Research & Reviews: Journal of Microbiology and Biotechnology

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Biosensor-Mediated Adaptive Laboratory Evolution of Escherichia coli for L-Serine Overproduction from Glycerol

Xiaomei Zhang[1], Dong Zhang[1], Yujie Gao[1], Xiaojuan Zhang[2,3], Guoqiang Xu[2,3], Hui Li[1], Jinsong Shi[1], Zhenghong Xu[2,3]*

[1]Laboratory of Pharmaceutical Engineering, School of Pharmaceutics Science, Jiangnan University, Wuxi 214122, China

[2]National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi 214122, China

[3]The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China

Research Article

Received date: 06/01/2020

Accepted date: 20/01/2020

Published date: 27/01/2020

***For Correspondence**

Zhenghong Xu, National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi 214122, China

E-mail: zhenghxu@jiangnan.edu.cn

Keywords: *Escherichia coli, Biosensor, Glycerol, Adaptive Laboratory Evolution, L-serine*

ABSTRACT

In this study, adaptive laboratory evolution (ALE) combined with biosensor were employed to improving L-serine yield. First, serine-biosensor was constructed in *E.coli* based on the transcriptional regulator NCgl0581 of *C. glutamicum*, furthermore, the validity and sensitivity of the serine-biosensor was studied, and the results showed that serine-biosensor pDser from *C. glutamicum* was effective in *E. coli* and only cellular L-serine biosynthesized was monitored by serine-biosensor. Then *E. coli* 4W capable of producing 1.1 g/L L-serine from glycerol was used as a starting strain, and L-serine degradation pathway to glycine of 4W was deleted by CRISPR/Cas9, resulting in strain 4WG, with L-serine titer of 2.01 g/L. 4WG was further evolved by using ALE combined with serine-biosensor, the evolved strain 4WGX was achieved and showed a yield of 0.41 g/g glycerol, and could produce 4.13 g/L L-serine, which was 105% and 275% higher than that of 4WG and 4W respectively, in addition, 4WGX showed better growth in the medium with 50 g/L L-serine addition, indicating its better L-serine tolerance. This work indicates that the serine-biosensor from *C. glutamicum* was useful in selecting serine over-producing *E. coli*, and this expanded the application of biosensor, and provided the more strategies for screening high performance strain.

INTRODUCTION

L-Serine is a non-essential amino acid that has an important physiological functions in the human body and is widely used in the pharmaceutical, food and cosmetic industries [1,2]. Most microbial L-serine production processes rely on Escherichia coli (*E. coli*) or Corynebacterium glutamicum (*C. glutamicum*) as production hosts [1-8]. However, *C. glutamicum* grows slowly, thus resulting in a long manufacturing cycle. Compared with *C. glutamicum*, *E.coli* has a higher growth rate, and the metabolic engineering method is well developed, thus suggesting that the production of L-serine by *E. coli* has great potential [1, 5,9].

Simultaneously, in addition to traditional fermentation substrates such as glucose, sucrose and other sugar raw materials, glycerol has become a very competitive new choice [10,11]. As non-renewable fossil energy is increasingly depleted, searching for new alternative energy sources, such as biodiesel, has become a top priority. However, the large-scale development and utilization of biodiesel has brought about another serious problem: the treatment and reuse of crude glycerol, the by-product of biodiesel manufacturing. According to statistics, every 10 kg of biodiesel produces approximately 1 kg of crude glycerol by-product [12,13]. If these by-products could be converted into high value-added chemicals, not only could the crude glycerol be reused but also the chemical cost could be decreased. Therefore, using glycerol as a carbon source to produce high value-added chemicals has become a major topic in the biodiesel industry [14,15].

The chemicals produced by using glycerol as a substrate mainly include shikimic acid, lactic acid, succinate, lysine, L-phenylalanine and 1,3-propanediol [11,13,16-19]. However, only our previous study had described the production of L-serine by glycerol as a substrate [20], and all studies on the production of L-serine by *E. coli* had focused on glucose. In the recombinant *E. coli* MG1655 strain, serine degradation was deleted, and eamA (a cysteine/acetyl serine transporter) overexpression was performed, the resulting strain produced 11.7 g/L L-serine from glucose with threonine addition [5]. Furthermore, an evolved strain obtained through adaptive laboratory evolution (ALE) accumulated 37 g/L L-serine from glucose with a yield of 0.24 g/g glucose

e-ISSN: 2320-3528
p-ISSN: 2347-2286

[1]. In a recent study, 50 g/L of L-serine was produced with glucose in *E. coli*, with a yield of 0.26-0.30 g/g glucose [21]. Compared with that from glucose, the metabolic pathway from glycerol to L-serine is shorter, and the carbon atom economy is also better [20]. The recombinant strain *E. coli* 4W, capable of producing 1.1 g/L of L-serine with glycerol as substrate, was constructed in our previous study [20]. However, its L-serine yield was relatively low, and L-serine severely inhibited the cell growth of strain 4W. To achieve a high-yield L-serine producing strain, besides modifying metabolic pathways, increasing the strain's serine tolerance and growth rate were also necessary.

Adaptive laboratory evolution, also called evolutionary engineering, has become a valuable tool in metabolic engineering for strain development and optimization by reliably facilitating microbial fitness improvement [22], ALE could be used to increase a strain's growth rate and tolerance, and to increase the product yield [23,24]. However, the traditional screening procedure was laborious and time-consuming. There need to develop an efficient method to screen high performance strain [25]. High-throughput screening (HTS) with biosensors had been used to screen overproducing strains [26-31]. In our previous study [32], the serine-biosensor of *C. glutamicum* was constructed and used to screen L-serine overproducing strains. However, little knowledge about the heterologous expression of the biosensor was also effective to screen the high-performance strain or not.

In this study, the validity and sensitivity of the serine-biosensor from *C. glutamicum* was studied in *E.coli* firstly, subsequently, to increase L-serine production, we used CRISPR/Cas9 gene editing technology to knock out the gene glyA, which encodes an L-serine degradation pathway enzyme (serine hydroxymethyl transferase) in *E. coli* 4W, thus resulting in strain 4WG. Then the serine-tolerant of strain *E. coli* 4WG was enhanced through ALE combined the high-throughput screening method based on serine-biosensor; and the evolved strain *E. coli* 4WGX was selected by this strategy. Moreover, the evolved strain 4WGX's tolerance to serine was studied, and the whole genome of the 4WGX was sequenced, and comparative genomics analysis and reverse mutation were performed **(Figure 1)**.

Figure 1. The protocol of constructing strain 4WGX over-producing L-serine from glycerol. GlyA encoded serine hydroxymethyl transferase, Red crosses on solid lines ( ) indicated genes that were deleted. 4W had been constructed with deletion of sdaA, sdaB, and tdcG (The three genes encoded L-serine deaminases) and the removal of feedback inhibition of serA (serA encoded 3-phosphoglycerate dehydrogenase) in our previous study.

MATERIALS AND METHODS

Strains and Plasmids

Strains and plasmids used in this study are summarized in **Table 1**. Primers for gene cloning and deleting are listed in **Table 2**. Strain 4W, carrying deletions of sdaA, sdaB and tdcG, was constructed in our previous study [20]. The serine-biosensor pDser from *C. glutamicum* was also constructed in our previous study [32]. The plasmids pTarget and pCas were used for knocking out the glyA gene.

Table 1. Strains and plasmids used in this study.

Strains or plasmids	Description	Sources
Strains		
E .coli JM109	recA1, endA1, gyrA96, thi-1, hsd R17(rk- mk+) supE44	Invitrogen
4W	W3110ΔtdcGΔsdaAΔsdaB serAdr	Invitrogen
4WG	4WΔglyA	This study
4W-pDer	4W harboring serine- biosensor pDser	This study
4WGX	A mutant derived from 4W	This study
4WG-pDer	4WG harboring serine- biosensor pDser	This study
Plasmids		
pCas	Carrying Cas9 and λRed System, kana	Invitrogen
pTargetF	Carrying N20 sequence, spc or smr	Invitrogen
pDser	Biosensor, kana	Invitrogen

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Table 2. Primers used in this study.

Primers	Sequence
pTargetF-ΔglyA1-F	ACTGTGGCAGGCTATGGAGCGTTTTAGAGCTAGAAATAGCAAGTT
	GCTCCATAGCCTGCCACAGTACTAGTATTATACCTAGGACTGAGC
pTargetF-ΔglyA2-F	AGAAGCCGAAGCGAAAGAACGTTTTAGAGCTAGAAATAGCAAGTTGTTCTTTCGCTTCGGCTTCTACTAGTATTATACCTAGGACTGAGC
pTargetF-ΔglyA2-R	AGCCCTGCAATGTAAATGGTT
glyA-U-F	ACAGCAAATCACCGTTTCGCCCGCATCTCCTGACTCAGCTA
glyA-U-R	AGCTGAGTCAGGAGATGCGGGCGAAACGGTGATTTGCTGTC
glyA-D-F	TCGCCAGACAGGATTTAACCC
glyA-D-R	CCCTGATTCTGTGGATAACCGTA
pTargetF: 1756F23	ACATCAGTCGATCATAGCACGAT
pTargetF: 78R23	

Growth medium and culture conditions

Luria-Bertani (LB) medium was used for plasmid construction. When appropriate, streptomycin (50 μg/mL) or kanamycin (50 μg/mL) was added. For L-serine fermentation, mineral AM1 medium [33] supplemented with 10.0 g/L glycerol, 8.6 g/L (NH4)2•HPO4, 3.9 g/L NH4H2PO4 and 1 g/L yeast extract was used.

E. coli was cultured according to our previous study [20].

CRISPR/Cas9 Genome engineering for knocking out the glyA gene

Using the 4W genome as a template, we obtained the upstream homologous arms of the glyA gene by PCR using primers glyA-U-F and glyA-U-R, and the downstream homologous arms of glyA gene by using primers glyA-D-F and glyA-D-R. Then both the upstream and downstream arms were used as templates and the homologous recombination repair templates were obtained by overlap extension PCR using primers glyA-U-F and glyA-D-R.

Plasmid pTarget was extracted from *E. coli* JM109 and amplified with primers pTargetF-ΔglyA1-F, pTargetF-ΔglyA1-R and pTargetF-ΔglyA2-R and pTargetF-glyA2-F. The template was degraded with the DpnI enzyme, and then PCR products were transferred to *E. coli* JM109 to repair the cyclization gap. After the clones were grown, pTargetF: 1756F23 and pTargetF:78R23 were used for PCR amplification. If the sequencing results were correct, then plasmids were amplified and extracted. Finally, two pTarget plasmids containing N20 sequences specifically targeting glyA were obtained. The N20 sequences were predicted in CHOPCHOP (chopchop.cbu.uib.no).

Finally, homologous arm fragments and two pTarget plasmids were electroporated into competent cells containing the pCas plasmid. Transformants were selected on kanamycin and streptomycin plates and verified by PCR using the corresponding primers glyA-U-F and glyA-D-R.

Construction and verification of the serine-biosensor

The pDser plasmid had been constructed in our previous study [32], and then the pDser plasmid was introduce in *E. coli* 4W, transformants were selected and verified, and strains containing the pDser plasmid had been achieved. And the verification of the serine-biosensor was according to our previous study [32].

Procedure of the biosensor-driven evolution experiment

For the strain evolution, *E. coli* 4WG harboring pDser was cultured in LB medium, transferring 10% (v/v) inoculum into fresh AM1 medium with 6 g/L L-serine, and growing for 10 generations. Then, the cells were transferred into fresh AM1 medium with 12 g/L L-serine. Successive rounds of ALE were carried out with the L-serine increased stepwise (6, 12, 25 and 50 g/L). Among the evolved strains, the most efficient strain was selected by using FACS according to our previous study [32]. The fluorescence distributions of five different time periods for *E. coli* 4WG-pDser and the parental strain were analyzed.

Genome sequencing

E. coli 4WGX genome was sequenced by GENEWIZ, Suzhou, China, with the Illumina HiSeq 10×platform, and identify sequence variants relative to the *E. coli* W3110 reference genome.

Analytical method

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Cell growth was measured as the OD600 (AOE UV-1200S, China). A triglyceride assay kit for measuring glycerol concentration was purchased from Nanjing Jiancheng Bioengineering Institute. First, a standard curve based on different concentrations of glycerol standard and the corresponding OD550 values was constructed, and then the actual glycerol concentration of each sample was calculated according to the OD550 value. The fluorescence intensity of bacteria was detected with a microplate reader with an excitation wavelength of 488 nm and emission wavelength of 530 nm. The concentrations of L-serine were determined with high-performance liquid chromatography (HPLC; Agilent 1100, USA) according to a previously reported method [32].

RESULTS

The construction and verification of serine-biosensor in *E.coli*

The serine-biosensor of *C. glutamicum* was constructed and used to screen L-serine overproducing strains in our previous study [32]. However, little study had reported that the heterologous expression of the biosensor was also effective to screen the high-performance strain or not. So the validity and sensitivity of the serine-biosensor from *C. glutamicum* was then studied in *E.coli*. The serine-biosensor pDser was transformed into *E. coli* 4W, 4W capable of producing 1.1 g/L L-serine from glycerol was constructed in our previous study [20], and 4W-pDser was constructed. Afterward, 4W and 4W-pDser were photographed with a laser scanning confocal microscope under visible light and UV light. As shown in **Figure 2**, the parent strains 4W showed no fluorescence signal. However, 4W-pDser showed substantial fluorescence intensity. This result indicated that the serine-biosensor was successfully expressed in *E. coli.*

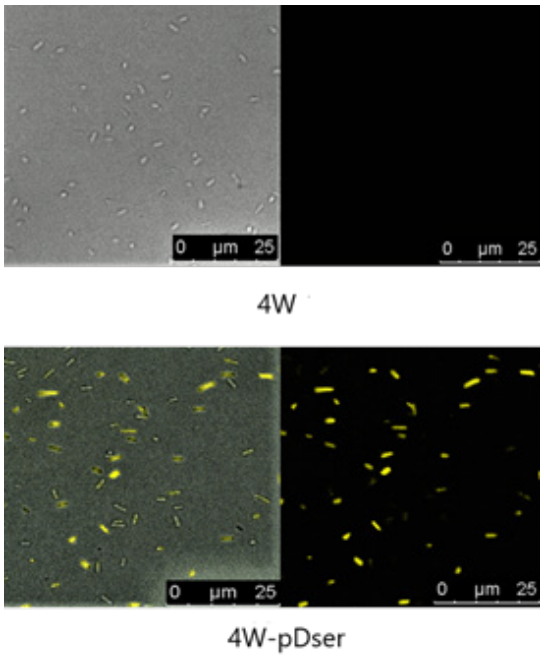

Figure 2. Identification of serine-biosensor pDser in *E. coli*. 4W-pDser clearly emitted yellow fluorescence, whereas the control *E. coli* 4W did not emit yellow fluorescence.

Then the relationship of fluorescence intensity and L-serine titer was studied, the result showed that, when L-serine titer increased, the fluorescence intensity increased, indicating that the fluorescence signal from the serine-biosensor correlated with the L-serine titer **(Figure 3)**. Moreover, in the next evolved experiment, L-serine was added to the medium, then the effect of the L-serine addition to the biosensor was studied, the result showed that there was no significant change in fluorescence intensity with varied amount of L-serine addition (data not shown), indicating that cellular L-serine biosynthesized was only monitored by serine-biosensor. These results demonstrated the functionality of the serine-biosensor from *C. glutamicum* in *E. coli*, then we used this method to screening the over-producing serine strain.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

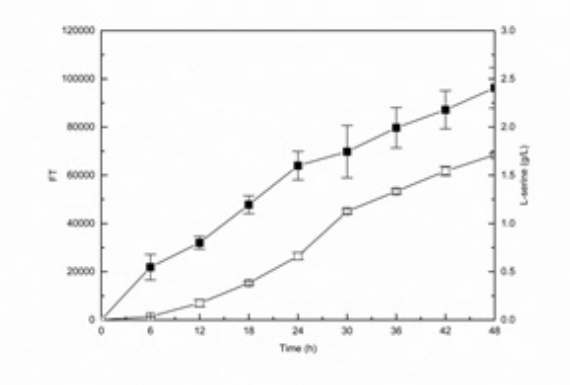

Figure 3. Comparison of L-serine accumulation and the fluorescence intensity of *E. coli* 4W-pDser. Squares represent fluorescence intensity, and open squares represent L-serine. Values denote the average of three independent experiments, and error bars indicate standard deviation.

Improved L-serine accumulation by decreasing serine degradation in *E. coli.*

To increase L-serine production of 4W further, we knocked out glyA in strain 4W by using CRISPR/Cas9, thus resulting in strain 4WG. As shown in **Figure 4B**, strain 4WG showed cell growth inhibition, with a maximum OD600 of 2.37. In contrast, the maximum OD600 of strain 4W was 5.73 **(Figure 4A)**, glyA deletion resulted in the cell growth significantly decrease. Correspondingly, the L-serine accumulation of strain 4WG was 0.75 g/L, a level significantly lower than that of the parental strain 4W (1.1 g/L). We inferred that intracellular glycine deficiency caused by knocking out the pathway of L-serine degradation resulted in poor growth status of the strain, thus leading to low L-serine accumulation.

According to a previous study [5], inactivation of SHMT in *E. coli* could effectively reduce the intracellular degradation of L-serine, exogenous glycine might be added to maintain the cell growth. Then 0.15 g/L (2 mM) glycine was added to the medium, the strain 4WG returned to normal growth with a maximum OD600 value of 4.87 **(Figure 4C)**. Meanwhile, L-serine accumulation also increased significantly, reaching 2.01 g/L after 54 h of fermentation, 53.4% higher that of the control strain 4W. Simultaneously, the substrate glycerol was completely consumed during fermentation for 48 h, a result identical to that for the parental strain 4W.

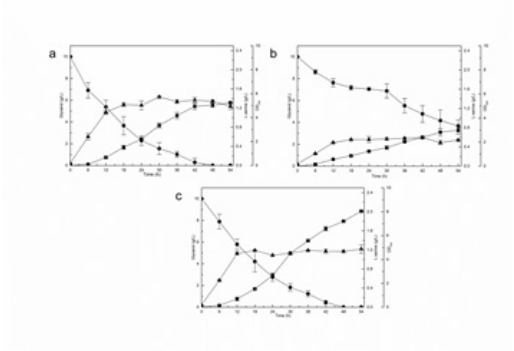

Figure 4. (A) CProfiles of glycerol consumption, cell growth and L-serine production in strain 4W. **(B)** Profiles of glycerol consumption, cell growth and L-serine production in 4WG. (C) Profiles of glycerol consumption, cell growth and L-serine production in strain 4WG with 0.15 g/L glycine added. Squares represent cell growth, circles represent residual glycerol, and triangles represent L-serine. Values denote the average of three independent experiments, and error bars indicate standard deviation.

Although L-serine titer of strain 4WG increased with the addition of glycine, L-serine was found to be highly toxic to this strain even at low concentrations. As shown in **Figure 5**, the cell growth of strain 4WG was significantly decreased by the addition of L-serine in the medium. When 6 g/L L-serine was added, the maximum OD600 was 2.62. When L-serine addition reached 12, 25 and 50 g/L, the strain 4WG showed negligible growth. How to increase the strain's tolerance to L-serine was the key to over-producing L-serine.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

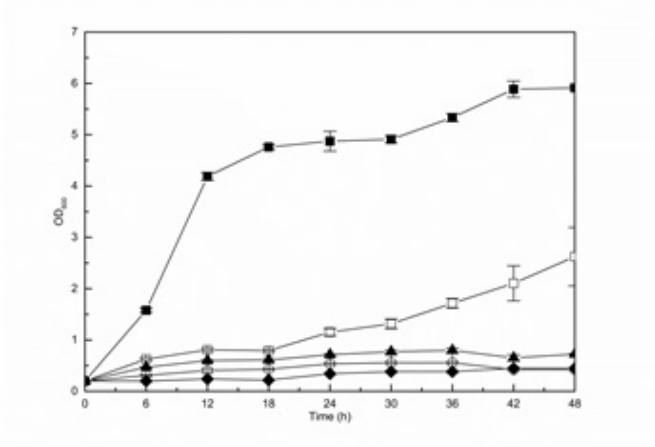

Figure 5. Growth profiles of strains 4WG in AM1 containing different concentrations of L-serine. Squares represent 0 g/L L-serine, open squares represent 6 g/L L-serine, triangles represent 12 g/L L-serine, open circles represent 25 g/L L-serine, and diamonds represent 50 g/L L-serine. Values denote the average of three independent experiments, and error bars indicate standard deviation.

Increased L-serine yield achieved by biosensor-driven evolution

ALE with biosensors was therefore performed to improve L-serine tolerance and L-serine production. Then the strain harbouring serine-biosensor (4WG-pDser) had been constructed, 4WG-pDser was repeatedly grown in culture medium containing L-serine at increasing concentrations (6, 12, 25 and 50 g/L), and the cells were continuously transferred into fresh medium during the exponential growth phase. At the end of the experiment, an appropriate amount of bacterial solution was absorbed from three parallel plates and then diluted to 10-5-10-6 times. Then 100 μL of diluted bacterial solution was coated on kanamycin plates and cultured overnight at 37°C, and single colonies were transferred to 96-well plates. After 24 hours of fermentation, the fluorescence intensity of the strain in each well was measured. As shown in **Figure 6**, the first one was the control strain 4WGX-pDser, and the remaining 95 strains were single colonies selected on the plates. By comparison of fluorescence intensity, 5 strains were selected with the highest intensity values for flask fermentation. The resultant ALE strain was named 4WGX. As shown in **Figure 7A**, after 48 h of fermentation, the maximum OD600 of strain 4WGX was 6.87; glycerol was completely consumed at 24 h; the L-serine titer was 4.13 g/L at 48 h, a value 105% higher than that of 4WG (2.01 g/L) and 275% higher than that of 4W (1.1 g/L); and the substrate conversion rate was 41.3%. Then, strain 4WGX was cultured in medium with the addition of 50 g/L L-serine; the cell growth was shown in **Figure 7B**, the maximum OD600 of strain 4WGX reached 3.65, and the parental strain 4WG showed almost no growth in the same medium **(Figure 5)**. To clarify the reasons for the greatly improved serine-tolerance of strain 4WGX, strain 4WGX was sent to Genewiz for whole genome resequencing. The sequencing results revealed a total of 11 single base mutations in the genome of strain 4WGX compared with strain 4W, including 10 non-synonymous mutations (bamA, brnQ, ybcJ, fepB, agp, dgcT, oppB, fliK, ygbN and eno) and 1 synonymous mutation (fdrA). We chose the two genes (agp encoded glucose-1-phosphatase, and eno encoded enolase) not involved in the membrane to study firstly, the reverse mutation of agp and eno were performed in the genome of 4WG, thus resulting in the mutant strains. However, we did not observe any significant change regarding to L-serine production, the cell growth, and glycerol consumption in both strains (data not shown). Further research would focus on understanding the reasons for the improvement in L-serine tolerance caused by these genomic mutations.

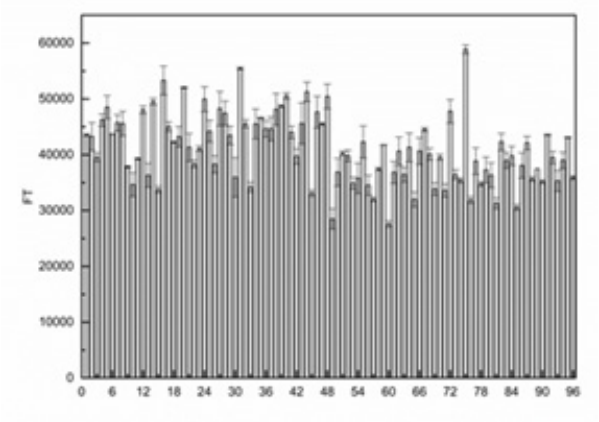

Figure 6. Comparison of the adaptive strains' fluorescence intensity. Cylinders represent fluorescence intensity. Values denote the average of three independent experiments, and error bars indicate standard deviation.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

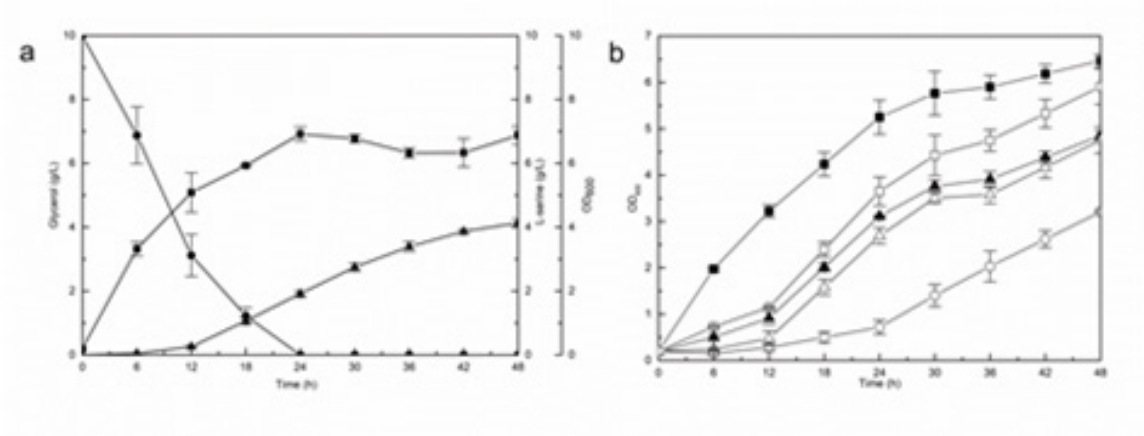

Figure 7. (A) Profiles of glycerol consumption, cell growth and L-serine production in strain 4WGX. Squares represent cell growth, circles represent residual glycerol, and triangles represent L-serine. Values denote the average of three independent experiments, and error bars indicate standard deviation. **(B)** Growth profiles of the evolved strains 4WGX in AM1 containing different concentrations of L-serine. Squares represent 0 g/L L-serine, open squares represent 6 g/L L-serine, triangles represent 12 g/L L-serine, open triangles represent 25 g/L L-serine, and open circles represent 50 g/L L-serine. Values denote the average of three independent experiments, and error bars indicate standard deviation.

DISCUSSION

In this study, we successfully established a biosensor-driven laboratory evolution approach using serine-biosensor from *C. glutamicum* for improving the L-serine tolerance and L-serine production of *E. coli*. Within several iterative rounds, *E. coli* 4WGX was isolated from a large evolved strain library, and strain produced 4.13 g/L L-serine, with the yield of 0.41g/g glycerol.

Degradation is a crucial issue in microbial L-serine accumulation. L-Serine has two main degradation pathways to either glycine or pyruvate. The conversion of L-serine to pyruvate in *E. coli* is catalyzed by three L-serine deaminases, sdaA, sdaB and tdcG. The conversion of serine to glycine through serine hydroxymethyl transferase (SHMT) is catalyzed by glyA. Strain 4W had deleted sdaA, sdaB and tdcG in *E.coli* W3110. In this study, to remove the L-serine degradation pathway in *E. coli* 4W, the gene glyA was knocked out only by using CRISPR/Cas9, thus resulting in strain 4WG, which produced 2.01 g/L L-serine with the addition of glycine. Decreasing SHMT activity strongly affects L-serine accumulation was observed in other studies [5-7]. However, in the present experiments, glyA deletion resulting the cell growth inhibition, and a lower glycerol consumption rate. These results were not completely consistent with Mundhada's study, in which the T1 strain (*E.coli* MG1655 with tdcG, sdaA and sdaB deletion) had a higher glucose consumption rate and a lower cell growth when compared to the Q1(strain T1 with glyA deletion) [5], namely with glyA deletion, the cell growth had not significantly change, and we inferred that this might be due to the different carbon source. And strain 4WG with lower OD600 was unsuitable for fed batch fermentation. To overcome this limitation, ALE was used to increase the strain's serine tolerance.

Biosensor has been widely used to construct high throughput screening method and optimize pathway expression [34-38]. In our previous study, the serine-biosensor pDser had been constructed in *C. glutamicum*, which was based on NCgl0581 (a transcription factor specifically responsive to L-serine in *C. glutamicum*), for high-throughput screening of high-yield L-serine strains. However, we didn't know whether this biosensor was fit for screening L-serine over-producing *E. coli* or not, because the heterologous expression of the transcriptional regulator might interfere with the host gene regulatory networks significantly. Moreover, the sensitivity of the biosensor was determined by the rate of occupation of a promoter by transcription factors through protein-protein interaction [39]. Therefore, the validity and sensitivity of serine-biosensor pDser was then verified in this study firstly, and the results showed that the biosensor from *C. glutamicum* was effective in selecting L-serine over-producing *E. coli*, based on this; the high-throughput screening method was constructed. Moreover, in the evolved experiment, L-serine was added to the medium, then the effect of the L-serine addition to the biosensor was studied, the result showed that there was no significant change in fluorescence intensity with varied amount of L-serine added (data not shown), indicating that cellular L-serine biosynthesized was only monitored by serine-biosensor.

In a recent study, 50 g/L of L-serine has been produced with glucose in *E. coli*, with a yield of 0.26-0.30 g/g glucose, and 50 g/L was the highest level of L-serine production reported to date. Compared with glucose for microbial L-serine production, more specific advantages of glycerol were its better carbon atomic economy and higher reduction degree, it was very promising to utilize glycerol as substrate for amino acid production [20,40]. Moreover, the performance of the recombinant strains on crude glycerol should be further examined. Efforts also need to focus on engineering strains for industrial implementation, especially for increased tolerance to toxic substances of crude glycerol.

CONCLUSION

e-ISSN: 2320-3528
p-ISSN: 2347-2286

This study demonstrated that the serine-biosensor from *C. glutamicum* was useful in selecting serine over-producing *E. coli*, and biosensor-mediated ALE was a valuable tool to improve the cell growth and productivity of microbial production strains. Although using glycerol as a substrate for microbial fermentative production remains more expensive than chemical production, owing to the lower production at present, glycerol is also an alternative substrate providing a variety of economic and metabolic advantages, with further engineering and optimization, fermentation directly using glycerol as carbon source could become competitive.

ACKNOWLEGEMENTS

This work was financially supported by the National Key Research and Development Program of China (2018YFA0901401). Program of National First-class Discipline Program of Light Industry Technology and Engineering (LITE2018-11).

CONFLICT OF INTEREST

The authors have no financial conflicts of interest to declare.

REFERENCES

1. Mundhada H, et al. Increased production of L-serine in Escherichia coil through Adaptive Laboratory Evolution. Metab Eng. 2017; 39:141-150.
2. Zhang XM, et al. Microbial production of L-serine from renewable feedstocks. Trends Biotechnol. 2018; 36: 700-712.
3. Gu PF, et al. Construction of an L-serine producing Escherichia coli via metabolic engineering. J Ind Microbiol Biotechnol. 2014; 41: 1443-1450.
4. Li Y, et al. Construction of Escherichia coli strains producing L-serine from glucose. Biotechnol Lett. 2012; 34: 1525-1530.
5. Mundhada H, et al. Engineering of high yield production of L-serine in Escherichia coli. Biotechnol Bioeng. 2016; 113: 807-816.
6. Peters-Wendisch P, et al. Metabolic engineering of Corynebacterium glutamicum for L-serine production. Appl Environ Microbiol. 2005; 71: 7139-7144.
7. Stolz M et al. Reduced folate supply as a key to enhanced L-serine production by Corynebacterium glutamicum. Appl Environ Microbiol. 2007; 73:750-755.
8. Zhu QJ, et al. L-serine overproduction with minimization of by-product synthesis by engineered Corynebacterium glutamicum. Appl Microbiol Biotechnol. 2005; 99: 1665-1673.
9. Wang Y et al. Evolving the L-lysine high-producing strain of Escherichia coli using a newly developed high-throughput screening method. J Ind Microbiol Biotechnol. 2016; 43:1227-1235.
10. Gottlieb K, et al. Improvement of L-phenylalanine production from glycerol by recombinant Escherichia coli strains: The role of extra copies of glpK, glpX, and tktA genes. Microb Cell Fact. 2014; 13: 16.
11. Nguyen-Vo TP et al. Development of 3-hydroxypropionic-acid-tolerant strain of Escherichia coli W and role of minor global regulator yieP. Metab Eng. 2019; 53:48-58.
12. Sprenger GA. Engineering of microorganisms for the production of chemicals and fuels from renewable resources. In: Gosset G (ed) Glycerol as carbon source for production of added-value compounds. Springer Nature Verlag. 2017; 4:93-123.
13. Luo X, et al. Value-added processing of crude glycerol into chemicals and polymers. Bioresource Technology. 2016; S0960852416303285.
14. Trondle J, et al. Fed-batch production of l-tryptophan from glycerol using recombinant Escherichia coli. Biotechnol Bioeng. 2018; 115:2881-2892.
15. Yang FX, et al. Value-added uses for crude glycerol-a byproduct of biodiesel production. Biotechnol Biofuels. 2012; 5:10.
16. Li Q, et al. Enhanced succinate production from glycerol by engineered Escherichia coli strains. Biores Technol. 2016; 218: 217-223.
17. Litsanov B, et al. Glycerol as a substrate for aerobic succinate production in minimal medium with Corynebacterium glutamicum. Microb Biotechnol. 2013; 6:189-195.
18. Mazumdar S, et al. Efficient synthesis of L-lactic acid from glycerol by metabolically engineered Escherichia coli. Microb Cell Fact. 2013; 12:11.
19. Weiner M, et al. Fed-batch production of L-phenylalanine from glycerol and ammonia with recombinant Escherichia coli. Biochem Eng J 2014; 83:62-69.
20. Zhang XM, et al. High-yield production of L-serine from glycerol by engineered Escherichia coli. J. Ind. Microbiol Biotechnol. 2019; 46: 883-885.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

21. Rennig M, et al. Industrializing a bacterial strain for L-serine production through translation initiation optimization. ACS Synth Biol. 2019; 8: 2347-2358.

22. Zhu ZM, et al. Evolutionary engineering of industrial microorganisms-strategies and applications. Appl Microbiol Biotechnol. 2018; 102: 4615-4627.

23. Leavitt JM, et al. Biosensor-enabled directed evolution to improve muconic acid production in Saccharomyces cerevisiae. Biotechnol J. 2017; 12:9.

24. Sandberg TE, et al. The emergence of adaptive laboratory evolution as an efficient tool for biological discovery and industrial biotechnology. Metab Eng. 2019; 56:1-16.

25. Zhang X, et al. Atmospheric and room temperature plasma (ARTP)as a new powerful mutagenesis tool. Appl. Microbiol. Biotechnol. 2014; 98: 5387-5396.

26. Binder S, et al. A high-throughput approach to identify genomic variants of bacterial metabolite producers at the single-cell level. Genome Biol. 2012; 13:12.

27. Chen W, et al. Design of an ectoine-responsive AraC mutant and its application in metabolic engineering of ectoine biosynthesis. Metab Eng. 2015; 30:149-155.

28. Cress BF, et al. Sensitive cells: enabling tools for static and dynamic control of microbial metabolic pathways. Curr Opin Biotechnol. 2015; 36:205-214.

29. Liu YN, et al. Developing a high-throughput screening method for threonine overproduction based on an artificial promoter. Microb Cell Fact. 2015; 14: 11.

30. Schulte J, et al. Development of a single-cell GlxR-based cAMP biosensor for Corynebacterium glutamicum. J Biotechnol. 2017; 258: 3-40.

31. Wang QZ, et al. Genetic biosensors for small-molecule products: Design and applications in high-throughput screening. Front Chem Sci Eng. 2017; 11: 15-26.

32. Liu C, et al. New intracellular shikimic acid biosensor for monitoring shikimate synthesis in Corynebacterium glutamicum. ACS Synth Biol. 2018; 7: 591-601.

33. Martinez A, et al. Low salt medium for lactate and ethanol production by recombinant Escherichia coli. B Biotechnol Lett. 2007; 29: 397-404.

34. Feist AM, et al. Model-driven evaluation of the production potential for growth-coupled products of Escherichia coli. Metab Eng. 2010; 12: 173-186.

35. Storek KM, et al. The Escherichia coli beta-barrel assembly machinery is sensitized to perturbations under high membrane fluidity. J Bacteriol. 2019; 201.

36. Eggeling L, et al. A giant market and a powerful metabolism: L-lysine provided by Corynebacterium glutamicum. Appl Microbiol Biotechnol. 2015; 99: 3387-3394.

37. Johnson AO, et al. Design and application of genetically-encoded malonyl-CoA biosensors for metabolic engineering of microbial cell factories. Metab Eng. 2017; 44: 253-264.

38. Xu P. Production of chemicals using dynamic control of metabolic fluxes. Curr Opin Biotechnol. 2018; 53: 12-19.

39. Mahr R, et al. Screening of an Escherichia coli promoter library for a phenylalanine biosensor. Appl Microbiol Biotechnol. 2016; 100: 6739-6753.

40. Chen Z, et al. Toward glycerol biorefinery: metabolic engineering for the production of biofuels and chemicals from glycerol. Biotechnol Biofuels. 2016; 9: 15.