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Lysine Catabolism in *Streptomyces* spp. Is Primarily through Cadaverine: β-Lactam Producers Also Make α-Aminoadipate

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Genetic and biochemical evidence was obtained for lysine catabolism via cadaverine and δ-aminovalerate in both the β-lactam producer *Streptomyces clavuligerus* and the nonproducer *Streptomyces lividans*. This pathway is used when lysine is supplied as the sole source of nitrogen for the organism. A second pathway for lysine catabolism is present in *S. clavuligerus* but not in *S. lividans*. It leads to α-aminoadipate, a precursor for β-lactam biosynthesis. Since it does not allow *S. clavuligerus* to grow on lysine as the sole nitrogen source, this pathway may be used exclusively to provide a precursor for β-lactam biosynthesis. β-Lactam producers were unable to grow well on α-aminoadipate as the only nitrogen source, whereas three of seven species not known to produce β-lactam grew well under the same conditions. Lysine ε-aminotransferase, the initial enzyme in the α-aminoadipate pathway for lysine catabolism, was detected in cell extracts only from the β-lactam producers. These results suggest that synthesis of α-aminoadipate is exclusively a secondary metabolic trait, present or expressed only in β-lactam producers, while genes governing the catabolism of α-aminoadipate are present or fully expressed only in β-lactam nonproducers.

Lysine catabolism in aerobic bacteria is notable for its biochemical diversity. In the genus *Pseudomonas*, there are four inducible lysine catabolic pathways. Two of these lead to δ-aminovalerate, one pathway initiated by decarboxylation of lysine to cadaverine (5, 18) and the other by a monooxygenase-catalyzed conversion of lysine to δ-aminovaleramide (13, 20). In the remaining pathways, lysine is transaminated either to 1-piperideine-6-carboxylate (5) or to pipecolate (12), both of which may be catabolized to α-aminoadipate. In *Flavobacterium lutescens*, lysine catabolism proceeds through 1-piperideine-6-carboxylate to α-aminoadipate (16, 17).

Romano and Nickerson (14) reported that lysine supported modest growth of *Streptomyces fradiae* when it was supplied as the sole source of nitrogen and carbon, but they provided no information on the pathway involved. Subsequently, the α-aminoadipyl side chain of β-lactam antibiotics was found to be derived from lysine, and the first enzyme catalyzing this conversion was identified as lysine ε-aminotransferase (10). Association of this enzyme with β-lactam production in cultures of *Streptomyces* (now *Nocardia*) *lactamdurans* and the isolation of mutants blocked in both β-lactam production and lysine ε-aminotransferase (10) suggested that the pathway for β-lactam antibiotic biosynthesis might start from lysine.

In this paper, we present biochemical and genetic evidence for the catabolism of lysine via cadaverine in both the β-lactam producer *Streptomyces clavuligerus* and in *Streptomyces lividans*, a species not known to produce β-lactam antibiotics. We also provide evidence that the pathway for conversion of lysine to α-aminoadipate is absent from *S. lividans*, suggesting that this pathway is principally involved in antibiotic production, and show that *S. lividans* can use α-aminoadipate as the sole source of nitrogen while *S. clavuligerus* cannot.

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MATERIALS AND METHODS

Organisms and growth conditions. Mutants unable to catabolize lysine (Lut$^-$) were isolated from *S. lividans* TK24 (8) and *S. clavuligerus* NRRL 3585 (6) after treatment with 1 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml in 0.05 M Tris–maleic acid buffer (pH 8.0) (7). To obtain the desired 5% survival (CFU), spores of *S. clavuligerus* and *S. lividans* were treated for 20 and 120 min, respectively. For the detection and characterization of lysine-nonutilizing mutants, defined minimal medium (7) without asparagine but containing lysine and MYM agar (19) was used. The defined liquid medium used in the analysis of intermediates in lysine catabolism was of the following composition: glucose, 30 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; KH_2PO_4, 10.5 g; lysine-HCl, 5.4 g; NaCl, 90 mg; $CaCl_2$, 90 mg; $ZnSO_4 \cdot 7H_2O$, 4.0 mg; $FeSO_4 \cdot 7H_2O$, 9.0 mg; $CuSO_4 \cdot 7H_2O$, 0.18 mg; H_3BO_3, 26 μg; $[NH_4]_3[MoO]_4 \cdot 4H_2O$, 17 μg; $MnSO_4 \cdot 4H_2O$, 27 μg; distilled water, 1,000 ml. Freshly harvested spores from MYM agar were used to inoculate liquid YEME medium containing 10% sucrose (7). After 48 h the mycelium was collected, washed twice with the defined liquid medium, resuspended in the medium, and grown at 30°C on a rotary shaker. Samples of the culture were taken at 24-h intervals for analysis of lysine metabolic products.

Isolation of lysine catabolic products. Samples (5 ml) of the culture were centrifuged at 900 × *g*, and the mycelium was washed twice with physiological saline. The mycelial pellet was suspended in 5 ml of 70% ethanol and was heated in boiling water for 10 min to extract amino acids. The resulting suspension was clarified by centrifugation at 10,000 × *g* for 10 min. The mycelium extract and the culture filtrate were each dried and suspended in 100 μl of distilled water.

Thin-layer chromatography and amino acid analyses. The amino acid extract and reference compounds were chromatographed on thin (250 μm) layers of Silica Gel G with acid, neutral, and alkaline solvent systems (3). Amines and amino acids were visualized with ninhydrin. The R_f values of δ-aminovalerate, cadaverine, glutamate, and lysine were

0.52, 0.18, 0.60, and 0.26, respectively, when methanol–30% ammonium hydroxide–acetic acid (7:3:0.1) was used as the solvent system. Amino acid analyses were obtained with a 119CL amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) by using a column of W-3H cation-exchange resin (Beckman) and sodium citrate (0.2 N Na^+ [pH 3.53], 0.4 N Na^+ [pH 4.12], and 1.0 N Na^+ [pH 6.40]) as sequential eluting buffers.

High-pressure liquid chromatography. Reference amino acids (2 mg) and mycelium extracts (10 to 20 μl) were derivatized for detection by being mixed with 10 mg of picryl sulfonic acid (2,4,6-trinitrobenzene sulfonic acid) in 0.5 ml of 2% $NaHCO_3$ as described by Vitt et al. (22). The solution was kept at room temperature in the dark for 2 h. Derivatized amino acids were applied to a Beckman Ultrasphere ODS (5 μm) column (0.46 by 25 cm) and were equilibrated with 35% acetonitrite in citric acid (0.098 M)–disodium hydrogen phosphate (0.016 M)–acetic acid (0.43 M) at pH 2.5. The column was developed isocratically with the solvent mixture at a flow rate of 1 ml/min (model 330 high-pressure liquid chromatograph [Beckman] and model 450 variable wavelength detector [Waters Associates, Inc., Milford, Mass.]). The output was monitored at 350 nm.

Lysine ε-aminotransferase and cadaverine aminotransferase assay. Cultures were grown in defined liquid medium and were harvested after 48 h. The mycelium was washed with 0.85% NaCl, suspended in 0.2 M potassium phosphate buffer (pH 7.5), and broken by sonic oscillation (Branson Sonifier, four to six 10-s pulses with a 1-min interval between each pulse). Cell debris was removed by centrifugation for 20 min at 4°C and 10,000 × *g*. The supernatant fluid was used as the cell extract.

The assay for lysine ε-aminotransferase was based on that of Kern et al. (10). The incubation mixture consisted of cell extract (1.0 ml), 40 μmol of lysine, 40 μmol of α-ketoglutarate, and 0.15 μmol of pyridoxal phosphate in a final volume of 2.0 ml. The assay for cadaverine aminotransferase was based on the method of Fothergill and Guest (5). The incubation mixture consisted of cell extract (1.0 ml), 300 μmol of cadaverine, 25 μmol of α-ketoglutarate, 0.25 μmol of pyridoxal phosphate, and 1 ml of 250 mM sodium carbonate buffer (pH 10.25). To stop both enzyme reactions, 0.8 ml of the reaction mixture was mixed with 0.4 ml of 5% trichloroacetic acid in absolute ethanol. Precipitated proteins were removed by centrifugation at 11,600 × *g*. The amounts of 1-piperideine-6-carboxylate and 1-piperideine in 1.0 ml of the deproteinized reaction mixture were measured by adding 1.5 ml of 4 mM *o*-aminobenzaldehyde in 0.2 M phosphate buffer (pH 7.5). The mixtures were heated at 37°C for 1 h, and the yellow-orange products were measured at 465 nm. To estimate the amounts of 1-piperideine-6-carboxylate and 1-piperideine formed, an extinction coefficient of 2,800 liters/mol per cm was used (5). Protein in cell extracts was determined by the method of Lowry et al. (11).

Bioassay. Cultures of *S. clavuligerus* were plated on defined fermentation medium (1) modified by addition of 1.5% agar. When single colonies had become visible, they were excised on agar plugs (7 by 5 mm) and were incubated in moist conditions at 30°C for 48 h. They were then transferred aseptically to trays (22.5 by 22.5 cm), each containing 100 ml of 3% tryptone soya agar seeded with the β-lactam-supersensitive strain *Escherichia coli* ESS (9). The trays were incubated at 37°C overnight, and zones of growth inhibition were then measured.

Chemicals. *o*-Aminobenzaldehyde, α-ketoglutaric acid, lysine, α-aminoadipic acid, cadaverine, δ-amino-*n*-valeric acid, and pyridoxal-5-phosphate were purchased from Sigma Chemical Co., St. Louis, Mo. High-pressure liquid chromatography-grade acetonitrile was from Fisher Scientific Co., Fairlawn, N.J. Other chemicals were of reagent grade. Silica Gel G thin-layer chromatography plates were from E. Merck AG, Darmstadt, Federal Republic of Germany.

TABLE 1. Early enzymes of lysine catabolism in prototrophic actinomycetes and growth on α-aminoadipate

Species	Sp act[a] for: Cadaverine amino-transferase	Sp act[a] for: Lysine ε-amino-transferase	Growth[b]
β-Lactam producers			
S. clavuligerus NRRL 3585	2.38	1.16	−
S. griseus NRRL 3851	1.92	0.85	+
N. lactamdurans NRRL 3802	0.64	0.21	+
β-Lactam nonproducers			
S. lividans TK24	2.10	0	++
S. phaeochromogenes B2119	2.48	0	+
S. viridochromogenes CUB416	1.67	0	−
S. glaucescens GLAO	1.16	0	++
S. venezuelae ISP5230	1.56	0	++
S. parvulus ISP5048	0.64	0	tr
S. rimosus NRRL 2234	1.1	0	tr

[a] Enzyme specific activity defined as nanomoles of product formed per minute per milligram of protein.

[b] α-Aminoadipate (0.05%, wt/vol) was added to minimal agar made without asparagine. ++, Growth as on asparagine; +, poor growth; tr, faint growth; −, no growth after 4 to 5 days at 30°C.

RESULTS

Lysine and cadaverine aminotransferases. Lysine ε-aminotransferase could not be detected in extracts of *S. lividans* cells but was present in extracts of *S. clavuligerus*. To test whether disruption of *S. lividans* cells released inhibitors or caused a loss of enzyme activity, cell extracts prepared from *S. lividans* and *S. clavuligerus* were mixed. Enzyme activity was present in the mixture in amounts that indicated no inhibition or rapid degradation but, rather, complete absence of the enzyme from the *S. lividans* extract. Of 10 actinomycetes tested, lysine ε-aminotransferase was detected only in the 3 β-lactam producers (Table 1). In contrast, cadaverine aminotransferase was detected in every strain.

Isolation and identification of intermediates in lysine catabolism. *S. lividans* was grown in a defined medium with lysine as the sole nitrogen source; the mycelium and culture filtrates were examined for accumulation of intermediates produced by lysine breakdown. Thin-layer chromatography of 70% ethanolic extracts from cells grown for 48, 72, 96, and 120 h showed four main ninhydrin-positive compounds, the R_f values of which matched those of cadaverine, glutamate, δ-aminovalerate, and lysine. As the culture aged, the intensities of the appropriate spots on thin-layer chromatography plates indicated that the δ-aminovalerate content also increased. When cell extracts from 48-h cultures were analyzed for amino acids by ion-exchange chromatography and reaction with ninhydrin, glutamate, δ-aminovalerate, and lysine were found at concentrations of 1.06, 0.28, and 1.25 μmol/ml, respectively. The procedure did not measure cadaverine. Examination of culture filtrates by thin-layer chromatography showed cadaverine to be the only ninhydrin-positive compound present other than lysine. Its accumulation in the culture correlated with disappearance of

TABLE 2. Growth of lysine-non-utilizing (Lut$^-$) mutants of both *S. lividans* and *S. clavuligerus*

Lut$^-$ group	Nitrogen source[a]		
	Lysine	Cadaverine	δ-Aminovalerate
I	−	+	+
II	−	−	+
III	−	−	−
Parent	+	+	+

[a] Supplied to minimal medium (7) without asparagine, at concentrations of 0.05% (wt/vol). +, Growth; −, no growth after 4 to 5 days of incubation at 30°C. All the *S. clavuligerus* strains produced β-lactam antibiotics (detected by bioassay). All *S. lividans* strains, but no *S. clavuligerus* strains, grew on α-aminoadipate.

lysine from the medium. Cell extracts from cultures grown for 48 and 120 h were derivatized with picryl sulfonic acid and were analyzed by high-pressure liquid chromatography. Results confirmed that glutamate and δ-aminovalerate were present (elution times of 5.43 and 1.43 min, respectively). It is noteworthy that α-aminoadipate could not be detected in any of the above analyses, although it was readily detected in cell extracts and culture filtrates of *S. clavuligerus* (S. Shapiro and R. L. White, unpublished observation).

Isolation and characterization of Lut$^-$ mutants. Mutants unable to grow with lysine as the sole source of nitrogen were isolated from *S. lividans* and *S. clavuligerus* after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. They were classified into three groups on the basis of their ability to grow when supplied with various intermediates (Table 2). One group grew on cadaverine and δ-aminovalerate, indicating that it comprised mutants blocked in the first step of lysine catabolism via the cadaverine pathway. A second group was unable to use lysine and cadaverine but grew on δ-aminovalerate. Mutants in the third group were unable to catabolize lysine, cadaverine, or δ-aminovalerate. Bioassays of the *S. clavuligerus* mutants showed that all were able to produce β-lactam antibiotics.

Catabolism of α-aminoadipate by different *Streptomyces* species. Several β-lactam-producing and -nonproducing actinomycetes were tested for their ability to catabolize α-aminoadipate in the absence of other nitrogen sources. Our intention was to discover possible differences in α-aminoadipate catabolism in β-lactam producers and nonproducers which might be exploited in the isolation of new β-lactam producers. The growth responses shown in Table 1 suggested that an ability to make efficient use of α-aminoadipate was correlated with an inability to make β-lactams. However, the converse was not true: some β-lactam nonproducers as well as producers showed relative (or total) inability to use α-aminoadipate for growth.

DISCUSSION

Since α-aminoadipate is a known precursor of β-lactam antibiotics and is also known to be an intermediate in lysine catabolism (17), we decided to test whether streptomycetes, β-lactam nonproducers as well as producers, could catabolize lysine via the pathway that includes α-aminoadipate. The presence of the same catabolic pathway leading to α-aminoadipate in both types would suggest that the α-aminoadipate pathway is involved in both primary and secondary metabolism. On the other hand, the absence of an α-aminoadipate pathway in β-lactam nonproducers and the presence of an entirely different pathway for lysine catabolism in all streptomycetes would suggest that the α-aminoadipate pathway is specifically for secondary metabolism.

Lysine ε-aminotransferase converts lysine to 1-piperideine-6-carboxylate in what can be considered the first step in β-lactam antibiotic biosynthesis. While this activity was readily detected in extracts of *S. clavuligerus*, it could not be detected in *S. lividans* grown under various conditions in which lysine utilization was expected to be mandatory. Since the same assay conditions for this enzyme were used for both *Streptomyces* spp., it is unlikely that an aminotransferase activity in *S. lividans* would have escaped detection. The possible presence of a highly active protease or strong enzyme inhibitor in cell extracts was ruled out by demonstrating enzyme activity in mixed cell extracts of *S. lividans* and *S. clavuligerus*. We therefore conclude that this enzyme activity is absent from *S. lividans* and is specific to β-lactam producers. Kern et al. (10) observed that lysine ε-aminotransferase activity peaked before the end of exponential growth and was correlated with cephamycin production in *S. lactamdurans*. The enzyme exhibited similar activity profiles during batch culture of *S. clavuligerus* (our unpublished observations). Vining et al. (21) observed that the activity of isopenicillin N synthetase, an early β-lactam pathway enzyme, peaked late in the exponential phase and rapidly declined afterwards. Other β-lactam biosynthetic enzymes also show this type of activity profile (2, 4).

Analysis of the contents of the mycelium and filtrates of cultures of *S. lividans* grown on lysine as the sole nitrogen source showed accumulation of cadaverine and δ-aminovalerate, indicating that the pathway for lysine catabolism involved these two intermediates. The isolation from both *S. lividans* and *S. clavuligerus* of phenotypically similar classes of mutants blocked in lysine catabolism (Table 2) provides further evidence that the cadaverine pathway functions in both species as the only route for using lysine as a nitrogen source. Presumably, mutants in group I lacked only lysine decarboxylase activity, while those in group II lacked cadaverine aminotransferase activity. However, group III mutants were more complex. They were probably deficient in step 2 of the pathway (Fig. 1) as well as in one or more of the steps after δ-aminovalerate, since production of glutamate from cadaverine might have permitted visible growth (given the presence of maltose). Therefore, group III mutants may bear multisite, polar, or regulatory mutations. These results strongly suggest that lysine catabolism in *Streptomyces* spp. in general proceeds via cadaverine and δ-aminovalerate (Fig. 1). This is similar to the pathway identified in *Pseudomonas aeruginosa* by Fothergill and Guest (5).

Cadaverine aminotransferase, the enzyme catalyzing the second step in lysine catabolism, was present in all nine *Streptomyces* spp. and one *Nocardia* sp. tested, whereas lysine ε-aminotransferase was present only in the three β-lactam producers (Table 1). This indicates that the cadaverine pathway is obligatory for complete lysine catabolism, whereas the aminoadipate pathway may only provide a precursor for β-lactam biosynthesis. The inability of Lut$^-$ mutants of *S. clavuligerus* to grow when supplied with lysine as their sole source of nitrogen despite their having a functional α-aminoadipate pathway is consistent with this pathway being used exclusively in secondary metabolism to provide a precursor for β-lactam antibiotic production. Romero et al. (15) isolated a mutant of *S. clavuligerus* which was deficient in lysine ε-aminotransferase but was similar to wild type in growth kinetics and in its ability to catabolize various amino acids including lysine. This phenotype indicated that the pathway involving lysine ε-aminotransferase was not necessary for exponential growth and hence is a secondary metabolic pathway enzyme.

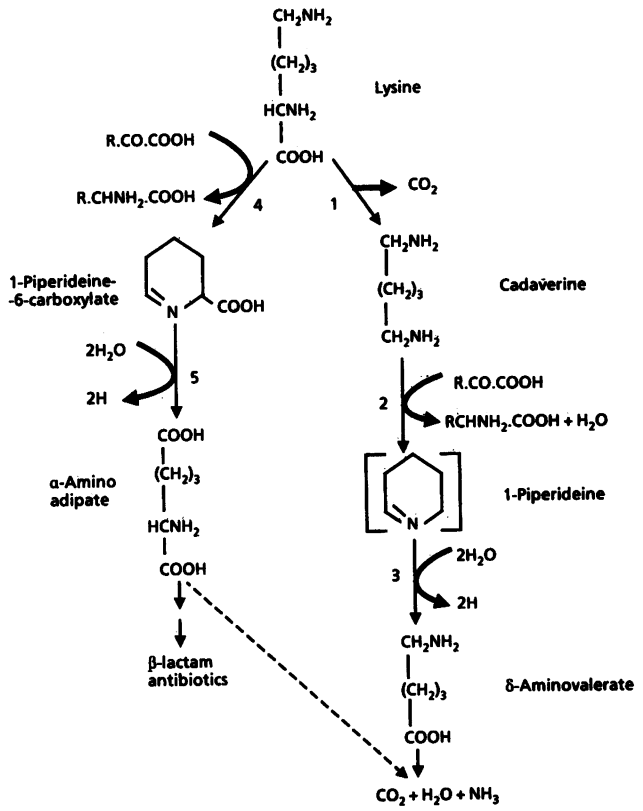

FIG. 1. Catabolic pathways for lysine in *Streptomyces* spp. Enzyme activities are: 1, lysine decarboxylase; 2, cadaverine aminotransferase; 3, 1-piperideine dehydrogenase; 4, lysine ε-aminotransferase; 5, 1-piperideine-6-carboxylate dehydrogenase. The pathway on the right is essential for the growth of *Streptomyces* spp. on lysine (5); the pathway on the left is present only in β-lactam-producing *Streptomyces* spp. Another pathway, for the catabolism of α-aminoadipate (---), exists in *S. lividans* but not in *S. clavuligerus*. Bracketed intermediate is hypothetical.

The absence of a clear correlation between the ability to produce β-lactam antibiotics and the failure to use α-aminoadipate as a source of nitrogen was disappointing. However, three of seven species which do not produce detectable amounts of β-lactam compounds grew well on α-aminoadipate, while three producers grew poorly or not at all. Whether the relative or total inability of β-lactam producers to catabolize α-aminoadipate is a general phenomenon and a prerequisite for β-lactam biosynthesis or, conversely, whether the efficient use of α-aminoadipate by some β-lactam nonproducers precludes its use for β-lactam biosynthesis are questions for further investigation.

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