

Production of Cadaverine in Recombinant *Corynebacterium Glutamicum* Overexpressing Lysine Decarboxylase (*IdcC*) and Response Regulator *dr1558*

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Short Report

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Abstract

In this study, the response regulator DR1558 from *Deinococcus radiodurans* was overexpressed in recombinant *Corynebacterium glutamicum* with lysine decarboxylase (*IdcC*). The recombinant *C. glutamicum* strain overexpressing *dr1558* and *IdcC* produced 5.9 g/L of cadaverine by flask cultivation, whereas the control strain overexpressing only *IdcC* produced 4.5 g/L of cadaverine. To investigate the mechanism underlying the effect of DR1558, the expression levels of genes related to central metabolism and lysine-biosynthesis were analyzed by quantitative-real time polymerase chain reaction. The results showed that phosphoenolpyruvate carboxykinase (*pck*) was downregulated, and pyruvate kinase (*pyk*) and other lysine biosynthesis genes were upregulated. Furthermore, in fed-batch fermentation, *C. glutamicum* coexpressing *dr1558* produced 25.14 g/L of cadaverine, a 1.25-fold increase in concentration relative to the control. These results suggested that the heterologous expression of *dr1558* may improve the production of biorefinery products by recombinant *C. glutamicum*.

Introduction

Recently, due to the depletion of fossil fuels and climate change, much research has focused on sustainable biorefinery processes as alternatives to chemical-based processes [1–4]. As a result, research on fermentation processes using metabolically engineered microorganisms to produce organic acids and various chemicals from biomass-derived sugars, such as glucose, has been continuously conducted [5–11].

Cadaverine, which can be used as a precursor for bio-based nylon production, is generated through an enzymatic reaction catalyzed by constitutive (*LdcC*) or inducible (*CadA*) lysine decarboxylase [12–14]. *CadA*, encoded by the *cadA* gene in the *cadBA* operon, is induced by low external pH, excess lysine, and low oxygen levels [15]. *CadA* functions only under acidic conditions, with an optimum pH of 5.7. In contrast, *LdcC* encoded by the *IdcC* is expressed constitutively, independent of pH changes [15]. *LdcC* is active across a relatively wide pH range, and the optimum pH for activity is 7.5. Here, *LdcC* was used for cadaverine biosynthesis because it is functional under fermentation conditions without pH manipulation.

Corynebacterium glutamicum is an industrial bacterial strain known for its ability to produce amino acids, such as L-lysine and glutamate, as well as organic acids, nucleotides, diamines, and polymers. However, *C. glutamicum* strains used to produce lysine, a precursor of cadaverine, are exposed to various environmental stresses during fermentation. These stresses, such as high osmotic pressure, accumulation of metabolites, and oxidation that occur during cultivation, have negative effects on cell growth and chemical productivity [16–19]. Many recent studies have reported that stress resistance could be improved by changing the physiological characteristics of the microorganisms [20–22].

Deinococcus radiodurans is a bacterium known for its resistance to various abiotic stresses, such as γ -radiation, reactive oxygen species, and oxidants [23]. DR1558, a response regulator of *D. radiodurans*, has been reported to improve stress resistance and cell growth when overexpressed in *Escherichia coli* [24].

Recently, the *dr1558* gene was introduced into *C. glutamicum* to improve L-lysine production [25]. Since DR1558 increased the production of L-lysine, a precursor of cadaverine, it is expected to also increase cadaverine biosynthesis in *C. glutamicum*.

In this study, we examined the effect of plasmid-based heterologous coexpression of *ldcC* and *dr1558* on cadaverine production by *C. glutamicum*. Cell growth, glucose consumption, and cadaverine production of the recombinant *C. glutamicum* strain were analyzed in comparison to a control strain expressing only *ldcC*. In addition, the expression levels of the genes involved in cadaverine biosynthesis were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) to determine the effect of DR1558 on the metabolic processes related to cadaverine biosynthesis in recombinant *C. glutamicum*.

Materials And Methods

Bacterial Strains, Plasmids, and Culture Medium

All bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* XL1-Blue (Stratagene Cloning Systems, La Jolla, CA, USA) was used for gene cloning. *Corynebacterium glutamicum* KCTC 1857 (Korean Collection for Type Cultures, Joengeup, Republic of Korea), a mutant strain of *C. glutamicum* ATCC 13032, was used as the host strain for cadaverine production [27]. *E. coli ldcC* and *D. radiodurans dr1558* were expressed under a synthetic P_{H30} promoter. Plasmids pCES208H30EcLdcC and pCES208H30EcLdcCdr1558 harboring the *E. coli ldcC* gene and both the *E. coli ldcC* gene and *D. radiodurans dr1558* were constructed as follows, respectively.

All DNA manipulations were performed according to standard procedures. *Corynebacterium glutamicum* KCTC 1857 was transformed with pCES208H30EcLdcC [26] to generate *C. glutamicum*(pCES208H30EcLdcC) (Cg_ldcC). The response regulator DR1558 was cloned into the plasmid pCES208H30EcLdcC. The *dr1558* gene was amplified by PCR using forward (attaccgggtaccaaagtaacttttcggt) and reverse primers (attaccgcgaattggagctccaccgcg) and was inserted into the BstZI71 site of pCES208H30EcLdcC. The final constructed strain, *C. glutamicum*(pCES208H30EcLdcCdr1558) (Cg_ldcC+dr1558), was used to investigate the effect of DR1558 on cadaverine biosynthesis.

Escherichia coli XL1-Blue was cultured at 37°C in Luria-Bertani medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl) for cloning. Shake-flask cultures of *C. glutamicum* were incubated at 30°C in a rotary shaker at 250 rpm. The CG-50 medium used to culture *C. glutamicum* contained (per liter): 50 g of glucose, 15 g of yeast extract, 15 g of (NH₄)₂SO₄·7H₂O, 0.5 g of KH₂PO₄, 0.5 g of MgSO₄·7H₂O, 0.01 g of MnSO₄·H₂O, and 0.01 g of FeSO₄·7H₂O. Calcium carbonate (15 g/L) was added to the culture medium for pH control along with kanamycin (30 mg/L).

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Transcriptional analysis was performed to analyze the changes in gene expression levels in the recombinant *C. glutamicum* strain overexpressing the *dr1558* gene. Cells were harvested during the mid-exponential growth phase (12 h) by centrifugation (16,000 × *g*, 10 min, 4°C). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. RNA was reverse-transcribed using a first-strand cDNA synthesis kit (TaKaRa, Kusatsu, Japan). The synthesized cDNA was subjected to qRT-PCR to determine the expression levels of the genes of interest and the housekeeping genes 16S rRNA and *sigA*. Real-time PCR (Illumina, San Diego, CA, USA) was performed using specific primers (Supplement Table 1) with TB Green Premix ExTaq (TaKaRa) for 40 cycles of 95°C for 10 s and 58°C for 30 s. All experiments were performed in triplicate. Data were analyzed using the $2^{-\Delta\Delta C_t}$ method [28].

Fed-batch Fermentation

Fed-batch fermentation was carried out at 30°C and 1,000 rpm in a 2.5-L jar fermenter (BioCNS, Daejeon, Republic of Korea) containing an initial volume of 500 mL of CG-100 medium [composition of 1 L of medium: 100 g of glucose, 30 g of yeast extract, 30 g of $(\text{NH}_4)_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$, 30 g of CaCO_3 , 0.5 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.01 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 mg of biotin, and 0.3 mg of thiamine-HCl]. During fed-batch fermentation, supplementation of the feeding solution was conducted to maintain glucose concentration at 10–50 g/L. The feeding solution contained (per liter) 400 g of glucose, 45 g of $(\text{NH}_4)_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 0.01 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The pH of the culture was maintained at 7.1 by automatic addition of 14% (*v/v*) NH_4OH . Foaming was suppressed by adding Antifoam 204 (Sigma-Aldrich, St. Louis, MO, USA), and cell growth was monitored by measuring the optical density at 600 nm (OD_{600}).

Analytical Procedures

Cell growth was monitored by measuring the OD_{600} using a UV/Vis spectrophotometer (Molecular Devices, San Jose, CA, USA). The concentrations of organic acids and glucose were determined by high-performance liquid chromatography using an Agilent Infinity 1260 system (Agilent Technologies, Santa Clara, CA, USA). The glucose concentration was determined using an Agilent Infinity 1260 System equipped with an Aminex HPX-87H ion exclusion column (BioRad, Hercules, CA, USA). The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min, and the column was maintained at 50°C. The concentrations of cadaverine and lysine were determined using an Agilent Infinity 1260 System equipped with a ZORBAX SB-C18 column (Agilent Technologies). The mobile phase consisted of 25 mM sodium acetate buffer (pH 4) and 1 M acetonitrile solution at a flow rate of 1 mL/min, and the column was maintained at 35°C. The concentrations of amino acids were measured after diethyl ethoxymethylenemalonate derivatization [29].

Results And Discussion

Cadaverine Production in Recombinant *C. glutamicum* with *dr1558* and *ldcC*

To determine the effect of *dr1558* expression in *C. glutamicum* on cadaverine production, the constructed recombinant strains with only *IdcC* (Cg_IdcC) and with both *IdcC* and *dr1558* (Cg_IdcC + dr1558) were cultivated in flasks for comparison (Fig. 1).

Both Cg_IdcC and Cg_IdcC + dr1558 recombinant strains grew similarly, but the growth rate of Cg_IdcC + dr1558 was higher than that of Cg_IdcC during the exponential phase. Additionally, the glucose consumption rate was higher in Cg_IdcC + dr1558. Glucose in the medium was completely consumed within 24 h of cultivation for both strains. Cadaverine was produced rapidly during the exponential phase in both strains. However, after 24 h, during the stationary phase, the growth of the Cg_IdcC quickly decreased, whereas that of Cg_IdcC + dr1558 gradually decreased. The amount of lysine remaining in the medium was similar for both strains. The lysine concentration was below 1 g/L during cultivation. However, final cadaverine production was increased by 15% (from 4.5 to 6.1 g/L) in Cg_IdcC + dr1558 compared with that in Cg_IdcC. Therefore, it was shown that *dr1558* overexpression led to enhanced cell growth and cadaverine production. To investigate the metabolic changes caused by *dr1558* expression in cadaverine-producing recombinant *C. glutamicum*, the expression levels of several genes involved in central and cadaverine biosynthesis pathways were compared.

Transcriptional Analysis of Cadaverine Biosynthesis Metabolism in Recombinant *C. glutamicum*

Comparative transcriptional analysis of Cg_IdcC + dr1558 and Cg_IdcC (the control strain) was performed to investigate the mechanism underlying the increased cadaverine production. Quantitative real-time PCR of 39 genes in three pathways, namely glycolysis, the tricarboxylic acid (TCA) cycle, and lysine-biosynthesis pathway, was performed (Fig. 2) [30, 31].

As expected, changes in the expression patterns of *pyc* and *pyk*, involved in oxaloacetate metabolism, were observed during the mid-exponential growth phase. The expression of *pyc*, which converts pyruvate to oxaloacetate, was upregulated 2.40-fold, while the expression of *pck*, which converts oxaloacetate to phosphoenolpyruvate, was downregulated 3.40-fold. The increase in *pyc* expression increased the flux into oxaloacetate, thereby increasing lysine-biosynthesis [32]. Furthermore, *ppc*, encoding phosphoenolpyruvate carboxylase, was slightly upregulated in Cg_IdcC + dr1558. Increasing the synthesis and reducing consumption of precursor were important factors because precursor availability was essential for producing the desired product. Cadaverine is synthesized from oxaloacetate produced in the TCA cycle [33–35]. Therefore, upregulation of genes in oxaloacetate biosynthesis would increase cadaverine production in recombinant *C. glutamicum* [33].

Other genes related to the TCA cycle, including *odhA*, *sdhA*, *sdhB*, *fumH*, and *mqO*, were upregulated, which would, in turn, increase the carbon flux into oxaloacetate in the TCA cycle. On the other hand, the upregulation of these genes was previously observed in a recombinant *C. glutamicum* strain with increased oxidative stress resistance [16]. In *E. coli*, it has been reported that *dr1558* bound to the promoter region of sigma factor *rpoS* and modulated its expression. Because the RpoS regulated various stress resistance-related genes, recombinant *E. coli* with *dr1558* was able to tolerate a low pH, a high

temperature, and high NaCl concentrations in addition to H₂O₂. The function of *dr1558* in *Corynebacterium* was not exhaustively investigated, but it may be possible that *dr1558* expression could improve oxidative stress tolerance and increase cell growth during cultivation by minimizing cell damage. Therefore, the improvement of tolerance could sustain the growth of Cg_IdsC + dr1558 during the stationary phase.

The expression levels of genes involved in the lysine-biosynthesis pathway were also investigated. In the lysine pathway, lysine is synthesized from oxaloacetate through seven reactions catalyzed by the products of the *aspC*, *lysC*, *asd*, *dapA*, *dapB*, *ddh*, and *lysA* genes [35] (Fig. 3). In the present study, the expression levels of *lysC*, *asd*, *dapA*, *dapB*, *ddh*, and *lysA* were upregulated. The changes in the expression levels of these genes could increase the oxaloacetate flux into lysine synthesis. There was a decrease of *IdsC* expression level in Cg_IdsC + dr1558, but the difference was statistically insignificant.

Figure 3 summarizes the metabolic changes that occurred in the recombinant *C. glutamicum* strain overexpressing *dr1558*. Among the metabolic pathways of *C. glutamicum*, expression of the response regulator DR1558 could affect the TCA cycle and the lysine-biosynthesis pathway; specifically, genes related to intracellular oxaloacetate supply in the TCA cycle and those related to lysine-biosynthesis were upregulated. However, the exact reason for the up-regulation of these genes was not thoroughly investigated, and further study of DR1558 in *Corynebacterium* at the molecular level is necessary.

Enhanced Cadaverine Production by Recombinant *C. glutamicum* in Fed-batch Culture

From the flask cultures, it was shown that a recombinant *C. glutamicum* strain, co-expressing DR1558, produced more cadaverine than the control strain. After confirming the expression patterns of genes related to cadaverine synthesis in *C. glutamicum* through qRT-PCR, cadaverine production by fed-batch cultivation was conducted. Previous studies have reported increased production of succinate [36], poly (3-hydroxybutyrate) [37], and 2,3-butanediol [38] in *E. coli* expressing DR1558. In particular, succinate production was increased in large-scale cultivation. Therefore, cadaverine production by the fed-batch cultivation of recombinant *C. glutamicum* coexpressing DR1558 was conducted. Fed-batch fermentation for cadaverine production was compared between Cg_IdsC + dr1558 and Cg_IdsC. The cell growth, glucose consumption, extracellular lysine concentration, and cadaverine concentration obtained during fed-batch fermentation are shown in Fig. 4. The results were similar to those of the flask cultures. Higher cell growth was observed with Cg_IdsC + dr1558 than with Cg_IdsC. Furthermore, after 45 h of cultivation, Cg_IdsC + dr1558 produced 25.1 g/L cadaverine (125% compared with that of Cg_IdsC). Similar lysine levels were accumulated in the culture medium with both strains, and no other byproducts were detected.

Conclusion

In the present study, the lysine-producing host strain *C. glutamicum* KCTC 1857 was genetically modified to co-express the *E. coli* lysine decarboxylase gene (*IdsC*) and *D. radiodurans* response regulator DR1558 (*dr1558*) to obtain the enhanced cadaverine production. According to the results, *dr1558* expression

improved the metabolic synthesis of cadaverine by improving oxaloacetate flux. Furthermore, *dr1558* expression resulted in a 1.25-fold increase in cadaverine production relative to the control strain during fed-batch fermentation. Therefore, the application of DR1558 may be of potential use for improving the yield of target products generated using microbial fermentation.

Declarations

Ethical Approval

Not applicable.

Consent to Participate

I confirm that the final manuscript has been seen and approved by all the authors. The undersigned author transfers all copyright ownership of the manuscript to Applied Biochemistry and Biotechnology in the event the work is published.

Consent to Publish

We hope that you will find our manuscript acceptable for publication in the above journal.

Contributions

S. Kang and J. Choi designed the experiments, analyzed the data, and wrote the paper.

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Competing Interests

The authors declare no competing interests.

Availability of data and materials

Not applicable.

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Tables

Table 1. Strains and plasmids used in the present study

Plasmid/strain	Relevant characteristics	Reference or source
Strains		
<i>E. coli</i> XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lac^f ZΔM15 Tn10 (Tet^R)</i>]	Stratagene
<i>C. glutamicum</i> KCTC 1857	L-lysine producing strain	KCTC
Plasmids		
pCES208H30GFP	pCES208 derivative, P _{H30} , eGFP, Km ^r	[25]
pCES208H30EcLdcC	pCES208 derivative, P _{H30} , <i>E. coli</i> <i>ldcC</i> gene, Km ^r	This study
pCES208H30EcLdcCdr1558	pCES208 derivative, P _{H30} , <i>E. coli</i> <i>ldcC</i> gene, <i>D. radiodurans</i> <i>dr1558</i> gene, Km ^r	This study

Figures

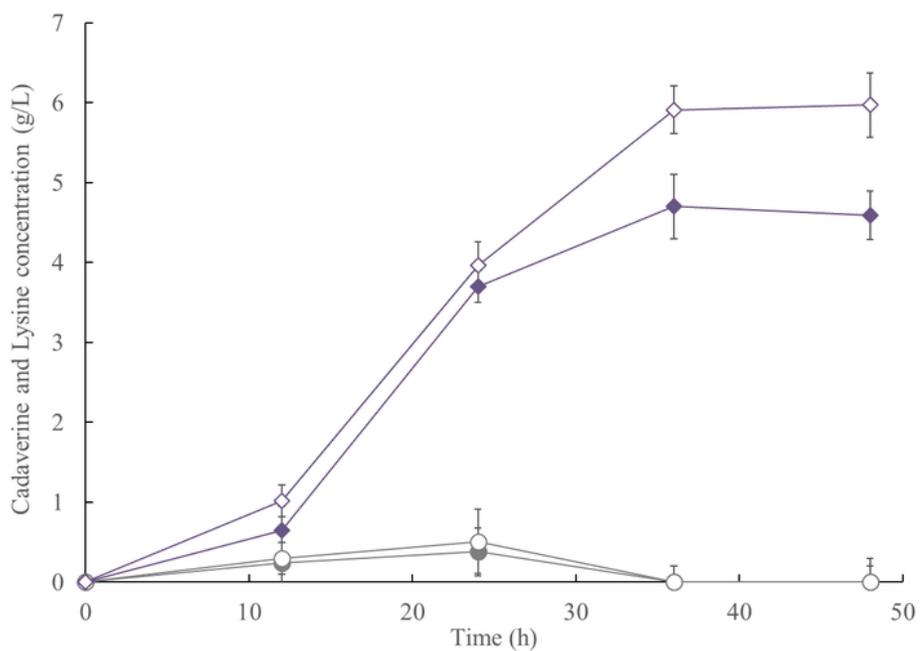
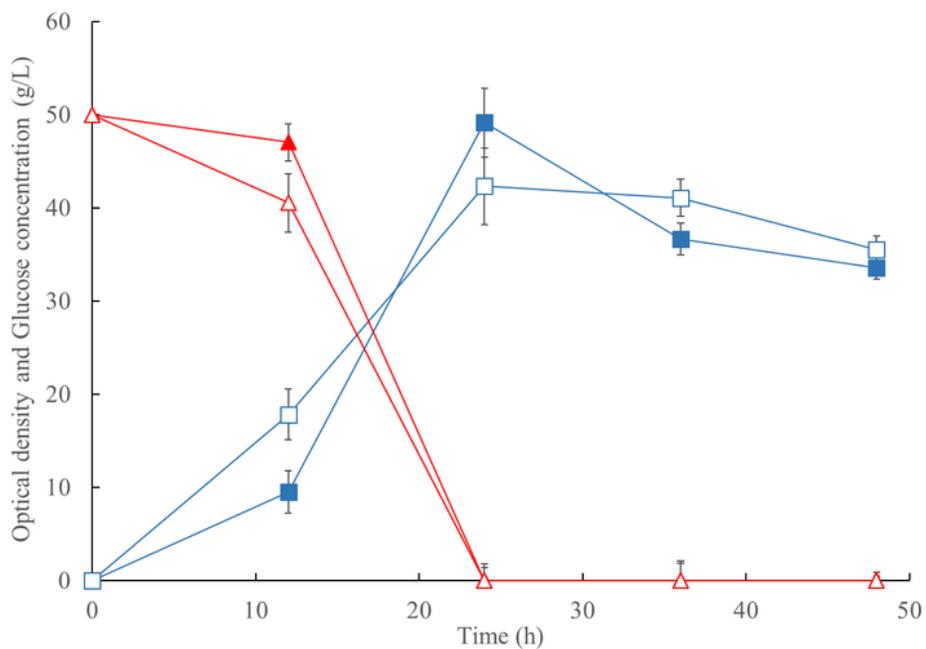


Figure 1

Time profiles of (A) glucose consumption (g/L, triangles) and cell growth (OD600, squares) and (B) the concentrations of cadaverine (g/L, diamonds) and lysine (g/L, circles) during fermentation of recombinant *C. glutamicum* KCTC 1857 expressing *E. coli* *ldcC* and *D. radiodurans* *dr1558* under the control of PH30. (open symbols: *C. glutamicum* KCTC 1857 harboring pCES208H30EcLdcCdr1558, filled symbols: *C. glutamicum* KCTC 1857 harboring pCES208H30EcLdc).

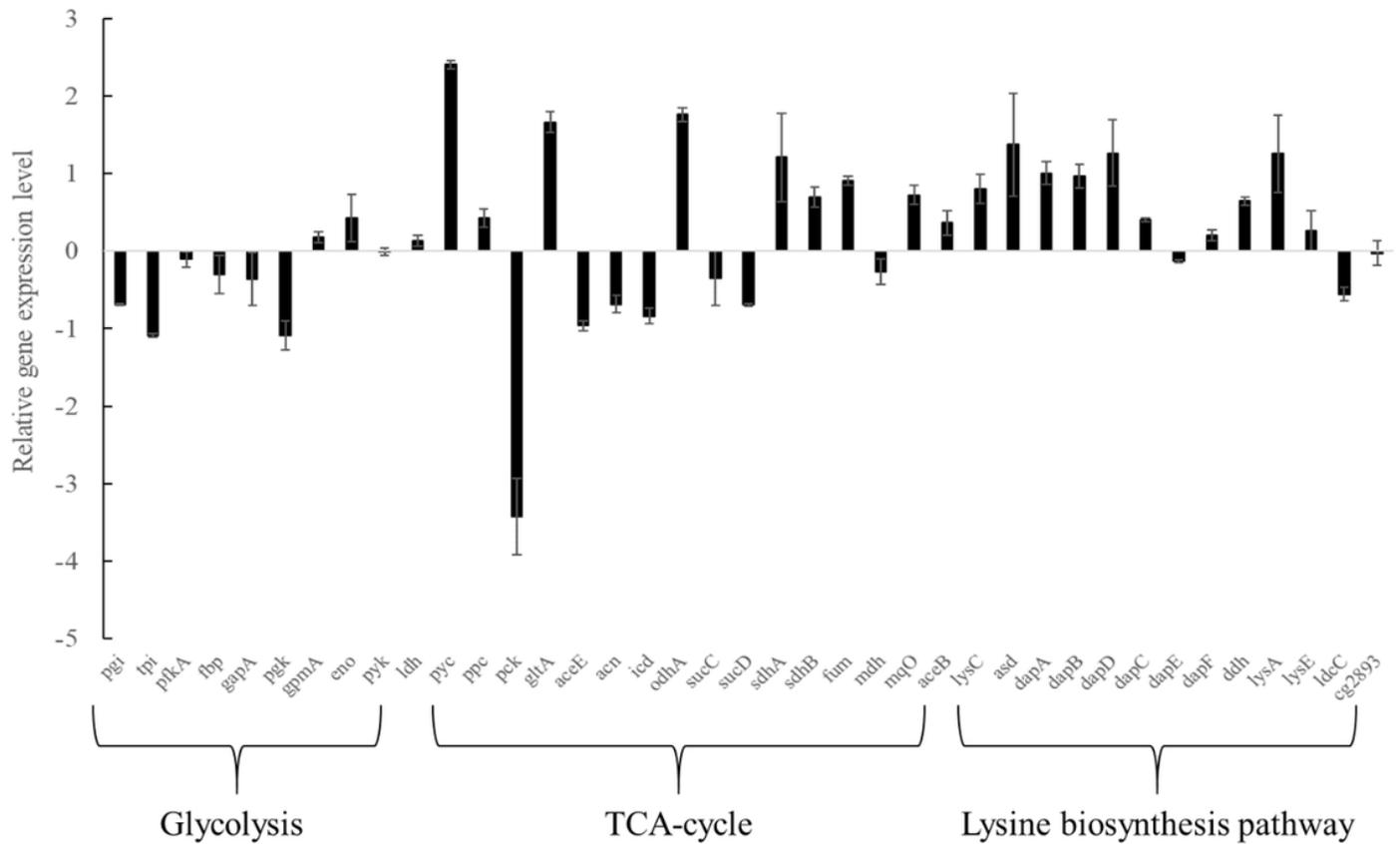


Figure 2

Relative expression levels of metabolic pathway genes in recombinant *C. glutamicum* expressing dr1558. The analyzed genes included the glycolytic pathway genes *pgi*, *tpi*, *pfkA*, *fbp*, *gapA*, *pgk*, *gpmA*, *eno*, and *pyk*, the TCA cycle genes *pyc*, *ppc*, *pck*, *gltA*, *aceE*, *acn*, *icd*, *odhA*, *sucC*, *sucD*, *sdhA*, *sdhB*, *fum*, *mdh*, *mqO*, and *aceB*, and the terminal pathway genes *lysC*, *asd*, *dapA*, *dapB*, *dapD*, *dapC*, *dapE*, *dapF*, *ddh*, *lysA*, *lysE*, *ldcC*, and *cg2893*. The data were analyzed by the $2^{-\Delta\Delta Ct}$ method. The histogram shows the mean of three biological replicates, and the error bars show the standard deviations.

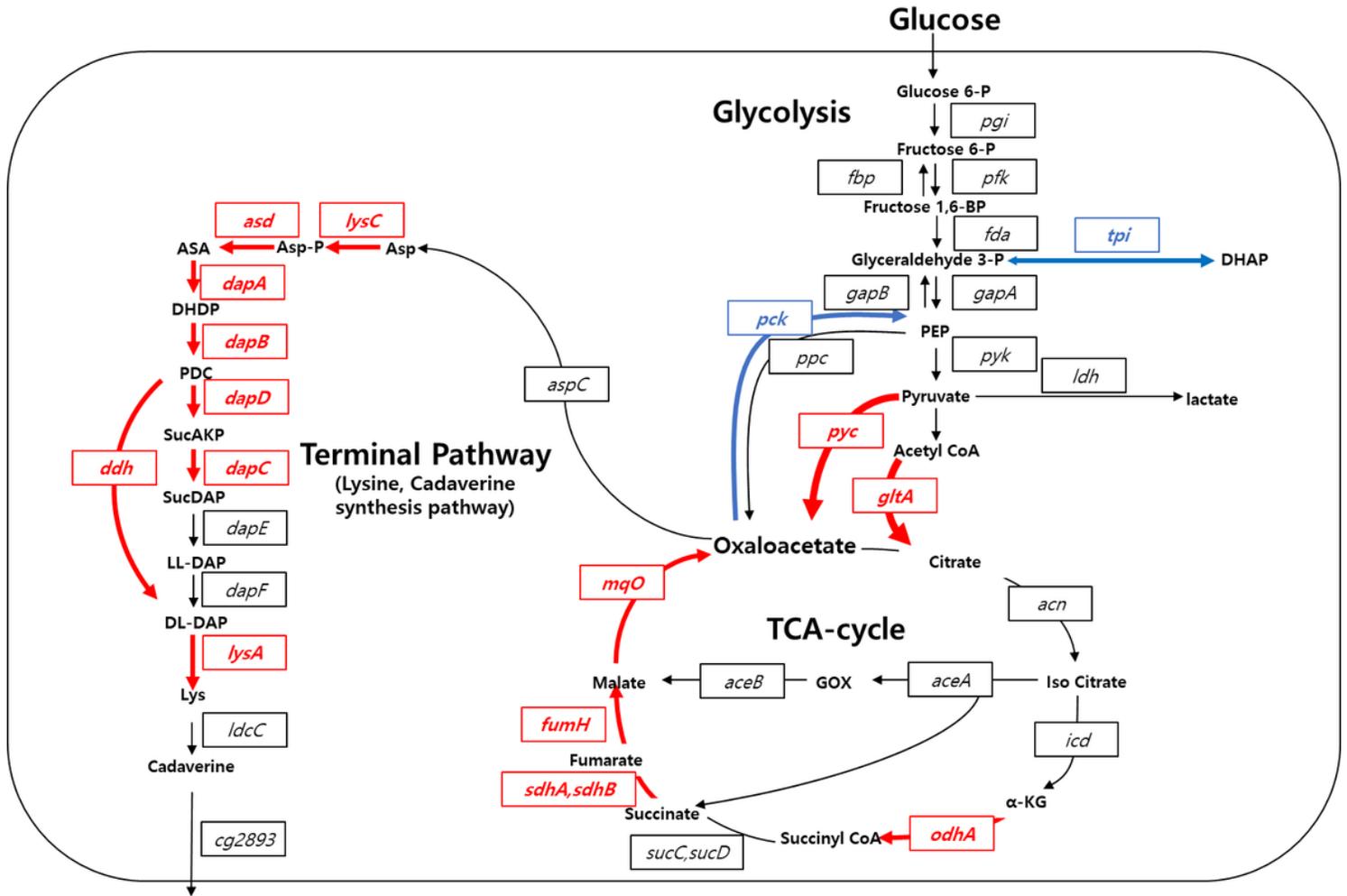


Figure 3

Schematic illustration of the expression levels of the genes related to central carbon metabolism in the recombinant *C. glutamicum* strain expressing *dr1558*. Black indicates no change in the expression level, blue indicates upregulation, and red indicates downregulation.

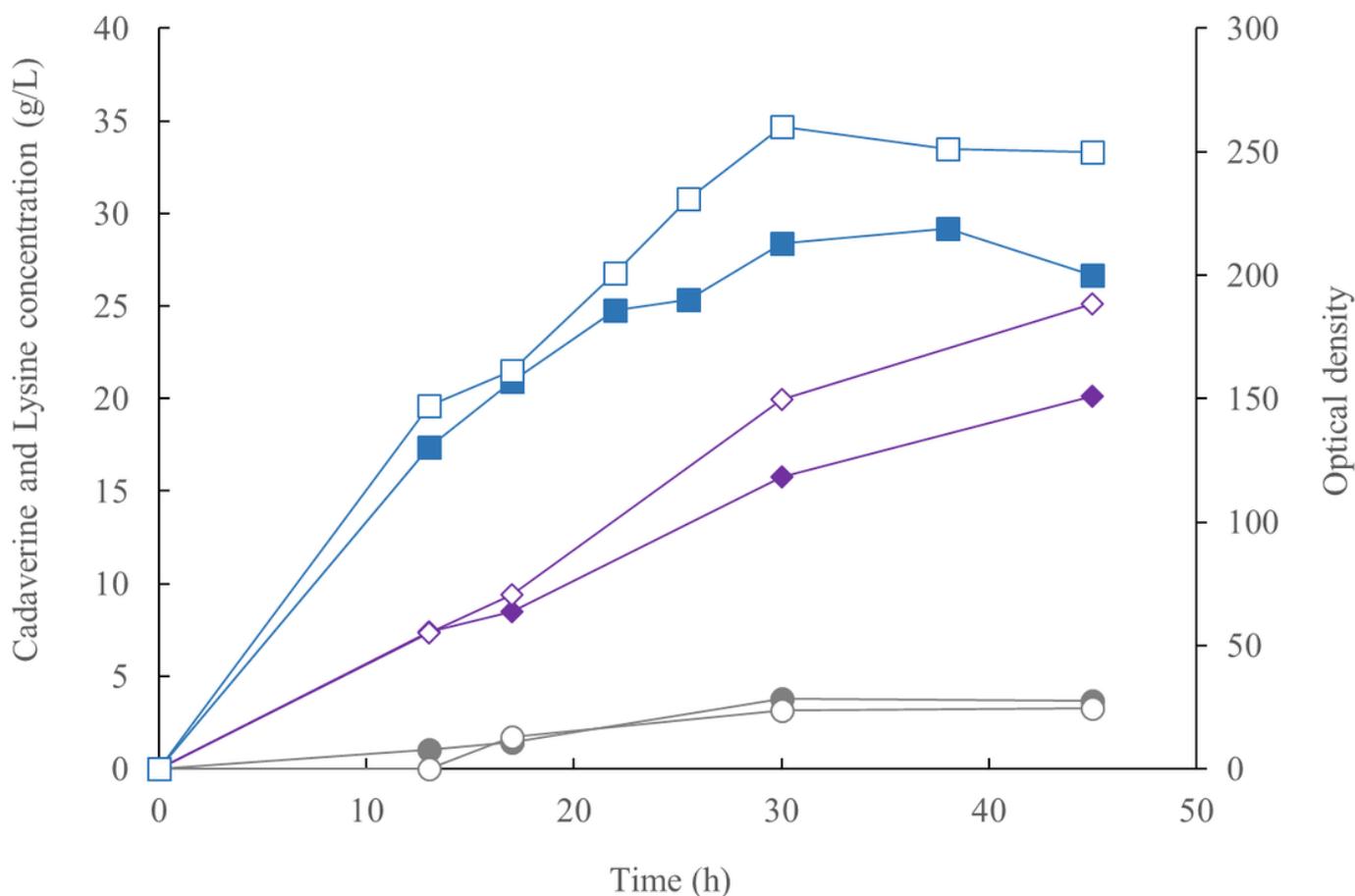


Figure 4

Time profiles of cell growth (OD600) (OD600, squares) and the concentrations of cadaverine (g/L, diamonds) and lysine (g/L, circles) in fed-batch cultivation of recombinant *C. glutamicum* KCTC 1857 expressing *E. coli* ldcC and *D. radiodurans* dr1558 under the control of the PH30 promoter. (Open symbols: *C. glutamicum* KCTC 1857 harboring pCES208H30EcLdcCdr1558, filled symbols: *C. glutamicum* KCTC 1857 harboring pCES208H30EcLdcC).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementTable1.docx](#)