a level of 0.01 mmol L^{-1}, suggesting that the rate-limiting enzyme, PGDH, might be a minor metabolic node for L-serine accumulation in *C. glutamicum* and further improvement of the metabolic flux toward L-serine biosynthesis was necessary. Strain SER-3 was engineered to harbor plasmid-based *pgk* (SER-5) or feedback-resistant $serA^r$ (SER-6). Use of these two strains further increased L-serine accumulation, suggesting that increasing the supply of precursors contributed to L-serine biosynthesis. Then, *pgk* and $serA^r$ were co-expressed in strain SER-3. The resulting strain, SER-8, showed a significant increase in L-serine accumulation, reaching a maximal level of (0.425±0.019) mmol L^{-1} during early fermentation after 9 h of cultivation, a 14.16-fold increase over the control strain SER-4 in shake flasks.

2.3 Physiological evaluation of SER-8 on an increased scale

2.3.1 The fermentation of SER-8 in a 7.5-L bioreactor

In order to assess the overall consequences of these five

genetic modifications on strain growth and L-serine production, a fed-batch fermentation in glucose minimal medium was performed in a 7.5-L bioreactor. As shown in Figure 2A, the fermentation process exhibited five distinct phases: Phase I, pure growth (0–15 h); Phase II, transition from pure growth to L-serine production (15–22 h); Phase III, L-serine accumulation at the expense of growth (22–28 h); Phase IV, L-serine productivity loss and a small degree of restoration of growth (28–37 h); and Phase V, death and L-serine yield stability (37–60 h). The specific growth of strain SER-8 was 0.26 h^{-1}, significantly lower than that of the control strain, SER-0 (μ_{max}=0.49 h^{-1}). The maximal CDM reached 16.71 g L^{-1} after 22 h of cultivation and the maximal L-serine production reached 1.21 mmol L^{-1} after 28 h.

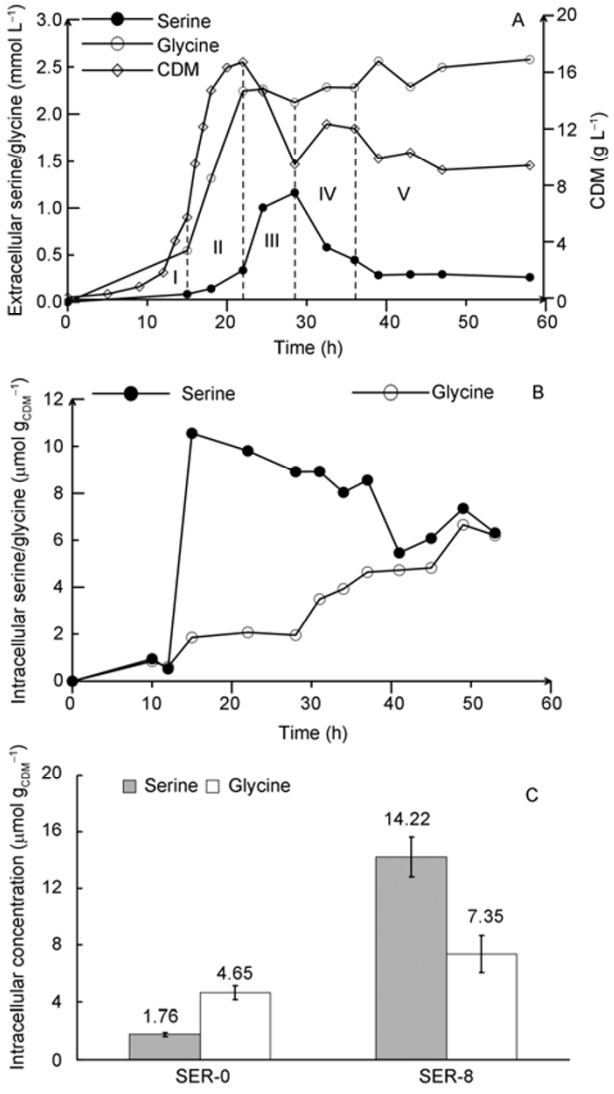

Figure 2 Physiological variation of SER-8 in a 7.5-L bioreactor. A, Performance of strain SER-8 in a fed-batch fermentation on CGX minimal medium with 20 g L^{-1} glucose showing growth, the accumulation of extracellular L-serine and glycine. B, Variations of intracellular L-serine and glycine levels depending on the incubation period in the medium. C, Intracellular pools of L-serine and glycine in mid-exponential phase; to reduce variation of feeding glucose, SER-0 and SER-8 were grown in CGX minimal medium containing 40 g L^{-1} glucose in a batch-fermentation. Error bars show the standard deviation for triple biological samples.

2.3.2 The extra- and intra-cellular variation of L-serine and glycine

Extracellular L-serine reached a maximum at 28 h and then decreased until the end of the fermentation. The extracellular glycine accumulated at the beginning of cultivation and reached as high as 2.24 mmol L^{-1}. After that, the glycine level kept increasing slightly along with the utilization of L-serine even after completion of Phase IV. For all fermentative phases, the extracellular L-serine level was much lower than glycine (Figure 2A). On the other hand, the intracellular L-serine level reached a maximum of 10.56 μmol g_{CDM}^{-1} after about 15 h of cultivation prior to its excretion (Figure 2B) and declined afterwards. On the contrary, the intracellular glycine was significant lower than the intracellular L-serine and increased continuously during the whole fermentation process (Figure 2B).

2.3.3 The intracellular pool of L-serine and glycine

A comparison of the intracellular pool of L-serine and glycine from SER-8 to that of SER-0 indicated that an 8.08-fold increase was achieved, i.e., from (1.76±0.11) to (14.22±1.41) μmol g_{CDM}^{-1} while the glycine pool increased by 34%, from (4.65±0.47) to (7.35±1.35) μmol g_{CDM}^{-1} (Figure 2C). The increased intracellular pool of both L-serine and glycine in SER-8 indicated that a higher flux was indeed directed into the L-serine biosynthesis pathway in the desired manner as designed. Interestingly, it was also noticed that the intracellular L-serine pool was much lower than glycine in SER-0 (Figure 2C). In contrast, SER-8 showed a higher intracellular pool of L-serine than glycine.

2.4 Comparative metabolic flux analysis of strains SER-0 and SER-8

To compare the flux distribution of the glycolysis pathway (EMP), the pentose phosphate pathway (PPP), the TCA cycle and the L-serine metabolism pathway in SER-0 and SER-8, the relative fluxes of the metabolic pathway were normalized to the glucose uptake rate (%) as shown in Figure 3. Significant changes in the flux distribution of EMP and PPP were observed between the SER-0 and SER-8 strains. The ratio of EMP flux in strain SER-8 significantly increased, implying the over-expression of PGK redirected more flux from PPP toward EMP. As a result of the increased EMP, SER-8 exhibited an almost 27% increase of the relative flux through the TCA cycle. Despite the fact that the supply of 3-phosphoglycerate increased, only a relatively small flux (0.68%) was assigned to the L-serine biosynthesis pathway. The flux toward L-serine biosynthesis was elevated to 2 fold, indicating the over-expression of $PGDH^r$ redirected more flux to the L-serine biosynthesis pathway. In addition, the flux from L-serine to glycine

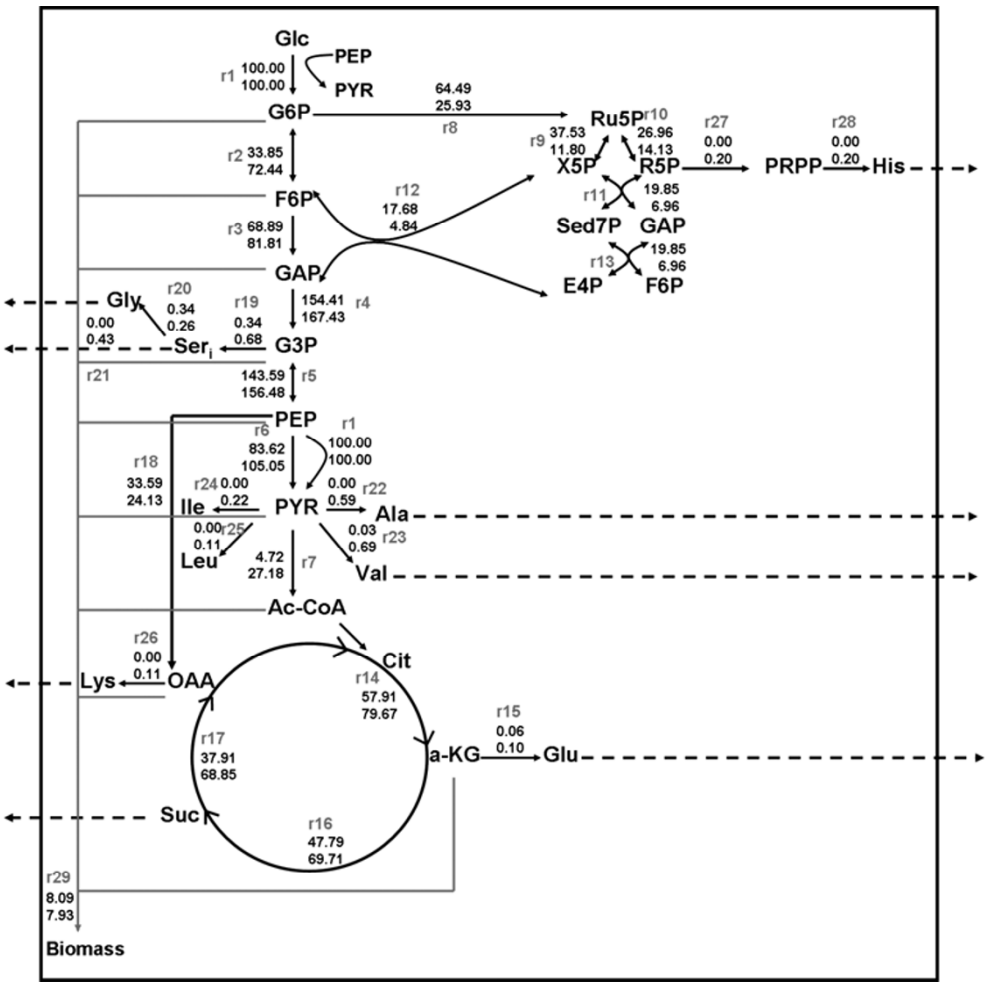

Figure 3 *In vivo* carbon flux distribution in the central metabolism of the control strain SER-0 (upper numbers of each pair) and the mutant SER-8 (lower numbers of each pair) during growth on glucose. All fluxes are expressed as the molar percentage of the mean specific glucose uptake rate (2.47 mmol g^{-1} h^{-1} for the control strain SER-0, 1.58 mmol g^{-1} h^{-1} for the mutant SER-8), which is defined as 100%. A replicate batch experiment showed consistent results with a standard deviation of 10%.

dropped by 24%, suggesting the conversion of L-serine to glycine was reduced by deleting the activator GlyR to down-regulate SHMT at the transcriptional level. Furthermore, the extracellular L-serine shunt flux increased to 0.43% in strain SER-8. In contrast, the total L-serine in strain SER-0 was utilized for the generation of glycine, resulting in no net L-serine outside the cell for SER-0.

3 Discussion

In this study, a systematical investigation was performed on *C. glutamicum* to determine the effects of different genetic modifications on L-serine production. The mutant SER-8, a combination of five modified genes in *C. glutamicum*, exhibited a higher L-serine production (Figure 2A). According to the changes in flux distribution between strain SER-8 and SER-0 (control strain), over-expressing PGK can "push" carbon flux into the G3P pool, whereas engineering of $PGDH^r$ can "pull" the carbon flux from the G3P pool into the L-serine biosynthesis pathway. This demonstrates that increases of the supply precursors are responsible for improving the flux distribution in L-serine biosynthesis.

The conversion of L-serine to glycine is the main degradation pathway of L-serine in *C. glutamicum*. It is impossible to disrupt the *glyA* gene since this reaction provides the majority of the C_1 units for cell growth [12]. When the expression of the *glyA* gene was controlled under the P_{tac} promoter, L-serine accumulation was obviously increased in the absence of IPTG and simultaneously the growth of cells was seriously reduced [20]. Nevertheless, strong inhibition of *glyA* expression resulted in apparent instability of the engineered strain [16]. Our results showed that despite the deletion of the activator GlyR was believed to be responsible for decreasing the conversion of L-serine to glycine to a certain degree, the ratio of flux toward glycine was still 38.2%. It further demonstrated that the conversion of L-serine to glycine is a rate-limiting step for L-serine accu-

mulation. Therefore, controlling the *glyA* expression under a promoter of relatively weak activity would further reduce the conversion of L-serine to glycine.

L-Serine synthesis and accumulation was probably induced by growth limitation in these strains. This notion was supported by the observation of the decreases in the specific growth rate and biomass yield. The genetic modifications in *C. glutamicum* had a significant impact on the maximum growth rate (μ_{max}) in accordance with previous reports [20]. Amino acid production under conditions of general growth limitations has been described elsewhere [18,33]. The decrease of the specific growth rate reflected a redirection of carbon flux due to genetic modifications of the L-serine biosynthetic pathway toward anabolic routes, and the co-metabolism of L-serine with glucose has shown that a distinct metabolic switch occurs—from the stage of incorporation of L-serine into biomass [9]. The growth maintenance during the stationary phase partially occurred at the expense of L-serine, which was evident from the gradual decrease in the concentration of L-serine and a concomitant sharp increase of glycine (Figures 2A and B). The positive effect of the genetic modifications on L-serine formation was diminished by simultaneous reutilization of L-serine to sustain physiological fitness. This suggests that L-serine biosynthesis occurred mainly at the expense of growth, and it would be optimal to decrease the specific growth rate for L-serine accumulation. Taken together, our findings imply that L-serine pool management was well correlated with the viability of *C. glutamicum*.

Interestingly, extracellular glycine continuously accumulated during all fermentative phases. For *E. coli*, the glycine cleavage (GCV) system converts glycine to 5,10-methylenetetrahydrofolate [34]. This system plays an important role in maintaining the intracellular homeostatic balance between glycine and concentrations of C_1 units [34]. However, glycine is an end-product without an efficient supply as a direct precursor in *C. glutamicum* [35]. Therefore, the export of redundant glycine might be an alternative way for *C. glutamicum* to maintain an intracellular glycine metabolic equilibrium. These findings make it possible to consume the intracellular redundant glycine by integrating the GCV system of *E. coli* into the *C. glutamicum* chromosome. In this way, the GCV system will generate more active C_1 units to provide a balance between cell growth and L-serine accumulation in *C. glutamicum*. Therefore, improving the generation of C_1 units by introducing the GCV system to degrade the excessive glycine is a promising target for the metabolic engineering of *C. glutamicum* for L-serine production.

In this study, several systems metabolic engineering strategies have been attempted to develop a L-serine production strain. Since the entire metabolic and regulatory networks are woven into a global system in an integrated manner, some target gene engineering is still necessary until an optimal strategy is obtained [36,37]. The work reported herein provides a framework of information on the direct fermentative production of L-serine from glucose.

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