Enhanced Cadaverine Production by Recombinant Corynebacterium Glutamicum with Response Regulator DR1558 at low pH Conditions

Soong-bin Kang  
Chonnam National University

Jong-Il Choi (choiji01@jnu.ac.kr)  
Chonnam National University  https://orcid.org/0000-0003-2204-6520

Research

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**Abstract**

**Background**: *Corynebacterium glutamicum* is used industrially to produce various bio-based organic acids. However, it is often cultivated under abiotic stress conditions, such as low pH, which can reduce both cell growth and the yield of the target compound. Here, a response regulator from *Deinococcus radiodurans*, DR1558, was introduced into a recombinant *C. glutamicum* strain expressing lysine decarboxylase (*cadA*) to enhance cadaverine production at acidic pHs.

**Results**: During batch cultivation under acidic conditions, 6.4 g/L of cadaverine was produced by the recombinant *C. glutamicum* strain expressing *cadA* and *dr1558*; this yield was 1.7-fold higher than that produced by a recombinant *C. glutamicum* strain expressing only *cadA*. Transcriptional analysis revealed altered expression levels of stress defense- and cadaverine biosynthesis-related genes in the recombinant *C. glutamicum* strain expressing *dr1558*. During fed-batch cultivation, the recombinant *C. glutamicum* strain expressing *cadA* and *dr1558* showed a 2.4-fold increase in cadaverine production compared to that produced by the recombinant *C. glutamicum* strain expressing only *cadA*. The cell growth of *C. glutamicum* expressing both *cadA* and *dr1558* increased markedly during fed-batch cultivation at acidic pH.

**Conclusion**: These results indicated that the response regulator *dr1558* altered the expression of genes involved in metabolic pathways and stress defense mechanisms in *C. glutamicum*. Furthermore, *C. glutamicum* expressing the *D. radiodurans* *dr1558* can be used to produce bio-based organic acids by fermentation in processes requiring acidic conditions.

**Background**

*Corynebacterium glutamicum* is a generally-recognized-as-safe (GRAS) gram-positive bacterium that is primarily used for the industrial production of amino acids, especially, the flavor enhancer L-glutamate and the feed additive L-lysine [1]. *C. glutamicum* can also be used as a microbial cell factory to produce other commercially relevant chemicals, such as cadaverine, succinate, iso-butanol, and ethanol [2]. Therefore, from a metabolic perspective, *C. glutamicum* is a promising producer of bio-chemicals.

Cadaverine, which is synthesized from lysine, is an important industrial platform-associated chemical that has a variety of applications, including the production of polyamides, polyurethanes, chelating agents, and additives. Additionally, it is used as a precursor for bio-based nylon synthesis, and cadaverine can replace hexamethylene diamine to produce nylon-5,4, nylon-5,6, nylon-5,10, or nylon-5,12 [3]. Cadaverine is produced through an enzymatic reaction catalyzed by either constitutive (LdcC) or inducible (CadA) lysine decarboxylase [4, 5]. Expression of CadA is induced by external acidic pH, excess lysine, and low oxygen [6]. CadA prefers acidic conditions, has an optimum pH of 5.7, and displays higher thermal stability and enzyme activity than LdcC. Therefore, the biotransformation of lysine to cadaverine by CadA was investigated under acidic conditions.
When generating a target product through microbial cultivation, the microbial strain may be exposed to various stresses, depending on the requirements for target production, including high osmotic pressure, high temperature, and an unfavorable pH [7–9]. Among these stresses, low pH is a common factor that can reduce the yield of bio-based compounds produced by fermentation [10]. The acidic pH required for enzyme activity during the production of cadaverine using CadA leads to decreased cell growth and lower productivity. Recently, a response regulator from Deinococcus radiodurans, DR1558, was introduced into a recombinant Escherichia coli strain to minimize the effects of stress under acidic conditions [11]. D. radiodurans is highly resistant to abiotic stresses, including gamma radiation, reactive oxygen species (ROS), and oxidants [11–12], and dr1558 is one of the genes responsible for its remarkable resistance. Introduction of dr1558 improved the stress resistance of E. coli [11]. The dr1558 gene has also been introduced into recombinant E. coli to improve the production of succinate, polyhydroxybutyrate, γ-aminobutyric acid, and 2,3-butanediol [13–16]. Since the expression of dr1558 increased the tolerance of E. coli cells to low pH, it was expected that it would also increase the productivity of cadaverine, especially at an acidic pH.

In this study, a recombinant plasmid that expressed both E. coli cadA and D. radiodurans dr1558 was constructed and transformed into C. glutamicum. Cadaverine biosynthesis by the C. glutamicum strain expressing cadA and dr1558 and a strain expressing only cadA was compared. To investigate the metabolic changes induced by introducing dr1558 into C. glutamicum, the changes in the transcriptome of recombinant C. glutamicum expressing dr1558 was analyzed. Finally, cadaverine production by the recombinant C. glutamicum strain expressing cadA and dr1558 was assessed in fed-batch cultivation at acidic pH.

**Materials And Methods**

**Strains, plasmids, and culture media**

All bacterial strains and plasmids used in this study are listed in Table 1. E. coli XL1-Blue (Stratagene, La Jolla, CA, USA) was used for general cloning. C. glutamicum KCTC 1857 was obtained from the Korean Collection for Type Cultures (KCTC; Joengeup, Republic of Korea). The plasmids used for the expression of the E. coli cadA and D. radiodurans dr1558 genes under the control of the synthetic H30 promoter were constructed as described below. Recombinant C. glutamicum KCTC 1857 strains were constructed to express cadA with or without dr1558.
Table 1
Strains and plasmids used in this study.

| Strain or plasmid | Relevant characteristics | Reference or source |
|-------------------|--------------------------|---------------------|
| **Strain**        |                          |                     |
| *E. coli* XL1-Blue | recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F’ proAB lacI ΔM15 Tn10 (TetR)] | Stratagene |
| *C. glutamicum* KCTC 1857 | L-lysine-producing bacterium | KCTC |
| **Plasmid**       |                          |                     |
| pCES208H30GFP     | pCES208 derivative; P_{H30}, eGFP, Km^{r} | [17] |
| pCES208H30dr1558  | pCES208 derivative; P_{H30}, *D. radiodurans* dr1558, Km^{r} | [17] |
| pCES208H30cadA    | pCES208 derivative; P_{H30}, *E. coli* cadA, Km^{r} | [18] |
| pCES208H30cadAdr1558 | pCES208 derivative; P_{H30}, *E. coli* cadA, *D. radiodurans* dr1558, Km^{r} | This study |

All DNA manipulations were performed according to standard procedures. A DNA fragment containing the *dr1558* gene under the H30 promoter was obtained from pCES208H30dr1558 [17] by digestion with the restriction endonuclease *Bam*HI. The fragment was inserted in plasmid pCES208H30cadA [18] at the *Bam*HI site to construct pCES208H30cadAdr1558. Each plasmid was transformed into *C. glutamicum* KCTC 1857 by electroporation. The transformed *C. glutamicum* KCTC 1857 strains were plated on medium containing kanamycin (Km) for selection.

*E. coli* XL1-Blue was cultured at 37°C in Lysogeny-broth medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl). *C. glutamicum* was grown in CG-50 medium (50 g/L glucose, 15 g/L yeast extract, 15 g/L (NH₄)₂SO₄·7H₂O, 0.5 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O, 0.01 g/L MnSO₄·H₂O, and 0.01 g/L FeSO₄·7H₂O, with 15 g/L CaCO₃ added for pH buffering). Km (30 mg/L) was added to the medium as needed.

**Fermentation**

Batch fermentations of recombinant *C. glutamicum* were carried out at 30°C and an initial agitation speed of 200 rpm in a 2.5-L jar fermenter (BioCNS, Daejeon, Republic of Korea) initially containing 500 mL of CG-100 medium (100 g/L glucose, 30 g/L yeast extract, 30 g/L (NH₄)₂SO₄·7H₂O, 0.5 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O, 0.01 g/L MnSO₄·H₂O, 0.01 g/L FeSO₄·7H₂O, 0.5 mg/L biotin, and 0.3 mg/L thiamine-HCl). When cultivating the recombinant strains, Km was added to the culture medium.
Fed-batch fermentations were carried out at 30°C and an initial agitation speed of 200 rpm in 2.5-L jar fermenters (BioCNS) initially containing 500 mL of CG-100 medium. The feeding solution contained (per liter): 400 g of glucose, 45 g of (NH₄)₂SO₄·7H₂O, 0.5 g of MgSO₄·7H₂O, 0.01 g of MnSO₄·H₂O, and 0.01 g of FeSO₄·7H₂O. The initial pH of the culture broth was 7.1, which was maintained for 8 h to promote cell growth. Then, the pH was changed to 5.7, which is the optimal pH for CadA activity, to promote the production of cadaverine. The fermentation pH was adjusted by automatic addition of 14% (v/v) NH₄OH and 1 M H₂SO₄. Foam formation was suppressed by adding Antifoam 204 (Sigma-Aldrich, St. Louis, MO, USA). The agitation speed was increased to maintain the dissolved oxygen (DO) level above 10%. Cell growth was monitored by measuring the optical density of the culture broth at 600 nm (OD₆₀₀).

**Rna Extraction And Quantitative Real-time Pcr (Qrt-pcr)**

A transcriptional analysis was performed to evaluate the gene expression changes in the *C. glutamicum* strain expressing *dr1558*. Cells were harvested by centrifugation (14,000 g, 10 min, 4°C) at 10 h during batch fermentation, and total RNA was extracted using the RNeasy Mini Kit (QIAGEN, Venlo, Netherlands), according to the manufacturer's protocol. The qRT-PCR analysis was performed using TB Green Premix ExTaq (TaKaRa Bio, Shiga, Japan) under the following cycling conditions: 40 cycles of 95°C for 10 s and 58°C for 30 s. The primers used are listed in Supplement Table 1. Data were analyzed using the 2⁻^ΔΔCt method, and 16S rRNA was used as an internal control [19]. The experiment was performed in triplicate using an applied Eco™ Real-Time PCR System (Illumina, Inc., San Diego, CA, USA).

**Analytical Procedures**

The concentrations of organic acids and glucose were determined by high-performance liquid chromatography using an Infinity 1260 system (Agilent Technologies, Santa Clara, CA, USA). The glucose concentration was determined using an Aminex HPX-87H ion exclusion column (Bio-Rad, Hercules, CA, USA). The mobile phase was 5 mM H₂SO₄, the flow rate was 0.6 mL/min, and the column was maintained at 50°C. The concentrations of cadaverine and lysine were determined using an ZORBAX SB-C18 column (Agilent Technologies). The mobile phase was 25 mM sodium acetate buffer (pH 4) and 1 M acetonitrile, the flow rate was 1 mL/min, and the column was maintained at 35°C. The concentrations of cadaverine and lysine were measured after diethyl ethoxymethylenemalonate (DEEMM) derivatization [20].

**Results And Discussion**

*Production of cadaverine by the recombinant C. glutamicum strain expressing cadA and dr1558 during batch fermentation*
To investigate the effects of the *D. radiodurans* response regulatory gene *dr1558* on *C. glutamicum*, cell growth, glucose consumption, and lysine and cadaverine production were compared between the recombinant strain expressing both *cadA* and *dr1558* (Cg-cadA + dr1558) and a recombinant strain expressing only *cadA* (Cg-cadA; control) (Fig. 1) during batch cultivation.

The results of the batch fermentation confirmed that the recombinant strain Cg-cadA + dr1558 showed significantly increased rates of cell growth and glucose consumption in batch fermentation, compared to those of Cg-cadA. Even after the pH of the medium was adjusted to 5.7, the growth rate of Cg-cadA + dr1558 continued to increase, whereas the growth rate of Cg-cadA decreased. Additionally, despite the acidic conditions, Cg-cadA + dr1558 consumed 100 g/L of glucose within 20 h. In contrast, glucose remained detectable in the medium of the control fermentation at 24 h. The amounts of cadaverine produced in the fermentations were 3.29 g/L for Cg-cadA and 6.39 g/L for Cg-cadA + dr1558, representing a 1.9-fold increase in cadaverine production. From Fig. 1, the maximum specific growth rate and specific cadaverine productivity of Cg-cadA + dr1558 were 0.302 g cells/L/h and 2.697 mg cadaverine/g cells/L/h, respectively. However, the maximum specific growth rate and specific cadaverine productivity of Cg-cadA were decreased to 0.0653 g cells/L/h and 1.071 mg cadaverine/g cells/L/h, respectively. This indicates that the specific growth rate was greatly increased by the expression of *dr1558* at acidic pH conditions.

It was previously reported that *E. coli* expressing *dr1558* showed greater resistance to acidic conditions as well as the enhanced production of polyhydroxybutyrate and 2,3-butanediol due to the altered expression of genes in metabolic pathways [14, 15]. Based on these findings, we expected changes in the expression levels of genes related to cadaverine production and glucose consumption. To investigate the alterations in the expression of these genes in the recombinant *C. glutamicum* strain expressing *dr1558*, a transcriptional analysis was carried out.

**Transcriptional analysis of the C. glutamicum strain expressing cadA and dr1558**

A transcriptional analysis of Cg-cadA + dr1558 and Cg-cadA was performed to investigate the reason for the observed increases in cadaverine production, cell density, and glucose consumption rate under acidic conditions. The analysis included 37 metabolism-related genes and 25 genes related to acid stress resistance (Figs. 2 and 3).

In Cg-cadA + dr1558, upregulation of the glycolysis-related genes *pfkA*, *eno*, and *pyk* may improve the carbon flux of the phosphotransferase system (PTS), thereby increasing glucose uptake. In the TCA cycle, changes in the expression levels of genes involved in the biosynthesis of oxaloacetate were also observed; *pck* was upregulated by 2.71-fold and *pyc*, *ppc*, and *pyk* were upregulated by 2.70-, 2.26-, and 1.5-fold, respectively. Thus, in the recombinant strain expressing *dr1558*, the flux of oxaloacetate is also increased; ultimately, this enhanced the synthesis of lysine, which is a precursor of cadaverine.

In the terminal pathway, no significant changes were observed in the expression levels of *dapB*, *dapD*, *dapC*, *dapE*, *dapF*, and *ddh*, which are directly involved in cadaverine biosynthesis. However, the
expression of cadA was significantly higher (3.39-fold) than that in the control strain. The lysine-dependent acid resistance (LDAR) system, which consists of lysine and the inducible lysine decarboxylase CadA [21], operates most efficiently under mild acid stress conditions [22, 23]. The LDAR system is a proton consumption-dependent system. The cadA expression was upregulated in the presence of dr1558; this enhanced the acid resistance of the dr1558-expressing C. glutamicum strain and promoted the conversion of lysine to cadaverine. The function of D. radiodurans dr1558 was investigated in E. coli [11]. It was reported that the foreign regulator DR1558 bound to the promoter regions of some sigma factors and modulated their expression levels. However, although the effect of dr1558 expression in Corynebacterium has not been investigated, DR1558 could alter the expression levels of several regulators and may indirectly increase the expression of cadA.

We investigated the expression of genes involved in pH homeostasis, which enables C. glutamicum to respond to, and survive under, acidic pH conditions. Recent studies revealed the physiological and biochemical processes involved in the defense mechanism against low pH in C. glutamicum [24–26]. A previous comprehensive analysis of pH homeostasis in C. glutamicum demonstrated a functional link between the pH response, oxidative stress, iron homeostasis, and metabolic shift [27]. Therefore, the changes in the expression levels of key genes related to the intracellular defense against acidic conditions were investigated. The expression levels of 25 genes related to acid resistance were examined via transcriptome analysis (Fig. 3). The expression of DNA-binding Proteins from Starved cells (Dps) [9] and KatA (catalase) is cooperatively regulated by intracellular ROS scavenging, and these proteins are required for resistance to low pH stress in C. glutamicum [28]. qRT-PCR analysis of Cg-cadA + dr1558 confirmed that dps expression was increased by about 1.5-fold. Given that an external acidic environment can lead to an accumulation of ROS in cells, elimination of ROS is a promising way to confer acid resistance [27].

In C. glutamicum, mycothiol peroxidase (MPx), mycothiol disulfide reductase (Mtr), and mycothiol glycosyltransferase (MshA) have been shown to promote adaptation to acid stress by regulating ROS homeostasis [29, 30]. qRT-PCR analysis showed that expression of the mtr gene was upregulated by 1.2-fold. ROS accumulation in the cells induced by the acidic conditions was likely effectively reduced by the upregulation of mtr, and like the upregulated levels of dps, may contribute to the increased growth that was observed under acidic conditions.

The qRT-PCR analysis also revealed that the mRNA expression of mcbR, which encodes a TetR-type transcriptional inhibitor of sulfur metabolism, was approximately 1.1-fold higher in Cg-cadA + dr1558 than in Cg-cadA. The accumulation of certain sulfur-containing intermediates, such as cysteine, can disrupt intracellular thiol homeostasis and cause oxidative damage by driving the Fenton reaction [31]. Inhibition of the sulfur anabolic pathway by McbR has been shown to contribute to a reduction in L-cysteine accumulation and have a beneficial effect on cell growth under acidic pH conditions [27].

The iron storage protein ferritin, which is encoded by ftn [32], was upregulated in Cg-cadA + dr1558. To protect the reducing environment of the cells from unwanted Fe³⁺/Fe²⁺ redox cycling, intracellular levels
of free Fe$^{2+}$ are maintained by both limiting external iron absorption and enhancing intracellular iron storage [28]. Thus, this increase in ftn expression may help protect cells from iron-mediated oxidative stress.

Cg1328, which encodes a copper chaperone, has been implicated in copper metabolism and trafficking [33]. This cytoplasmic protein functions to specifically deliver copper to copper proteins in plant, bacterial, yeast, and animal cells. The cg1328 gene also promotes cell survival under acid stress conditions, which is consistent with the interplay between acid stress and copper toxicity reported in some bacteria. In this study, qRT-PCR analysis confirmed that the expression of the cg1328 gene was upregulated. Thus, intracellular acid resistance may also involve enhanced intracellular copper metabolism and transport. In addition, slight upregulation of sucE, a putative succinate exporter that has not yet been functionally characterized, was also observed. In addition, the expression of cg1k, which was reported to encode a protein that is essential for pH homeostasis in the presence of acidic pHs in the absence of K$^+$, was downregulated. However, since potassium was added to the culture medium, the function of the putative channel protein CgIK may not be important. Most researchers consider a log$_2$ fold change of 2 in expression as the cutoff for a differentially expressed gene. However, to consider all the changes in gene expression to understand the mechanism underlying the enhanced cadaverine production and cell growth, a less strict condition, i.e., a log$_2$ fold change of 1, was used for the analysis in this study.

These findings indicate that the expression of dr1558 in C. glutamicum influences the expression of metabolic pathway-related genes and genes related to the defense against acidic stress. These changes in gene expression enhance pH homeostasis, leading to increases in the cell growth rate and cadaverine production.

**Fed-batch fermentation for the production of cadaverine by recombinant C. glutamicum expressing dr1558 and cadA at an acidic pH**

Cadaverine production by the recombinant C. glutamicum strain expressing dr1558 was enhanced in batch fermentation. To further investigate the effect of DR1558 on the production of cadaverine, a fed-batch fermentation was carried out. When the glucose concentration in the broth decreased to below 1 g/L, an appropriate amount of feeding solution was added to adjust the glucose concentration to 50 g/L. The time profiles of cell growth and the concentrations of glucose, lysine, and cadaverine during the fed-batch fermentation of Cg-cadA + dr1558 and Cg-cadA are shown in Fig. 4.

During the culture of Cg-cadA + dr1558, the pH was adjusted from an initial value of 7.1 to 5.7 when the OD$_{600}$ of the culture reached 50. Even at this acidic pH, additional glucose was consumed, and at the end of the fermentation (35 h), 10.3 g/L of cadaverine was produced (Fig. 4). Cell growth also continued for 35 h, even after the pH was adjusted to 5.7. In contrast, the control strain Cg-cadA, which did not express dr1558, displayed lower rates of glucose consumption and cell growth at the acidic pH (Fig. 4). The strain expressing dr1558 and cadA showed a 1.5-fold increase in cadaverine production, compared to that of the control strain after 35 h.
Conclusions

In this study, enhanced cadaverine production was observed in a recombinant *C. glutamicum* strain co-expressing *dr1558* and *cadA*. The addition of the *dr1558* gene altered the expression levels of metabolism-related genes under acidic conditions. The metabolic changes induced in the recombinant *C. glutamicum* strain as a result of *dr1558* expression are summarized in Fig. 5. The expression levels of genes related to glycolysis, the TCA cycle, and terminal pathways were altered. Some genes involved in defense mechanisms, including *dps*, *mcbR*, *mtr*, *cg1328*, and *ftn*, were also upregulated during cultivation at acidic pH. These genes, which are related to mechanisms underlying the defense against low pH, may be associated with the positive effects on cell growth. The exact mechanisms underlying the upregulation of the *cadA* gene and other stress-related genes following overexpression of *dr1558* are still under investigation. However, *dr1558* might bind to some regulator genes and thus change the expression level of genes involved in cadaverine biosynthesis and acid tolerance. Furthermore, these results suggest the possible application of *dr1558* for the enhanced production of biochemicals under acidic conditions.

Declarations

Acknowledgments

Not applicable.

Authors’ contributions

Conceptualization, J.C.; methodology, S.B.; software, S.B.; validation, S.B.; formal analysis, S.B.; investigation, S.B.; resources, J.C.; data curation, S.B.; writing—original draft preparation, S.B.; writing—review and editing, J.C.; visualization, S.B.; supervision, J.C.; project administration, J.C.; funding acquisition, J.C.. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

**Competing interests**

The authors declare that they have no competing interest.

**Author details**

Department of Biotechnology and Bioengineering, Interdisciplinary Program of Bioenergy and Biomaterials, Chonnam National University, Gwangju 61186, Republic of Korea

**References**

1. Kalinowski J, Bathe B, Bartels D, Bischoff N, Bott M, Burkovski A, Dusch N, Eggeling L, Eikmanns BJ, Gaigalat L. The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *J Biotechnol.* 2003;104(1–3):5–25.

2. Woo HM, Park JB. Recent progress in development of synthetic biology platforms and metabolic engineering of *Corynebacterium glutamicum*. *J Biotechnol.* 2014;180:43–51.

3. Qian ZG, Xia XX, Lee SY. Metabolic engineering of *Escherichia coli* for the production of cadaverine: a five carbon diamine. *Biotechnol Bioeng.* 2011;108(1):93–103.

4. Kind S, Kreye S, Wittmann C. Metabolic engineering of cellular transport for overproduction of the platform chemical 1, 5-diaminopentane in *Corynebacterium glutamicum*. *Metab Eng.* 2011;13(5):617–27.

5. Mimitsuka T, Sawai H, Hatsu M, Yamada K. Metabolic engineering of *Corynebacterium glutamicum* for cadaverine fermentation. *Biosci Biotech Bioch.* 2007;71(9):2130–5.

6. Krithika G, Jothi Arunachalam HP, Indulekha K. The two forms of lysine decarboxylase; kinetics and effect of expression in relation to acid tolerance response in *E. coli*. *J Exp Sci.* 2010;1(12):10–21.

7. Beales N. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Compr Rev Food Sci Food Saf.* 2004;3(1):1–20.

8. Touati D. Iron and oxidative stress in bacteria. *Arch Biochem Biophys.* 2000;373(1):1–6.

9. Bremer E, Krämer R. Responses of microorganisms to osmotic stress. *Annu Rev Microbiol.* 2019;73:313–34.

10. Akcil A, Koldas S. Acid Mine Drainage (AMD): causes, treatment and case studies. *J Clea Prod.* 2006;14(12–13):1139–45.

11. Appukuttan D, Singh H, Park SH, Jung JH, Jeong S, Seo HS, Choi YJ, Lim S. Engineering synthetic multistress tolerance in *Escherichia coli* by using a deinococcal response regulator, DR1558. *Appl. Environ. Microb.* 2016;82(4):1154–1166.

12. Krisko A, Radman M. Biology of extreme radiation resistance: the way of *Deinococcus radiodurans*. *Cold Spring Harbor perspectives in biology* 2013:5(7):a012765.
13. Guo S, Yi X, Zhang W, Wu M, Xin F, Dong W, Zhang M, Ma J, Wu H, Jiang M. Inducing hyperosmotic stress resistance in succinate-producing *Escherichia coli* by using the response regulator DR1558 from *Deinococcus radiodurans*. Process Biochem. 2017;61:30–37.

14. Park SH, Kim GB, Kim HU, Park SJ, Choi J. Enhanced production of poly-3-hydroxybutyrate (PHB) by expression of response regulator DR1558 in recombinant *Escherichia coli*. Int J Biol Macromol. 2019;131(15):29–35.

15. Park SJ, Sohn YJ, Park SJ, Choi J. Enhanced production of 2, 3-butanediol in recombinant *Escherichia coli* using response regulator DR1558 derived from *Deinococcus radiodurans*. Biotechnol Bioprocess Eng. 2020;25(1):45–52.

16. Park SH, Sohn YJ, Park SJ, Choi J. Effect of DR1558, a *Deinococcus radiodurans* response regulator, on the production of GABA in the recombinant *Escherichia coli* under low pH conditions. Microb Cell Fact. 2020;19(1):1–12.

17. Kim SM, Lim S, Park SJ, Joo JC, Choi J. Enhancement of lysine production in recombinant *Corynebacterium glutamicum* through expression of *Deinococcus radiodurans* pprM and dr1558 genes. Microbiol. Biotechnol. Lett. 2017;45(3):271–275.

18. Oh YH, Choi JW, Kim EY, Song BK, Jeong KJ, Park K, Kim IK, Woo HM, Lee SH, Park SJ. Construction of synthetic promoter-based expression cassettes for the production of cadaverine in recombinant *Corynebacterium glutamicum*. App Biochem Biotechnol. 2015;176(7):2065–75.

19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001;25(4):402–8.

20. Kim YH, Kim HJ, Shin JH, Bhatia SK, Seo HM, Kim YG, Lee YK, Yang YH, Park K. Application of diethyl ethoxymethylenemalonate (DEEMM) derivatization for monitoring of lysine decarboxylase activity. J Mol Catal B: Enzym. 2015;115:151–4.

21. Kanjee U, Houry WA. Mechanisms of acid resistance in *Escherichia coli*. Annu Rev Microbiol. 2013;67:65–81.

22. Foster JW. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat Rev Microbiol. 2004;2(11):898–907.

23. Kanjee U, Gutsche I, Alexopoulos E, Zhao B, El Bakkouri M, Thibault G, Liu K, Ramachandran S, Snider J, Pai EF. Linkage between the bacterial acid stress and stringent responses: the structure of the inducible lysine decarboxylase. EMBO J. 2011;30(5):931–44.

24. Follmann M, Ochrombel I, Krämer R, Trötschel C, Poetsch A, Rückert C, Hüser A, Persicke M, Seiferling D, Kalinowski J. Functional genomics of pH homeostasis in *Corynebacterium glutamicum* revealed novel links between pH response, oxidative stress, iron homeostasis and methionine synthesis. BMC Genom. 2009;10(1):621.

25. Guo J, Ma Z, Gao J, Zhao J, Wei L, Liu J, Xu N. Recent advances of pH homeostasis mechanisms in *Corynebacterium glutamicum*. World J Microbiol Biotechnol. 2019;35(12):192.

26. Liu Y, Yang X, Yin Y, Lin J, Chen C, Pan J, Si M, Shen X. Mycothiol protects *Corynebacterium glutamicum* against acid stress via maintaining intracellular pH homeostasis, scavenging ROS, and...
27. Gao C, Xu P, Ye C, Chen X, Liu L. Genetic circuit-assisted smart microbial engineering. Trends Microbiol. 2019;27(12):1011–24.

28. Xu N, Lv H, Wei L, Liang Y, Ju J, Liu J, Ma Y. Impaired oxidative stress and sulfur assimilation contribute to acid tolerance of *Corynebacterium glutamicum*. Appl Microbiol Biotechnol. 2019;103(4):1877–91.

29. Si M, Zhao C, Zhang B, Wei D, Chen K, Yang X, Xiao H, Shen X. Overexpression of mycothiol disulfide reductase enhances *Corynebacterium glutamicum* robustness by modulating cellular redox homeostasis and antioxidant proteins under oxidative stress. Sci Rep. 2016;6(1):1–14.

30. Wang T, Gao F, Kang Y, Zhao C, Su T, Li M, Si M, Shen X. Mycothiol peroxidase MPx protects *Corynebacterium glutamicum* against acid stress by scavenging ROS. Biotechnol Lett. 2016;38(7):1221–8.

31. Park S, Imlay JA. High levels of intracellular cysteine promote oxidative DNA damage by driving the fenton reaction. J Bacteriol. 2003;185(6):1942–50.

32. Rivera M. Bacterioferritin: structure, dynamics, and protein–protein interactions at play in iron storage and mobilization. Acc Chem Res. 2017;50(2):331–40.

33. Harrison MD, Jones CE, Dameron CT. Copper chaperones: function, structure and copper-binding properties. J Biol Inorg Chem. 1999;4(2):145–53.

**Figures**

![Figure 1](image)
Time profiles of cell growth, the production of cadaverine and lysine, and glucose consumption during batch cultivation by (A) a recombinant C. glutamicum strain expressing cadA and dr1558 (Cg-cadA+dr1558) and (B) a recombinant C. glutamicum strain expressing cadA (Cg-cadA). The cells were harvested for mRNA preparation at 10 h (indicated by arrows).

Figure 2

Relative expression levels of metabolic pathway-related genes in recombinant C. glutamicum expressing cadA and dr1558 compared to those in recombinant C. glutamicum expressing only cadA. Glycolytic pathway genes: pgi, tpi, pfkA, fbp, pgk, gpmA, eno, and pyk; TCA cycle genes: pyc, ppc, pck, gltA, aceE, acn, icd, odhA, sucC, sucD, sdhA, sdhB, fum, mdh, mqO, and aceB; and Central pathway genes: lysC, asd, dapA, dapB, dapC, dapE, dapF, ddh, lysE, cadA, and cg2893. Data were analyzed using the 2−ΔΔCt method. The histogram shows the mean of three biological replicates, and the error bars represent the standard deviations. The names of the genes are shown in the Supplementary Table.
Figure 3

Relative expression levels of genes related to the acid resistance defense mechanism of recombinant C. glutamicum expressing cadA and dr1558 compared to those in recombinant C. glutamicum expressing only cadA. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method. The histogram shows the mean of three biological replicates, and the error bars represent the standard deviations.
Figure 4

Time profiles of cell growth, the production of cadaverine and lysine, and glucose consumption during fed-batch cultivation by (A) recombinant C. glutamicum expressing cadA and dr1558 (Cg-cadA+dr1558) and (B) recombinant C. glutamicum expressing cadA (Cg-cadA).

Figure 5
Schematic illustration of the changes in the expression levels of genes related to central metabolism and the acid resistance mechanism of recombinant C. glutamicum expressing dr1558. Genes with upregulated expression levels in the strain expressing dr1558 are shown in red.

**Supplementary Files**

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