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ORIGINAL ARTICLE

Characterization, modification, and overexpression of 3-phosphoglycerate dehydrogenase in *Corynebacterium glutamicum* for enhancing L-serine production

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Abstract The direct fermentative production of L-serine from renewable biomass using *Corynebacterium glutamicum* is attracting increasing attention. In this study, wild-type *C. glutamicum* SYPS-062 produced up to 6.65±0.23 g/L L-serine; to further improve L-serine production, the *serA* gene was cloned, and the C-terminal domain of 3-phosphoglycerate dehydrogenase (PGDH) from this strain was truncated. When expressed in *Escherichia coli*, the resultant mutein SerAΔ197 showed a specific PGDH activity of 1.092±0.05 U/mg protein, representing a decrease of 25.87 % from that encoded by *serA*, and was no longer sensitive to high concentrations of L-serine. When $serA^{\Delta 591}$ was overexpressed in *C. glutamicum* SYPS-062, the activity of PGDH in *C. glutamicum* pJC1-*tac*-$serA^{\Delta 591}$ increased by 47.72 %, and the resultant strain *C. glutamicum* pJC1-*tac*-$serA^{\Delta 591}$ could accumulate 7.69±0.22 g/L L-serine. Furthermore, when $serA^{\Delta 591}$ was overexpressed in *C. glutamicum* SYPS-062*ΔsdaA*, the resultant strain could accumulate 8.84 ±0.23 g/L L-serine at 102 h, and the yield of L-serine on cells (Y p/x) improved by 60 % when compared with that noted in the control. These results demonstrate that L-serine production in *C. glutamicum* SYPS-062 could be improved by overexpressing a C-terminal truncation of PGDH in combination with other genetic modifications.

Keywords L-Serine · *Corynebacterium glutamicum* · PGDH · Feedback inhibition · Fermentation

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Introduction

L-Serine is used widely as a third-generation compound amino acid infusion and nutritional supplement in the pharmaceutical industry, and as an excellent moisturizing agent in anti-aging skin care cosmetics. Additionally, it is utilized as a building block for chemical and biochemical purposes and as an animal feed to promote growth and development in cattle. The demand for L-serine is about 300 tons per year (Peters-Wendisch et al. 2005). Currently, L-serine production still relies mainly on extraction from protein hydrolysates as well as on enzymatic and cellular conversion from the precursor glycine along with a C1 compound (Hagishita et al. 1996; Hsiao and Wei 1986; Izumi et al. 1993). In recent years, the direct fermentative production of L-serine from renewable biomass has attracted increasing attention. *Sarcina albida* was first described to have been used for L-serine production from glycine (Ema et al. 1979; Omori et al. 1983). In addition, *Escherichia coli* has also been genetically engineered to produce L-serine from glucose intracellularly (Li et al. 2012). *Corynebacterium glutamicum*, a Gram-positive bacterium, is particularly well suited to the production of cellular metabolites such as amino acids, and has been used for the industrial production of different amino acids, especially for the production of L-glutamate (Eggeling and Sahm 1999) and L-lysine (Vallino and Stephanopoulos 1994). A wealth of knowledge about the physiology, biochemistry, and genetics of these microorganisms has been accumulated, which forms the basis for efficient strain development by metabolic engineering (Eggeling and Bott 2005; Sauer and Eikmanns 2005).

The synthesis of L-serine is carried out in three steps, starting from 3-phosphoglycerate. The corresponding L-serine synthesis genes are *serA*, *serC*, and *serB*. The *serA* gene encodes 3-phosphoglycerate dehydrogenase (PGDH), the *serC* gene encodes phosphoserine aminotransferase, and the *serB* gene encodes phosphoserine phosphatase. It has been reported that PGDH is inhibited by L-serine in *E. coli* (Grant et al. 1996) and *Bacillus subtilis* (Saski and Pizer 1975). PGDH proteins, encoded by *serA*, belong to the family of D-isomer 2-hydroxyacid dehydrogenases. The primary structure of SerA polypeptides is characterized by two highly conserved D-2-hydroxyacid binding motifs in their N-terminal part (Grant 1989). On analyzing the three-dimensional (3D) structure of *E. coli* PGDH, a homotetramer with four active sites and four effector-binding sites was found. In addition, it has been noted that the PGDH from *B. subtilis* and plants are most likely to be homotetramers (Ho et al. 1999; Saski and Pizer 1975). These proteins have been reported to share a very similar 3D structure with that of PGDH from *E. coli* owing to their high sequence identity over a long N-terminal part with the *E. coli* enzyme (Schuller et al. 1995). In *C. glutamicum* ATCC 13032, the *serA* gene encoding PGDH has been isolated and functionally characterized (Peters-Wendisch et al. 2002). Overexpression of *serA* in *C. glutamicum* has been observed to result in a 16-fold increase in specific PGDH activity, reaching a value of 2.1 U/mg protein, with the activity being inhibited by high concentration of L-serine; however, the mutein SerAΔ197 showed a specific PGDH activity of 1.3 U/mg protein, with the activity no longer being sensitive to L-serine (Peters-Wendisch et al. 2002). In addition, *C. glutamicum* has been reported to possess a high capacity to degrade L-serine in the presence of glucose and L-serine dehydratase (L-SerDH) encoded by *sdaA*, which is involved in L-serine degradation (Netzer et al. 2004). The strain SYPS-062, which can directly convert sugar to L-serine, has been isolated and identified as *C. glutamicum* in our laboratory.

The goal of the present study was to isolate the *serA* gene from wild-type *C. glutamicum* SYPS-062, and compare its sequences with that of the *serA* gene from *C. glutamicum* ATCC 13032. Subsequently, the effect of truncation of the C-terminal domain of this gene, analysis of PGDH activity in *E. coli*, and examination of the enzyme's sensitivity to L-serine were investigated. Then, to further improve L-serine production, the C-terminal region truncated PGDH was overexpressed in *C. glutamicum* SYPS-062 and *C. glutamicum* SYPS-062Δ*sdaA*.

Materials and methods

Strains, plasmids, and culture conditions

The wild-type strain *C. glutamicum* SYPS-062, which can produce L-serine directly from sugar, was screened naturally and stored at −80 °C in our laboratory, and was identified by using 16S rDNA sequence analysis, the GenBank accession number is KJ 525753, and the deposited number is CGMCC No.1843. The bacterial strains and plasmids used in this study are listed in Table 1.

Luria-Bertani (LB) medium (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl) was used for the purification of plasmids obtained from *E. coli*. The medium used for the seed culture of *C. glutamicum* contained the following: 20 g/L glucose, 10 g/L tryptone, 10 g/L yeast extract, 10 g/L beef extract, and 3 g/L NaCl. The fermentation medium consisted of the following: 60 g/L sucrose, 30 g/L $(NH_4)_2SO_4$, 3 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.02 g/L $FeSO_4 \cdot 7H_2O$, 0.02 g/L $MnSO_4 \cdot 7H_2O$, 50 μg/L biotin, 10 μg/L streptomycin, 450 μg/L thiamine, and 30 g/L calcium carbonate. The selective medium contained 10 g/L peptone, 10 g/L beef extract, 5 g/L yeast extract, 3 g/L NaCl, 20 g/L agar, and 40 mg/L kanamycin. Prior to use, the pH of these media were set to 7.0 with NaOH and they were heat sterilized for 20 min at 121 °C, except the fermentation medium, which was heat sterilized for 10 min.

The seed culture was cultivated in a 250-mL flask containing 20 mL medium at 30 °C in an orbital shaker at 120 rpm. Fermentation was carried out in a 250-mL flask containing 20 mL medium. The inoculation size was 5 % (v/v), and all experiments were carried out in triplicate.

Construction of plasmids and strains

The genes *serA* and $serA^{\Delta 591}$ were amplified by PCR using the genome of *C. glutamicum* SYPS-062 as template. The reaction conditions employed for *serA* were as follows: incubation at 94 °C for 5 min, 30 cycles at 94 °C for 45 s, 57 °C for 45 s, and 72 °C for 90 s. The reaction conditions used for $serA^{\Delta 591}$ were as follows: incubation at 94 °C for 5 min, 30 cycles at 94 °C for 45 s, 58 °C for 45 s, and 72 °C for 60 s. A 1,593-bp fragment of the *serA* gene and 1,002-bp fragment of the $serA^{\Delta 591}$ gene were obtained. All primers used in this study are listed in Table 2.

The *serA* and $serA^{\Delta 591}$ gene fragments obtained were ligated to pMD18-T Simple Vector to create pMD18-T-*serA* and pMD18-T-$serA^{\Delta 591}$, respectively. Subsequently, both these vectors and plasmid pET-28a were digested with *Bam*HI and *Xba*I, and ligated together to create the pET-28a-*serA* and pET-28a-$serA^{\Delta 591}$, respectively. The plasmids were introduced into *E. coli* BL21 cells using the $CaCl_2$ transformation method, and the corresponding transformants BL21(pET-28a-*serA*) and BL21(pET-28a-$serA^{\Delta 591}$) were obtained. Similarly, the plasmids were introduced into *C. glutamicum* SYPS-062 via electroporation, and the corresponding transformants SYPS-062 (pJC1-*tac*-$serA^{\Delta 591}$) and SYPS-062 (pJC1-*tac*-*serA*) were obtained.

Table 1 Plasmids and strains used in this study

Plasmids/strains	Relevant characteristics	Source
Plasmids		
pET-28a	Vector, Kan^r	Laboratory collection
PET-28a-*serA*	pET-28a with 1,593 bp PCR product containing *serA*	This work
pET-28a-$serA^{\Delta 591}$	pET-28a with 999 bp PCR product containing $serA^{\Delta 591}$	This work
pMD18-T vector	Cloning vector, Ap^r	Purchased from Takara (Kyoto, Japan)
pK18mob*sacB*	*E. coli–C. glutamicum* shuttle vector, Tet^r	–
pK18mob*sacB*-Δ*sdaA*	pK18mob*sacB* with *sdaA* deleting cassette	This work
Corynebacterium glutamicum strains		
SYPS-062	Can directly produce L-serine from sugar	Laboratory collection
SYPS-062 (pJC1-*tac-serA*)	SYPS-062 with *serA* overexpression	This work
SYPS-062 (pJC1-*tac-*$serA^{\Delta 591}$)	SYPS-062 with $serA^{\Delta 591}$ overexpression	This work
SYPS-062Δ*sdaA*	SYPS-062 carrying a deletion in the Δ*sdaA* gene	This work
SYPS-062Δ*sdaA* (pJC1-*tac-*$serA^{\Delta 591}$)	SYPS-062Δ*sdaA* with $serA^{\Delta 591}$ overexpression	This work
Escherichia coli strains		
BL21	DE3, λ	Laboratory collection
BL21 (pET-28a-*serA*)	BL21 with plasmid pET-28a-*serA*	This work
BL21 (pET-28a-$serA^{\Delta 591}$)	BL21 with plasmid pET-28a- $serA^{\Delta 591}$	This work

To enable *sdaA* deletion, pK18mob*sacB*-Δ*sdaA* was constructed. For this purpose, primers P-A1 and P-A2 were used to amplify a 427-bp fragment of the 5′-end of *sdaA*, and primers P-A3 and P-A4 were employed to amplify a 521-bp fragment of the 3′-end of *sdaA*. The resulting fragments were used in a second PCR with P-A1 and P-A4 as primers. The 957-bp fragment obtained was ligated into pMD18-T vector to create pMD18-T-*sdaA*; subsequently, this plasmid was digested with *Sal*I and *Eco*RI, and ligated to the vector pK18mob*sacB*, which was digested with the same enzymes, to generate pK18mob*sacB*-Δ*sdaA*. The generated pK18mob*sacB*-Δ*sdaA* was used to replace the intact chromosomal *sdaA* gene in *C. glutamicum* SYPS-062 with the truncated *sdaA* gene, resulting in the strain *C. glutamicum* SYPS-062Δ*sdaA*.

Table 2 Primers used in this study

Primer	Sequence (5′ -3′)
PserAF	ATAGGATCCATGAGCCAGAATGGCCGT
PserAR	GGGGTCTAGATTAGTCAAGATCAACCTGG
$PserA^{\Delta 591}F$	ATAGGATCCATGAGCCAGAATGGCCGT
$PserA^{\Delta 591}R$	TGTTCTAGATTAAGCCAGATCCATCCACACAGC
P-A1	ATGGCTATCAGTGTTGTTGA
P-A2	CCCATCCACTAAACTTAAACACGTCATAATGA ACCCACC
P-A3	TGTTTAAGTTTAGTGGATGGGCCGACTAATGG TGCTGCG
P-A4	CGGGAAGCCCAAGGTGGT

Protein analysis

The recombinants *E. coli* BL21(pET-28a-*serA*) and BL21 (pET-28a-$serA^{\Delta 591}$) were inoculated into 20 mL Luria-Bertani (LB) medium containing 100 μg/mL ampicillin and grown at 28 °C. The expression of PGDH was induced with 0.4 mM isopropyl β-D-L-thiogalactopyranoside when the optical density at 600 nm (OD_{600}) reached 0.3; the broth was then harvested after 3 h culture, and centrifuged at 8,000 rpm for 10 min. The cells were suspended in phosphate buffer saline, and disrupted by ultrasonic mixing. Crude enzyme solution in culture supernatants were obtained by centrifugation. The crude enzyme solution was purified using affinity chromatography on a Ni-NTA agarose column, and a relatively high purity of PGDH could be obtained by this method. Finally, SDS-PAGE was performed to analyze the molecular weight of these proteins.

PGDH assay

Crude extracts were prepared by sonication and subsequent centrifugation of cells grown in minimal medium and harvested in the exponential growth phase. The extracts were passed over an FF column and equilibrated with 50 mM phosphate buffered saline (PBS) at pH 7.4. The PGDH activity was determined in an assay containing 1 mL 40 mM PBS (pH 7.5), 1 mM dithiothreitol, 0.25 mM NADH, and crude extract (10–300 μg protein) (Zhao and Winkler 1996). The reaction was started with 5 mM α-ketoglutaric acid and the reduction of NADH

was measured spectrophotometrically at 340 nm and 30 °C for 10 min. One unit of enzyme activity was defined as the reduction of 1 mM NADH in 1 min.

Analytical methods

The cell density was determined by measuring the optical density of the culture broth at 562 nm (OD_{562}) using a spectrophotometer-UV2100 (Third Analytical Instrument Factory, Shanghai, China). The cell concentration was expressed as dry cell weight (DCW), calculated according to an experimentally determined formula: DCW (g/L)= $0.1925 \times OD_{562}$.

Sucrose was assayed using the resorcin-spectrometric method. The concentrations of L-serine and other amino acids (L-alanine, L-valine, glycine, L-threonine, and lysine) were determined using an Agilent 1100 Series HPLC (Agilent Technologies, Santa Clara, CA), equipped with a Hypersil ODS-C18 column (4×125 mm) and a fluorescence detector (FID). The column was maintained at 40 °C and elution was performed using 20 mM buffer [sodium acetate:methanol:acetonitrile=1:2:2 (v/v)] as the mobile phase at a flow rate of 1.0 mL/min.

Results

Isolation of the *serA* gene from *C. glutamicum* SYPS-062

The *serA* gene from *C. glutamicum* SYPS-062 was cloned and a 1,593-bp fragment of this gene was obtained (Fig. 1a), and found to encode a PGDH polypeptide of 530 amino acids. This gene fragment has been submitted to GenBank under accession no. HQ329183. The nucleotide sequence alignments of the 1,593-bp *serA* gene fragment showed high identity (99.62 %) with the sequence of the *serA* gene from *C. glutamicum* ATCC 13032. Further sequence analysis showed that alanine was replaced by threonine ($Ala^{43}Thr$) and methionine was replaced by leucine ($Met^{93}Leu$). The other mutations of the nucleotide sequence led to no change in amino acid.

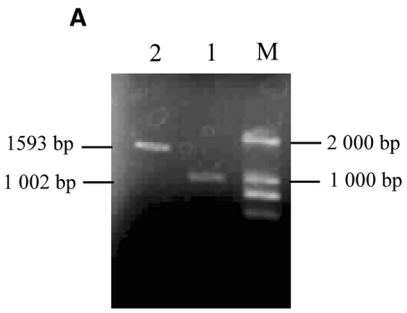
Truncation of the C-terminal domain and analysis of PGDH activity in *E. coli*

To remove the feedback inhibition of PGDH by L-serine in *C. glutamicum* SYPS-062, we also focused on the C-terminal region to derive PGDH muteins that are not sensitive to L-serine. A mutein of *serA* was constructed by truncating 591 nucleic acid bases at the 3′-terminal end of the gene (Fig. 1a). When expressed in *E. coli*, as shown in Fig. 1b and Fig. 2a, the mutein SerAΔ197 showed a specific PGDH activity of 1.092±0.08 U/mg protein—a decreased of 25.87 % compared to the PGDH encoded by *serA*.

To determine whether the mutein was inhibited by L-serine, a PGDH assay was carried out in the presence of different concentrations of L-serine. As shown in Fig. 2b, under these conditions, the PGDH activity decreased dramatically with the increase in L-serine concentration (from 0 to 40 mM), and only 48.56 % of the original activity was observed when the L-serine concentration increased to 40 mM. A further increase in L-serine concentration (from 40 to 150 mM) led to a slight decrease in enzyme activity. On the other hand, with regard to the mutein of PGDH, the activity exhibited a slight decrease when L-serine concentration was increased from 0 to 40 mM, and almost no decrease in activity was observed when the L-serine concentration was increased from 40 to 150 mM. These results indicated that feedback inhibition of PGDH by L-serine in *C. glutamicum* SYPS-062 could be removed by truncating 591 nucleic acid bases at the 3′-terminal end of *serA*.

Overexpression of $serA^{\Delta 591}$ or *serA* in *C. glutamicum* SYPS-062

In order to improve L-serine production, the endogenetic genes *serA* and $serA^{\Delta 591}$ were overexpressed in *C. glutamicum* SYPS-



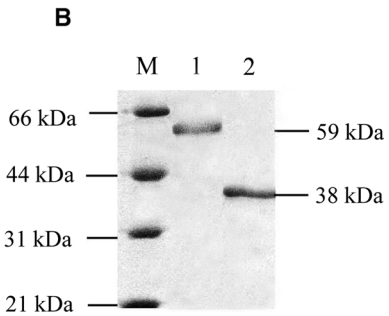


Fig. 1a,b Modification and expression of the *serA* gene from *Corynebacterium glutamicum* SYPS-062. **a** Agarose gel electrophoresis of *serA* and $serA^{\Delta 591}$ PCR fragments. Lanes: *M* DNA marker, *1 serA*, *2* $serA^{\Delta 591}$. **b** SDS-PAGE of protein after purification from crude extracts of *Escherichia coli* strains overexpressing *serA* or $serA^{\Delta 591}$. Lanes: *M* Protein molecular weight marker, *1* protein encoded by *serA* from *E. coli* BL21 (pET-28a-*serA*), *2* protein encoded by $serA^{\Delta 591}$ from BL21 (pET-28a-$serA^{\Delta 591}$)

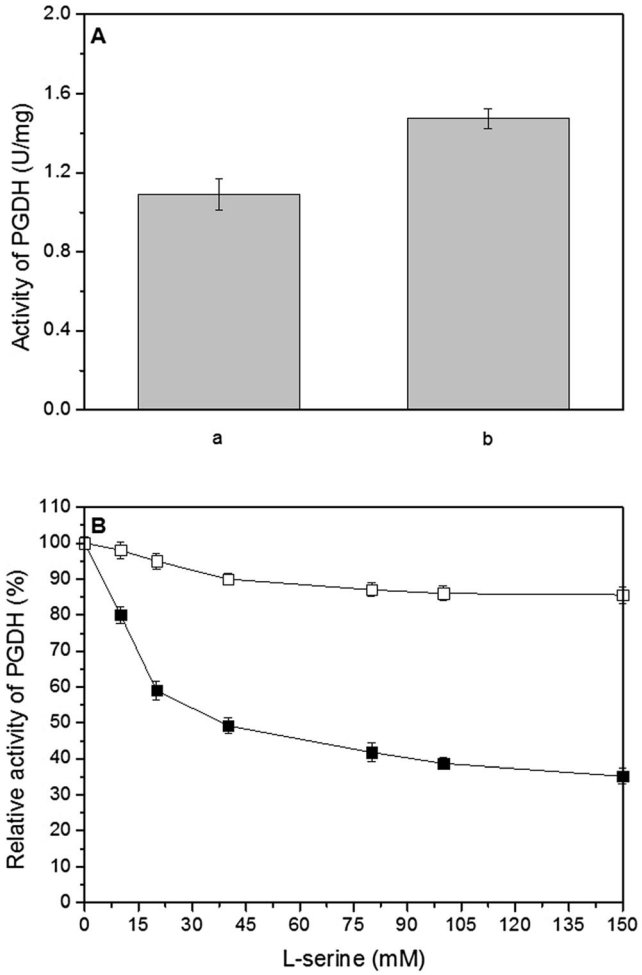


Fig. 2a,b 3-Phosphoglycerate dehydrogenase (PGDH) activity and characteristics in BL21 (pET-28a-*serA*) and BL21 (pET-28a-*serA*$^{\Delta 591}$). **a** *a* BL21 (pET-28a-*serA*$^{\Delta 591}$), *b* BL21 (pET-28a-*serA*). **b** *Closed squares* BL21 (pET-28a-*serA*), *open squares* BL21 (pET-28a-*serA*$^{\Delta 591}$)

062. Enzyme assay showed that the activities of the engineered strains *C. glutamicum* SYPS-062 (pJC1-*tac-serA*$^{\Delta 591}$) and SYPS-062 (pJC1-*tac-serA*) were 0.827±0.04 and 0.939±0.05 U/mg, which were noted to be 47.42 and 67.38 % higher than that of the parent strain *C. glutamicum* SYPS-062, respectively. The fermentation profile showed that, when compared with the parent strain, *C. glutamicum* SYPS-062 (pJC1-*tac-serA*$^{\Delta 591}$) could accumulate 7.69±0.22 g/L L-serine (15.64 % higher than the parent strain); however, a lower increase was observed in *C. glutamicum* SYPS-062 (pJC1-*tac-serA*) (7.21±0.21 g/L).

Overexpression of *serA*$^{\Delta 591}$ in *C. glutamicum* SYPS-062Δ*sdaA*

Based on an earlier report indicating a significant contribution of *sdaA*-encoded L-serine dehydrogenase to L-serine degradation in *C. glutamicum* ATCC 13032 (Netzer et al. 2004), to further improve L-serine production we constructed the *sdaA* deletion strain *C. glutamicum* SYPS-062Δ*sdaA* and investigated the effect of *serA*$^{\Delta 591}$ overexpression on L-serine accumulation in this background. The resultant strain *C. glutamicum* SYPS-062Δ*sdaA* (pJC1-*tac-serA*$^{\Delta 591}$) exhibited a low growth rate with a maximum DCW of 5.22±0.20 g/L, which was 17.1 % lower than that of the control (Fig. 3a). However, when compared with the control, this strain could accumulate up to 8.84±0.23 g/L L-serine after 102 h of cultivation, which was 32.93 % higher (Fig. 3b), and the yield of L-serine on cell (Yp/x) improved by 60 % (Fig. 3c). In addition, the recombinant strain *C. glutamicum* SYPS-062Δ*sdaA* (pJC1-*tac-serA*$^{\Delta 591}$) exhibited a weak ability to consume sucrose as compared to the parent strain *C. glutamicum* SYPS-062 (Fig. 3d).

Discussion

The goal of this study was to isolate the *serA* gene of the wild-type *C. glutamicum* SYPS-062, investigate whether the feedback inhibition could also be removed by truncating the C-terminal domain, and examine the possibility of improving L-serine production by overexpressing PGDH with the truncated C-terminal domain. We succeeded in characterization of the wild-type *serA* gene, in which alanine was replaced by threonine (Ala^{43}Thr) and methionine was replaced by leucine (Met^{93}Leu). In addition, we found that the C-terminal-domain-truncated PGDH was no longer sensitive to high concentrations of L-serine. Overexpression of *serA*$^{\Delta 591}$ in *C. glutamicum* SYPS-062 led to a slight increase in L-serine production, whereas a dramatic increase in L-serine production and Yp/x was observed when *serA*$^{\Delta 591}$ was overexpressed in *C. glutamicum* SYPS-062Δ*sdaA*.

In this study, we isolated the *serA* gene from *C. glutamicum* SYPS-062, whose length was similar to that of the *serA* gene from *C. glutamicum* ATCC 13032. However, alanine was replaced by threonine (Ala^{43}Thr) and methionine was replaced by leucine (Met^{93}Leu). Overexpression of *serA* in *E. coli* BL21 resulted in specific PGDH activity of up to 1.473±0.05 U/mg protein, and the enzyme activity was inhibited by high concentrations of L-serine. On the other hand, when the mutein *serA*$^{\Delta 591}$ was overexpressed in *E. coli* BL21, the specific PGDH activity decreased to 1.092±0.08 U/mg protein, and the enzyme activity was almost no longer inhibited by L-serine. These results showed that similar phenomena were observed when the C-terminal part of PGDH encoded by *serA* was removed both in *C. glutamicum* ATCC 13032 and *C. glutamicum* SYPS-062.

The wild-type *C. glutamicum* SYPS-062 was used to examine the direct fermentative production of L-serine from sugar (Zhang et al. 2010), while *C. glutamicum* ATCC 13032 produced almost no L-serine. Our previous study showed that

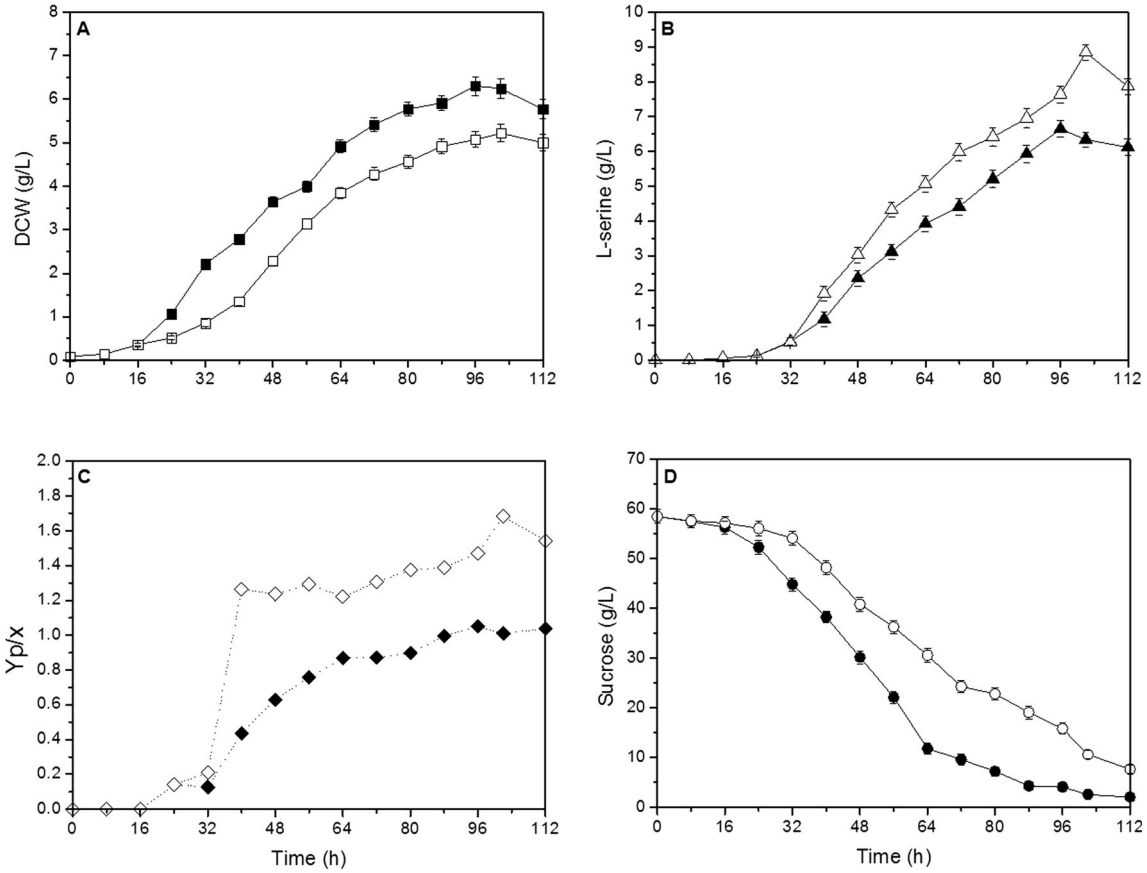


Fig. 3a–d Fermentation curve of *C. glutamicum* SYPS-062*ΔsdaA* (pJC1-*tac-serA*$^{\Delta 591}$) and *C. glutamicum* SYPS-062. **a** Growth, **b** L-serine titer, **c** Yp/x, **d** sucrose. *Squares* Cell growth (dry cell weight, *DCW*), *triangles* L-serine titer, *circles* residual sucrose, *diamonds* Yp/x (yield of L-serine to DCW). *Closed symbols* Parent strain *C. glutamicum* SYPS-062, *open symbols* recombinant strain *C. glutamicum* SYPS-062*ΔsdaA* (pJC1-*tac-serA*$^{\Delta 591}$)

L-serine hydroxymethyltransferase (SHMT) activity in *C. glutamicum* SYPS-062 was only about 34.4 % of that observed in *C. glutamicum* ATCC 13032 (data not shown), which could be one of the reasons for the ability of *C. glutamicum* SYPS-062 to produce L-serine. It has been reported that SHMT catalyzes the interconversion of glycine and L-serine. SHMT is essential in *C. glutamicum* and may thus be the only enzyme that makes activated C1 units available in the form of 5,10-methylene tetrahydrofolate (Schweitzer et al. 2009). After truncation of the C-terminal domain of PGDH encoded by *serA*, no obvious improvement in L-serine production was observed when *serA*$^{\Delta 591}$ was overexpressed alone, which is consistent with previous reports indicating that overexpression of the *serC* and *serB* genes, together with the third biosynthetic *serA* gene, *serA*$^{\Delta 591}$, in *C. glutamicum* ATCC 13032 yielded only traces of L-serine (Peters-Wendisch et al. 2005). One reason for this could be the fact that L-serine has a key position in the precursor supply and has to be regarded as an intermediate of central metabolism (Stauffer 1996), and that it is essential for synthesis not only of protein, but also of glycine, cysteine, tryptophan, and phospholipid as well as for C1 unit generation (Stauffer 1996). In *C. glutamicum*, about 7.5 % of the total carbon flux toward L-serine is utilized for this purpose (Marx et al. 1996; Stauffer 1996). In a previous study, through ^{13}C-labeled L-serine analysis of cell-derived metabolites using nuclear magnetic resonance spectroscopy, it was reported that L-serine was consumed at high rates in *C. glutamicum* ATCC 13032 during growth on glucose, and that the carbon skeleton of L-serine was converted mainly to pyruvate-derived metabolites such as L-alanine (Netzer et al. 2004). Thus, no obvious effect of *serA*$^{\Delta 591}$ overexpression could be found, and further metabolism of L-serine needs to be weakened. L-SerDH encoded by *sdaA* catalyzes the conversion of L-serine to pyruvate. In the present study, when *serA*$^{\Delta 591}$ was overexpressed in *C. glutamicum* SYPS-062*ΔsdaA*, interestingly, the L-serine titer increased by 32.93 % and Yp/x improved by 60 % compared with those observed in the control. However, the growth rate decreased, which is consistent with the findings of previous reports indicating that deletion of *sdaA* decreased the rate of L-serine co-metabolism with glucose by 47 %, but still results in degradation of L-serine to pyruvate (Netzer et al. 2004). Furthermore, it has been reported that attenuation of

the conversion of L-serine to pyruvate and glycine, in combination with the co-expression of phosphoglycerate kinase and feedback-resistant PGDH, could dramatically improve L-serine production (Lai et al. 2012). Therefore, it can be concluded that enhancement of L-serine production not only requires overexpression of the C-terminal truncated PGDH in the L-serine biosynthesis pathway, but also reduction of the L-serine degradation pathway in *C. glutamicum* SYPS-062.

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