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Development of engineered *Escherichia coli* whole-cell biocatalysts for high-level conversion of L-lysine into cadaverine

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Abstract A whole-cell biocatalytic system for the production of cadaverine from L-lysine has been developed. Among the investigated lysine decarboxylases from different microorganisms, *Escherichia coli* LdcC showed the best performance on cadaverine synthesis when *E. coli* XL1-Blue was used as the host strain. Six different strains of *E. coli* expressing *E. coli* LdcC were investigated and recombinant *E. coli* XL1-Blue, BL21(DE3) and W were chosen for further investigation since they showed higher conversion yield of lysine into cadaverine. The effects of substrate pH, substrate concentrations, buffering conditions, and biocatalyst concentrations have been investigated. Finally, recombinant *E. coli* XL1-Blue concentrated to an OD_{600} of 50, converted 192.6 g/L (1317 mM) of crude lysine solution, obtained from an actual lysine manufacturing process, to 133.7 g/L (1308 mM) of cadaverine with a molar yield of 99.90 %. The whole-cell biocatalytic system described herein is expected to be applicable to the development of industrial bionylon production process.

Keywords Cadaverine · L-Lysine · Whole-cell conversion · Bio-catalyst · Recombinant *E. coli*

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Introduction

Due to the growing concerns about environmental problems caused by petroleum resources and fossil resource depletion problems and unstable cost of fossil fuels, production of industrial products such as fuels, organic acids, and other building blocks in bio-based process becomes inevitable. Among these bio-based products, diamines, dicarboxylic acids, and aminocarboxylic acids recently gain more attention since they can be used as monomers for the synthesis of bio-nylons whose mechanical properties are expected to be compatible with those of petroleum-based plastics. Indeed, it has been demonstrated that bio-based nylon 5, 10 synthesized using biomass-derived cadaverine and sebacic acid as monomers has mechanical properties that even surpass those of commercial nylon 6 and nylon 6.6 in some aspects [7].

Cadaverine, C5 diamine, can be biologically produced from L-lysine by a one-step enzymatic conversion of L-lysine catalyzed by the lysine decarboxylase (LDC). *Escherichia coli* CadA and LdcC are the most examined

lysine decarboxylases (LDCs) in the microbial cadaverine production. Following the report that *Corynebacterium glutamicum* could be engineered to efficiently produce cadaverine from renewable carbon sources using inherent metabolite, L-lysine as a substrate for lysine decarboxylase by the expression of heterologous lysine decarboxylase gene, dozens of studies have been conducted for the production of cadaverine using recombinant *C. glutamicum* strains [2, 5, 6, 11]. Since initial studies have employed wild-type *C. glutamicum* strains that have low L-lysine production capacity as host strains for the production of cadaverine, rather low cadaverine titers and yields have been achieved [24]. Therefore, *C. glutamicum* engineered to highly produce L-lysine have been used as host strains for the production of cadaverine in quite higher titers and yields. For example, cadaverine has been produced up to 88.0 g/L (861 mM) with a molar yield of 50 % by fed-batch fermentation of an L-lysine hyper-producing *C. glutamicum* expressing the codon optimized *E. coli* LdcC [7]. In addition, high cadaverine concentration of 72.0 g/L (705 mM) could be achieved by fed-batch cultivation of recombinant *C. glutamicum* LU11271, an L-lysine hyper-producer, which expresses *E. coli* LdcC, even in large-scale 5000 L fermenter [25, 27].

On the other hand, whole cell bioconversion can be another promising way for the production of cadaverine along with gamma-aminobutyrate and 5-aminovalerate, all of which can be converted from corresponding amino acid substrates, L-glutamate and L-lysine [12, 14, 17]. A Japanese L-lysine supplier, Ajinomoto Co., Ltd., has proved the possibility of producing cadaverine using whole-cell biocatalysts in its patent. Using a recombinant *E. coli* expressing the *cadA* gene, cadaverine of 69.0 g/L (675 mM) was produced from L-lysine in a 50-L jar fermenter [12]. Compared to the direct fermentative production of cadaverine from renewable carbon sources, it is distinguished that the reaction conditions are less complicated and thus the following separation/purification process of cadaverine would be simpler than that of direct fermentation process. Therefore, it should be carefully considered which process is more economical and suitable for the commercial production cadaverine.

In this study, we have constructed different strains of *E. coli* that express lysine decarboxylase encoded by *E. coli ldcC* gene to examine a whole-cell biocatalytic conversion for the production of cadaverine from L-lysine, in which *E. coli ldcC* gene was chosen for further investigation among five different kinds of LDCs from *E. coli*, *Ralstonia eutropha*, *Psuedomonas aeruginosa,* and *P. putida* [3, 9, 23, 26]. After the reaction conditions for the conversion of L-lysine into cadaverine have been determined by varying concentration of *E. coli* cells employed for reaction, initial concentration of L-lysine in the reaction, and reaction buffer condition, highly concentrated L-lysine solution that is provided by Paik Kwang Industry, a Korean lysine manufacturing company, has been employed for the production of cadaverine to investigate the possibility of the whole-cell biocatalytic process for industrial applications.

Materials and methods

Bacterial strains, plasmids, and genes

All bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* XL1-Blue (Stratagene Cloning Systems, La Jolla, CA, USA) was used for general gene cloning studies. Recombinant *E. coli* strains expressing lysine decarboxylase were used as whole-cell biocatalysts for the conversion of L-lysine into cadaverine. Plasmid pKE112-MCS, the expression vector containing the *tac* promoter, has previously been described [15, 16].

Plasmid construction

All DNA manipulations were performed following standard procedures. Polymerase chain reaction (PCR) was performed with the C1000 Thermal cycler (Bio-Rad, Hercules, CA, USA). Primers used in this study (Table 2) were synthesized at Bioneer (Daejeon, Korea). Plasmids pKE112-CadA and pKE112-LdcC were constructed by cloning the *E. coli cadA* and *ldcC* genes into pKE112-MCS at *Bam*HI and *Sbf*I sites, respectively. Plasmids pKE112-RELDC, pKE112-PALDC, and pKE112-PPLDC were constructed by cloning the *R. eutropha ldc* gene, the *P. aeruginosa ldc* gene, and the *P. putida ldc* gene into pKE112-MCS at *Bam*HI and *Sbf*I sites, respectively.

Culture conditions

E. coli XL1-Blue was cultured for the gene cloning procedure at 37 °C in Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl). For the synthesis of cadaverine, recombinant *E. coli* strains expressing different kinds of LDCs were cultured in LB medium and MR medium supplemented with 20 g/L of glucose and 10 g/L (68 mM) of lysine at 30 °C in a rotary shaker at 250 rpm. MR medium (pH 7.0) contains per liter the following: 6.67 g KH_2PO_4, 4 g $(NH_4)_2HPO_4$, 0.8 g $MgSO_4 \cdot 7H_2O$, 0.8 g citric acid, and 5 ml trace metal solution. The trace metal solution contains per liter of 0.5 M HCl the following: 10 g $FeSO_4 \cdot 7H_2O$, 2 g $CaCl_2$, 2.2 g $ZnSO_4 \cdot 7H_2O$, 0.5 g $MnSO_4 \cdot 4H_2O$, 1 g $CuSO_4 \cdot 5H_2O$, 0.1 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0.02 g $Na_2B_4O_7 \cdot 10H_2O$. Glucose and $MgSO_4 \cdot 7H_2O$ were separately sterilized. Ampicillin (Ap, 50 μg/mL) was added to the medium.

Table 1 Lists of bacterial strains and plasmids used in this study

Plasmid	Relevant characteristics	Reference or source
Strains		
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI^qZΔM15* Tn*10* (Tet^R)]	Stratagene
BL21(DE3)	*E. coli* B F⁻ *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)	NEB strain catalog no. C2527
BL21(DE3)star	A derivative of BL21 with a mutation in RNAseE F–Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1	Novagen
DH-5α	hsdR17 (rK−, mK+) phoA supE44 λ–thi-1 gyrA96 relA1	Invitrogen™
WL3110	W3110 ΔlacI	[13]
W	Wild type	ATCC
Plasmids		
pKE112-MCS	Expression vector; *tac* promoter, *Ralstonia eutropha* PHA biosynthesis genes transcription terminator; Ap^r	[15]
pKE112-LdcC	pKE112-MCS derivative, *tac* promoter, the *E. coli ldcC* gene, Ap^r	This study
pKE112- CadA	pKE112-MCS derivative, *tac* promoter, the *E. coli cadA* gene, Ap^r	This study
pKE112-RELDC	pKE112-MCS derivative, *tac* promoter, the *R. eutropha ldc* gene, Ap^r	This study
pKE112-PALDC	pKE112-MCS derivative, *tac* promoter, the *P. aeruginosa ldc* gene, Ap^r	This study
pKE112-PPLDC	pKE112-MCS derivative, *tac* promoter, the *P. putida ldc* gene, Ap^r	This study

Table 2 List of primers used in PCR experiments

Target gene	Primer	Primer sequence
E. coli cadA gene	Primer1	**GGTACC** TTTCACACAGGAAACAGACC ATGAACATCATTGCCATTATG
	Primer2	**CCTGCAGG** TTATCCCGCCATTTTTAG
E. coli ldcC gene	Primer3	**GGATCC** TTTCACACAGGAAACAGACC ATGAACGTTATTGCAATATTG
	Primer4	**CCTGCAGG** TTATTTTTTGCTTTCTTCTTTCAATACC
R. eutropha ldc gene	Primer5	**GGATCC** TTTCACACAGGAAACA ATGAAATTCCGTTTCCCCGTC
	Primer6	**CCTGCAGG** TCAGCCGCCCTGCTTCACGC
P. aeruginosa ldc gene	Primer7	**GGATCC** TTTCACACAGGAAACA ATGTACAAGGACCTCAAGTTCC
	Primer8	**CCTGCAGG** TCACGCGCATTCCGTG
P. putida ldc gene	Primer9	**GGATCC** TTTCACACAGGAAACA ATGTATAAAGACCTCAAATTTCCC
	Primer10	**CCTGCAGG** TCATTCCTTTATGCATTCAACG

Restriction enzyme sites are shown in bold

Conversion of L-lysine into cadaverine by whole-cell biocatalysts

Overnight cultures of recombinant *E. coli* strains grown in LB medium were inoculated into MR medium supplemented with 20 g/L of glucose and 10 g/L (68 mM) of L-lysine. They were cultured at 30 °C in a rotary shaker at 250 rpm. Recombinant *E. coli* strains showing higher conversion of L-lysine into cadaverine were further investigated according to the following procedures.

Overnight cultures of recombinant *E. coli* strains in LB medium were collected by a centrifuge (5702R, Eppendorf, Hamburg, Germany) at 4 °C and 4000 rpm for 10 min. The pellets were washed twice with deionized water (DW) and were resuspended in 20 mL of 100 mM Tris–HCl buffer (pH 6.8) or DW to have appropriate values of OD_{600}. After the whole-cell biocatalysts were prepared, filtered L-lysine solution was added to have appropriate concentrations of L-lysine in the reaction mixtures. When it is required, an appropriate amount of HCl was added to the substrate solution to adjust the pH of the solution to pH 6.8 before filtering the substrate solution. The reaction mixtures were incubated at 37 °C in a rotary shaker at 250 rpm.

Highly concentrated L-lysine solution was provided by Paik Kwang Industry, a lysine-manufacturing company in Korea. Highly concentrated L-lysine solution was diluted with DW to have appropriate concentrations of L-lysine in the reaction mixtures.

Analytical procedures

Concentrations of cadaverine and L-lysine were determined by HPLC fitted with a Optimapak C18 column (RStech, DaeJeon, Korea) as previously described [4]. Whole-cell lysates of recombinant *E. coli* strains and their respective soluble and insoluble fraction were analyzed by 12 % (*w*/*v*) SDS-PAGE.

Results

Synthesis of cadaverine by recombinant *E. coli* stains expressing lysine decarboxylase from different microorganisms

Recombinant *E. coli* XL1-Blue strain harboring pKE112-LdcC, pKE112-CadA, pKE112RELDC, pKE112-PALDC, or pKE112-PPLDC, which expresses the *E. coli cadA* gene, the *E. coli ldcC* gene, the *R. eutropha ldc* gene, the *P. aeruginosa ldc* gene, or the *P. putida ldc* gene, respectively, was constructed for the conversion of L-lysine into cadaverine. These recombinant *E. coli* XL1-Blue strains were investigated for the synthesis of cadaverine from L-lysine. As shown in Fig. 1, *E. coli* LdcC showed a superior performance in the synthesis of cadaverine, which resulted in the production of cadaverine of 6.93 (67.8 mM) g/L by consuming 9.92 g/L (67.9 mM) of L-lysine with the molar yield of 99.9 % in the MR medium supplemented with 20 g/L of glucose and 10 g/L (68 mM) of L-lysine. On the other hand, only a small amount of L-lysine was converted into cadaverine by other lysine decarboxylases. SDS-PAGE analysis of the whole cell extracts of recombinant *E. coli* XL1-Blue strains and their respective soluble fraction supported these results that only the polypeptide band corresponding to *E. coli* LdcC was clearly identified (indicated by red arrows on Fig. S1).

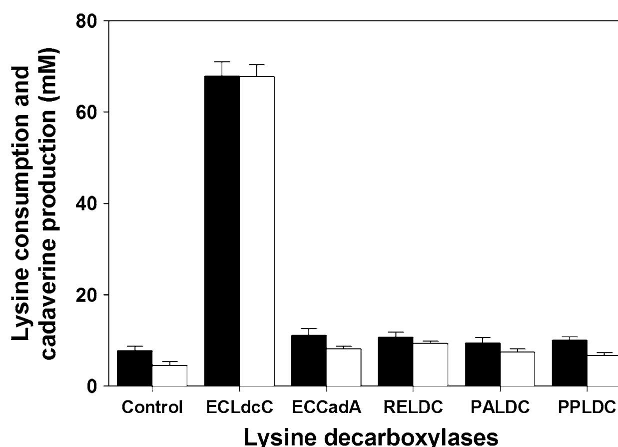

Fig. 1 Synthesis of cadaverine by recombinant *E. coli* XL1-Blue expressing *E. coli* LdcC, *E. coli* CadA, *R. eutropha* LDC, *P. aeruginosa* LDC, or *P. putida* LDC from 10 g/L of lysine solution in 48 h. Recombinant *E. coli* strains were cultured in LB medium 24 h and transferred into MR medium containing 20 g/L of glucose and 10 g/L of lysine. The concentrations of lysine and cadaverine are written in molar concentrations. Data represent mean values from three independent experiments with corresponding deviations. (Symbols: *filled bars*, lysine concentrations; *open bars*, cadaverine concentrations)

Synthesis of cadaverine using different *E. coli* strains expressing *E. coli* LdcC as the whole-cell biocatalysts

Recombinant *E. coli* XL1-Blue, *E. coli* BL21(DE3), *E. coli* BL21(DE3)star, *E. coli* DH-5α, *E. coli* WL3110, or *E. coli* W, harboring pKE112-LdcC individually, was investigated for the synthesis of cadaverine in Tris–HCl buffer containing 10 g/L (68 mM) of L-lysine (Fig. 2). All of recombinant *E. coli* strains harboring pKE112-LdcC consumed more than 80 % of L-lysine existing in the media to synthesize cadaverine in 120 h except *E. coli* BL21(DE3)star harboring pKE112-LdcC. The highest conversion of L-lysine was achieved in *E. coli* W (pKE112-LdcC), which resulted in the production of 6.81 g/L (66.6 mM) of cadaverine with a molar yield of 98.9 % by consuming 9.85 g/L (67.4 mM) of L-lysine in 96 h. The rate of L-lysine consumption and cadaverine production was fastest in the early stage of the reaction (0–48 h) when recombinant *E. coli* XL1-Blue harboring pKE112-LdcC was used as a whole-cell biocatalyst. But *E. coli* BL21(DE3)star harboring pKE112-LdcC consumed only about 50 % of L-lysine in the medium to produce 2.75 g/L (26.9 mM) of cadaverine in 120 h. Four *E. coli* strains, *E. coli* XL1-Blue, BL21(DE3), WL3110, and W, harboring pKE112-LdcC individually, were chosen for further investigations on the production of cadaverine.

Determination of the reaction conditions for the synthesis of cadaverine

Reaction conditions using *E. coli* strain expressing *E. coli* LdcC as a whole cell biocatalyst for efficient conversion of L-lysine into cadaverine were investigated employing *E. coli* XL1-Blue, *E. coli* BL21(DE3), *E. coli* WL3110, and *E. coli* W, as the host strains. The recombinant *E. coli* strains cultured in LB medium overnight were harvested and washed with deionized water (DW). And then they were resuspended in 100 mM Tris–HCl buffer (pH 6.8) to have an OD_{600} of 10 and then were used as whole-cell biocatalysts for the synthesis of cadaverine. L-Lysine solutions with or without pH adjustment were added into each of reaction tubes to have 50 g/L (343 mM) of initial L-lysine concentration in the reaction mixtures. As shown in Fig. 3, both L-lysine consumption and cadaverine production were improved when the pH of L-lysine substrate solution was adjusted to 6.8. In the reactions using recombinant *E. coli* XL1-Blue, *E.*

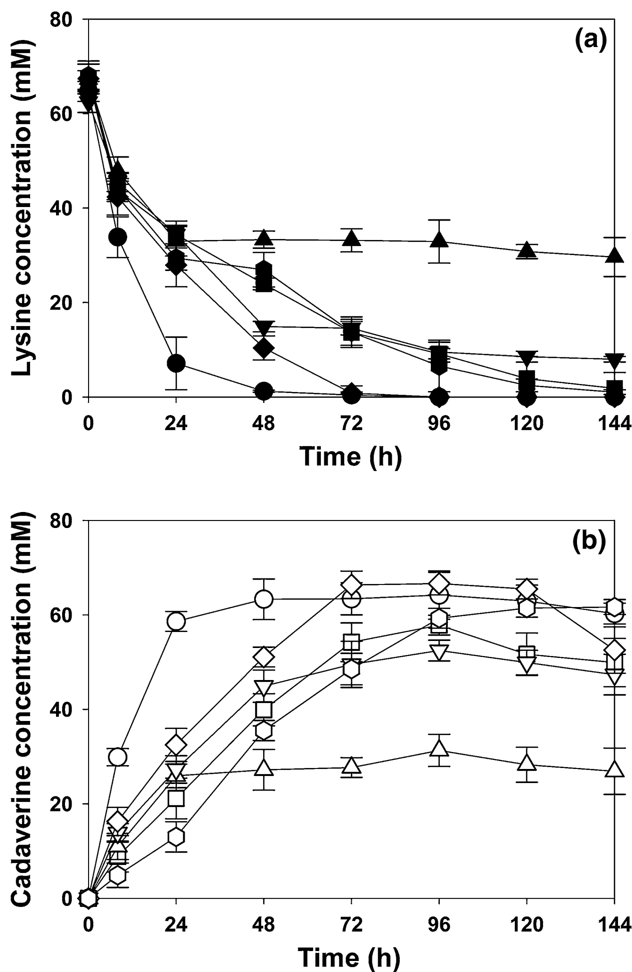

Fig. 2 Time profiles of **a** lysine and **b** cadaverine in the reactions of cadaverine synthesis by recombinant *E. coli* strains harboring pKE112LdcC. Recombinant *E. coli* strains were cultured in LB medium 24 h and transferred into MR medium containing 20 g/L of glucose and 10 g/L of lysine. The concentrations of lysine and cadaverine are written in molar concentrations. Data represent mean values from three independent experiments with corresponding deviations. (Symbols: *filled shapes*, lysine concentrations; *open shapes*, cadaverine concentrations; *circle*, *E. coli* XL1-Blue; *square*, *E. coli* BL21(DE3); *triangle up*, *E. coli* BL21(DE3)star; *triangle down*, *E. coli* DH-5α; *diamond*, *E. coli* W; *hexagon*, *E. coli* WL3110)

coli BL21(DE3), and *E. coli* WL3110, harboring pKE112-LdcC individually, most of L-lysine was consumed to produce almost equal molar amount of cadaverine in 20 h. For instance, recombinant *E. coli* XL1-Blue harboring pKE112-LdcC converted 48.50 g/L (331.8 mM) of L-lysine into 33.47 g/L (327.6 mM) of cadaverine in 100 mM Tris–HCl buffer (pH 6.8) with a molar yield of 98.73 %. On the other hand, recombinant *E. coli* WL3110 harboring pKE112-LdcC showed lower rates of L-lysine consumption and cadaverine production compared to other strains.

Cadaverine production in water was further investigated with the pH-adjusted L-lysine substrate solution. The

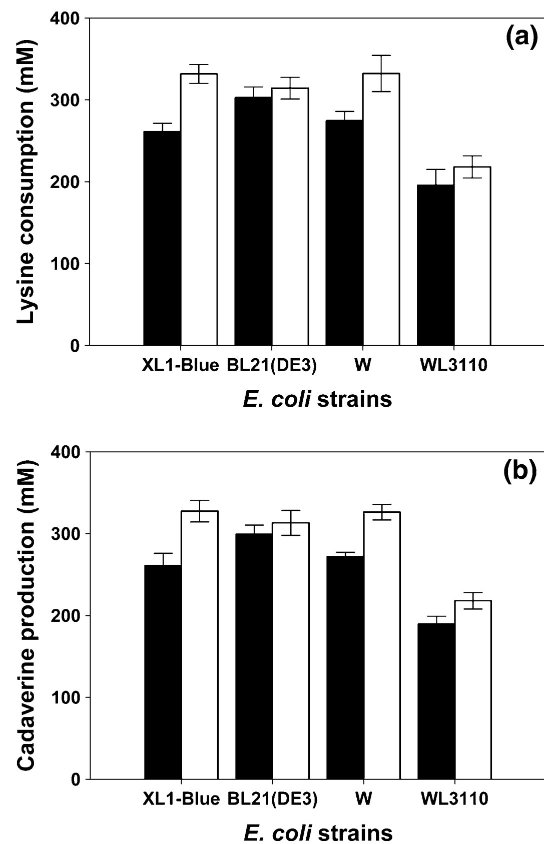

Fig. 3 Effects of pH adjustment of lysine substrate on cadaverine production. Different concentrations of **a** lysine were consumed to synthesize, **b** cadaverine by different strains of *E. coli* expressing the *E. coli ldcC* gene in 20 h. The reactions were performed in Tris–HCl buffer (pH 6.8) and recombinant *E. coli* strains concentrated to OD_{600} of 10 were used as whole-cell biocatalysts. The concentrations of lysine and cadaverine are written in molar concentrations. Data represent mean values from three independent experiments with corresponding deviations. (Symbols: *filled bars*, without pH adjustment of the substrate; *open bars*, with pH adjustment of the substrate)

recombinant *E. coli* strains prepared by the same procedure described above were used as the whole-cell biocatalysts for the synthesis of cadaverine either in 100 mM Tris–HCl buffer (pH 6.8) or in pure water. As shown in Fig. 4, the concentrations and molar yields of cadaverine produced in water were comparable to those obtained in 100 mM Tris–HCl buffer. Except recombinant *E. coli* WL3110 harboring pKE112-LdcC that showed the lowest rates of substrate consumption as well as product formation in water, most of *E. coli* strains expressing LdcC did not show much difference in the conversion of lysine into cadaverine in water and Tris–HCl buffer. The final pHs of the whole cell biocatalyst processes were in the range of pH 8.1–8.4 whether in 100 mM Tris–HCl buffer (pH 6.8) or in pure water (Table S1). Based on these results, *E. coli* XL1-Blue, BL21(DE3), and W were selected for further investigation in cadaverine production in pure water.

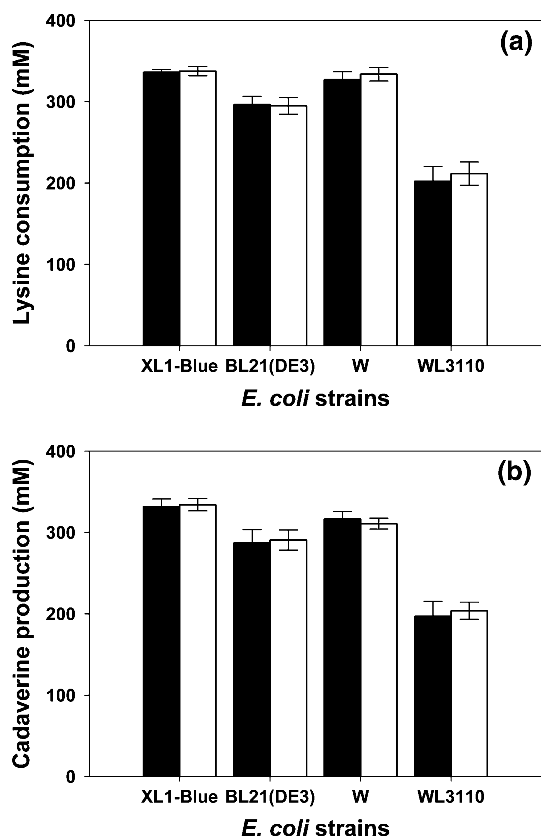

Fig. 4 Effects of reaction condition on cadaverine production. Different concentrations of **a** lysine were consumed to synthesize, **b** cadaverine by different strains of *E. coli* expressing the *E. coli ldcC* gene in 24 h. Recombinant *E. coli* strains concentrated to OD_{600} of 10 were used as whole-cell biocatalysts. L-Lysine solutions whose initial pHs were adjusted to 6.8 were used as substrate solutions. The concentrations of lysine and cadaverine are written in molar concentrations. Data represent mean values from three independent experiments with corresponding deviations. (Symbols: *filled bars*, reaction conducted in 100 mM Tris–HCl buffer (pH 6.8); *open bars*, reaction conducted in deionized water)

Effects of biocatalyst and substrate concentrations on cadaverine synthesis

Whole-cell biocatalytic conversions of higher concentrations of L-lysine into cadaverine were carried out in pure water. The optical density (OD_{600}) of recombinant *E. coli* XL1-Blue, *E. coli* BL21(DE3), and *E. coli* W, which expresses *E. coli* LdcC, respectively, was adjusted to 10 in pure water and then was used as a whole-cell biocatalyst for the synthesis of cadaverine from 50 g/L (342 mM), 100 g/L (684 mM), or 150 g/L (1026 mM) of L-lysine. The results are summarized on Table 3. More than 80 % of L-lysine was converted into cadaverine in 24 h and was almost consumed in 48 h to produce an equal molar amount of cadaverine when the L-lysine was added to the concentration of 50 g/L (342 mM). Recombinant *E. coli* XL1-Blue harboring pKE112-LdcC showed the fastest initial rate cadaverine production than those obtained by the other *E. coli* strains. When L-lysine concentration was increased to 100 g/L (684 mM), about 60 g/L (587 mM) of cadaverine was produced by the recombinant strains of *E. coli*. However, rate of cadaverine production and L-lysine consumption were reduced, and even L-lysine could not be completely consumed in longer reaction times up to 96 h in all cases (data not shown). Further increase of initial L-lysine concentration up to 150 g/L (1026 mM) resulted in significant decreases in L-lysine consumptions as well as cadaverine production by the whole-cell biocatalysts (Table 3).

Optical densities of recombinant *E. coli* strains employed for the cadaverine production were increased to 30 and 50 to convert higher concentrations of L-lysine into cadaverine. The results are also summarized on Table 3. Recombinant *E. coli* strains concentrated to OD_{600} of 50 showed faster L-lysine consumption and cadaverine production than those obtained by recombinant *E. coli* strains having an OD_{600} of 30 (data not shown). Final cadaverine titers were also higher when recombinant *E. coli* strains concentrated to OD_{600} of 50 were used than when those concentrated to OD_{600} of 30 were used as biocatalysts. Compared with the results obtained with recombinant *E. coli* strains concentrated to OD_{600} of 10, most of L-lysine added to the reaction medium was consumed by increasing concentration of *E. coli* strains employed for the reaction, which resulted in about 100 % molar yields of cadaverine synthesized from L-lysine. Among the recombinant *E. coli* strains, recombinant *E. coli* XL1-Blue harboring pKE112-LdcC showed the fastest rates of L-lysine consumption and cadaverine production in the early stages of the reactions, but the final cadaverine concentrations produced by recombinant *E. coli* strains harboring pKE112-LdcC were not much different from each other (Table 3). The highest concentrations of cadaverine achieved by recombinant *E. coli* XL1-Blue harboring pKE112-LdcC that was concentrated to OD_{600} of 50 were 118.47 g/L (1159.4 mM) and 135.64 g/L (1327.5 mM) from 150 g/L (1026 mM) and 200 g/L (1368 mM) of L-lysine, respectively.

Synthesis of cadaverine from highly concentrated L-lysine solution prepared by an industrial L-lysine-production process

Highly concentrated L-lysine solution, prepared from an actual L-lysine-production process of a L-lysine-manufacturing company, was used as the feedstock for the synthesis of cadaverine. Using recombinant *E. coli* XL1-Blue, *E. coli* BL21(DE3), or *E. coli* W, harboring pKE112-LdcC individually, concentrated to an OD_{600} of 50, crude L-lysine solution, which corresponds to initial L-lysine concentration of 200 g/L (1368 mM) in the reaction mixture, was applied to the reaction mixtures. As same as the previous reactions,

Table 3 Effects of biocatalyst and substrate concentrations on cadaverine production

Strain	*E. coli* XL1-Blue						
Lysine (g/L)	50	100	150			200	
OD_{600}	10	10	10	30	50	30	50
Biocatalyst ratio[a]	0.2	0.1	0.07	0.2	0.33	0.15	0.25
Initial reaction rate (mM/h)[b]	13.70 ± 1.08[d]	16.05 ± 2.25	0.49 ± 0.00	22.71 ± 1.86	39.15 ± 2.74	27.11 ± 4.01	40.42 ± 2.15
Final concentration (mM)[c]	335.6 ± 21.6	582.7 ± 31.6	15.95 ± 2.06	872.7 ± 40.2	1159.4 ± 42.7	1201.4 ± 34.9	1327.5 ± 35.5
Molar yield (%)	99.40 ± 0.41	98.78 ± 0.55	3.30 ± 0.08	82.86 ± 0.57	99.74 ± 0.13	98.42 ± 0.37	99.84 ± 0.13
Strain	*E. coli* BL21(DE3)						
Lysine	50	100	150			200	
OD_{600}	10	10	10	30	50	30	50
Biocatalyst ratio[a]	0.2	0.1	0.07	0.2	0.33	0.15	0.25
Initial reaction rate (mM/h)[b]	11.35 ± 1.27	16.64 ± 3.43	0.29 ± 0.00	15.95 ± 4.21	32.88 ± 1.66	12.53 ± 2.06	35.92 ± 2.25
Final concentration (mM)[c]	336.6 ± 14.8	618.0 ± 22.0	12.43 ± 1.66	998.9 ± 30.8	1088.4 ± 26.2	1221.3 ± 33.4	1338.7 ± 28.1
Molar yield (%)	98.91 ± 0.38	98.77 ± 0.41	3.95 ± 0.06	98.96 ± 0.32	99.45 ± 0.38	99.35 ± 0.23	99.27 ± 0.21
Strain	*E. coli* W						
Lysine	50	100	150			200	
OD_{600}	10	10	10	30	50	30	50
Biocatalyst ratio[a]	0.2	0.1	0.07	0.2	0.33	0.15	0.25
Initial reaction rate (mM/h)[b]	13.02 ± 2.06	10.08 ± 1.27	0.39 ± 0.00	19.08 ± 2.64	30.63 ± 7.14	19.96 ± 5.19	32.98 ± 2.35
Final concentration (mM)[c]	324.9 ± 14.0	597.1 ± 20.7	14.88 ± 0.88	1025.9 ± 25.1	1013.3 ± 20.9	1213.9 ± 40.1	1200.2 ± 26.7
Molar yield (%)	98.83 ± 0.25	99.47 ± 0.21	3.81 ± 0.04	99.62 ± 0.27	99.63 ± 0.31	99.88 ± 0.11	98.99 ± 0.18

The reactions were performed in DW using recombinant *E. coli* strains expressing the *E. coli ldcC* gene concentrated to OD_{600} of 10, 30, or 50 as whole-cell biocatalysts. 50, 100, 150, or 200 g/L of lysine whose initial pH was adjusted to 6.8 was added into the reaction mixtures. The reaction was stopped when it reached equilibrium

[a] Biocatalyst ratio, ratio of cell density (OD_{600}) to substrate concentration

[b] Initial reaction rate, rate of cadaverine production produced during early exponential phase (first 24 h) of a reaction

[c] Final concentration, final cadaverine concentration when a reaction reaches equilibrium

[d] Data represent mean values from three independent experiments with corresponding deviations

recombinant *E. coli* XL1-Blue harboring pKE112-LdcC showed the fastest rates of L-lysine consumption and cadaverine production in the early stages (Fig. 5). Comparison of Fig. 5 and Table 3 indicates that the crude L-lysine solution from Paik Kwang Industry was suitable for the efficient cadaverine synthesis, which is comparable to pure L-lysine available as a chemical reagent. In 120 h, 133.75 g/L (1309.0 mM) of cadaverine was synthesized from 200 g/L (1368 mM) of L-lysine from Paik Kwang Industry by recombinant *E. coli* XL1-Blue harboring pKE112-LdcC.

Discussion

L-Lysine that is produced by microbial fermentation processes has many applications such as human nutrients, animal feed, chemical agent, and medicament [1]. According to a report by Transparency Market Research, L-lysine had a global market size of about 1.7 million tons in 2011 and the market size is expected to increase up to about 2.5 million tons in 2018. With this market increase and global need for developing alternatives of petroleum-based chemicals, amino acids such as L-glutamate and L-lysine regain a lot of interests from industry since they can be used as precursors of valuable chemicals including C4 and C5 bio-nylon monomers such as gamma-aminobutyrate (GABA), 5-aminovalerate, and cadaverine [7, 10, 14, 15, 17, 21, 22, 24].

Recently, a whole-cell biocatalytic conversion of L-lysine into 5-aminovaleric acid, the promising C5 nylon monomer precursor, has been developed using recombinant *E. coli* expressing *P. putida* delta-aminovaleramidase and lysine 2-monooxygenase as whole-cell biocatalyst in which 90.59 g/L (773.3 mM) of 5-aminovalerate was synthesized from 120 g/L (820 mM) of L-lysine, with a weight yield of 0.755, which corresponds to a molar yield of 0.942 [17]. In addition, a bio and chemical hybrid process for the production of bio-based nylon has been suggested and carefully

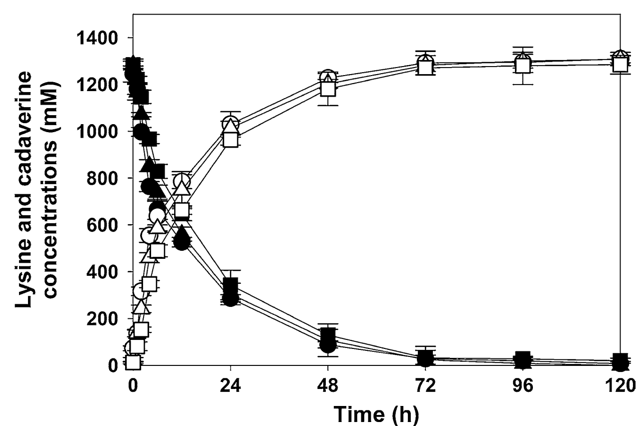

Fig. 5 Time profiles of cadaverine synthesis from 200 g/L of crude lysine solution using three different strains of recombinant *E. coli* expressing *E. coli* LdcC as the whole-cell biocatalysts. The reactions were performed in DW and recombinant *E. coli* strains concentrated to OD_{600} of 50 were used as whole-cell biocatalysts. L-Lysine solutions whose initial pHs were adjusted to 6.8 were used as substrate solutions. The concentrations of lysine and cadaverine are written in molar concentrations. Data represent mean values from three independent experiments with corresponding deviations. (Symbols are: *filled shapes*, lysine concentrations; *open shapes*, cadaverine concentrations; *circle*, *E. coli* XL1-Blue; *square*, *E. coli* BL21(DE3); *diamond*, *E. coli* W)

considered for its industrial application, in which L-lysine is converted into 5-aminovalerate by whole-cell biocatalyst and then δ-valerolactam, the exact monomer for nylon synthesis, is prepared from 5-aminovalerate and used for nylon synthesis [17]. In other study, an enzymatic conversion of L-lysine into 5-aminovaleric acid by employing purified delta-aminovaleramidase and lysine 2-monooxygenase has also been examined [18]. Under the optimum conditions, 13.4 g/L (114 mM) of 5-aminovaleric acid was synthesized from 17.6 g/L (120 mM) of L-lysine with a molar yield of 0.95 [18]. Although the enzymatic synthesis has many possibilities, this process at present is far from commercialization due to its complexities and high-costs [17, 18].

On the other hand, production of cadaverine, which is another promising C5 bionylon monomer, has not been carefully examined by such biocatalytic conversions. Instead, most of studies about cadaverine production have focused on the development of engineered microbial strains that can produce cadaverine from glucose. Since it is needed to consider various processes to support economical production of target chemicals for the development of commercialization process, at this moment, it would not be too excessive to suggest that biocatalytic conversion of L-lysine into cadaverine should be examined for its industrial applications. For this purpose, herein we have developed a biocatalytic conversion system for the production of cadaverine using different strains of recombinant *E. coli* expressing lysine decarboxylase as the whole-cell biocatalysts.

First of all, five different LDCs were investigated for the synthesis of cadaverine using *E. coli* XL1-Blue as the host strain. As mentioned in the Introduction, *E. coli* CadA and LdcC are the most well-known LDCs. In addition to these LDCs, three different kinds of LDCs from *R. eutropha*, *P. aeruginosa*, and *P. putida* were examined for the synthesis of cadaverine. Basic local alignment search tool (BLAST) analysis conducted at the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) web site revealed that the percent homologies in amino acid sequence of *R. eutropha*, *P. aeruginosa*, and *P. putida* are 38, 45, and 46 %, respectively, compared to *E. coli* LdcC. SDS-PAGE analysis of these LDCs (Fig. S1) indicated that these LDCs were much less expressed than *E. coli* LdcC was in the host strain. The follow-up experiments were carried out with this *E. coli* LDC.

The expression of *E. coli ldcC* by the six different strains of recombinant *E. coli* has been analyzed by SDS-PAGE (Fig. S2). Comparison of Fig. 2 and Fig. 2S indicates that the capabilities of different host strains expressing the lysine decarboxylase are not directly correlated with those converting lysine into cadaverine (i.e., one of the powerful cadaverine-synthesizing biocatalyst, *E. coli* XL1-Blue harboring pKE112-LdcC showed the lowest expression of *E. coli* LdcC as shown in SDS-PAGE analysis). The capability of synthesizing a desired product in a microbial strain seems to be determined by a comprehensive interaction of related factors rather than by the strong expression of a key enzyme. For example, in this case, the uptake or secretion of lysine and conversion of lysine into cadaverine are affected by related actions of at least four enzymes such as *E. coli* CadA, CadB, CadC, and CadR [20].

As shown in Fig. 3, the reaction preferred moderate pH conditions than high pH conditions. *E. coli* LdcC is known to have an optimum pH range of 6–8 [9]. Above the optimum range, it showed a sharp decrease in the LDC activity [9]. Therefore, considering that both L-lysine and cadaverine have high pI values, addition of pure L-lysine solution to the reaction mixture might affect the conversion efficiencies by increasing the pHs in the reaction mixtures. Indeed, such effects tended to increase along with the increase of substrate concentrations (data not shown). In addition, since the initial molar concentration of lysine was 342 mM, which exceed the buffering capacity of 100 mM Tris–HCl (pH 6.8), this pH adjustment was compulsory. As the pH of substrate is adjusted, no more buffering compounds were required. The conversion reaction occurred normally in DW and even brought better results in DW than in Tris–HCl buffer. After the fundamental conditions for the conversion reaction were determined, the three recombinant strains of *E. coli*, which showed better production performances than

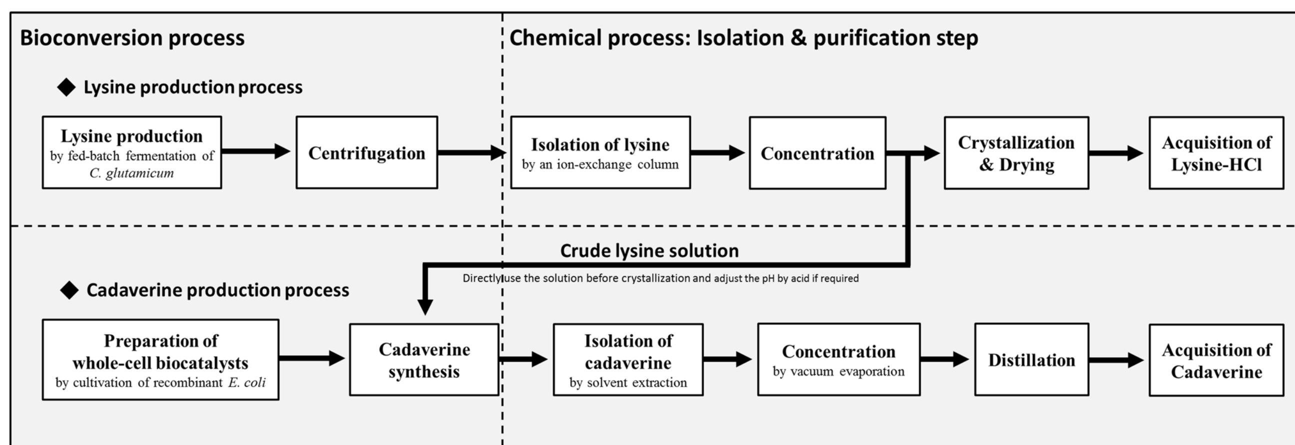

Fig. 6 A schematic illustration of the integrated process: lysine is produced by an industrial lysine producer by fed-batch fermentation and the isolated and purified lysine is further converted to cadaverine by whole-cell biocatalytic process

the other three strains, were further investigated. Recombinant *E. coli* XL1-Blue, *E. coli* BL21(DE3), and *E. coli* W, which expresses *E. coli* LdcC, respectively, showed comparable results in most cases in terms of final cadaverine titers and yields. As to be expected, higher concentrations of the whole-cell biocatalysts were required to convert higher concentrations of the substrates. While the recombinant *E. coli* strains harboring pKE112-LdcC having an OD_{600} of 10 could consume less than 30 % of the substrate from the initial concentration of 150 g/L (1026 mM, data not shown), they could consume most of the substrate to produce equal molar amount of cadaverine when their concentrations increased up to an OD_{600} of 30 or 50 (Table 3). In terms of biocatalyst ratio in Table 3, at least 0.1 of the ratio was required to convert most of substrate into cadaverine. In addition, the higher the biocatalyst ratios, the higher the initial reaction rates achieved. *E. coli* XL1-Blue harboring pKE112-LdcC tended to consume the substrate as well as to produce cadaverine faster than the other two strains.

Therefore, using the recombinant *E. coli* strains harboring pKE112-LdcC, the possibility of connecting the actual L-lysine production process with the whole-cell bioconversion process was examined by employing crude L-lysine solution provided from Paik Kwang Industry into the conversion process. Although the crude L-lysine solution might contain undetermined impurities, it did not adversely affect the conversion performances of the whole-cell biocatalysts. Indeed, the whole-cell biocatalysts converted L-lysine into cadaverine more efficiently from crude L-lysine solution than from pure L-lysine solution, but the exact reason of these results needs to be solved for further development of bioconversion process connecting L-lysine synthesis with other valuable chemicals that can be derived from L-lysine.

A virtual process of integrating commercial L-lysine production process and cadaverine production process is demonstrated in Fig. 6. Conventionally, L-lysine-HCl is the final product of the L-lysine manufacturing process, which is mainly composed of fermentation, separation, concentration, crystallization, and drying [8]. In addition to the crude L-lysine solution investigated in this study, the untreated fermentation supernatant or the more crude L-lysine solution produced before the concentration step in Fig. 6, which typically contains about 150 g/L (1026 mM)–200 g/L (1368 mM) of L-lysine, obtained by simple removal of cells and other impurities from the fermentation broth is likely to be applied directly to cadaverine synthesis as the feedstock. In that case, the final cadaverine titer would decrease due to the low substrate concentration. If the fermentation supernatant is used as the feedstock for cadaverine synthesis, pH adjustment of the substrate seems not to be required because the pH of L-lysine fermentation process is usually controlled by around 7 and thus the solution already has a suitable pH value for cadaverine synthesis. However, the conjugate acid of L-lysine can still act as the conjugate acid of cadaverine that has a higher pI value, which may contribute on reserving the reaction conditions suitable for cadaverine synthesis. Thus, it should be carefully considered whether HCl is added to the crude L-lysine solution or not before using it as the substrate. Otherwise, di-carboxylic acids that can be used as the pair monomers of cadaverine for the synthesis of bionylons can be the alternatives. Indeed, such a method has already been demonstrated by Ajinomoto Co., Ltd. using pure L-lysine adipate, L-lysine succinate, and L-lysine sebacate to produce the corresponding cadaverine di-acids [12]. In this case, the follow-up processes after the cadaverine synthesis in Fig. 6, which are based on the published reports on cadaverine production, should be replaced by the alternative purification processes including crystallization and filtration [7, 12, 19]. Considering that biological production of such di-carboxylic acids

is already being carried out by several companies or at least it is under consideration, integration of the di-carboxylic acid production process with the process in Fig. 6 is also likely to be taken into account for the manufacturers if they intend to develop a commercial bionylon production process.

Conclusion

A whole-cell bioconversion process for the production of cadaverine from L-lysine has been developed by employing recombinant *E. coli* strains expressing lysine decarboxylase. Since *E. coli* LdcC showed the best performance in cadaverine synthesis, further development for the conversion of L-lysine into cadaverine was carried out based on the recombinant *E. coli* strains expressing *E. coli* LdcC. Among six recombinant *E. coli* strains, XL1-Blue, BL21(DE3), BL21(DE3)star, DH-5α, WL3110, and W, which express *E. coli* LdcC, *E. coli* XL1-Blue expressing *E. coli* LdcC was superior to other recombinant *E. coli* strains in the conversion of L-lysine into cadaverine. It was also demonstrated that crude L-lysine solution obtained from a L-lysine manufacturing process of Paik Kwang Industry could successfully be applied to the whole-cell bioconversion process developed in this study to produce equal molar amount of cadaverine from L-lysine. Whole-cell biocatalytic conversion of L-lysine into cadaverine should be useful for the development of bio-based nylon synthesis strategies and this can be also widely applicable for the construction of bio-based process to produce valuable chemicals using amino acids as starting resources.

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