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Biotransformation of L-Lysine to L-Pipecolic Acid Catalyzed by L-Lysine 6-Aminotransferase and Pyrroline-5-carboxylate Reductase

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The enzyme involved in the reduction of Δ^1-piperideine-6-carboxylate (P6C) to L-pipecolic acid (L-PA) has never been identified. We found that *Escherichia coli* JM109 transformed with the *lat* gene encoding L-lysine 6-aminotransferase (LAT) converted L-lysine (L-Lys) to L-PA. This suggested that there is a gene encoding ''P6C reductase'' that catalyzes the reduction of P6C to L-PA in the genome of *E. coli*. The complementation experiment of *proC32* in *E. coli* RK4904 for L-PA production clearly shows that the expression of both *lat* and *proC* is essential for the biotransformation of L-Lys to L-PA. Further, We showed that both LAT and pyrroline-5-carboxylate (P5C) reductase, the product of *proC*, were needed to convert L-Lys to L-PA *in vitro*. These results demonstrate that P5C reductase catalyzes the reduction of P6C to L-PA. Biotransformation of L-Lys to L-PA using *lat*-expressing *E. coli* BL21 was done and L-PA was accumulated in the medium to reach at an amount of 3.9 g/l after 159 h of cultivation. It is noteworthy that the ee-value of the produced pipecolic acid was 100%.

Key words: *lat*; L-pipecolic acid (L-PA); *proC*; Δ^1-pyrroline-5-carboxylate (P5C) reductase; Δ^1-piperideine-6-carboxylate (P6C)

L-Pipecolic acid (L-PA) is an amino acid not observed in proteins, and is an important component and a precursor of many microbial and plant secondary metabolites such as immunosuppressants,[1] peptide antibiotics,[2] and piperideine alkaloids.[3] The biosynthesis of L-PA has been investigated in terms of L-lysine (L-Lys) metabolism for a long time. It has been known that L-PA is synthesized biologically by the deaminative cyclization of L-Lys, by the reduction of Δ^1-piperideine-2-carboxylate (P2C), or by the reduction of Δ^1-piperideine-6-carboxylate (P6C). In *Streptomyces hygroscopicus*, which produces rapamycin, an immunosuppressant containing L-PA as a component, lysine cyclodeaminase catalyses the deaminative cyclization of L-Lys to L-PA.[1] The *rapL* gene encoding lysine cyclodeaminase is located in the gene cluster for rapamycin.[4] In *Pseudomonas putida*, L-Lys is initially converted to D-lysine, metabolized to P2C, and then P2C is converted to L-PA by P2C reductase.[5] P6C, which is the reversible cyclic form of α-aminoadipic semialdehyde, was identified as the product of the deamination of L-Lys that was catalyzed by L-lysine 6-aminotransferase (LAT, E.C.2.6.1.36) in *Flavobacterium lutescens* IFO3084[6] and β-lactam-producing actinomycetes[7] (Fig. 1). In addition, P6C is also produced from L-Lys by lysine 6-dehydrogenase in *Agrobacterium tumefaciens*[8] and from saccharopine by saccharopine oxidase (EC 1.5.1.10) in *Rhizoctonia leguminicola*.[9] The activity of the reduction of P6C to L-PA was found in cell-free enzyme systems of microorganisms.[9] However, no information about the enzyme involved in the reduction of P6C has been ever obtained, because the substrate, P6C, is chemically unstable and the lack of available P6C precluded the characterization of the enzyme.

We have investigated the biosynthesis of L-α-aminoadipic acid (L-AAA) from L-Lys in *F. lutescens* IFO3084 and cloned the *lat* gene encoding LAT and the *pcd* gene encoding P6C dehydrogenase from *F. lutescens* IFO3084.[10,11] Our next attention was focused on the ''P6C reductase'' that catalyzes the reduction of P6C to L-PA to produce L-PA from L-Lys. We found that ''P6C reductase'' exists in *Escherichia coli*. Further our works *in vivo* and *in vitro* demonstrate that the enzyme is pyrroline-5-carboxylate (P5C) reductase (EC 1.5.1.2) encoded by *proC*, which catalyzes the reduction of Δ^1-pyrroline-5-carboxylate to L-proline and is found in almost all organisms.[12,13]

In this paper we describe the reduction of P6C to L-PA catalyzed by P5C reductase and the biotransformation of L-Lys to L-PA using *lat*-expressing *E. coli*.

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Abbreviations: L-AAA, L-α-aminoadipic acid; LAT, L-lysine 6-aminotransferase; L-Lys, L-lysine; L-PA, L-pipecolic acid; P2C, Δ^1-Piperideine-2-carboxylate; P5C, Δ^1-pyrroline-5-carboxylate; P6C, Δ^1-Piperideine-6-carboxylate

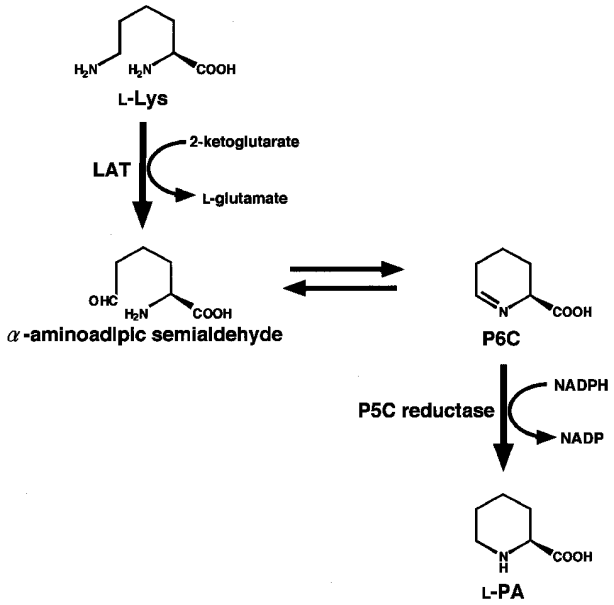

Fig. 1. Conversion of L-Lys to L-PA by LAT and P5C Reductase.

Materials and Methods

Bacterial strains. E. coli JM109 was used for the isolation of chromosomal DNA and the production of L-PA. *E. coli* RK4904 (CGSC#: 6404), which is a *proC32* mutant obtained from the *E. coli* Genetic Stock Center (Yale University, USA), was used as the host strain for a complementation experiment of *proC32*. *E. coli* TOP10 (Invitrogen) was used for protein expression. *E. coli* BL21 was used as the host strain for the production of L-PA.

Construction of plasmids. A *lat*-expressing plasmid pRH124 was constructed as follows: using *F. lutescens* IFO3084 chromosomal DNA as a template, a 1571-bp fragment containing *lat* was amplified by PCR. The primers were designed on the basis of the sequence of *lat* and contained a 5′ *Hin*dIII site in primer I (5′-ATAAGCTTGTCCCTTCTTGCCCCGCTCGC-3′) and a 5′ *Bam*HI site in the primer II (5′-GCGGATCCTGTTGCCGCTGGTGCCGGGCAG-3′). The amplified PCR product was digested with *Hin*dIII and *Bam*HI, and ligated into *Hin*dIII-*Bam*HI-digested pUC19. A *lat* and *proC* expressing plasmid pRH134 was constructed as follows: using *E. coli* JM109 chromosomal DNA as a template, a 1443-bp fragment containing *proC* was amplified by PCR with the primers that contained a 5′ *Kpn*I site in each (5′- CCGGTACCATAAAATCGCGCATCGTCAGGC-3′ and 5′-CCGGTACCGCCACAGGTAACTTTACGGATT-3′). The *Kpn*I digested PCR product was ligated into the *Kpn*I site of pRH124. A *proC*-expressing plasmid pRH135 was also constructed as follows: pRH134 was digested with *Hin*dIII and *Bam*HI, filled with T4 polymerase, and self-ligated with T4 ligase. A *proC*-expressing plasmid pTrcP5CR was constructed as follows: two primers, primer EX1 and primer EX2, were prepared for PCR to obtain *proC*. Primer EX1 was designed on the basis of the N-terminal sequence of *proC* and contained a 5′ *Bam*HI site (5′-ATGGATCCGAAAATCGGTTTTATTGGCT-3′). Primer EX2 was designed on the basis of the C-terminal sequence of *proC* and contained a 5′ *Eco*RI site (5′-TCGAATTCCAGGATTTGCTGAGTTTTTCT-3′). Using *E. coli* JM109 chromosomal DNA as a template, an amplification by PCR was done. The PCR product was digested with *Bam*HI and *Eco*RI and then ligated into pTrcHis2A (Invitrogen) digested with *Bam*HI and *Eco*RI. The resulting plasmid was designated as pTrcP5CR.

Expression of lat in E. coli JM109. E. coli JM109 harboring pRH124 was cultivated at 32°C with shaking in L-broth (polypeptone 1.0%, yeast extract 0.5%, NaCl 0.5%, glucose 0.1%, pH 7.2) containing 50 μg of ampicillin sodium per ml. Thirty μl of overnight-grown culture was added to 3 ml of L-broth containing 50 μg ampicillin sodium per ml. The culture was grown at 32°C with shaking, and the expression of LAT was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM, final concentration) when the absorbance at 660 nm of the culture reached 1.0. After 4 h of cultivation, 30 μl of 50% glycerol and 30 μl of 50% L-lysine HCl were added to the culture, and the culture was cultivated for 15 h more. The supernatants of these cultures were analyzed by TLC and HPLC to estimate the amount of L-PA.

Complementation experiment of proC32. E. coli RK4904 harboring pUC19, pRH124, pRH134, or pRH135 was cultivated at 32°C with shaking in L-broth containing 50 μg of ampicillin sodium per ml. Two hundred and seventy five μl of the broth was added to 25 ml of TB (polypeptone 3.2%, yeast extract 2.4%, glycerol 0.4%, 100 mM phosphate buffer (pH 7.4)) containing 50 μg ampicillin sodium per ml. After 8 h of cultivation, 500 μl of 50% glycerol and 500 μl of 50% L-lysine HCl were added to the culture broth and the cultivation was continued for 40 h more. The supernatants of these cultures were analyzed by TLC and HPLC to estimate the amount of L-PA.

Fermentation experiment. E. coli BL21 harboring pRH124 was cultured in the same manner as the cultivation of *E. coli* RK4904 described above. One hundred μl of each culture was collected after 15, 39, 63, 87, 111 and 159 h of cultivation.

Expression and purification of recombinant proteins. Recombinant LAT and recombinant P5C

reductase were expressed and purified using *E. coli* TOP10/pTrcLAT and *E. coli* TOP10/pTrcP5CR, respectively, by the method described previously.[10]

Cooperative enzymatic transformation of L-Lys to L-PA in vitro. Reaction mixture contained 0.2 mmol of sodium phosphate buffer (pH 7.2), 1 μmol of L-lysine HCl, 10 μmol of 2-ketoglutarate, 37.5 pmol of pyridoxsal phosphate, 2 μmol of NADPH, 1 μg of LAT, and 5 μg of P5C reductase in a final volume of 1 ml. The reaction mixture was incubated at 32°C. At regular times, a sample (100 μl) of the reaction mixture was collected and added 900 μl of acetonitrile/water (5:4) to stop the enzymatic reactions. The amounts of L-Lys and L-PA in the reaction mixture were measured by ESI LC/MS.

TLC analysis. The culture broth was centrifuged and the resulting supernatant was used for quantitative analysis with TLC as described previously.[11]

HPLC analysis. Pipecolic acid and lysine were labeled with N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-leucineamide (L-FDLA),[14] and the L-FDLA derivatives of pipecolic acid and lysine were analyzed by HPLC on a YMC-Pack s-5 60A ODS (4.6 × 150 mm) column maintained at 35°C using a acetonitrile concentration gradient in 1% acetic acid aqueous solution from 40% to 80% for 20 minutes with a flow rate of 1.0 ml per minute. The derivatives were detected by UV-monitoring at 340 nm. The enantiomeric purity of pipecolic acid was also determined by this HPLC method.

LC/MS analysis. The cooperative enzymatic transformation from L-Lys to L-PA by LAT and P5C reductase was monitored by liquid chromatography/mass spectrometry (LC/MS). HPLC conditions were as follows: column, YMC J's phere ODS (4.5 × 75 mm); mobile phase, methanol/2 mM ammonium acetate solution containing 5 mM nonafluorovaleric acid (30:70); flow rate, 0.5 ml/minute; column temperature, 35°C. The mass spectrometer used was a Finnigan AQA (Thermo Quest, UK). The protonated molecules $[M+H]^+$ of L-Lys and L-PA were detected in positive ion mode to give peaks at m/z 147 and 130, respectively. Under these LC/MS conditions, the retention times of L-PA and L-Lys were 4.01 and 6.72 min, respectively.

Results

Production of L-PA by E. coli transformed with lat gene

In the course of our studies on *pcd*, we found that the *pcd* deficient mutant of *F. lutescens* IFO3084 slightly converted L-Lys to L-PA in phosphate buffer (pH 7.0) containing L-Lys and glycerol (data not shown). This result suggested that there is the gene in the genome of *F. lutescens* IFO3084 encoding "P6C reductase" that catalyzes the reduction of P6C to L-PA. To clone the gene encoding "P6C reductase", we first tried to construct the gene library of *F. lutescens* IFO3084, introduce the gene library into *lat*-expressing *E. coli* JM109/pRH124, and isolate the clone in which L-Lys is converted to L-PA. However, to our surprise, *E. coli* JM109/pRH124 itself converted L-Lys to L-PA (Fig. 2A). Since LAT catalyzes the conversion of L-Lys to P6C,[6,10] this result implies that there is a gene encoding another "P6C reductase" also in the genome of *E. coli* JM109.

Complementation of proC32 for L-PA production

The structural similarity between P6C and P5C brought us an idea that "P6C reductase" may be similar to P5C reductase. However, we failed to find any additional protein with high similarity to P5C reductase in *E. coli* by a BLAST search (data not shown), suggesting that P5C reductase itself catalyzes the reduction of P6C as well as P5C. To examine whether P5C reductase catalyzes the reduction of P6C to L-PA, we did a complementation experiment of *proC32*, a mutation of *proC* encoding P5C reductase. We transformed *E. coli* RK4904 (*proC32*) with pUC19, a *lat* expression plasmid pRH124, a *lat* and *proC* expression plasmid pRH134, or a *proC* expression plasmid pRH135, and cultured these transformants in TB added with L-Lys. L-PA was not detected by TLC and HPLC analysis in the culture broth of *E. coli* RK4904/pUC19 (lat^-, $proC^-$), *E. coli* RK4904/pRH124 (lat^+, $proC^-$), and *E. coli* RK4904/pRH135 (lat^-, $proC^+$) (Fig. 2(B)). On the other hand, L-PA was detected in the culture broth of *E. coli* RK4904/pRH134 (lat^+, $proC^+$) (Fig. 2(B)). These results clearly show that the expression of both *lat* and *proC* is essential for the biotransformation of L-Lys to L-PA.

Reduction of P6C to L-PA by P5C reductase

To verify that P5C reductase, the *proC* gene product, catalyzes the reduction of P6C to L-PA *in vitro*, we tried the cooperative enzymatic transformation of L-Lys to L-PA by LAT and P5C reductase. First, *proC* of *E. coli* JM109 was amplified and ligated into pTrcHis2A to construct a recombinant P5C reductase expression plasmid of pTrcP5CR. The expressed and purified recombinant P5C reductase gave a single band on SDS-PAGE, corresponding to a molecular mass of about 32 kDa (Fig. 3), which is in good agreement with the value (31.4 kDa) estimated from the deduced amino acid sequence. By using the recombinant P5C reductase and the recombinant LAT,[10] L-Lys was stoichiometrically converted to L-PA *in vitro*. Figure 4 shows that the number of moles of consumed L-Lys was almost equal to that of

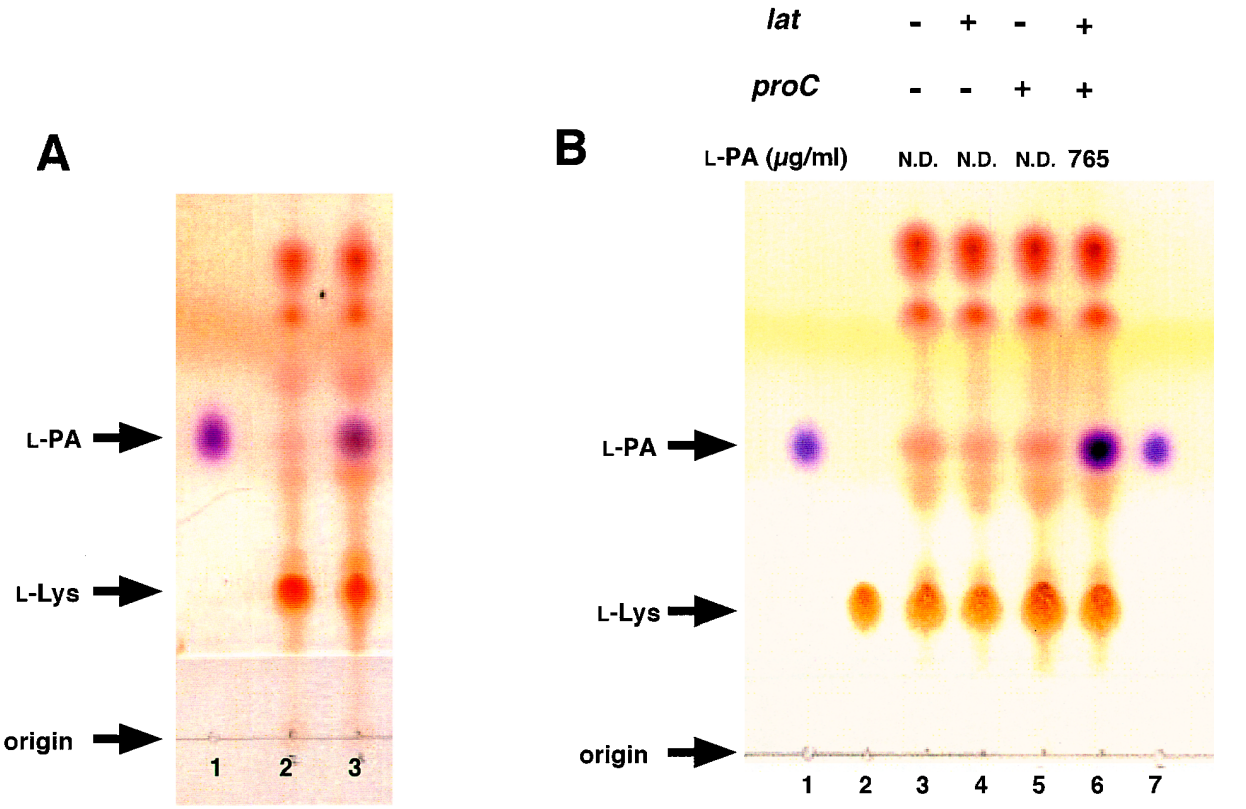

Fig. 2. Biotransformation of L-Lys to L-PA.

(A) TLC analysis of the culture broth of *lat*-expressing *E. coli* JM109. Lane 1, L-PA (500 μg/ml); lane 2, JM109/pUC19; lane 3, JM109/pRH124.

(B) TLC and HPLC analysis of the culture broth of *lat*-expressing *E. coli* RK4904. Lane 1 and 7, L-PA (500 μg/ml); lane 2, L-Lys (500 μg/ml); lane 3, RK4904/pUC19; lane 4, RK4904/pRH124; lane 5, RK4904/pRH135; lane 6, RK4904/pRH134. N.D., not detected.

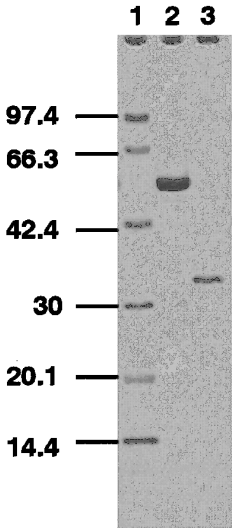

Fig. 3. SDS-PAGE Analysis of Recombinant LAT and Recombinant P5C Reductase.

Lane 1, molecular mass standards (sizes in kilodaltons are indicated at the left); lane 2, recombinant LAT; lane 3, recombinant P5C reductase.

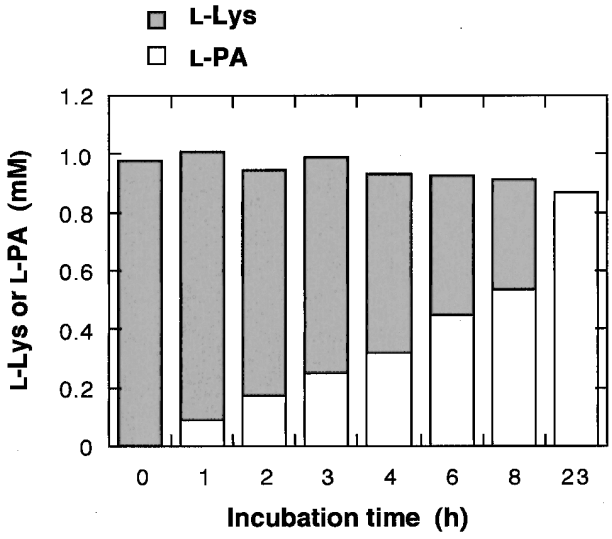

Fig. 4. Cooperative Enzymatic Transformation of L-Lys to L-PA by LAT and P5C Reductase.

Cooperative enzymatic transformation of L-Lys to L-PA by LAT and P5C reductase was done and the amounts of L-Lys (■) and L-PA (□) in the reaction mixture were measured as described in Materials and Methods.

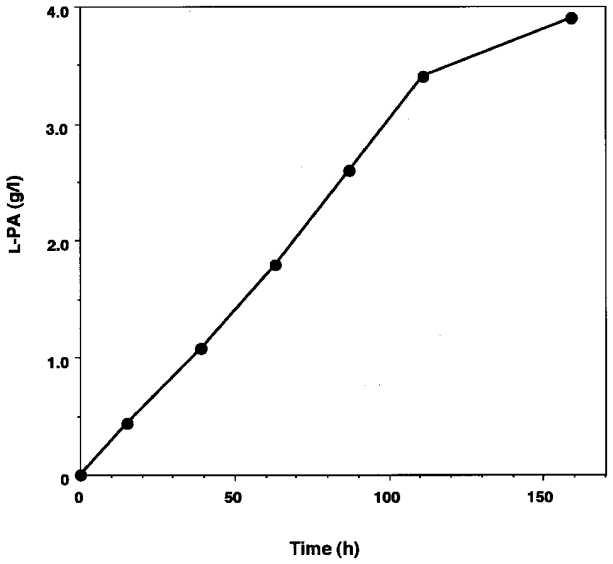

Fig. 5. Fermentation Experiment for L-PA Production.
E. coli BL21/pRH124 was cultured and the amount of L-PA in the culture broth was measured as described in Materials and Methods.

produced L-PA in each incubation time. In addition, the formation of L-glutamate was observed in the reaction mixture (data not shown).

Fermentation experiment for L-PA production

We have described above that L-PA was accumulated in the culture broth of *lat*-expressing *E. coli* JM109. Further, we transformed *E. coli* JM109, C600, and BL21 with pRH124 or pRH134 and examined L-PA production using these transformants. We found that *E. coli* BL21/pRH124 produced the largest amount of L-PA of these six transformants (data not shown). Then, a fermentation experiment for L-PA production using *E. coli* BL21/pRH124 was done. *E. coli* BL21/pRH124 was cultured and excessive L-Lys and glycerol were added to the culture medium. L-PA was accumulated in the medium and reached at an amount of 3.9 g/l after 159 h of cultivation (Fig. 5). The ee-value of the produced pipecolic acid was 100%.

Discussion

We here demonstrated for the first time that the P5C reductase from *E. coli* catalyzes the reduction of P6C to L-PA by *in vivo* and *in vitro* experiments (Fig. 1). This finding suggests that in the microorganisms that produce L-PA *via* P6C from L-Lys, such as *R. leguminicola*[9] and *F. lutescens*, P5C reductase is responsible, at least in part, for the reduction of P6C to L-PA, because P5C reductase is present in almost all organisms.

It is difficult to analyze quantitatively the enzymatic reaction of P6C, because P6C is chemically unstable, tends to undergo polymerization, and is reversibly converted to α-aminoadipic semialdehyde in aqueous solution.[15,16] However, our data suggest that following reaction steps are involved in the biotransformation of L-Lys to L-PA:

(i) L-Lys + 2-ketoglutarate → α-aminoadipic semialdehyde + L-glutamate
(ii) α-aminoadipic semialdehyde → P6C + H_2O
(iii) P6C + NADPH + H^+ → L-PA + NADP

Step (i) is the known transamination by LAT.[6,10] Step (ii) is a spontaneous chemical reaction. Step (iii) is the novel reduction by P5C reductase. The instability of P6C at pH above 5.0 may be the reason why the number of moles of produced L-PA was not completely equal to the number of moles of added L-Lys at the incubation time of 23 h.[15,16]

It is currently unclear why *E. coli* BL21/pRH124 produced a larger amount of L-PA than *E. coli* BL21/pRH134. It seems possible that in this biotransformation system of L-Lys to L-PA the reduction of P6C to L-PA is not the rate-limiting step and the excess expression of *proC* has a bad influence.

L-PA is an important compound as a component of many drugs. Recently, much attention has been focused on the importance of chirality as the key to the efficacy of many drug products. The current route to the pure enantiomer of pipecolic acid is the classical resolution of the racemate by fractional crystallization of diastereomeric salts[17] or the stereoselective transformation of the racemate by biocatalytical process.[18] However, it is difficult to obtain the 100% ee of L-PA. Pipecolic acid produced by our biotransformation system was (*S*)-pipecolic acid (L-PA), and its ee-value was 100%. It is noteworthy that our biotransformation of L-Lys to L-PA by the *lat*-expressing *E. coli* preserved the chirality. On the basis of this biotransformation, highly efficient production of L-PA will become possible in the near future.

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References

1) Khaw, L. E., Bohm, G. A., Metcalfe, S., Staunton, J., and Leadlay, P. F., Mutational biosynthesis of novel rapamycins by a strain of *Streptomyces hygroscopicus* NRRL 5491 disrupted in *rapL*, encoding a putative lysine cyclodeaminase. *J. Bacteriol.*, **180**, 809–814 (1998).
2) Takayama, S., Isogai, A., Nakata, M., Suzuki, H., and Suzuki, A., Structure of Cyl-1, a novel cyclotetrapeptide from *Cylindrocladium scoparium*.

Agric Biol. Chem., **48**, 839–842 (1984).
3) Clevenstine, E. C., Walter, P., Harris, T. M., and Broquist H. P., Biosynthesis of slaframine, (1*S*,6*S*,8a*S*)-1-acetoxy-6-aminooctahydroindolizine, a parasympathomimetic alkaloid of fungal origin. 4. Metabolic fate of ethyl pipecolylacetate, 1,3-dioxooctahydroindolizine, and 1-hydroxyoctahydroindolizine in *Rhizoctonia leguminicola*. *Biochemistry*, **18**, 3663–3667 (1979).
4) Molnar, I., Aparicio, J. F., Haydock, S. F., Khaw, L. E., Schwecke, T., Konig, A., Staunton, J., and Leadlay, P. F., Organisation of the biosynthetic gene cluster for rapamycin in *Streptomyces hygroscopicus*: analysis of genes flanking the polyketide synthase. *Gene*, **169**, 1–7 (1996).
5) Payton, C. W. and Chang, Y. F., Delta1-piperideine-2-carboxylate reductase of *Pseudomonas putida*. *J. Bacteriol.*, **149**, 864–871 (1982).
6) Soda, K., Misono, H., and Yamamoto, T., L-lysine: α-ketoglutarate aminotransferase. I. Identification of a product, Δ-1-piperideine-6-carboxylic acid. *Biochemistry*, **7**, 4102–4109 (1968).
7) Madduri, K., Stuttard, C., and Vining, L. C., Cloning and location of a gene governing lysine ε-aminotransferase, an enzyme initiating β-lactam biosynthesis in *Streptomyces* spp. *J. Bacteriol.*, **173**, 985–988 (1991).
8) Misono, H. and Nagasaki, S., Occurrence of L-lysine epsilon-dehydrogenase in *Agrobacterium tumefaciens*. *J. Bacteriol.*, **150**, 398–401 (1982).
9) Wickwire, B. M., Harris, C. M., Harris, T. M., and Broquist, H. P., Pipecolic acid biosynthesis in *Rhizoctonia leguminicola*. I. The lysine saccharopine, delta 1-piperideine-6-carboxylic acid pathway. *J. Biol. Chem.*, **265**, 14742–14747 (1990).
10) Fujii, T., Narita, T., Agematu, H., Agata, N., and Isshiki, K. Characterization of L-lysine 6-aminotransferase and its structural gene from *Flavobacterium lutescens* IFO3084. *J. Biochem.*, **128**, 391–397 (2000).
11) Fujii, T., Narita, T., Agematu, H., Agata, N., and Isshiki K., Cloning and characterization of *pcd* encoding Δ^1-piperideine-6-carboxylate dehydrogenase from *Flavobacterium lutescens* IFO3084. *J. Biochem.*, **128**, 975–982 (2000).
12) Leisinger, T., Biosynthesis of proline. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanic, B., Schaechter, M., and Umbarger, H. E., *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. In ''American Society for Microbiology'', Washington, DC, pp. 345–351 (1987).
13) Smith, R. J., Downing, S. J., Phang, J. M., Lodato, R. F., and Aoki, T. T., Pyrroline-5-carboxylate reductase activity in mammalian cells. *Proc. Natl. Acad. Sci. USA*, **77**, 5221–5225 (1980).
14) Fujii, K., Ikai, Y., Oka, H., Suzuki, M., and Harada, K., A nonempirical method using LC/MS for determination of the absolute configuration of constituent amino acids in a peptide: combination of Marfey's method with mass spectrometry and its practical application. *Anal. Chem.*, **69**, 5146–5151 (1997).
15) Soda, K. and Misono, H., L-lysine: α-ketoglutarate aminotransferase. II. Purification, crystallization, and properties. *Biochemistry*, **7**, 4110–4119 (1968).
16) Rodwell, V. W., Methods in Enzymology. Vol. XVIIB, pp. 188–199 (1971).
17) Hasegawa, G., JP patent no. P2000–178253A.
18) Eichhorn, E., Roduit, J. P., Shaw, N., Heinzmann, K., and Kiener, A., Preparation of (*S*)-piperazine-2-carboxylic acid, (*R*)-piperazine-2-carboxylic acid, and (*S*)-piperidine-2-carboxylic acid by kinetic resolution of the corresponding racemic carboxamides with stereoselective amidases in whole bacterial cells. *Tetrahedron*: *Asymmetry*, **8**, 2533–2536 (1997).