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Whole-cell catalytic synthesis of cadaverine by recombinant *Corynebacterium glutamicum* using corncob residue as carbohydrate feedstock

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ABSTRACT

Industrial production of cadaverine primarily relies on whole-cell catalysis of lysine using engineered *Escherichia coli* as host cells. The growing interest in cadaverine as a monomer of biobased polyamides requires inexpensive, non-food carbohydrate feedstock for its bioproduction, rather than using food-derived sugars as feedstock. This study used corncob residue as the starting carbohydrate feedstock followed by enzymatic hydrolysis to obtain the sugars. Since *E. coli* was not tolerant to even minor inhibitor residues in corncob residue hydrolysate, the robust *Corynebacterium glutamicum* was used as the host bacterium followed by the metabolic modifications of secretory expression of lysine decarboxylase via *Ncgl1289* and *cgR_0949*, and cadaverine degradation pathway knockout. The final whole-cell catalysis of *C. glutamicum* recombinant using corncob hydrolysate as carbohydrate feedstock achieved a record-high 78.19 g/L of cadaverine with a conversion yield of 91 %.

1. Introduction

Cadaverine is a diamine with a similar structure to hexamethylenediamine used for co-polymerization with dicarboxylic acids to produce bio-based polyamides [1–4]. Cadaverine is synthesized either by whole-cell catalysis from lysine using *Escherichia coli* with lysine decarboxylase expression [5–8], or by fermentation from glucose using lysine-producing microbes with lysine decarboxylase expression [9–12]. Using cheap non-food carbohydrate feedstock is crucially important for cost effective production of cadaverine. Ikeda et al. [13] produced 0.62 g/L of cadaverine from cellobiose using β-glucosidase (BGL) and lysine decarboxylase (CadA) expressing *E. coli*. Matsuura et al. [14] produced 27.00 g/L of cadaverine by fed-batch fermentation of cellobiose and glucose using secretive β-glucosidase and lysine decarboxylase expressing *C. glutamicum*. Few studies directly used lignocellulosic feedstock as a carbon source for cadaverine production.

A major obstacle for cadaverine production from lignocellulose feedstock is the inhibitor existence from pretreatment operation of lignocellulose biorefinery, such as hydroxymethylfurfural, furfural, phenolic inhibitors, organic acid, etc, have effects on microbial growth and fermentation, including inhibition of enzymes involved in metabolic processes, reduction of intracellular pH, and disruption of cell membrane integrity, which leads to slower growth of the strain and reduced fermentation performance. [15,16]. Even after the detoxification and enzymatic hydrolysis steps, minor phenolic inhibitors from lignin over-degradation still exist in the lignocellulose hydrolysate [17]. Minor phenolic inhibitors are still present in lignocellulosic hydrolysate after detoxification, which have a strong inhibitory effect on microorganisms such as *E. coli* [15,18]. *C. glutamicum* is not only highly tolerant to phenolic inhibitors in lignocellulosic hydrolysate, but also capable of producing lysine as the precursor of cadaverine synthesis. The engineered *C. glutamicum* had been used as the host strain for the construction of various recombinant strains for fermentative production of glutamic acid [19–21], lysine [22,23], PHB [24–26], hydrocarbons [27] using lignocellulosic feedstocks after biorefinery processing.

This study constructed an engineered *C. glutamicum* as whole cell catalyst for the decarboxylation of lysine to cadaverine by expressing lysine decarboxylase. Corncob residue was used as carbohydrate feedstock for the cell growth of *C. glutamicum* as whole cell catalyst. Corncob residue is an available byproduct of corncob processing industry for production of xylitol or furfural with high annual output up to one million metric tons in China. The other lignocellulose feedstocks such as corn stover, wheat straw, or rice straw have to be collected to the processing line and pretreated before they can be used as carbohydrate

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feedstock. Till now, no available pretreated corn stover, wheat straw, or rice straw are available as carbohydrate feedstock on the market. Therefore, corncob residue was selected as lignocellulose feedstock for production of cadaverine in this study. Several strategies on modifying *C. glutamicum* were proposed including secretive lysine decarboxylase expression, lysine-cadaverine transporter protein expression on enhancement of cadaverine transport, and knockout of N-acetyl-cadaverine pathway. The final recombinant *C. glutamicum* produced 78.19 g/L of cadaverine by whole-cell catalysis with a conversion yield of 91 %. In this study, the whole-cell catalysis of cadaverine was carried out for the first time using *C. glutamicum* as the host bacterium, the first expression of lysine decarboxylase by secretory expression, and the synthesis of cadaverine from lignocellulosic feedstock was achieved in a real sense.

2. Materials and methods

2.1. Feedstock

Corncob residue was obtained from Shandong Longlive Biotech Co., Jining, Shandong, Chian, as the byproduct of xylose production from corncob. The cellulose content was 82 % and the hemicellulose content was 5 % based on the dry basis of corncob residue. The total phenolic content of corncob residue was 16.8 mg/g dry matter, and the main phenolics included 1.7 g guaiacol/100 mL hydrolysate, 2.7 g 2-furoic acid /100 mL hydrolysate,0.1 g p-coumaric acid /100 mL hydrolysate, 0.5 g p-hydroxybenzoic acid /100 mL hydrolysate and 0.6 g ferulic acid /100 mL hydrolysate [28].

Corn steep liquor (CSL) was purchased from Xiwang Group, Zouping, Shandong, China with the solid content of 37 % (w/w).

The cellulase enzyme Cellulase Cellic CTec2 was purchased from Novozymes (China), Beijing, China, and the protein content of the enzyme was 86.3 mg proteins/mL by the Bradford method. The cellulase activity was 256 FPU/mL according to the National Renewable Energy Laboratory (NREL) LAP-006 method.

Pyridoxal 5-phosphate (PLP) was purchased from Aladdin, Shanghai, China. Other reagents were purchased from Titan Tech, Shanghai, China.

2.2. Strains and media

E. coli DH5α was used for plasmid construction and cultured in LB medium, which contained 5 g/L of yeast extract, 10 g/L of peptone, and 10 g/L of NaCl. The synthetic medium for *C. glutamicum* contained 100 g/L of glucose, 30 g/L of xylose, 1 g/L of KH_2PO_4, 20 g/L of $(NH_4)_2SO_4$, 0.6 g/L of $MgSO_4$•7 H_2O, 3.6 mg/L $FeSO_4$•7 H_2O, 2 mg/L $MnSO_4$. 40 g/L of corn steep liquor (CSL, dry base), 0.5 g/L of threonine, and 0.5 g/L methionine. 50 μg/mL of kanamycin was added into the media if needed.

Corncob residue hydrolysate was prepared by hydrolyzing corncob residue at the solid content of 20 % with cellulase at the dosage of 4 mg total proteins per gram of dry corncob residue at 50 ℃ for 48 h in a 5 L fermenter (Baoxing Biotech, Shanghai, China). The insoluble residue was removed by centrifugation at 8000 rpm for 15 minutes. The centrifuged supernatant was sterilized at 115 ℃ for 20 min, and then filtered to obtain the corncob residue hydrolysate. The corncob residue hydrolysate was supplemented with 1 g/L of KH_2PO_4, 20 g/L of $(NH_4)_2SO_4$, 0.6 g/L of $MgSO_4$•7 H_2O, 3.6 mg/L of $FeSO_4$•7 H_2O, 2 mg/L of $MnSO_4$, 40 g/L of corn steep liquor (CSL, dry base), 0.5 g/L of threonine, 0.5 g/L of methionine for subsequent fermentation.

2.3. Construction of plasmids and recombinant strains

To construct the synthesis pathway of cadaverine, the fragments of lysine decarboxylase cadA and ldcC gene were cloned from *E. coli* K12 genome. The CadA$_{\text{Aerdh}}$, CadA$_{\text{Aerso}}$, CadA$_{\text{Haf}}$, and CadA$_{\text{Et}}$ genes were synthesized in Tsingke Biotech Co., Shanghai, China based on the gene sequence information as shown in Supplementary Material.

The lysine decarboxylase genes were inserted into the plasmids pH36mob under the control of promoter H36, or pEftumob under the control of promoter Eftu to obtain the expression plasmids pH36-CadA, pH36-LdcC, pH36-CadA$_{\text{Aerdh}}$, pH36-CadA$_{\text{Aerso}}$, pH36-CadA$_{\text{Haf}}$, pH36-CadA$_{\text{Et}}$, pEftu-CadA, and pEftu-LdcC. The plasmids were introduced to *C. glutamicum* LJ01 by electroporation. The cadaverine-producing recombinants LJ01-pH36-CadA, LJ01-pH36-LdcC, LJ01-pH36-CadA$_{\text{Aerdh}}$, LJ01-pH36-CadA$_{\text{Aerso}}$, LJ01-pH36-CadA$_{\text{Haf}}$, LJ01-pH36-CadA$_{\text{Et}}$, LJ01-pEftu-CadA, and LJ01-pEftu-LdcC were obtained as showed in Table 1. The kits used for plasmid construction were purchased from Shanghai Generay Biotech, Shanghai, China.

The signal peptide sequences of *Ncgl1289* and *cgR_0949* were amplified from *C. glutamicum* ATCC 13032 and *C. glutamicum* S9114,

Table 1
Strains and plasmids used.

Strains	Characteristics	Sources
E. coli DH5α	Host for plasmid construction	Lab stock
C. glutamicum LJ01	Lysine production strains	Lab stock
C. glutamicum LJ01-pH36-CadA	Expression of CadA under the control of pH36	This study
C. glutamicum LJ01-pH36-Ldcc	Expression of Ldcc under the control of pH36	This study
C. glutamicum LJ01-pH36-CadA$_{\text{Et}}$	Expression of CadA$_{\text{Et}}$ under the control of pH36	This study
C. glutamicum LJ01-pH36-CadA$_{\text{Haf}}$	Expression of CadA$_{\text{Haf}}$ under the control of pH36	This study
C. glutamicum LJ01-pH36-CadA$_{\text{Aerdh}}$	Expression of CadA$_{\text{Aerdh}}$ under the control of pH36	This study
C. glutamicum LJ01-pH36-CadA$_{\text{Aerso}}$	Expression of CadA$_{\text{Aerso}}$ under the control of pH36	This study
C. glutamicum LJ01-pEftu-CadA	Expression of CadA under the control of pEftu	This study
C. glutamicum LJ01-pEftu-Ldcc	Expression of Ldcc under the control of pEftu	This study
C. glutamicum LJ01-pH36-CadBA	Co-expression of CadA and CadB under the control of pH36	This study
C. glutamicum LJ01-pH36-NcglCadA	Co-expression of Ncgl and CadA under the control of pH36	This study
C. glutamicum LJ01-pH36-CGRCadA	Co-expression of CGR and CadA under the control of pH36	This study
C. glutamicum LJ01-Δ06425-pH36-NcglCadA	Knockout of *SB89_06425* and co-expression of Ncgl and CadA under the control of pH36	This study
C. glutamicum LJ01-Δ06425-pH36-CGRCadA	Knockout of *SB89_06425* and co-expression of CGR and CadA under the control of pH36	This study
C. glutamicum LJ01-Δ07875-pH36-NcglCadA	Knockout of *SB89_07875* and co-expression of Ncgl and CadA under the control of pH36	This study
C. glutamicum LJ01-Δ07875-pH36-CGRCadA	Knockout of *SB89_07875* and co-expression of CGR and CadA under the control of pH36	This study
C. glutamicum LJ02-pH36-NcglCadA	Knockout of *SB89_06425* and *SB89_07875* and co-expression of Ncgl and CadA under the control of pH36	This study
C. glutamicum LJ02-pH36-CGRdCadA	Knockout of *SB89_06425* and *SB89_07875* and co-expression of CGR and CadA under the control of pH36	This study
C. glutamicum LJ01-pXMJ19-NcglCadA	Co-expression of Ncgl and CadA under the control of pXMJ19	This study
C. glutamicum LJ01-Δ06425-pXMJ19-NcglCadA	Knockout of *SB89_06425* and co-expression of Ncgl and CadA under the control of pXMJ19	This study
C. glutamicum LJ01-Δ07875-pXMJ19-NcglCadA	Knockout of *SB89_07875* and co-expression of Ncgl and CadA under the control of pXMJ19	This study
C. glutamicum LJ02-pXMJ19-NcglCadA	Knockout of *SB89_06425* and *SB89_07875* and co-expression of Ncgl and CadA under the control of pXMJ19	This study

respectively [29–31]. The lysine-cadaverine transporter protein CadB gene was cloned from *E. coli* K12. Then the signal peptide sequences of Ncgl1289 and the fragment of CadA gene were overlapped together and inserted into pH36mob, resulting in the plasmid pH36-NcglCadA. Similarly, the plasmids were constructed by fusing membrane protein sequences of *cgR_0949* and *cadB* in front of CadA to obtain the plasmids pH36-CGRCadA and pH36-CadBA, respectively. The above plasmids were electro-transferred into the *C. glutamicum* LJ01 to obtain the recombinants LJ01-pH36-NcglCadA, LJ01-pH36-CGRCadA, and LJ01-pH36-CadBA.

The up- and down-fragments of *SB89_06425* and *SB89_07875* were cloned from *C. glutamicum* LJ01, and then inserted into pK18mobsacb, resulting in the pK18-Δ*06425* and pK18-Δ*07875* plasmids, respectively. The plasmids were verified by sequencing and transformed into *C. glutamicum* LJ01 by electroporation. The correct recombinant mutant was isolated through two rounds of homologous recombination and verified by colony PCR [32].

2.4. Fermentations

The seed culture was prepared by taking one vial of the preserved *C. glutamicum* stock from the −80 ℃ refrigerator and streaked on an LB plate for 48 hours. One single colony was picked up and inoculated into 10 mL of synthetic medium and incubated at 30 ℃, 200 rpm, pH 7.0 for 12 hours. The culture broth was used for inoculation of subsequent fermentation experiments.

For shake flask fermentation, the seed culture was inoculated into 250 mL flasks containing 30 mL of the fresh synthetic medium at 5 % inoculum and incubated at 30 °C, 200 rpm, pH 7.0 for 72 h. The pH was adjusted to 7.0 every 12 h using 5 mol/L NaOH.

The batch fermentation was performed in a 3 L fermenter (3BG-4, Baoxing Biotech, Shanghai, China) at 30 ℃, 1.4 vvm aeration, and 600 rpm. The pH was maintained at 7.0 by adding 25 % (w/v) ammonium hydroxide solution and 5 mol/L NaOH. The seed culture was inoculated at 10 % (v/v) inoculum ratio.

2.5. Whole-cell catalysis

Lysine decarboxylase is screened by catalysis of cell-free extracts. *C. glutamicum* was cultured for 36 hours according to the shake flask fermentation method described above. Cells were collected at 4 ℃, 4000 rpm, and washed twice with PBS buffer of pH 7.0. Cells were resuspended using PBS buffer and broken using an ultrasonic cell pulverizer SCIENTZ-IID (Scientz Biotech Co, Ningbo, Zhejiang, China) at the condition of power 75 %, 2 s sonication, 3 s intervals for 30 minutes. 0.1 M of lysine-HCl, 0.1 mM of PLP were added to the broken cell suspension and catalyzed for 12–24 h at 30 °C, 200 rpm.

For whole-cell catalysis, *C. glutamicum* was cultured for 36–48 h as described above in shake flasks. The cells were collected at 4000 rpm, 4 °C and washed twice with PBS buffer using pH 7.0. The final cell suspension obtained was supplemented with 0.1 M lysine-HCl, 0.1 mM PLP, and catalysis for 12–24 h at 30 °C, 200 rpm.

2.6. Analysis of products

Glucose and xylose were determined by high-performance liquid chromatography (HPLC) (Shimadzu Corporation (Japan), model LC-20D with a differential refractometer RID-10A), the chromatographic column was Aminex HPX-87H (300 mm × 7.8 mm) from Bio-Rad, Hercules, USA at 65 ℃, the mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. lysine was detected using a biosensor analyzer SBA-90 (Shandong Academy of Sciences, Jinan, Shandong, China).

Cadaverine concentration was detected by pre-column derivatization of HPLC (SPD-20A, Shimadzu, Kyoto, Japan). Cadaverine was derived by diethyl ethoxymethylenemalonate (DEEMM) [33], 50 μL of sample was derived with 3 μL of DEEMM, 47 μL of deionized water, 100 μL of methanol and 300 μL of borate buffer (50 mM, pH 9.0) for 2 h at 70 °C. The derivatized cadaverine was detected by C18 column (YMC-Park ODS-A, 150 mm × 4.6 I.D.), with a column temperature of 35 °C and a detection wavelength of 284 nm. The mobile phase was composed of 25 mM sodium acetate at pH 4.8 (phase A) and 100 % acetonitrile (phase B). The flow rate was maintained at 1 mL/min with the following composition gradient: 0–2 min, 20–25 %; 2–10 min, 25–70 %; 10–15 min, 70–20 %; 15–20 min, 20 % [34].

3. Result and discussion

3.1. Evaluation of host strains

The growth behaviors of *C. glutamicum* and *E. coli* in corncob residue hydrolysate were evaluated as shown in Fig. 1. The results indicate that the lysine-producing strain *C. glutamicum* LJ01 grew well in corncob residue hydrolysate with the optical density at 600 nm (OD_{600}) reached ~15 after 24 h of culture. On the other hand, the control strain *E. coli* BL21 showed a poor growth rate in corncob residue hydrolysate with the maximum OD_{600} at ~4.50, approximately 1/3 of that of *C. glutamicum* LJ01, this result verifies the conclusion in previous studies that *E. coli* is sensitive to inhibitors such as organic acid and phenolic compounds of lignocellulosic origin, and therefore cannot grow well [15–18]. Therefore, *C. glutamicum* was selected as the host strain for the consequent metabolic engineering, fermentation or whole-cell catalysis on cadaverine production using corncob residue because of its excellent growth characteristics in corncob residue hydrolysate.

3.2. Screening the lysine decarboxylase genes and promoters for cadaverine synthesis

The host bacterium *C. glutamicum* LJ01 was used for the heterologous expression of lysine decarboxylase as a catalyst to convert lysine to cadaverine. The principle diagram of metabolic engineering of *C. glutamicum* LJ01 for cadaverine synthesis is shown in Fig. 2.

The cadaverine synthesis pathway as shown in Fig. 2**(a)**, six lysine decarboxylase (LDC) genes were selected including CadA from *E. coli* K12 (NCBI Gene ID 948643), LdcC from *E.coli* K12 (NCBI Gene ID 944887), $\text{CadA}_{\text{Aerdh}}$ from *Aeromonas dhakensis* KOR1 (NCBI Gene ID 58198185), $\text{CadA}_{\text{Aerso}}$ from *Aeromonas sobria* CECT4245 (NCBI Gene ID 58922194), CadA_{Et} from *Edwardsiella tarda* KC-Pc-HB1 (NCBI Gene ID 60779409) , CadA_{Haf} from *Hafnia alvei* A23BA (NCBI Gene ID 56893104). These lysine decarboxylase genes were inserted into the expression plasmid pH36mob under the control of promoter H36 or

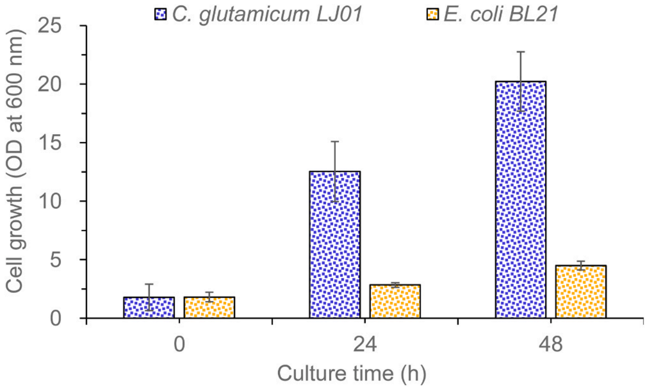


Fig. 1. Cell growth performance of *C. glutamicum* LJ01 and *E. coli* BL21 in corncob residue hydrolysate. The cultures were conducted in 250 mL shake flasks at 200 rpm, 30 ℃, and pH 7.0. The ingredients added including 1 g/L KH_2PO_4, 20 g/L $(NH_4)_2SO_4$, 0.6 g/L $MgSO_4 \bullet 7\ H_2O$, 3.6 mg/L $FeSO_4 \bullet 7\ H_2O$, 2 mg/L $MnSO_4$, 40 g/L corn steep liquor (CSL), 0.1 mM PLP, 0.5 g/L threonine, 0.5 g/L methionine.

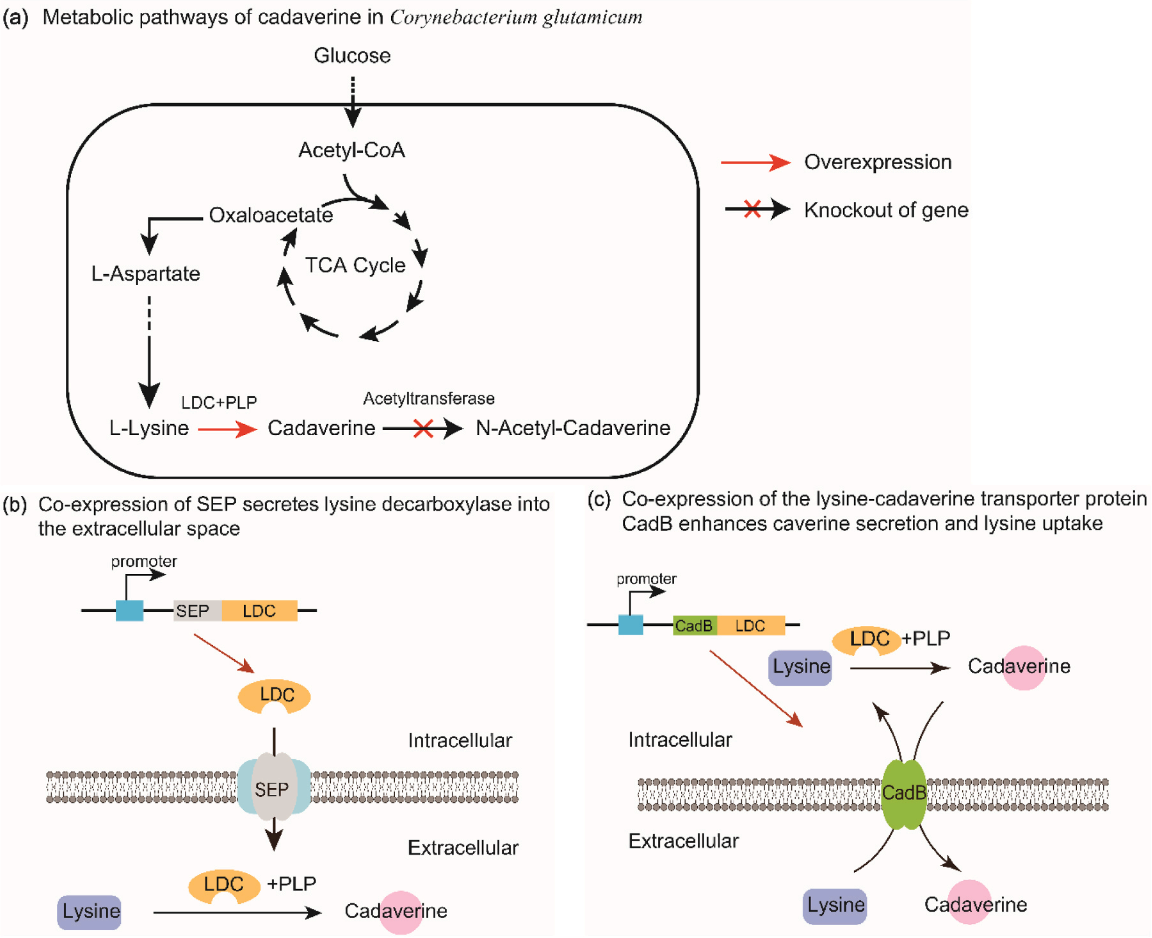


Fig. 2. Diagram of metabolic engineering of *C. glutamicum* LJ01 for cadaverine synthesis. (a) Cadaverine synthesis pathway in *C. glutamicum* LJ01. (b) Secretion expression pathway (SEP) of lysine decarboxylase, transporting lysine decarboxylase to the extracellular space for cadaverine synthesis. (c) Enhancement of cadaverine secretion and lysine uptake by expressing the transporter protein CadB. LDC, lysine decarboxylase. PLP, pyridoxal 5′-phosphate. SEP, secretory expressed protein include Ncgl and CGR. CadB, Lysine-cadaverine transporter protein.

pEftumob under the control of promoter Eftu and then introduced into *C. glutamicum* LJ01 by electroporation to obtain the recombinant *C. glutamicum* strains. The catalysis of cell-free extracts was conducted as described in Section 2.3.

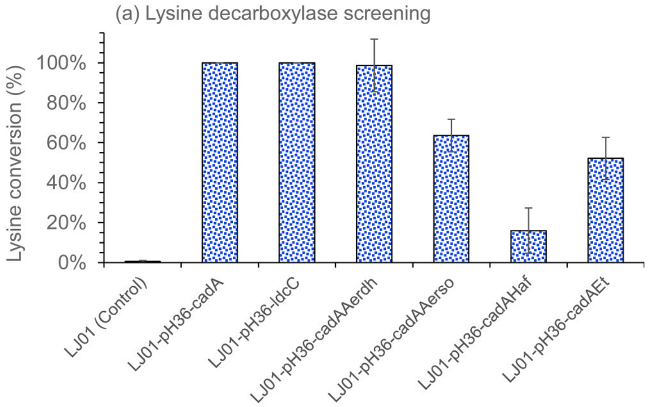
Fig. 3(a) indicates that the lysine decarboxylases encoded by CadA, LdcC and $\text{CadA}_{\text{Aerdh}}$ showed the relatively high catalytic activity on complete conversion of lysine to cadaverine. On the other hand, the lysine decarboxylases encoded by $\text{CadA}_{\text{Aerso}}$, CadA_{Et}, CadA_{Haf} showed the low catalytic activity with less potentials for applications, it may be that these three enzymes are not suitable for the expression system of *C. glutamicum*, resulting in lower expression. Fig. 3(b) showed the screening of promoters, H36 and Eftu, for the two lysine decarboxylase genes CadA and LdcC. The obtained recombinant *C. glutamicum* LJ01-pH36-CadA, LJ01-pH36-LdcC, LJ01-pEftu-CadA, and LJ01-pEftu-LdcC were used as the fermentation strains for cadaverine synthesis in 3 L fermenter. Fig. 3(b) showed that the H36 promoter was more favorable for cadaverine synthesis from lysine by using LJ01-pH36-LdcC. In summary, the H36 promoter is more favorable for the expression of lysine decarboxylase, CadA and ldcC have similar catalytic activities, of which the expression of CadA in *C. glutamicum* has been widely and deeply studied with good catalytic activity, so CadA is mainly used as the target of the subsequent study. The pH activity of lysine decarboxylases from different sources is usually distributed between 5 and 9 [35]. Considering that the optimum growth pH of *C. glutamicum* was 7.0, the fermentation of cadaverine was carried out at pH 7.0 in order to ensure a good growth condition of the host strain.

The low lysine generation by fermentation way (~3.00 g/L) indicated that the supply of lysine by *C. glutamicum* LJ01 might be insufficient. Therefore, the whole-cell catalysis by the recombinant *C. glutamicum* cells with lysine as precursor was adopted using lignocellulose as the carbon source to cultivate the engineered bacteria as a whole-cell catalyst. Fig. 3(a) shows that lysine decarboxylase CadA and LdcC have strong and relatively similar catalytic abilities, so one of them, lysine decarboxylase CadA, was selected for subsequent whole-cell catalysis experiments.

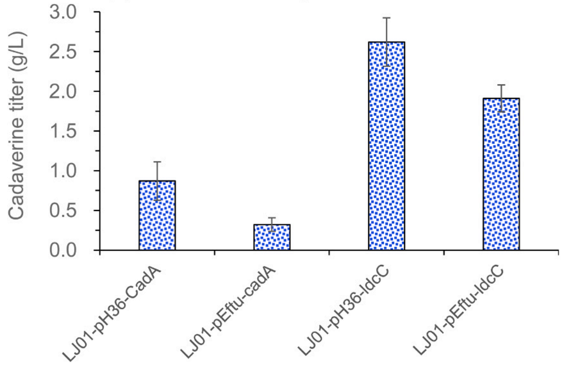
3.3. *Expressions of secretory expressed protein Ncgl, CGR and lysine-cadaverine transporter CadB*

Due to the alkaline nature of cadaverine, the neutral pH in the intracellular space of *C. glutamicum* may be affected by the accumulation of cadaverine inside the cells, and the transmembrane transportation of cadaverine may also cause cell membrane damage. In this study, lysine decarboxylase was secreted and the decarboxylation of lysine was conducted in extracellular space to overcome these cadaverine synthesis difficulties (Fig. 2).

Two signal peptides, Ncgl1289 and CGR_0949, mediating the Sec pathway and the TAT pathway, respectively, were fused with lysine decarboxylase to construct two plasmids pH36-NcglCadA and pH36-CGRCadA (Fig. 2(b)), lysine decarboxylase is transferred extracellularly through the Sec pathway and the TAT pathway, and the extracellular catalytic reaction avoids the accumulation of the product



(a) Lysine decarboxylase screening



(b) Promoter screening

Fig. 3. Screening of lysine decarboxylase genes and promoters in the engineered *C. glutamicum.* (a) Lysine decarboxylase gene screening by catalysis of cell-free extracts. *C. glutamicum* LJ01 as control, the other six strains were *C. glutamicum* LJ01 expressing lysine decarboxylase CadA, LdcC, CadAAerdh, CadAAerso, CadAHaf, CadAEt using the vector pH36mob. (b) Promotor screening of pH36 and Eftu for expression of lysine decarboxylases. LJ01-pH36-CadA, LJ01-pEftu-CadA, LJ01-pH36-ldcC, LJ01-pEftu-ldcC were developed by harboring the expression vectors pH36-CadA, pEftu-CadA, pH36-ldcC, pEftu-ldcC in *C. glutamicum* LJ01 fermented in 3 L fermenters at 600 rpm, 1.2 vvm aeration, 30 ℃, pH 7.0 with 10 % inoculum of the seed culture.

intracellularly. On the other hand, the endogenous lysine-cadaverine transporter protein CadB was co-expressed in *C. glutamicum* LJ01 with lysine decarboxylase to construct the plasmid pH36-CadBA and transport lysine to intracellular and cadaverine to extracellular (Fig. 2**(c)**). Three recombinant *C. glutamicum* LJ01-pH36-NcglCadA, LJ01-pH36-CGRCadA, and LJ01-pH36-CadBA were obtained and fermented in a 3 L fermenter using the synthetic medium under the conditions of 30 ℃, 600 rpm, 1.4 vvm, and the pH 7.0.

Fig. 4 shows that with the recombinant strain LJ01-pH36-CadA as the control, the fusion expression of the secreted expression proteins Ncgl and CGR with the lysine decarboxylase increased the cadaverine generation by 57 % and 98 %, respectively. The results indicate that the secretive expression of lysine decarboxylase significantly improved the lysine conversion to cadaverine. The co-expression of the lysine-cadaverine transporter protein CadB did not increase cadaverine generation, indicating that the transportation of lysine and cadaverine was not the rate-limiting step of lysine conversion to cadaverine.

3.4. Knockout of by-product synthesis genes of cadaverine to acetyl-cadaverine

There are two acetyltransferase genes *SB89_06425* and *SB89_07875* in *C. glutamicum* LJ01 by gene mining in NCBI database responsible for converting cadaverine to a byproduct acetyl-cadaverine (Fig. 2(a)). In this study, the two genes were knocked out to obtain three recombinants *C. glutamicum* LJ01-Δ06425 with the knockout of *SB89_06425*,

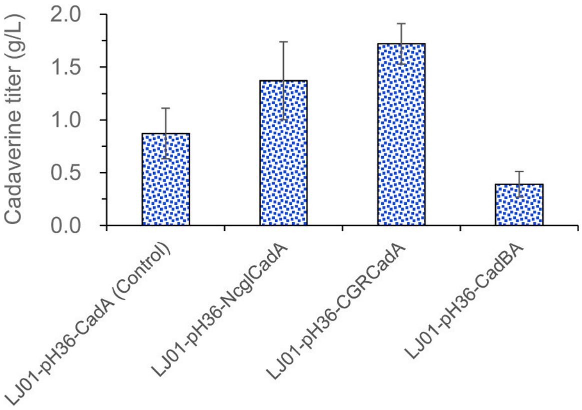


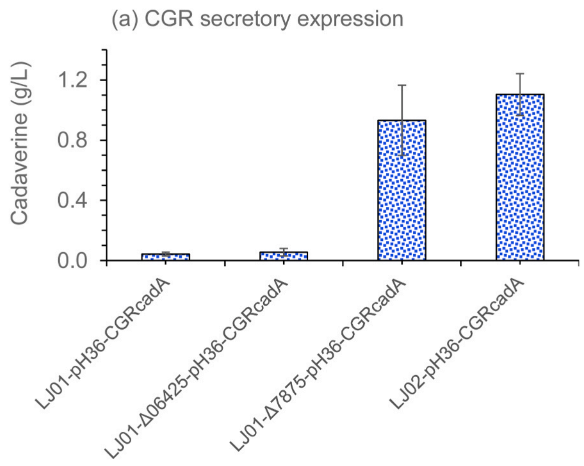
Fig. 4. Evaluation of the impact of secretory expressed protein Ncgl, CGR and lysine-cadaverine transporter protein CadB on cadaverine synthesis. The control strain was LJ01-pH36-CadA. The strains LJ01-pH36-NcglCadA, LJ01-pH36-CGRCadA, and LJ01-pH36-CadBA were *C. glutamicum* LJ01 with the co-expression of lysine decarboxylase CadA with the secreted signal peptides Ncgl, CGR and lysine-cadaverine transporter protein CadB, respectively. Fermentation was carried out in a 3 L fermenter at 600 rpm, 1.2 vvm, 30 ℃, pH 7.0 with 10 % inoculum.

C. glutamicum LJ01-Δ07875 with the knockout of *SB89_07875*, and *C. glutamicum* LJ02 with the knockout of both *SB89_06425* and *SB89_07875*. Then the lysine decarboxylase expression plasmids pH36-NcglCadA and pH36-CGRCadA were introduced into the above three recombinants to obtain the six new *C. gluctamicum* recombinants LJ01-Δ06425-pH36-NcglCadA, LJ01-Δ07875-pH36-NcglCadA, LJ01-Δ06425-pH36-CGRCadA, LJ01-Δ07875-pH36-CGRCadA, LJ02-pH36-NcglCadA, and LJ02-pH36-CGRCadA.

These recombinants were used to ferment in shake flasks at 30 ℃, pH 7.0 for 72 h and the final cadaverine obtained was shown in Fig. 5. The results show that the knockout of the acetyltransferase gene *SB89_07875* significantly promoted the synthesis of cadaverine, while the knockout of *SB89_06425* had little effect on the production of cadaverine. The production of cadaverine in the recombinant strain LJ02-pH36-CGRCadA with the double knockout of the two cadaverine degradation genes was 13-fold higher than that in the control strain, indicating the knockout of acetyltransferases favored the accumulation of cadaverine. The results in Fig. 5 indicate that knockout of the by-product synthesis pathway significantly increased the production of cadaverine, regardless of whether the Sec pathway or the TAT pathway was used for secretory expression of lysine decarboxylase.

3.5. Cadaverine production by whole-cell catalysis in C. glutamicum

In the results of previous experiments, the production of cadaverine by fermentation did not reach a high level, but the concentration of the residual precursor lysine in the fermentation broth was very low, so we deduced that it was the insufficient supply of the precursor that led to the low yield of cadaverine, and that whole-cell catalyzing can solve the above problem. For whole-cell catalysis, *C. glutamicum* was cultured for 36–48 h in shake flasks. The cells were collected at 4000 rpm, 4 °C and washed twice with PBS buffer using pH 7.0. The final cell suspension obtained was supplemented with 0.1 M lysine-HCl, 0.1 mM PLP, and catalysis for 12–24 h at 30 °C, 200 rpm.In addition to the above problems, the problem of plasmid loss occurred in the study, the antibiotic marker might develop resistance in prolonged cultures and lose the pH36 plasmid under kanamycin [36–39]. Therefore, the plasmid with pXMJ19 with a more sensitive chloramphenicol marker was introduced into *C. glutamicum* LJ01. The fragment of NcglCadA was amplified from pH36-NcglCadA, inserted it into pXMJ19, resulting in pXMJ19-NcglCadA, and transformed into *C. glutamicum* LJ01, LJ01-Δ06425, LJ01-Δ07875, and LJ02 by electroporation, respectively,



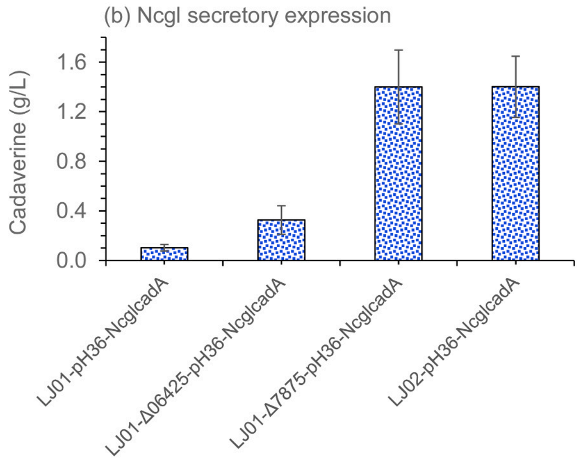


Fig. 5. Evaluation of the knockout of acetyltransferase genes *SB89_06425* and *SB89_07875* on cadaverine generation. LJ01-Δ06425 and LJ01-Δ07875 were knocked out of the genes *SB89_06425* and *SB89_07875*, respectively. LJ02 was knocked out of both the genes. (a) Secreted expression protein CGR with the byproduct gene knockout. LJ01-pH36-CGRCadA was the control; the test recombinants were LJ01-Δ06425 and LJ01-Δ07875 carrying the expression plasmid pH36-CGRCadA. (b) Secreted expression protein Ncgl with the byproduct gene knockout. LJ01-pH36-NcglCadA was the control; the rest recombinants were LJ01-Δ06425 and LJ01-Δ07875 carrying the expression plasmid pH36-NcglCadA. The tests were carried out in 250 mL shake flasks at 30 ℃, 200 rpm, pH 7.0 with 5 % inoculum.

to give the recombinants LJ01-pXMJ19-NcglCadA, LJ01-Δ06425-pXMJ19-NcglCadA, LJ01-Δ07875-pXMJ19-NcglCadA, and LJ02-pXMJ19-NcglCadA.

To ensure the stability and the physiological state during whole-cell catalysis, the catalysis was carried out in PBS buffer using the cells cultured in a synthetic medium, initial lysine concentration of 100 g/L. Fig. 6(a) shows that the recombinant LJ02-pXMJ19-NcglCadA with the knockout of two acetyltransferases *SB89_06425* and *SB89_07875* significantly improved the cadaverine production to 79.41 g/L with 91 % of the molar conversion yield from lysine to cadaverine, representing 35 % enhancement in conversion efficiency than the control LJ01-pXMJ19-NcglCadA (52.50 g/L of cadaverine and a molar conversion yield of 61 %).

To further investigate the feasibility of whole-cell catalysis using lignocellulosic feedstock, the recombinants were cultured in corncob residue hydrolysate to harvest the cells as the whole-cell catalysts. The recombinant cells were subjected to whole-cell catalysis according to the same conditions above. Fig. 6(b) shows that the recombinant LJ02-pXMJ19-NcglCadA led to the cadaverine production to 78.19 g/L with a lysine conversion yield of 91 %, representing the cadaverine increase of 30 % compared to the control LJ01-pXMJ19-NcglCadA (60.28 g/L). On the other hand, the recombinants LJ01-Δ06425-pXMJ19-NcglCadA and LJ01-Δ07875-pXMJ19-NcglCadA did not show a significant increase in cadaverine production and conversion compared to the control.

4. Conclusion

This study developed a cadaverine-producing recombinant strain *C. glutamicum* LJ02-pXMJ19-NcglCadA by co-expression of secretively expressed lysine decarboxylase and knockout of the cadaverine degradation pathway. Which can effectively convert lysine to cadaverine as the whole-cell catalyst using corncob residue hydrolysate. Corncob residue was used as the carbohydrate feedstock for producing the recombinant LJ02-pXMJ19-NcglCadA cells as the whole-cell catalyst for production of cadaverine from lysine. A high 78.19 g/L of cadaverine was generated with a lysine conversion yield of 91 % using the corncob residue cultured whole cells of the recombinant.

The biosynthesis of cadaverine is primarily divided into two ways: de novo synthesis and whole-cell catalysis. The de novo synthesis of cadaverine in *C. glutamicum* has been extensively studied. Stefanie et al. [4] used *C. glutamicum* as a host bacterium to construct a synthesis pathway for cadaverine, and the production of cadaverine from a fed-batch fermentation was 88 g/L. However, most of the studies related to de novo synthesis use glucose as the carbon source. Matsuura et al. [14] constructed *C. glutamicum* that could express β-glucosidase for the de novo synthesis of cadaverine using cellulose-derived sugars, but the production was only 27 g/L. Whole-cell catalysis of cadaverine mainly with *E. coli* as the host bacterium. Leong et al. [40] increased the production of cadaverine to 145 g/L by whole-cell catalytic. Guo et al. [41] used the carbon dioxide released from lysine decarboxylation during whole-cell catalysis to regulate the pH of the conversion system, and the concentration of cadaverine generated was 208.2 g/L at an initial lysine concentration of 300.0 g/L. Whole-cell catalyzed production of cadaverine usually has high production because the production of cadaverine depends on the concentration of the precursor, and high production of cadaverine can be achieved with the addition of a sufficiently large amount of precursor. However, whole-cell catalytic production of cadaverine using *E. coli* also requires glucose as a carbon source and the strain is unable to grow in lignocellulosic hydrolysis, so in this study, we used *C. glutamicum* as the host bacterium for whole-cell catalytic production of cadaverine. The raw material of corncob residue used in this study reduces the production cost of cadaverine, and the production of cadaverine through whole-cell catalysis also avoids the insufficient supply of precursor to limit the synthesis of the product. The results provided a practical biocatalyst for industrial production of cadaverine using lignocellulosic feedstock.

There are still massive challenges in using lignocellulosic biomass as carbohydrates, including full utilization of fermentable sugars such as xylose, arabinose, mannose, galactose; complete removal of inhibitors without causing high wastewater or solvent discharge; high conversion levels to overcome the low technical performance and compete with corn starch based conversions. The wild-type lysine decarboxylase can be further improved the catalytic activity by targeted modification, the cell stability can be strengthened by immobilization of the cells, and the time cost can be reduced by the recycling of the cells. In addition, the better lysine producing *C. glutamicum* host strain may help the direct utilization of glucose or xylose to cadaverine.

CRediT authorship contribution statement

Xu Ying-Ying: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Bao Jie:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Zhang Bin:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition.

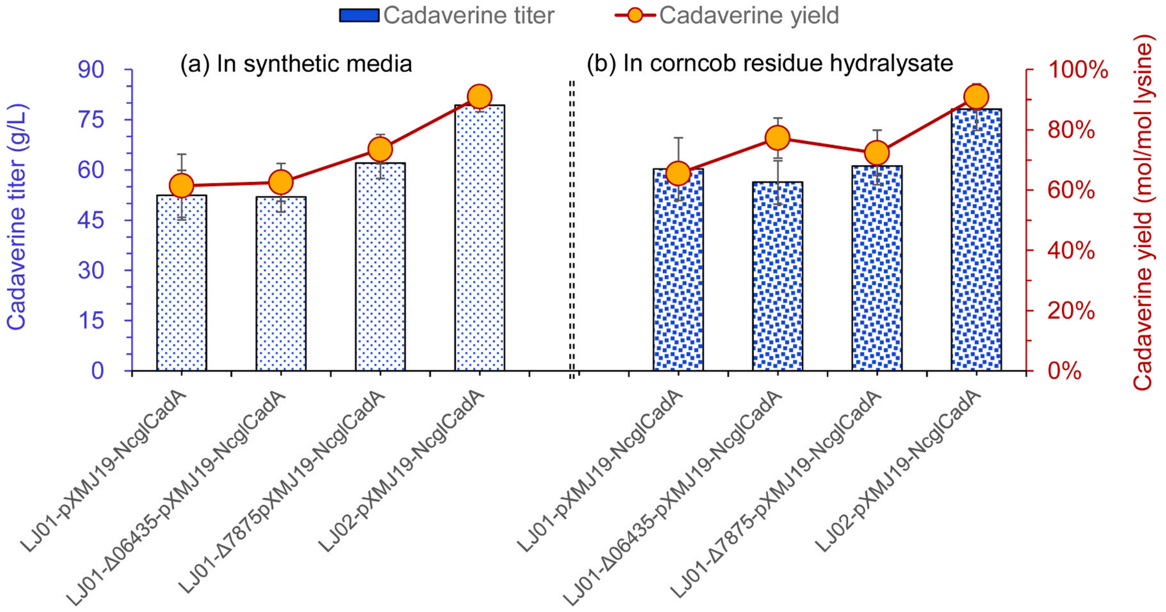


Fig. 6. Whole-cell catalysis of the recombinants with the knockout of acetyltransferases *SB89_06425* and *SB89_07875* using corncob residue hydrolysate as carbohydrate feedstock. (a) the recombinant cells were cultured in a synthetic medium. (b) the recombinant cells were cultured in corncob residue hydrolysate. LJ01-pXMJ19-NcglCadA was the control. The test recombinants included LJ01-Δ06425, LJ01-Δ07875, and LJ02 harboring the expression plasmids pXMJ19-NcglCadA. Whole-cell catalysis is described in Section 2.5 in shake flasks. The cells were collected at 4000 rpm, 4 °C and washed twice with PBS buffer using pH 7.0. The final cell suspension obtained was supplemented with 0.1 M lysine-HCl, 0.1 mM PLP, and catalysis for 12–24 h at 30 °C, 200 rpm. Chloramphenicol concentration in cell culture is 25 μL/mL.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2025.109760.

Data availability

Data will be made available on request.

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