High-yield production of L-serine through a novel identified exporter combined with synthetic pathway in *Corynebacterium glutamicum*

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**Abstract**

**Background:** L-Serine has wide and increasing applications in industries with fast-growing market demand. Although strategies for achieving and improving L-serine production in *Corynebacterium glutamicum* (C. glutamicum) have focused on inhibiting its degradation and enhancing its biosynthetic pathway, L-serine yield has remained relatively low. Exporters play an essential role in the fermentative production of amino acids. To achieve higher L-serine yield, L-serine export from the cell should be improved. In *C. glutamicum*, ThrE, which can export L-threonine and L-serine, is the only identified L-serine exporter so far.

**Results:** In this study, a novel L-serine exporter NCgI0580 was identified and characterized in *C. glutamicum* ΔSSAAI (SSAAI), and named as SerE (encoded by serE). Deletion of serE in SSAAI led to a 56.5% decrease in L-serine titer, whereas overexpression of serE compensated for the lack of serE with respect to L-serine titer. A fusion protein with SerE and enhanced green fluorescent protein (EGFP) was constructed to confirm that SerE localized at the plasma membrane. The function of SerE was studied by peptide feeding approaches, and the results showed that SerE is a novel exporter for L-serine and L-threonine in *C. glutamicum*. Subsequently, the interaction of a known L-serine exporter ThrE and SerE was studied, and the results suggested that SerE is more important than ThrE in L-serine export in SSAAI. In addition, probe plasmid and electrophoretic mobility shift assays (EMSA) revealed NCgI0581 as the transcriptional regulator of SerE. Comparative transcriptomics between SSAAI and the NCgI0581 deletion strain showed that NCgI0581 is a positive regulator of NCgI0580. Finally, by overexpressing the novel exporter SerE, combined with L-serine synthetic pathway key enzyme serAΔ197, serC, and serB, the resulting strain presented an L-serine titer of 43.9 g/L with a yield of 0.44 g/g sucrose, which is the highest L-serine titer and yield reported so far in *C. glutamicum*.

**Conclusions:** This study provides a novel target for L-serine and L-threonine export engineering as well as a new global transcriptional regulator NCgI0581 in *C. glutamicum*.

**Background**

L-serine has been identified as one of the top 30 most interesting building blocks for a range of chemicals and materials, and is used in cosmetic, pharmaceutical, and food industries [1, 2]. Metabolic engineering of *C. glutamicum* for L-serine production has been focused on its terminal synthesis pathways and degradation pathways, and proven to be very useful for improving L-serine production in this bacterium [3-6]; however, the L-serine productivity achieved is still low for large-scale L-serine production, and the highest reported L-serine titer is 42.62 and 50 g/L with an yield of 0.21 g/g sucrose and 0.36 g/g glucose in *C. glutamicum* and *Escherichia coli* (*E. coli*), respectively. Besides, L-serine can also be potentially produced from sugar via fermentation with a very high theoretical yield (1.17 g/g glucose, 1.22 g/g sucrose) [2].

For enhancing L-serine production, an improvement in L-serine export from the cell should be considered. Export system plays an essential role in metabolic engineering strategies for the production of amino acids [7], because it reduces intracellular amino acid concentrations, thereby alleviating feedback inhibition and circumventing toxicity problems [1, 8-10]. In recent decades, several export systems have been identified for excreting amino acids, such as L-lysine, L-cysteine, L-glutamate, L-threonine, L-arginine, L-methionine, and branched-chain amino acids, in *C. glutamicum* and *E. coli*. However, to the best of our knowledge, except for ThrE (L-threonine and L-serine exporter) [11-17], no other L-serine exporters have been reported in *C. glutamicum* so far. In *E. coli*, Mundhada et al. found that intracellular L-serine accumulation was toxic to the engineered strain modified to produce L-serine, and that following overexpression of *eamA*, which encodes L-cysteine exporter in *E. coli*, the engineered strain exhibited increased tolerance toward L-serine with higher L-serine productivity [2]. Therefore, L-serine exporter in *C. glutamicum* could be a potential target for strain optimization to further improve L-serine production.

It has been reported that homologs similar to the exporters in *E. coli* might fulfil a comparable function in *C. glutamicum* [17, 19, 20]. Accordingly, we hypothesized that the homolog to EamA (L-serine exporter in *E. coli*)
might be involved in L-serine export in *C. glutamicum*. In the present study, three homologs to EamA, namely, NCgl2050, NCgl2065, and NCgl0580, were determined, and their functions were identified by targeted gene deletion, respectively. The results showed that one of the genes, NCgl0580 gene, was involved in L-serine export. Subsequently, localization and function of NCgl0580 were investigated, and the interaction of a known L-serine exporter ThrE (encoded by *thrE*) and the novel exporter NCgl0580 was studied. Furthermore, the transcriptional regulator of NCgl0580 was identified and studied. Finally, the effects of overexpression of L-serine exporter in combination with L-serine synthetic pathway enzyme on L-serine production were evaluated.

### Results

#### Exploring putative L-serine exporters in *C. glutamicum*

In previous studies, homologs of *E. coli* exporters have been shown to have similar functions in *C. glutamicum* [17, 19, 20]. Therefore, we hypothesized that the *C. glutamicum* homolog to EamA (L-serine exporter in *E. coli*) [2] might be involved in L-serine export in *C. glutamicum*. According to the NCBI database, EamA belongs to the RhaT superfamily, and 15 records of related proteins associated with RhaT superfamily in *C. glutamicum* ATCC13032 were obtained. After eliminating duplicate records, three related genes, NCgl2050, NCgl2065, and NCgl0580 genes, were obtained, which might be involved in L-serine export in *C. glutamicum*.

To verify the function of these putative proteins in *C. glutamicum* SSAAI (SSAAI), NCgl2050, NCgl2065, and NCgl0580 were deleted in this strain respectively. The results showed that the deletion of NCgl2050 and NCgl2065 did not produce any changes in cell growth and L-serine titer (Fig. 1a and 1b). Strikingly, deletion of NCgl0580 significantly reduced the L-serine titer in SSAAI, but did not affect the growth of the strain (Fig. 1c). SSAAI ΔNCgl0580 produced 11.31 g/L L-serine, which was 56.5% lower than that noted in SSAAI (Fig. 1c, P < 0.001). However, plasmid-borne overexpression of NCgl0580 compensated for the lack of NCgl0580 with respect to L-serine titer, resulting in 26.76 g/L L-serine titer, similar to that generated by the parent strain SSAAI (Fig. 1d). As shown in Fig. 1d and 1e, when compared with SSAAI, the strain harboring the plasmid grew slowly to some extent in the logarithmic growth phase, finally reaching cell growth similar to that of SSAAI. This finding suggested that NCgl0580 might act as the L-serine exporter in *C. glutamicum*, and was named as SerE and its function was further investigated.

![Fig. 1](image)

**Fig. 1** Effect of NCgl2050, NCgl2065, and NCgl0580 deletion and complemented strain on SSAAI. (a) NCgl2050 deletion strain SSAAIΔNCgl2050 (open symbols) and SSAAI (solid symbols). (b) NCgl2065 deletion strain SSAAIΔNCgl2065 (open symbols) and SSAAI (solid symbols). (c) NCgl0580 deletion strain SSAAIΔNCgl0580 (open symbols) and SSAAI (solid symbols). (d) Complemented strain SSAAIΔNCgl0580-NCgl0580 (open symbols) and SSAAI (solid symbols). Squares and circles indicate cell growth OD<sub>562</sub> and L-serine titer, respectively. (e) Growth rates of the complemented strain SSAAIΔN0580-NCgl0580 (red) and SSAAI (black).

#### Localization and function of SerE

According to the NCBI, SerE was presumed to be a hypothetical membrane protein of 301 amino acids, similar to permease of the drug/metabolite transporter (DMT) superfamily. The transmembrane helices of SerE were predicted by TMHMM Server v. 2.0, and SerE exhibited ten transmembrane-spanning helices with both amino- and carboxy-terminal ends in the cytoplasm.

To confirm the localization of SerE, SerE-EGFP fusion protein was expressed in SSAAI. Confocal microscopic observations of SSAAI-egfp and SSAAI-serE-egfp confirmed that EGFP and SerE-EGFP fusion proteins were successfully expressed, respectively (Fig. S1). To further verify the localization of SerE, membrane and cytoplasmic proteins from these two strains were extracted by ultrasonication, and the fluorescence of these proteins was determined using a fluorescence spectrophotometer. The fluorescence of the cytoplasmic proteins of SSAAI-egfp and membrane proteins of SSAAI-serE-egfp (Fig. 2a) affirmed that SerE was localized at the plasma membrane in SSAAI.

To substantiate the function of SerE, a peptide feeding approach was employed by incubating SSAAI and SerE
deletion strain, SSAAI ΔserE, with 2 mM of the dipeptide Ser-Ser, respectively, and measuring the concentration of extracellular L-serine. As shown in Fig. 2b, a higher L-serine concentration was detected in SSAAI, when compared with that in SSAAI ΔserE, confirming that SerE is a novel exporter of L-serine in *C. glutamicum*.

**Fig. 2** Fluorescence of cytoplasmic proteins and membrane proteins, and the result of amino acid export of SerE by using peptide feeding approach in SSAAI. (a) Fluorescence of cytoplasmic proteins and membrane proteins of SSAAI-10 (SSAAI harboring plasmid pDXW-10 only, gray bar with slash), SSAAI-egfp (SSAAI overexpressing EGFP protein with pDXW-10, gray bar), and SSAAI-serE-egfp (SSAAI overexpressing SerE-EGFP fusion protein with pDXW-10, white bar). (b) Extracellular concentration of L-serine in SSAAI (solid squares) and serE deletion strain SSAAI ΔserE (solid circles) with 2 mM of the dipeptide Ser-Ser. Extracellular concentration of L-serine in SSAAI (empty squares) without the dipeptide Ser-Ser. (c) Extracellular concentration of L-threonine in SSAAI (solid squares) and serE deletion strain SSAAI ΔserE (solid circles) with 2 mM of the dipeptide Thr-Thr. Extracellular concentration of L-threonine in SSAAI (empty squares) without the dipeptide Thr-Thr.

It is known that L-cysteine export system in *E. coli* (encoded by *eamA*) also catalyzes L-serine export [2], and that L-threonine exporter in *C. glutamicum* (encoded by *thrE*) also transports L-serine [15]. We therefore analyzed whether the novel exporter SerE could export L-cysteine or L-threonine. The export experiments with dipeptides (Thr-Thr, Cys-Cys) were performed using SSAAI and SSAAI ΔserE. The dipeptides were added at a concentration of 2 mM to the medium, and the extracellular amino acid concentrations at different time intervals were determined by HPLC. The results revealed that the concentration of L-cysteine was comparable in both strains and did not significantly change (data not shown), indicating that SerE might not export L-cysteine.

Interestingly, the concentrations of L-threonine in SSAAI ΔserE were lower than those in SSAAI (Fig. 2c), indicating that SerE might be also an exporter of L-threonine in *C. glutamicum*.

**Interaction of a known exporter ThrE and a novel exporter SerE**

It is well known that *thrE* encodes ThrE that can export L-threonine and L-serine in *C. glutamicum* ATCC13032 [15]. To understand the interaction between ThrE and SerE on L-serine export, *thrE* was deleted in SSAAI (SSAAI ΔthrE), which did not produce any significant change in L-serine titer in the deletion mutant (Fig. 3a and 3b). In contrast, deletion of SerE significantly reduced the L-serine titer in SSAAI, and resulted in little change in cell growth (Fig. 1c). The SSAAI ΔserE produced 11.31 g/L L-serine, which was 56.5% lower than that produced by SSAAI (Fig. 1c). Subsequently, *thrE* and *serE* double deletion mutant was constructed, which exhibited cell growth comparable to that of SSAAI, and produced 10.34 g/L L-serine, which was 60% lower than that observed in SSAAI (Fig. 3a and Fig. 3b).

Furthermore, *thrE* and *serE* were overexpressed alone or in combination in SSAAI to obtain SSAAI-*thrE*, SSAAI-*serE*, and SSAAI-*serE-thrE*. While L-serine accumulation in SSAAI-*thrE* was similar to that in SSAAI, the production of L-serine in SSAAI-*serE* reached 28.67 g/L, which was 10.5% higher than that noted in SSAAI (Fig. 3a and 3c). However, a decrease in cell growth was observed in SSAAI-*serE* before 72 h of fermentation, when compared with that found in SSAAI (Fig. 3d). Furthermore, no significant difference in L-serine titer was found in the time courses of both SSAAI-*serE* and SSAAI-*serE-thrE*, and SSAAI-*serE-thrE* exhibited lower cell growth than SSAAI-*thrE* before 96 h of fermentation (Fig. 3c and 3d). These observations might be due to the inhibition of cell growth resulting from L-serine over-efflux, metabolic burden of overexpression of two membrane-binding proteins, or inhibition of cell growth by L-threonine over-efflux. Taken together, these findings suggested that SerE plays a more important role than ThrE for L-serine export in SSAAI.

**Fig. 3** Effect of the exporters *thrE* and *serE* deletion or overexpression on SSAAI. (a) Cell growth (gray bar with slash) and L-serine titer (white bar) of SSAAI, *thrE* deletion strain SSAAI ΔthrE, serE deletion strain SSAAI ΔserE, *thrE* and *serE* deletion strain SSAAI ΔserE ΔthrE, *thrE* overexpression strain SSAAI-*thrE*, *serE* overexpression strain SSAAI-*serE*, and *thrE* and *serE* double overexpression strain SSAAI-*serE-thrE*. (b) Cell growth (open symbols) and L-serine titer (solid symbols) of SSAAI (squares), *serE* deletion strain SSAAI ΔserE (circles), *thrE* deletion strain SSAAI ΔthrE (triangles), and *thrE* and *serE* deletion strain SSAAI ΔserE ΔthrE (rhombus). (c) Cell growth (open symbols) and L-serine titer (solid symbols) of SSAAI (squares), *serE* overexpression strain SSAAI-*serE* (circles), *thrE* overexpression strain SSAAI-*thrE* (triangles), and *thrE* and *serE* double overexpression strain SSAAI-*serE-thrE* (rhombus). (d) The growth rates of SSAAI-*serE-thrE* (red) and SSAAI (black).
Transcriptional regulator of the novel exporter SerE

The gene NEWCgl0581, located upstream of *serE* and divergently transcribed from *serE* (Fig. S2), and its product (consisting of 303 amino acids) was found to be a member of the LysR-type transcriptional regulators (LTTRs) family. It must be noted that LTTRs were initially described as regulators of divergently transcribed genes [21]. In a previous study on *C. glutamicum*, LysG, located upstream of L-lysine exporter gene *lysE*, was observed to encode a LysR-type transcriptional regulator, confirming that LysG is a positive transcriptional regulator of *lysE* [22]. Accordingly, we speculated that NCgl0581 might be involved in the control of *serE* transcription.

To determine the function of NCgl0581, a mutant strain with NCgl0581 deletion was constructed. As shown in Fig. 4a, the growth of SSAAI ΔNCgl0581 was similar to that of the parent strain SSAAI. However, the L-serine titer of SSAAI ΔNCgl0581 was 11.08 g/L, which was 57.4% lower than that of the parent strain (P<0.001), indicating that NCgl0581 played an important role in L-serine production. Subsequently, the effect of NCgl0581 on *serE* expression was further investigated by using the probe plasmid pDXW-11. Two recombinant strains, SSAAI ΔNCgl0581-1 (harboring the plasmid pDXW-11-1, Fig. 4b) and SSAAI ΔNCgl0581-0 (harboring the plasmid pDXW-11-0, Fig. 4c) were constructed, and their fluorescence during fermentation was measured. The fluorescence of SSAAI ΔNCgl0581-1 was stronger than that of SSAAI ΔNCgl0581-0 during the fermentation process (Fig. 4d), revealing that NCgl0581 functioned as a positive regulator of *serE* expression. To verify whether the regulatory protein NCgl0581 binds to the upstream region of SerE, EMSA was performed by using the DNA probe labeled with biotin, and the result clearly indicated that NCgl0581 binds to this region (Fig. 4e).

**Fig. 4** Verification of the function of NCgl0581. (a) The cell growth (squares) and L-serine titer (circles) of SSAAI (solid symbols) and NCgl0581 deletion strain SSAAI ΔNCgl0581 (open symbols), respectively. (b) Plasmid pDXW-11-1 containing fragments of NCgl0581 (gray), intergenic region between NCgl0581 and NCgl0580 (black), and EGFP (green). (c) Plasmid pDXW-11-0 containing fragments of the intergenic region between NCgl0581 and NCgl0580 (black) and EGFP (green). (d) Fluorescence of the two strains, SSAAI ΔNCgl0581-1 (gray bar with slash) and SSAAI ΔNCgl0581-0 (white bar). (e) Verification of NCgl0581 binding to the upstream region of SerE by using EMSA. Lane 1: the nuclear extracts with activated specific TF (positive control), Lane 2: the nuclear extracts without activated TF (negative control), Lane 3: Sample.

To confirm whether NCgl0581 is a specific regulator of SerE, transcriptome sequencing was performed using SSAAI and NCgl0581 deletion strain. The findings showed that the transcription levels of 115 genes were altered, including 56 upregulated genes and 59 downregulated genes, in response to NCgl0581 deletion, indicating that NCgl0581 is a global transcriptional regulator in *C. glutamicum*. The genes with significant transcriptional change (≥4-fold) are shown in Tables 1 and 2.

Table 1 Genes significantly upregulated by NCgl0581 deletion
| Gene id | SSAI Δ0581 | SSAI | Fold change | Protein function                                      |
|---------|------------|------|-------------|------------------------------------------------------|
| NCgl2897 | 701.56     | 71.07| 9.87        | Starvation-inducible DNA-binding protein              |
| NCgl0546 | 17.78      | 2.75 | 6.45        | Hypothetical protein                                  |
| NCgl1405 | 15.94      | 2.71 | 5.88        | ABC transporter periplasmic component                 |
| NCgl1302 | 10.05      | 1.96 | 5.13        | Aldo/keto reductase                                   |
| NCgl1344 | 286.87     | 55.96| 5.12        | Ornithine carbamoyltransferase                        |
| NCgl1343 | 280.65     | 57.24| 4.9         | Acetylornithine aminotransferase                     |
| NCgl0746 | 43.30      | 9.04 | 4.7         | Hypothetical protein                                  |
| NCgl1342 | 134.70     | 29.07| 4.63        | Acetylglutamate kinase                                |
| NCgl2946 | 672.93     | 155.87| 4.31       | Hypothetical protein                                  |
| NCgl1022 | 89.53      | 21.28| 4.20        | Cysteine sulfinate desulfinase                        |
| NCgl1023 | 368.88     | 88.67| 4.15        | Nicotinate-nucleotide pyrophosphorylase               |
| NCgl1341 | 108.49     | 27.09| 4.00        | Bifunctional ornithine acetyltransferase/N-ace...    |

Table 2 Genes significantly downregulated by NCgl0581 deletion
| Gene id   | SSAAI Δ0581 | SSAAI | Fold change | Protein function                                      |
|----------|-------------|-------|-------------|------------------------------------------------------|
| NCgl0580 | 18.40       | 5152.54 | 280.02      | Hypothetical protein                                  |
| NCgl0638 | 1.71        | 20.97  | 12.22       | ABC transporter permease                              |
| NCgl0639 | 11.00       | 82.47  | 7.49        | ABC transporter periplasmic component                 |
| NCgl2943 | 207.03      | 1355.55| 6.54        | Hypothetical protein                                  |
| NCgl0943 | 16.19       | 103.52 | 6.39        | AraC family transcriptional regulator                 |
| NCgl0484 | 2.32        | 14.57  | 6.28        | ABC transporter permease                              |
| NCgl2942 | 283.52      | 1776.15| 6.26        | NADH:flavin oxidoreductase                            |
| NCgl0166 | 13.41       | 79.70  | 5.94        | Hypothetical protein                                  |
| NCgl0324 | 2.11        | 11.87  | 5.61        | Zn-dependent alcohol dehydrogenase                   |
| NCgl0282 | 5.19        | 28.25  | 5.44        | 4-Hydroxyphenyl-beta-ketoacyl-CoA hydrolase           |
| NCgl1975 | 102.94      | 503.75 | 4.89        | Hypothetical protein                                  |
| NCgl2893 | 1.25        | 6.08   | 4.84        | Efflux system protein                                 |
| NCgl0155 | 9.11        | 43.69  | 4.79        | 5-Dehydro-2-deoxygluconokinase                        |
| NCgl0014 | 10.02       | 47.76  | 4.76        | Hypothetical protein                                  |
| NCgl2953 | 7.68        | 35.80  | 4.66        | Sugar permease                                        |
| NCgl2744 | 12.26       | 55.19  | 4.50        | Hypothetical protein                                  |
| NCgl2970 | 15.22       | 67.51  | 4.43        | ABC transporter periplasmic component                 |
| NCgl0608 | 23.06       | 100.35 | 4.35        | ABC transporter permease                              |
| NCgl0258 | 4.51        | 19.50  | 4.32        | Arsenite efflux pump ACR3                             |
| NCgl0281 | 16.83       | 67.69  | 4.02        | Dehydrogenase                                         |

The transcriptional level of serE was significantly decreased by 280-fold following NCgl0581 deletion, revealing that NCgl0581 is a positive regulator of serE. Furthermore, NCgl0581 deletion downregulated the two ABC transporter permeases (NCgl0638 and NCgl0484) and ABC transporter periplasmic component (NCgl0639) by 12-, 6.3-, and 7.5-fold, respectively, and upregulated ABC transporter periplasmic component (NCgl1405) by 5.88-fold, suggesting that NCgl0581 is involved in the synthesis of substances transported through ABC transporter.

**Overexpression of SerE and NCgl0581**

As NCgl0581 could activate the expression of SerE in SSAAI, the overexpression of NCgl0581, serE, or their co-expression was studied, and strains SSAAI-NCgl0581 and SSAAI-NCgl0581-serE were constructed respectively. As shown in Fig. 5a and Fig. 5b, a decrease in cell growth was observed in SSAAI-NCgl0581-serE and SSAAI-NCgl0581 before 96 h of fermentation, and SSAAI-NCgl0581-serE showed the lowest growth rate, the time courses for L-serine production were similar in all the strains. Furthermore, the yield of L-serine to biomass (Yp/x) increased in both SSAAI-NCgl0581-serE and SSAAI-NCgl0581 (Fig. 5c and 5d), suggesting that overexpression of a novel exporter SerE and its transcriptional regulator NCgl0581 was beneficial for L-serine efflux, but not for cell growth. Besides, SSAAI-NCgl0581-serE and SSAAI-NCgl0581 exhibited 9.67% (p < 0.05) and 19.17% higher Yp/x in 96 h (p<0.01), respectively, when compared with SSAAI. A similar decrease in cell growth was observed in SSAAI-serE (Fig. 3c); however, the L-serine titer was 28.67 g/L, which was 10.5% higher than that noted in SSAAI. This decrease in cell growth in the recombinant strain could be due to the transportation of the...
synthesized L-serine out of the cell, resulting in inadequate intracellular L-serine for cell growth. Therefore, our subsequent investigation involved replenishment of L-serine by overexpressing L-serine synthetic pathway key enzyme.

**Fig. 5** Effect of serE and NCgl0581 deletion or overexpression on SSAAI. (a) Cell growth (open symbols) and L-serine titer (solid symbols) of SSAAI (squares), NCgl0581 overexpression strain SSAAI-NCgl0581 (circles), and NCgl0581 and serE double overexpression strain SSAAI-NCgl0581-serE (triangles). (b) The growth rates of SSAAI-NCgl0581 (red), SSAAI-NCgl0581-serE (blue) and SSAAI (black). (c) Yp/x of SSAAI (gray bar with slash) and NCgl0581 overexpression strain SSAAI-NCgl0581 (white bar). (d) Yp/x of SSAAI (gray bar with slash) and NCgl0581 and serE double overexpression strain SSAAI-NCgl0581-serE (white bar).

**High yield production of L-serine through SerE combined with synthetic pathway**

To direct more flux to L-serine synthesis, L-serine exporter SerE and L-serine synthetic pathway key enzyme (containing a feedback insensitive serAΔ197, serC, and serB encoding the deregulated 3-phosphoglycerate dehydrogenase, phosphoserine phosphatase, and phosphoserine aminotransferase, respectively) were co-overexpressed in SSAAI to obtain SSAAI-serE-serAΔ197-serC-serB. The recombinant strain shared similar typical growth curves as the parent strain SSAAI, and achieved a final L-serine titer of 32.8 g/L, which was 22.1% higher than that noted in SSAAI (p<0.001). Subsequently, L-serine exporter serE, serAΔ197, serC and serB were overexpressed in strain A36 to obtainA36-serE-serAΔ197-serC-serB. A36 was stemmed from SSAAI by using ARTP mutation, which produced 34.78 g/L L-serine [23]. As shown in Fig. 6, the tandem expression strain A36-serE-serAΔ197-serC-serB shared similar typical growth curves as the parent strain A36 in the overall process. Furthermore, the sucrose level decreased with time in a similar pattern in both the strains. Interestingly, when the incubation time of batch cultivations exceeded 72 h, the cell growth and L-serine titer of A36-serE-serAΔ197-serC-serB were higher than those of the parent strain A36. After 120 h of cultivation, A36-serE-serAΔ197-serC-serB consumed all of the sucrose and achieved a final L-serine titer of 43.9 g/L, with a conversion rate of 0.44 g/g. These results demonstrated that overexpression of L-serine exporter in combination with L-serine synthetic pathway could facilitate L-serine production in C. glutamicum.

**Fig. 6** Fermentation process of strain A36 and strain A36-serE-serAΔ197-serC-serB. The cell growth (open symbols), L-serine titer (solid symbols), and residual sucrose (gray symbols) of strain A36 (squares) and A36-serE-serAΔ197-serC-serB (circles) are presented. Three parallel experiments were performed. Error bars indicate standard deviations of the results from three parallel experiments.

**Discussion**

Transport engineering is becoming an attractive strategy for strain improvement [11, 16, 17]. However, only a relatively limited number of exporters of amino acids have been identified in C. glutamicum (Table S1) [8, 12, 14-17, 24-27]. In this study, SerE was identified as a novel L-serine exporter in C. glutamicum. Further analysis showed that SerE could also export L-threonine (Fig. 2c), but not L-cysteine, similar to ThrE, which could export both L-serine and L-threonine in C. glutamicum [15]. It was assumed that the presence of -OH in both L-serine and L-threonine might be the reason for these exporters to transport the two substrates. Based on homology search, SerE was found to be similar to a member of the DMT superfamily. Although DMT superfamily proteins are involved in the transport of a wide range of substrates, there are only a few reports available on their structures and mechanisms of substrate transport. Christian et al. performed structural and functional analyses of YddG, a DMT protein, and provided insight into the common transport mechanism shared among the DMT superfamily members [28]. It has been reported that analyses of the crystal structure data of exporters could help to elucidate the elusive transport mechanism [29], and in the future, we will further investigate the SerE structures and mechanisms of substrate transport.

To explore the interaction between the known L-serine exporter ThrE and the novel exporter SerE on L-serine export, ThrE and SerE single and double mutants were constructed. The results showed that serE and thrE double deletion mutant could still accumulate 10.34 g/L L-serine (Fig. 3b), suggesting that C. glutamicum might also possess other L-serine exporter systems. The evolution of multiple exporter systems for a single substrate is
beneficial for the survival of bacteria in variable environment [7, 30]. It must be noted that overexpression of serE in SSAAI resulted in 10.5% increase in L-serine titer, but a decrease in cell growth. This could be due to the use of constitutive-type promoter to overexpress SerE, causing higher L-serine efflux. As sufficient L-serine content is important to maintain cell growth, a decrease in cell growth was noted as a stress response to serE overexpression. In future studies, better tuning of the serE expression must be achieved in SSAAI by testing different promoters and RBS. When thrE and serE were co-overexpressed in SSAAI, SSAAI-serE-thrE exhibited lower cell growth than SSAAI, but an L-serine titer similar to that of SSAAI-serE (Fig. 3c). A severe decline in cell growth was observed in all exporter overexpression strains, which may be caused by the accumulation of L-serine in the medium as well as additional burden on the cell overexpressing the exporters, similar to that reported by Mundhada et al. [31].

NCgl0581 was identified as the transcriptional regulator of the novel L-serine exporter SerE, and EMSA was performed to confirm the binding sites of NCgl0581 with the promoter of SerE. A previous study reported that the first member of the protein-gene pairs, ArgP-argO in E. coli and LysG-lysE in C. glutamicum, is a LysR-type transcriptional regulator, while the second member is its target gene encoding an amino acid exporter [22, 32, 33]. Similarly, NCgl0581-serE might also be a protein-gene pair sharing the same regulation mechanism. A serine biosensor based NCgl0581 was reported by Binder et al. [34], and accordingly, we constructed a biosensor for L-serine and found that NCgl0581 activated NCgl0580 (SerE) expression in the presence of L-serine, with expression of SerE enhancing with increasing L-serine titer [23]. However, NCgl0581 did not activate the expression of SerE in the presence of L-alanine and L-valine. To further confirm whether SerE could export L-alanine and L-valine, peptide feeding assays were employed using dipeptides (Ala-Ala, Val-Val) with SSAAI and SSAAI ΔserE. The results revealed that SerE could neither export L-alanine nor L-valine (data not shown). Moreover, transcriptome sequencing showed that NCgl0581 regulated 115 genes in C. glutamicum, suggesting that NCgl0581 was a novel global transcriptional regulator in C. glutamicum. Transcriptional regulators and their roles in expression control of target genes are important for metabolic engineering of C. glutamicum for industrial applications [35], and the present study provided a new member of transcriptional regulator family.

Overexpression of SerE alone resulted in 10.8% increase in L-serine titer and a simultaneous decrease in cell growth, implying that the synthesized L-serine was transported out of the cell, and that the intracellular L-serine was not adequate for cell growth. When L-serine was replenished by overexpressing L-serine synthetic pathway key enzyme, the cell growth was restored and L-serine titer increased to 43.9 g/L, with an L-serine yield of 0.44 g/g sucrose, which are the highest yield reported so far for C. glutamicum. These results indicated that serAΔ197, serC, and serB overexpression ensured sufficient L-serine supply preventing cell growth inhibition. In previous studies by Mundhada et al., 37 g/L L-serine was produced with a yield of 0.24 g/g glucose in E. coli [2], and 11.7 g/L L-serine titer was achieved with the highest yield of 0.43 g/g glucose [31]. Interestingly, in the present study, we found that the L-serine titer significantly increased with serB overexpression in A36, producing an L-serine titer of 37.9 g/L, which was 24% higher than that in A36. It must be noted that serB encodes phosphoserine phosphatase (PSP, EC 3.1.3.3), which catalyzes the last step of L-serine biosynthesis. However, L-serine titer did not significantly change when serAΔ197 and serC were respectively overexpressed in A36 (with L-serine titer of 31.1 and 32.78 g/L, respectively). In a recent study, 50 g/L L-serine was produced with glucose as the carbon source in E. coli, which is the highest L-serine production reported so far; however, the yield was 0.36 g/g glucose [36], which is lower than that obtained in the present study (0.44 g/g sucrose). Therefore, fine controlling of the three enzymes of L-serine biosynthesis pathway could possibly further enhance L-serine production.

**Conclusion**

In the present study, a novel exporter SerE and its positive regulator NCgl0581 were identified in C. glutamicum. SerE exhibited the ability to export L-threonine and NCgl0581 acting as a novel global transcriptional regulator in C. glutamicum, and by overexpressing this novel exporter along with L-serine synthetic pathway enzyme, significant increase in L-serine yield could be achieved. These results enrich our understanding of amino acid transport and can provide additional targets for exporter engineering in C. glutamicum.


Materials And Methods

Strains, plasmids, and growth conditions

The strains and plasmids used in this study are listed in Table 3. *E. coli* JM109 was used as the cloning host, and was grown in lysogeny broth (LB) medium (containing 5.0 g/L yeast extract, 10.0 g/L tryptone, and 10.0 g/L NaCl) at 37°C and 220 rpm. The engineered SSAAI (CGMCC No.15170) was selected as the original strain, which was constructed in our laboratory by knocking out 591 bp of the C-terminal domain of *serA*, deleting *sdaA*, *avtA*, and *alaT*, as well as attenuating *ilvBN* in the genome of *C. glutamicum* SYPS-062-33a (CGMCC No. 8667). Strain A36 derived from SSAAI by ARTP mutation, with higher L-serine titer and yield than SSAAI. The seed and fermentation media for *C. glutamicum* were prepared as described previously [5]. The *C. glutamicum* strains were pre-incubated in the seed medium overnight to an optical density (OD$_{562}$) of about 25, and then inoculated at an initial concentration of OD$_{562}$=1 into a 250 mL flask containing 25 mL of the fermentation medium at 30°C and 120 rpm. The antibiotic kanamycin (50 mg/L) was added when necessary. Samples were withdrawn periodically for the measurement of residual sugar, amino acids, and OD$_{562}$ as described previously [5].

Table 3 Strains and plasmids used in this study.

| Strain/Plasmid          | Description                                      | Sources or reference |
|-------------------------|--------------------------------------------------|----------------------|
| *E. coli*               |                                                  |                      |
| JM109                   | *recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17*, *supE44*, *relA1* | Laboratory strain    |
| *C. glutamicum*         |                                                  |                      |
| SSAAI                   | *C. glutamicum* SYPS-33a with deletion of the 591 bp in the C-terminus of *serA*, deletion of *sdaA*,*alaT*,*avtA* and attenuation of *ilvBN* | [5]                   |
| A36                     | SSAAI mutant strain                              | [23]                 |
| SSAAI-thrE              | SSAAI harboring plasmid pDXW-10-thrE             | This study           |
| SSAAIΔthrE              | SSAAI with deletion of *thrE*                    | This study           |
| SSAAIΔNCgl2050          | SSAAI with deletion of NCgl2050                  | This study           |
| SSAAIΔNCgl2065          | SSAAI with deletion of NCgl2065                  | This study           |
| SSAAIΔNCgl0580          | SSAAI with deletion of NCgl0580                  | This study           |
| SSAAI-10                | SSAAI harboring plasmid pDXW-10                  | This study           |
| SSAAI-egfp              | SSAAI harboring plasmid pDXW-10-egfp             | This study           |
| SSAAI-serE-egfp         | SSAAI harboring plasmid pDXW-10-serE-egfp        | This study           |
| SSAAI-NCgl0581          | SSAAI harboring plasmid pDXW-10- NCgl0581        | This study           |
| SSAAI-NCgl0581-serE     | SSAAI harboring plasmid pDXW-10- NCgl0581-serE   | This study           |
| SSAAIΔNCgl0581          | SSAAI with deletion of NCgl0581                  | This study           |
| SSAAIΔNCgl0581-1        | SSAAIΔNCgl0581 harboring pDXW-11-1               | This study           |
| SSAAIΔNCgl0581-0        | SSAAIΔNCgl0581 harboring pDXW-11-0               | This study           |
| SSAAIΔNCgl0580-NCgl0580 | SSAAIΔserE harboring plasmid pDXW-10-serE (NCgl0580) | This study           |
| SSAAI-serE              | SSAAI harboring plasmid pDXW-10-serE (NCgl0580)  | This study           |
ATCC13032 Wild type Laboratory strain
ATCC13032ΔserE ATCC13032 with deletion of serE (NCgl0580) This study

Integration vector, oriV, oriT, mob, sacB, Km⁻

pK18mobsacB carrying the up- and downstream homologous fragments of thrE gene for thrE deletion
pK18mobsacBΔthrE

This study

pK18mobsacBΔNCgl2050 fragments of NCgl2050 gene for NCgl2050 deletion
This study

pK18mobsacBΔNCgl2065 fragments of NCgl2065 gene for NCgl2065 deletion
This study

pK18mobsacBΔNCgl0580 fragments of NCgl0580 gene for NCgl0580 deletion
This study

pK18mobsacBΔNCgl0581 fragments of NCgl0581 gene for NCgl0581 deletion
This study

pDXW-10 E. coli-C. glutamicum shuttle vector, tacM promoter, Km⁻
[38]

pDXW-10-thrE pDXW-10 carrying the gene of thrE
This study

pDXW-10-serE pDXW-10 carrying the gene of serE
This study

pDXW-10-egfp pDXW-10 carrying the gene of egfp
This study

pDXW-10-egfp-serE pDXW-10 carrying the gene of egfp and serE for the expression of fusion protein EGFP-SerE
This study

pDXW-10- NCgl0581 pDXW-10 carrying the gene of NCgl0581
This study

pDXW-10- NCgl0581-serE pDXW-10 carrying the gene of NCgl0581 and serE
This study

pDXW-11 E. coli-C. glutamicum shuttle vector, probe plasmid, Km⁻
[39]

pDXW-11-1 pDXW-11 carrying the fragments of NCgl0581, the intergenic region between NCgl0581 and NCgl0580, and egfp
This study

pDXW-11-0 pDXW-11 carrying the fragments of the intergenic region between NCgl0581 and NCgl0580, and egfp
This study

Km⁻, kanamycin resistance.

**Construction of plasmids and strains**

The primers used in this study for gene expression/deletion are listed in Table S2. Gene deletion was performed using the nonreplicable deletion vector pK18mobsacB, as reported previously [37]. For example, to achieve thrE deletion, the homologous-arm fragments for thrE deletion were amplified from SSAAI chromosome using the primer pairs thrE1/2 for the upstream fragment and thrE3/4 for the downstream fragment. Then, with the two fragments as templates, a crossover PCR was performed using the primer pair thrE1/4. The truncated product of thrE was digested with XbaI and HindIII and ligated to the vector pK18mobsacB that was similarly treated. The recombinant plasmid pK18mobsacBΔthrE was transformed into SSAAI competent cells by electroporation, and chromosomal deletion was performed by selecting cells that were kanamycin resistant and sucrose nonresistant, and verified by PCR.
The pDXW-10 and pDXW-11 plasmids were used to overexpress genes in *C. glutamicum* [38, 39]. The recombinant plasmids were constructed as follows: the genes *thrE* and *serE* were amplified, digested, and ligated to the pDXW-10 plasmid that was digested with *Hind*III/*Bgl*II. The plasmid harboring the fusion protein, EGFP-SerE (enhanced green fluorescent protein), was constructed by using the method reported in a previous study [19]. To confirm the role of NCgl0581 on NCgl0580 expression, the fragment consisting of intergenic region of NCgl0581 and NCgl0580 and EGFP with or without NCgl0581 was ligated to the plasmid pDXW-11 by Clon Express MultiS One Step Cloning Kit (Vazyme, Nanjing, China). The strains were constructed by electroporation with the corresponding plasmids.

The genes, *serAΔ197*, *serC*, and *serB*, were PCR amplified from SSAAI using primers shown in Table S2. To construct plasmid pDXW-10-*serAΔ197*, the resultant fragment of *serAΔ197* was digested with *EcoR*I and *Not*I and cloned into pDXW-10. To construct plasmid pDXW-10-*serAΔ197-serC-serB*, the PCR fragments of *serB* and *serC* were digested with appropriate restriction enzymes and successively cloned into the corresponding plasmids to form plasmid pDXW-10-*serAΔ197-serC-serB*. The resulting plasmid (pDXW-10-*serAΔ197-serC-serB*) was then subjected to double digestion by *Nde*I and *Pac*I for cloning of NCgl0580 to obtain pDXW-10-*serE-serAΔ197-serC-serB*.

**Confocal microscopic observation**

The strains SSAAI-10 (SSAAI-harboring plasmid pDXW-10), SSAAI-*egfp*, and SSAAI-*serE-egfp* were grown in the seed medium and harvested during the exponential phase. The cells were washed twice and maintained in PBS (pH 7.4), mounted on a microscope slide, and observed under a Leica laser scanning confocal microscope (Leica, TCS SP8; Leica, Wetzlar, Germany) equipped with a HC PL Apo 63x/1.40 Oil CS2 oil-Immersion objective, with excitation filter at 488 nm and emission filter at 510-550 nm. The digital images were acquired and analyzed with Leica Application Suite X 2.0.

**Membrane and cytoplasmic protein extraction and fluorescence measurements**

The strains SSAAI-10, SSAAI-*egfp*, and SSAAI-*serE-egfp* were used for extracting membrane and cytoplasmic proteins to determine SerE localization. The extraction was performed using Membrane and a Cytoplasmic Protein Extraction Kit according to the manufacturer’s protocol (Beyotime, Nanjing, China). The cells were washed twice with PBS (pH 7.4) and disrupted by ultrasonication on ice (pulse, 4 s; interval, 6 s; total duration, 30 min) (Sonics Vibra-Cell™, Sonics, Newtown, CT, USA). The supernatant containing cytoplasmic proteins was collected by centrifugation (700 × *g*, 4°C for 10 min), and the precipitate was used for extracting membrane proteins. The protein concentration was determined with a Modified BCA Protein Assay Kit (Sangon, China). After extraction, the fluorescence intensity (excitation at 488 nm, emission at 517 nm) of the membrane and cytoplasmic proteins was determined using fluorescence spectrophotometer (Synergy H4; BioTek, Winooski, VT, USA).

**Amino acid export assay**

For ascertaining the function of *serE*, a dipeptide Ser-Ser addition assay was performed [15]. In brief, the pre-incubated cells (in seed medium) were washed once with CGXII minimal medium [40], inoculated into CGXII minimal medium with 2 mM Ser-Ser (other dipeptide), and incubated for 2 h at 30°C. Then, the cells were harvested, washed once with cold CGXII minimal medium, and resuspended in CGXII minimal medium. Amino acid excretion was initiated by adding 2 mM Ser-Ser (other dipeptide). HPLC was used to determine the concentrations of amino acids [19].

**Analytical procedures**

Cell density (OD	extsubscript{562}) was measured using an AOE UV-1200S UV/vis spectrophotometer (AOE Instruments Co. Inc., Shanghai, China). Sugar concentration was determined using SBA-40E glucose analyzer (Biology Institute of Shandong Academy of Sciences, China). For measurement of extracellular L-serine concentration in shake-flask fermentation, 500 μL of the culture were centrifuged at 700 ×*g* for 5 min, and the supernatant was used for detection after appropriate dilution. To ascertain intracellular L-serine concentration, 300 μL of the culture were
centrifuged at 700 xg and 4°C for 10 min, and 300 μL of water were added to the cells. The cells were disrupted by FastPrep-24 5G instrument (5 m/s, 120 s, MP Biomedicals, Shanghai, China). The cytoplasmic volume was assumed to be 2 μL/mg dry cell weight [27]. The titers of intracellular and extracellular L-serine and other amino acids were analyzed by HPLC using phenyl isothiocyanate as a precolumn derivatization agent, according to our previously study [8].

**EMSA**

To identify the binding site of NCgl0581 in the NCgl0580 promoter region, EMSA was conducted by using Non-Radioactive EMSA Kits with Biotin-Probes User’s Manual VER. 5.11 (Viogene Biotech Inc, Changzhou, China), according to the manufacturer’s instruction. The consensus oligonucleotides were BIO-JNZXM-TP (5’-AACACGCAA CTATAGTTAAGTAATA-3’) and BIO-JNZXM-BM (5’-TATTACTTAACTATAGTTGGCTGTTT-3’). The positive control was the nuclear extracts with activated specific TF, and the negative control was the nuclear extracts without activated TF.

### Abbreviations

*C. glutamicum*: glutamicum; **SSAAI**: *C. glutamicum* ΔSSAAI; **EGFP**: Enhanced green fluorescent protein; **EMSA**: Electrophoretic mobility shift assays; **DMT**: Drug/metabolite transporter superfamily; **Thr-Thr**: L-Threonine dipeptides; **Cys-Cys**: L-Cysteine dipeptides; **Ala-Ala**: L-Alanine dipeptides; **Val-Val**: L-Valine dipeptides; **LTTRs**: LysR-type transcriptional regulators family; **Yp/x**: Yield of L-serine to biomass; **PSP**: Phosphoserine phosphatase; **LB**: Lysogeny broth; **ARTP**: Atmospheric and room temperature plasma.

### Declarations

#### Authors’ contributions

XM Z, YJ G, and ZW C conceived and designed the experiments; XM Z led the working of the experiments; YJ G and ZW C performed the experiments; XM Z, GQ X, and XJ Z analyzed the data; XM Z, YJ G, and ZW C wrote the manuscript; and JS S, MK, and ZH X gave suggestions for the experiments and revised the manuscript. All the authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

#### Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author on request.

#### Consent for publication

Not applicable.

#### Ethics approval and consent to participate

Not applicable.

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Effect of NCgI2050, NCgI2065, and NCgI0580 deletion and complemented strain on SSAAI. (a) NCgI2050 deletion strain SSAAIΔNCgI2050 (open symbols) and SSAAI (solid symbols). (b) NCgI2065 deletion strain SSAAIΔNCgI2065 (open symbols) and SSAAI (solid symbols). (c) NCgI0580 deletion strain SSAAIΔNCgI0580 (open symbols) and SSAAI (solid symbols). (d) Complemented strain SSAAIΔNCgI0580-NCgI0580 (open symbols) and SSAAI (solid symbols). Squares and circles indicate cell growth OD562 and L-serine titer, respectively. (e) Growth rates of the complemented strain SSAAIΔN0580-NCgI0580 (red) and SSAAI (black).
Figure 2

Fluorescence of cytoplasmic proteins and membrane proteins, and the result of amino acid export of SerE by using peptide feeding approach in SSAAI. (a) Fluorescence of cytoplasmic proteins and membrane proteins of SSAAI-10 (SSAAI harboring plasmid pDXW-10 only, gray bar with slash), SSAAI-egfp (SSAAI overexpressing EGFP protein with pDXW-10, gray bar), and SSAAI-serE-egfp (SSAAI overexpressing SerE-EGFP fusion protein with pDXW-10, white bar). (b) Extracellular concentration of L-serine in SSAAI (solid squares) and serE deletion strain SSAAI ΔserE (solid circles) with 2 mM of the dipeptide Ser-Ser. Extracellular concentration of L-serine in SSAAI (empty squares) without the dipeptide Ser-Ser. (c) Extracellular concentration of L-threonine in SSAAI (solid squares) and serE deletion strain SSAAI ΔserE (solid circles) with 2 mM of the dipeptide Thr-Thr. Extracellular concentration of L-threonine in SSAAI (empty squares) without the dipeptide Thr-Thr.
Figure 3

Effect of the exporters thrE and serE deletion or overexpression on SSAAI. (a) Cell growth (gray bar with slash) and L-serine titer (white bar) of SSAAI, thrE deletion strain SSAAI ΔthrE, serE deletion strain SSAAI ΔserE, thrE and serE deletion strain SSAAI ΔserE ΔthrE, thrE overexpression strain SSAAI-thrE, serE overexpression strain SSAAI-serE, and thrE and serE double overexpression strain SSAAI-serE-thrE. (b) Cell growth (open symbols) and L-serine titer (solid symbols) of SSAAI (squares), serE deletion strain SSAAI ΔserE (circles), thrE deletion strain SSAAI ΔthrE (triangles), and thrE and serE deletion strain SSAAI ΔserE ΔthrE (rhombus). (c) Cell growth (open symbols) and L-serine titer (solid symbols) of SSAAI (squares), serE overexpression strain SSAAI-serE (circles), thrE overexpression strain SSAAI-thrE (triangles), and thrE and serE double overexpression strain SSAAI-serE-thrE (rhombus). (d) The growth rates of SSAAI-serE-thrE (red) and SSAAI (black).
Figure 4

Verification of the function of NCgl0581. (a) The cell growth (squares) and L-serine titer (circles) of SSAAI (solid symbols) and NCgl0581 deletion strain SSAAIΔNCgl0581 (open symbols), respectively. (b) Plasmid pDXW-11-1 containing fragments of NCgl0581 (gray), intergenic region between NCgl0581 and NCgl0580 (black), and EGFP (green). (c) Plasmid pDXW-11-0 containing fragments of the intergenic region between NCgl0581 and NCgl0580 (black) and EGFP (green). (d) Fluorescence of the two strains, SSAAI ΔNCgl0581-1 (gray bar with slash) and SSAAIΔNCgl0581-0 (white bar). (e) Verification of NCgl0581 binding to the upstream region of SerE by using EMSA. Lane 1: the nuclear extracts with activated specific TF (positive control), Lane 2: the nuclear extracts without activated TF (negative control), Lane 3: Sample.
Figure 5

Effect of serE and NCgl0581 deletion or overexpression on SSAAI. (a) Cell growth (open symbols) and L-serine titer (solid symbols) of SSAAI (squares), NCgl0581 overexpression strain SSAAI-NCgl0581 (circles), and NCgl0581 and serE double overexpression strain SSAAI-NCgl0581-serE (triangles). (b) The growth rates of SSAAI-NCgl0581 (red), SSAAI-NCgl0581-serE (blue) and SSAAI (black). (c) Yp/x of SSAAI (gray bar with slash) and NCgl0581 overexpression strain SSAAI-NCgl0581 (white bar). (d) Yp/x of SSAAI (gray bar with slash) and NCgl0581 and serE double overexpression strain SSAAI-NCgl0581-serE (white bar).
Figure 6

Fermentation process of strain A36 and strain A36-serE-serAΔ197-serC-serB. The cell growth (open symbols), L-serine titer (solid symbols), and residual sucrose (gray symbols) of strain A36 (squares) and A36-serE-serAΔ197-serC-serB (circles) are presented. Three parallel experiments were performed. Error bars indicate standard deviations of the results from three parallel experiments.

Supplementary Files

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- Supplementarymaterials.pdf