J Ind Microbiol Biotechnol (2006) 33: 401–407
DOI 10.1007/s10295-006-0078-3

REVIEW PAPER

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Pipecolic acid in microbes: biosynthetic routes and enzymes

Received: 4 August 2005 / Accepted: 31 December 2005 / Published online: 18 January 2006
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Abstract Pipecolic acid is an important precursor of many useful microbial secondary metabolites. Pipecolic acid-derived moieties are often crucial for the biological activities of some microbial natural products with pharmaceutical applications. Understanding the biogenesis of pipecolic acid in microorganisms would be a significant step toward the mutasynthesis of novel analogs of choice. This review focuses on various microbial pathways and enzymes for pipecolic acid synthesis, especially those related to the origination of pipecolic acid moieties in secondary metabolites.

Keywords Pipecolic acid · Biosynthesis · Lysine metabolism · Microbial secondary metabolites

Introduction

L-Pipecolic acid is a non-proteingenic amino acid widely distributed in plants, animals, and microorganisms. It is an important precursor of many useful microbial secondary metabolites, such as the immunosuppressant rapamycin, the antitumor agent swainsonine, the peptide antibiotic virginiamycin, and the anthelmintic agent marcfortine (Fig. 1). In fact, the pipecolic acid-derived moieties often play important roles in the biological activities of some pharmaceutically important compounds. Elucidation of the origination of pipecolic acid thus serves as the first step toward generating novel analogs of these compounds for the structure-activity relationship studies.

Biosynthesis of pipecolic acid has been extensively investigated in animals and plants, mainly because of its close relationship with lysine metabolism and certain disorders related to lysine metabolism [1, 2]. These studies have established two basic routes for converting lysine into pipecolic acid, distinguishable at the loss of a specific amino group of lysine. One route is through the loss of the α-amino group of lysine and the incorporation of the ε-nitrogen into pipecolic acid (shown in the left branch of Fig. 2). The alternative route is via the loss of ε-nitrogen and the incorporation of α-nitrogen into pipecolic acid (shown in the right branch of Fig. 2). The important intermediates in these two pathways, Δ^1-piperideine-2-carboxylic acid (P2C) and Δ^1-piperideine-6-carboxylic acid (P6C), are isomers and exist in chemical equilibrium with their respective open-chain hydrated forms, α-keto-ε-amino-caproic acid and α-aminoadipic-δ-semialdehyde [2]. Although both routes have been reported to account for the biogenesis of pipecolic acid in microorganisms, variations of certain conversion steps and penultimate origin other than lysine have also been observed, as reviewed here. Experimental evidences supporting the existence of these various routes in microorganisms, as well as specific enzymes involved in some catalytic steps will also be discussed.

P2C pathway

The P2C route (reactions 1 and 2 in Fig. 2) for pipecolic acid synthesis has been studied in detail in *Pseudomonas putida* as a part of D-lysine catabolic pathway. *Pseudomonas* strains use different routes to metabolize L-lysine and D-lysine for carbon and nitrogen sources [3–5]. Whereas L-lysine is degraded primarily through the δ-aminovalerate pathway [5], D-lysine is metabolized through the transamination of the α-amino group of lysine, resulting in the formation of P2C, which is reduced into pipecolic acid [4, 6]. The latter is ultimately metabolized into glutamate. One of the most important experiments to elucidate this route was the identification of the enzyme catalyzing the reduction of P2C into pipecolic acid (reaction 2 in Fig. 2). In the 1980s, Payton and Chang [7] reported the purification and initial characterization of a reductase possessing such activity

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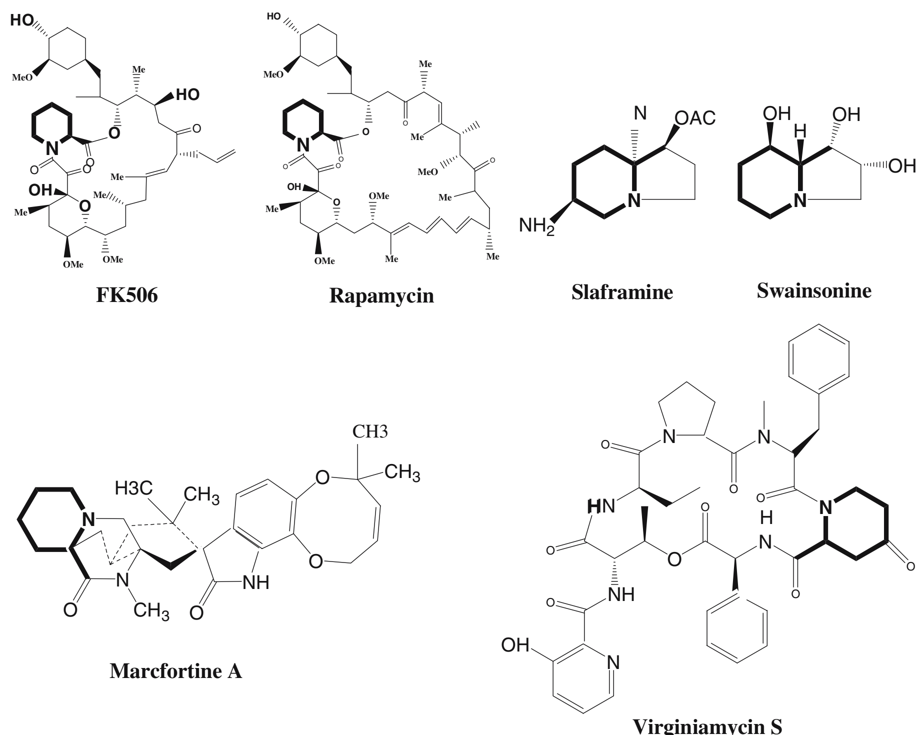
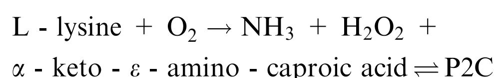


Fig. 1 Microbial secondary metabolites containing pipecolic acid derived moieties (pipecolate moieties are shown in *bold-faced type* in the drawing)

from *P. putida* ATCC 15070. At that time, information on the protein sequence or the gene encoding this enzyme was not provided. Almost 20 years later, Muramatsu et al. [8] surprisingly found that the product of a *P. putida* ATCC12633 gene, *dpkA*, which had previously been annotated as a malate/L-lactate dehydrogenase, was actually an NADPH-dependent P2C reductase. DpkA irreversibly catalyzed the NADPH-dependent reduction of P2C to form L-pipecolic acid, as well as the reduction of Δ^1-pyrroline-2-carxoylate into L-proline. The dual functions of this enzyme have been unambiguously demonstrated by in vivo genetic knockout experiment and in vitro biochemical assay using purified enzyme [8]. Sequence analysis has revealed that this enzyme represents a novel subclass in an NAD (P)-dependent oxidoreductase superfamily, with close homologs also found in *P. syringae* and *P. aeruginosa* [8].

The specific enzyme for conversion of D-lysine to P2C (reaction 1 in Fig. 2) has not been reported in *Pseudomonas*, although the D-amino acid aminotransferase from *Bacillus sphaericus* has been demonstrated to be capable of catalyzing the α-transamination of D-lysine with pyruvate to yield P2C [9]. A L-lysine α-oxidase catalyzing the oxidative deamination of the α-amino group of L-lysine has been isolated from fungi *Trichoderma viride* Y244-2 [10] and *T. harzianum* Rifai [11]. The enzyme has been characterized as a flavoprotein with two identical subunits, each containing one molecule of FAD as the coenzyme. It exhibits a high stereospecificity, being absolutely inert toward D-lysine. In the reactions catalyzed by this enzyme, the α-amino group of L-lysine is oxidized to yield α-keto-ε-amino-caproic acid, which is spontaneously converted into the dehydrated cyclic form, P2C [10, 11]:



$$\text{L - lysine} + O_2 \rightarrow NH_3 + H_2O_2 +$$
$$\alpha\text{ - keto - }\varepsilon\text{ - amino - caproic acid} \rightleftharpoons \text{P2C}$$

It is noteworthy that a L-lysine 2-aminotransferase (L2AT) has also been reported to catalyze the α-transamination reaction of L-lysine in *Streptomyces tendae* Tu901, an actinomycete strain which produces peptidyl nucleoside antibiotic nikkomycin D [12]. Nikkomycin D does not contain any pipecolate moiety, but has a picolinic acid moiety which is also derived from L-lysine through loss of α-amine [13]. In the biosynthetic gene cluster of nikkomycin D, a gene has been identified to encode a protein (NikC), which belongs to a novel class of pyridoxamine or pyridoxal-phosphate-dependent dehydrases and aminotransferases. The function of NikC as L-lysine 2-aminotransferase has been clearly demonstrated by the *o*-aminobezaldehyde assay, which allows differentiation between the α-transamination and ε-transamination of lysine [12].

Lysine cyclodeamination pathway

This route (reaction 3 in Fig. 2) arose from the studies of the biosynthesis of rapamycin, FK506 and FK520 (ascomycin), a group of closely related actinomycete secondary metabolites with potent immunosuppressive, neurotrophic, and antifungal activities [14, 15]. A common structural feature of these heterocyclic polyketide

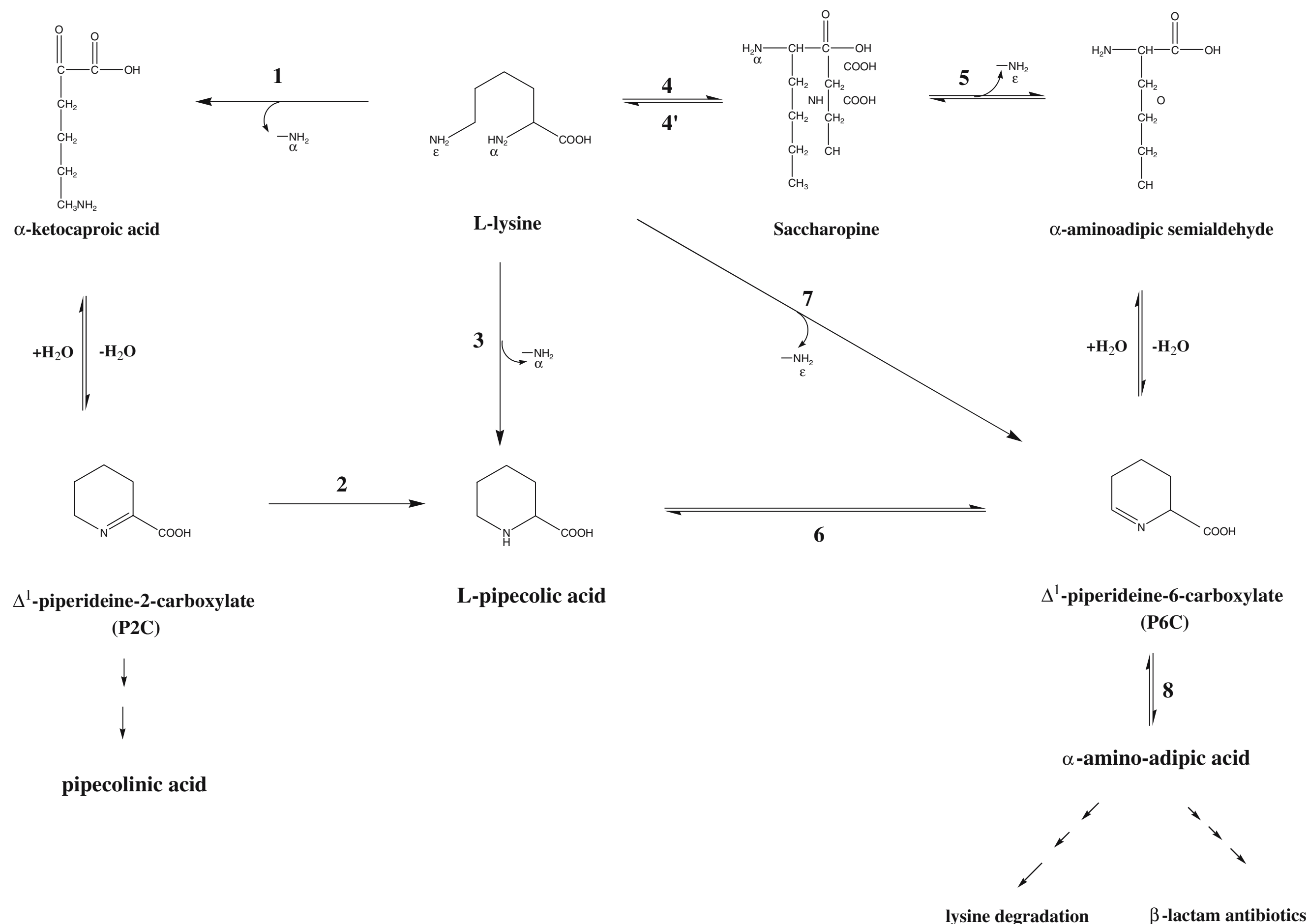


Fig. 2 Various routes of pipecolic acid biosynthesis in microorganisms

compounds is a so-called "FKBP12-binding motif", which is composed of a pipecolate ester and a cyclic hemiketal moiety [16]. This structural motif is believed to be indispensable for the biological activities of these compounds, because it is involved in the binding of their initial in vivo target, FKBP12 [17–19]. Labeling experiments using DL-lysine-α-^{15}N and DL-lysine-ε-^{15}N clearly indicated the loss of α-nitrogen atom and the incorporation of ε-nitrogen into the final FK520 macrolide ring [20]. Later, a gene (*rapL*) encoding a putative enzyme called lysine cyclodeaminase has been identified in the completely sequenced biosynthetic gene cluster of rapamycin [21, 22]. The deduced protein product (RapL) of *rapL* gene exhibits strong sequence similarity with two isoenzymes of ornithine deaminase from *Agrobacterium tumefaciens*, which catalyze the deaminative cyclization of ornithine to proline [23, 24]. Based on this homology, Molnar et al. [21] proposed that RapL catalyzes the analogous conversion of L-lysine to L-pipecolic acid. Khaw et al. [25] have confirmed the involvement of *rapL* in the biosynthesis of rapamycin by genetic disruption of this gene in *Streptomyces hygroscopicus* NRRL 5491, which resulted in significant decrease in rapamycin production and allowed the replacement of pipecolate with proline to generate prolyrapamycin. Close homologs of *rapL* gene have also been found in the biosynthetic gene clusters of FK506 [26] and FK520 [27], indicating a common route for supplying pipecolate in the biosynthesis of these related compounds. Since ornithine deaminase catalyzes the cyclization of ornithine to proline via the loss of α-amino group of ornithine [28], it is likely that RapL and its homologs function accordingly on lysine, a mechanism consistent with the results from earlier labeling experiment with FK520 [20]. However, the precise enzymatic reactions catalyzed by RapL or its homologs need to be established by future in vitro biochemical characterization using expressed and purified protein. It would also be interesting to investigate whether P2C is generated as an intermediate in the reactions catalyzed by RapL.

Interestingly, both L-lysine 2-aminotransferase and lysine cyclodeaminase have been implicated in the biosynthesis of virginiamycin S in *Streptomyces virginiae* [29]. Virginiamycin S, a cyclohexadepsipeptide antibiotic, has two unusual amino acid residues, 4-oxopipecolic acid, and 3-hydroxypicolinic acid [30, 31]. Both amino acids are originally derived from lysine [32], and the first steps in the biogenesis of these two amino acids

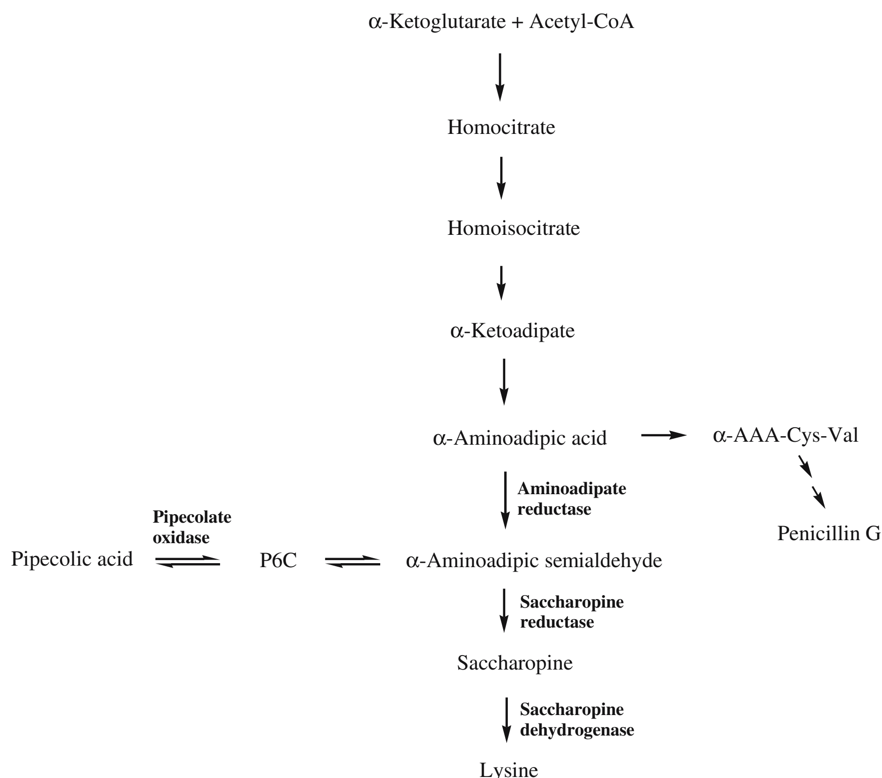


Fig. 3 Relationship of pipecolic acid formation and the biosynthesis of lysine and penicillin in *P. chrysogenum* (modified from reference 54)

are presumably the same: cyclization of L-lysine with loss of α-nitrogen [33]. Two genes that have been identified in the virginiamycin biosynthetic gene cluster could be responsible for this reaction: *visC* encoding a cyclodeaminase similar to RapL, and *visA* encoding a L-lysine 2-maminotransferase with high homology to NikC [29]. Namwat et al. [34] provided convincing evidence to suggest that the L2AT activity encoded by *visA* is dedicated to supplying 3-hydroxypicolinic acid from lysine. Therefore, pipecolic acid, the precursor of 4-oxopipecolic acid, is likely to be generated by the cyclodeaminase encoded by *visC*.

P6C pathway

This pathway has been intensively studied in fungi parasite *Rhizoctonia leguminicola*, because the synthesis of pipecolic acid via this route represents the initial steps in the production of two toxic octahydroindolzine alkaloids, slaframine, and swainsonine [35]. In the investigation of the origin of pipecolic acid in this fungus using cell-free enzyme systems, Wickwire et al. [36, 37] have established a chain of reactions through which L-lysine was converted to saccharopine, which was in turn converted to P6C through oxidative cleavage. The latter was then readily reduced to pipecolic acid (reactions 4, 5, and 6 in Fig. 2). A previously unrecognized flavin enzyme, saccharopine oxidase, has been identified in their study, which oxidatively cleaves saccharopine to yield P6C as follows:

$$\text{Saccharopine} + O_2 \rightarrow \text{P6C} + \text{glutamate} + H_2O_2$$

Since saccharopine is a major metabolite in lysine degradation in *R. leguminicola*, saccharopine oxidase apparently functions to shunt saccharopine into secondary metabolism pathway to supply precursor (pipecolic acid) for slaframine and swainsonine production [37]. It is interesting that in aerobic red yeast *R. glutinis*, the reverse reactions of this pathway are operative too, through which pipecolic acid is converted to lysine via P6C, α-aminoadipic semialdehyde, and saccharopine [38, 39].

The direct formation of P6C from lysine through ε-transamination has also been observed in some microorganisms. In β-lactam producing actinomycetes, P6C is synthesized from lysine by L-lysine ε-aminotransferase (LAT, E.C.2.6.1.36), and then converted into L-α-aminoadipic acid, the common precursor of all penicillins, cephalosporins, and cephamycins [40]. Gene encoding LAT is located in the β-lactam antibiotic biosynthetic gene clusters in both *S. clavuligerus* and *N. lactamdurans* [41, 42], but is absent from the genome of other non-β-lactam producing actinomycetes, indicating that LAT is specific for secondary metabolism [43]. LAT has also been found in gram-negative bacterium *Flavobacterium lutescens* [44, 45]. At the present time, no example can be given to show the direct involvement of LAT in pipecolic acid biosynthesis in a naturally occurring microorganism; however, *lat* gene has been used to engineer recombinant *E. coli* strains for pipecolic acid production (see below).

Not much information about the enzyme dedicated to the second step of P6C pathway, the reduction of P6C into pipecolic acid, is available at current stage. In their

search for this enzyme in *F. lutescens*, Fujii et al. [46] serendipitously found that *E. coli* pyrroline-5-carboxylate (P5C) reductase (EC 1.5.1.2) (encoded by *proC* gene) acted efficiently with *F. lutesens* LAT to convert L-lysine into L-pipecolic acid. They have then genetically engineered a recombinant *E. coli* strain for massive L-pipecolic acid production through combined expression of the *lat* gene from *F. lutescens* and the *proC* gene from *E. coli* [46, 47]. This biotransformation system provided the pure S enantiomer of pipecolic acid (L-pipecolic acid), with ee-value (enantiomeric excess, a measure for how much of one enantiomer is present compared to the other) was 100%. It is noteworthy that P5C reductase is present in almost all organisms, catalyzing the terminal step in proline biosynthesis—conversion of Δ^1-pyrroline-5-carboxylate into proline [48–51]. It is possible that in the microorganisms that produce L-pipecolic acid via P6C pathway, the universally conserved P5C reductase is actually responsible, at least in part, for the reduction of P6C into L-pipecolic acid [46].

α-Aminoadipic acid pathway

In some filamentous fungi, it appeared that pipecolic acid is derived from α-aminoadipic acid (α-AAA). The first indication of this route came from Aspen and Meister's [52] observation that some lysine auxotroph mutants of *Aspergillus nidulans* can convert α-aminoadipic acid into pipecolic acid. In their experiments using radio labeled α-AAA and lysine, the carbon skeleton and the nitrogen atom of pipecolic acid were found to be predominantly derived from that of α-AAA rather than that of lysine. This observation prompted Naranjo et al. to investigate the pipecolic acid synthesis in *Penicillium chrysogenum* since this fungus has a very active lysine biosynthesis pathway to provide α-AAA for penicillin production [53]. In their initial study, pipecolic acid was found to be associated with lysine biosynthesis via a chain of reactions catalyzed by pipecolate oxidase, saccharopine reductase, and saccharopine dehydrogenase [54] (Fig. 3). Later on, this group observed the accumulation of pipecolic acid (along with P6C) upon inactivation of the gene encoding saccharopine reductase, and its origin was determined to be from α-AAA instead of lysine through comparison of the intracellular accumulation of pipecolic acid with another mutant which lacks α-AAA reductase [55]. Here, pipecolic acid appeared to be an incidental intermediate in lysine biosynthesis, derived from the spontaneous chemical equilibrium of α-AAA-semialdehyde into P6C, and the easy conversion of P6C to pipecolic acid (probably catalyzed by the universally existing P5C reductase). In addition, *P. chrysogenum* does not naturally produce any secondary metabolite containing pipecolic acid-derived moiety. However, the discovery of this α-aminoadipic acid pathway was an evidence to show that this fungus could be genetic engineered as a host for producing pipecolic acid-containing complex secondary metabolites because of its potential capability to provide pipecolic acid [54].

Concluding remarks

Pipecolic acid is an important non-proteingenic amino acid that serves as a component or precursor of many microbial and plant secondary metabolites. Studies reviewed herein illustrated the multiple routes that could lead to the production of this amino acid in different microorganisms. Whereas some microorganisms have dedicated pathways to supply pipecolic acid for the biosynthesis of specific secondary metabolites, others might just accumulate this amino acid as an intermediate or shunt product from a primary metabolic pathway. Information available at current stage only provided an incomplete picture of each route, either lacking the identification of the enzyme responsible for a specific reaction step, or requiring more experimental evidences to establish the function of an identified enzyme in a native pipecolic producing microorganism. In terms of identifying the specific biogenesis route for the pipecolate moiety of microbial secondary metabolites, feeding experiment using ^{15}N-labled lysine was a powerful tool to differentiate the two plausible routes in converting L-lysine to pipecolic acid. However, a specific pipecolic acid biosynthetic route cannot be readily assigned to a strain solely based on the identification of the loss of the α-amino or *β*-amino group of lysine. For example, even though it has been demonstrated clearly by NMR and MS studies that the marcfortine A producing *Penicillium* strain incorporates L-lysine by losing the α-amino group to generate pipecolate moiety [56], it is not clear at the present time that whether a L-lysine α-oxidase or a lysine 2-aminotransferase along with a P2C reductase, or a lysine cyclodeaminase alone, is responsible for the synthesis of pipecolic acid in this strain. Complete sequencing of the biosynthetic gene cluster of microbial secondary metabolites will allow the identification of pathway-dedicated gene(s), if they exist, for pipecolic acid supply, and therefore provide the starting point for mutasynthesis of novel analogs with the pipecolate moiety being replaced by other amino acids.

Acknowledgements The author thanks Drs. Hao Jiang, John Hucul, Edmund Graziani, Frank Koehn, and Mr. Bradley Haltli for valuable discussions and comments.

References

1. Broquist HP (1991) Lysine-pipecolic acid metabolic relationships in microbes and mammals. Annu Rev Nutr 11:435–448
2. Gupta RN, Spenser ID (1969) Biosynthesis of the piperidine nucleus. The mode of incorporation of lysine into pipecolic acid and into piperidine alkaloids. J Biol Chem 244:88–94

3. Chang YF, Adams E (1974) D-Lysine catabolic pathway in *Pseudomonas putida*: interrelations with L-lysine catabolism. J Bacteriol 117:753–764
4. Chang YF, Adams E (1971) Induction of separate catabolic pathways for L- and D-lysine in *Pseudomonas putida*. Biochem Biophys Res Commun 45:570–577
5. Fothergill JC, Guest JR (1977) Catabolism of L-lysine by *Pseudomonas aeruginosa*. J Gen Microbiol 99:139–155
6. Miller DL, Rodwell VW (1971) Metabolism of basic amino acids in *Pseudomonas putida*. Catabolism of lysine by cyclic and acyclic intermediates. J Biol Chem 246:2758–2764
7. Payton CW, Chang YF (1982) Δ^1-piperideine-2-carboxylate reductase of *Pseudomonas putida*. J Bacteriol 149:864–871
8. Muramatsu H, Mihara H, Kakutani R, Yasuda M, Ueda M, Kurihara T, Esaki N (2005) The putative malate/lactate dehydrogenase from *Pseudomonas putida* is an NADPH-dependent Δ^1-piperideine-2-carboxylate/delta1-pyrroline-2-carboxylate reductase involved in the catabolism of D-lysine and D-proline. J Biol Chem 280:5329–5335
9. Yonaha K, Misono H, Yamamoto T, Soda K (1975) D-Amino acid aminotransferase of *Bacillus sphaericus*: enzymologic and spectrometric properties. J Biol Chem 250:6983–6989
10. Kusakabe H, Kodama K, Kuninaka A, Yoshino H, Misono H, Soda K (1980) A new antitumor enzyme, L-lysine α-oxidase from *Trichoderma viride*. Purification and enzymological properties. J Biol Chem 255:976–981
11. Lukasheva EV, Berezov TT (2002) L-Lysine α-oxidase: physicochemical and biological properties. Biochemistry (Moscow) 67:1152–1158
12. Bruntner C, Bormann C (1998) The *Streptomyces tendae* Tu901 L-lysine 2-aminotransferase catalyzes the initial reaction in nikkomycin D biosynthesis. Eur J Biochem 254:347–355
13. Jordan B, Schmidt RM, Pape H (1984) Nikkomycin formation and lysine metabolism in *Streptomyces tendae*, In: The 3rd European congress on biotechnology. 1:451–455. Verlag Chemie, Weinheim
14. Cardenas ME, Zhu D, Heitman J (1995) Molecular mechanisms of immunosuppression by cyclosporine, FK506, and rapamycin. Curr Opin Nephrol Hypertens 4:472–477
15. Sanglier JJGB, Dreyfuss M, Fehr T, Traber R, Schreier MH (1993) Immunosuppressants of microbial origin, Developments in industrial microbiology series, vol. 32. Wm, C. Brown Publisher, Dubuque, pp 1–27
16. Paiva NL, Demain AL, Roberts MF (1993) The immediate precursor of the nitrogen-containing ring of rapamycin is free pipecolic acid. Enzyme Microb Technol 15:581–585
17. Banaszynski LA, Liu, CW, Wandless TJ (2005) Characterization of the FKBP.rapamycin.FRB ternary complex. J Am Chem Soc 127:4715–4721
18. Choi J, Chen J, Schreiber SL, Clardy J (1996) Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 273:239–242
19. Meadows RP, Nettesheim DG, Xu RX, Olejniczak ET, Petros AM, Holzman TF, Severin J, Gubbins E, Smith H, Fesik SW (1993) Three-dimensional structure of the FK506 binding protein/ascomycin complex in solution by heteronuclear three- and four-dimensional NMR. Biochemistry 32:754–765
20. Byrne KM, Shafiee A, Nielsen J, Arison B, Monaghan RL, Kaplan L (1993) The biosynthesis and enzymology of an immunosuppressant, immunomycin, produced by *Streptomyces hygroscopicus* var. *ascomyceticus*. Dev Ind Microbiol 32:29–45
21. Molnar I, Aparicio JF, Haydock SF, Khaw LE, Schwecke T, Konisg A, Staunton J, Leadlay PF (1996) Organization of the biosynthetic gene cluster for rapamycin in *Streptomyces hygroscopicus*: analysis of genes flanking the polyketide synthase. Gene 169:1–7
22. Schwecke T, Aparicio JF, Molnar I, Konig A, Khaw LE, Haydock SF, Oliynyk M, Caffrey P, Cortes J, Lester JB et al (1995) The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin. Proc Natl Acad Sci USA 92:7839–7843
23. Sans N, Schindler U, Schroder J (1988) Ornithine cyclodeaminase from Ti plasmid C58: DNA sequence, enzyme properties and regulation of activity by arginine. Eur J Biochem 173:123–130
24. Schindler U, Sans N, Schroder J (1989) Ornithine cyclodeaminase from octopine Ti plasmid Ach5: identification, DNA sequence, enzyme properties, and comparison with gene and enzyme from nopaline Ti plasmid C58. J Bacteriol 171:847–854
25. Khaw LE, Bohm GA, Metcalfe S, Staunton J, Leadlay PF (1998) 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
26. Motamedi H, Shafiee A (1998) The biosynthetic gene cluster for the macrolactone ring of the immunosuppressant FK506. Eur J Biochem 256:528–534
27. Wu K, Chung L, Revill WP, Katz L, Reeves CD (2000) The FK520 gene cluster of *Streptomyces hygroscopicus var. ascomyceticus* (ATCC 14891) contains genes for biosynthesis of unusual polyketide extender units. Gene 251:81–90
28. Muth WL, Costilow RN (1974) Ornithine cyclase (deaminating). III. Mechanism of the conversion of ornithine to proline. J Biol Chem 249:7463–7467
29. Namwat W, Kamioka Y, Kinoshita H, Yamada Y, Nihira T (2002) Characterization of virginiamycin S biosynthetic genes from *Streptomyces virginiae*. Gene 286:283–290
30. Paris JM, Barriere JC, Smith C, Bost PE (1990) The chemistry of pristinamycins. Recent progress in the chemical synthesis of antibiotics. Springer, Berlin Heidelberg New York, pp 183–248
31. Yamada Y, Nihira T, Sakuda S (1997) Butyrolactone autoregulators, inducers of virginiamycin in *Streptomyces virginiae*: their structures, biosynthesis, recepter proteins, and induction of virginiamycin biosynthesis. In: Strolhl WR (ed) Biotechnology of antibiotics. Marcel Dekker, New York, pp 63–79
32. Molinero AA, Kingston DGI, Reed JW (1989) Biosynthesis of antibiotics of the virginiamycin family. 6. Biosynthesis of virginiamycin S1. J Nat Prod 52:99–108
33. Reed JW, Purvis MB, Kingston DGI, Biot A, Gossele F (1989) Biosynthesis of antibiotics of the virginiamycin family. 7. Stereo-and regiochemical studies on the formation of the 3-hydroxypicolinic acid and pipecolic acid units. J Org Chem 54:1161–1165
34. Namwat W, Kinoshita H, Nihira T (2002) Identification by heterologous expression and gene disruption of *VisA* as L-lysine 2-aminotransferase essential for virginiamycin S biosynthesis in *Streptomyces virginiae*. J Bacteriol 184:4811–4818
35. Broquist HP (1985) The indolizidine alkaoids, slaframine and swainsonine: contaminants in animal forages. Annu Rev Nutr 5:391–409
36. Wickwire BM, Harris CM, Harris TM, Broquist HP (1990) Pipecolic acid biosynthesis in *Rhizoctonia leguminicola*. I. The lysine saccharopine, Δ^1-piperideine-6-carboxylic acid pathway. J Biol Chem 265:14742–14747
37. Wickwire BM, Wagner C, Broquist HP (1990) Pipecolic acid biosynthesis in *Rhizoctonia leguminicola*. II. Saccharopine oxidase: a unique flavin enzyme involved in pipecolic acid biosynthesis. J Biol Chem 265:14748–14753
38. Kinzel JJ, Bhattacharjee JK (1979) Role of pipecolic acid in the biosynthesis of lysine in *Rhodotorula glutinis*. J Bacteriol 138:410–417
39. Kurtz M, Bhattacharjee JK (1975) Biosynthesis of lysine in *Rhodotorula glutinis*: role of pipecolic acid. J Gen Microbiol 86:103–110
40. Rius N, Demain AL (1997) Lysine ε-aminotransferase, the initial enzyme of cephalosporin biosynthesis in actinomycetes. J Microbiol Biotechnol 7:95–100
41. Aharonowitz Y, Cohen G, Martin JF (1992) Penicillin and cephalosporin biosynthetic genes: structure, organization, regulation, and evolution. Annu Rev Microbiol 46:461–495

42. Tobin MB, Kovacevic S, Madduri K, Hoskins JA, Skatrud PL, Vining LC, Stuttard C, Miller JR (1991) Localization of the lysine ε-aminotransferase (lat) and Δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthetase (pcbAB) genes from *Streptomyces clavuligerus* and production of lysine ε-aminotransferase activity in *Escherichia coli*. J Bacteriol 173:6223–6229
43. Madduri K, Stuttard C, Vining LC (1989) Lysine catabolism in *Streptomyces spp*. is primarily through cadaverine: β-lactam producers also make α-aminoadipate. J Bacteriol 171:299–302
44. Fujii T, Narita T, Agematu H, Agata N, Isshiki K (2000) Characterization of L-lysine 6-aminotransferase and its structural gene from *Flavobacterium lutescens* IFO3084. J Biochem 128:391–397
45. Soda K, Misono H, Yamamoto T (1968) L-Lysine:α-ketoglutarate aminotransferase. I. Identification of a product, Δ^1-piperideine-6-carboxylic acid. Biochemistry 7:4102–4109
46. Fujii T, Mukaihara M, Agematu H, Tsunekawa H (2002) Biotransformation of L-lysine to L-pipecolic acid catalyzed by L-lysine 6-aminotransferase and pyrroline-5-carboxylate reductase. Biosci Biotechnol Biochem 66:622–627
47. Fujii T, Aritoku Y, Agematu H, Tsunekawa H (2002) Increase in the rate of L-pipecolic acid production using lat-expressing *Escherichia coli* by *lysP* and *yeiE* amplification. Biosci Biotechnol Biochem 66:1981–1984
48. Brandriss MC, Falvey DA (1992) Proline biosynthesis in *Saccharomyces cerevisiae*: analysis of the PRO3 gene, which encodes Δ^1-pyrroline-5-carboxylate reductase. J Bacteriol 174:3782–3788
49. Delauney AJ, Verma DP (1990) A soybean gene encoding Δ^1-pyrroline-5-carboxylate reductase was isolated by functional complementation in *Escherichia coli* and is found to be osmoregulated. Mol Gen Genet 221:299–305
50. Leisinger T (1987) Biosynthesis of proline. In: Neidhardt FC, Ingraham JL, Low KB, Magasanic B, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, pp 345–351
51. Smith RJ, Downing SJ, Phang JM, Lodato RF, Aoki TT (1980) Pyrroline-5-carboxylate reductase activity in mammalian cells. Proc Natl Acad Sci USA 77:5221–5225
52. Aspen AJ, Meister A (1962) Conversion of α-aminoadipic acid to L-pipecolic acid by *Aspergillus nidulans*. Biochemistry 1:606–612
53. Casqueiro J, Bañuelos O, Sutiérrez S, Martín JF (2001) Metabolic engineering of the lysine pathway for β-lactam overproduction in *Penicillium chrysogenum*. In: Van Broedkhoven A, Anne J, Shapiro F (eds) Focus on biotechnology, vol.1. Novel frontiers in the production of compounds for biomedical use. Kluwer Academic Publishers, Dordrecht, pp 147–159
54. Naranjo L, Martin de Valmaseda E, Banuelos O, Lopez P, Riano J, Casqueiro J, Martin JF (2001) Conversion of pipecolic acid into lysine in *Penicillium chrysogenum* requires pipecolate oxidase and saccharopine reductase: characterization of the *lys7* gene encoding saccharopine reductase. J Bacteriol 183:7165–7172
55. Naranjo L, Martin de Valmaseda E, Casqueiro J, Ullan RV, Lamas-Maceiras M, Banuelos O, Martin JF (2004) Inactivation of the *lys7* gene, encoding saccharopine reductase in *Penicillium chrysogenum*, leads to accumulation of the secondary metabolite precursors piperideine-6-carboxylic acid and pipecolic acid from α-aminoadipic acid. Appl Environ Microbiol 70:1031–1039
56. Kuo MS, Yurek DA, Mizsak SA, Cialdella JI, Baczynskyj L, Marshall VP (1999) Biosynthesis of the pipecolate moiety of marcfortine A. J Am Chem Soc 121:1763–1767