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METABOLIC ENGINEERING AND SYNTHETIC BIOLOGY - REVIEW

Expanding lysine industry: industrial biomanufacturing of lysine and its derivatives

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Abstract

L-Lysine is widely used as a nutrition supplement in feed, food, and beverage industries as well as a chemical intermediate. At present, great efforts are made to further decrease the cost of lysine to make it more competitive in the markets. Furthermore, lysine also shows potential as a feedstock to produce other high-value chemicals for active pharmaceutical ingredients, drugs, or materials. In this review, the current biomanufacturing of lysine is first presented. Second, the production of novel derivatives from lysine is discussed. Some chemicals like L-pipecolic acid, cadaverine, and 5-aminovalerate already have been obtained at a lab scale. Others like 6-aminocaproic acid, valerolactam, and caprolactam could be produced through a biological and chemical coupling pathway or be synthesized by a hypothetical pathway. This review demonstrates an active and expansive lysine industry, and these green biomanufacturing strategies could also be applied to enhance the competitiveness of other amino acid industry.

Keywords Lysine · Metabolic engineering · Synthetic biology · Strain improvement · δ-Valerolactam

Abbreviations

Lys	Lysine
L-PA	L-Pipecolic acid
LCD	Lysine cyclodeaminase
LDC	L-Lysine decarboxylase
GDH	Glucose dehydrogenase
LysR	Lysine racemase
6ACA	6-Aminocaproic acid
5AVA	5-Aminovalerate

Introduction

Amino acids are the basic constituent units of many biological functional macromolecules, especially the proteins needed for animal survival. Traditionally, amino acid manufacturing methods include acid hydrolysis of proteins or chemical synthesis [32, 79]. However, recently, innovative fermentation has emerged as an alternative of the classical manufacturing methods, and the large production volume pushes the amino acid industry to progressively gain a prevailing share in the international market. Accompanying the emerging technologies such as metabolic engineering and synthetic biology, establishing new value chains from amino acids to high-value-added chemicals, such as fuels, materials, and drugs, should be focused on more closely. On one hand, this activity could lead to more economic boom in the production of novel and high-value-added chemicals, thus leading to new jobs. On the other hand, the extension of the amino acid industry could help to maintain the price of the original amino acid in the international market, thus protecting the effective market operations and stabilizing the profit of the amino acids industry and the workers.

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Lysine (Lys) is reported as a kind of imperative nutrition for human and animal. The largest commercial usage is relying on its promotion of the growth of poultry and other animals as a feed additive. However, it could also be used as special chemicals in food, pharmaceutical, and chemical industries. The calculated global Lys market is about 2.5 million metric tons in 2016, and is still growing at a rate of about 7% per year [2, 33, 91]. Due to the market competition from industrial capacity and demand, and also from the natural Lys resources such as sardine, swine blood, and maize, the per kilogram price of Lys has decreased to around $1.5 since 2013 [11, 29] (Fig. 1). However, the gross annual value of the output is maintained around $2.8 billion, continue showing attraction for investors. Here, first, we want to summarize the great efforts for low-cost Lys production, which include strain improvement, fermentation optimization, and downstream processing. Some of the characteristics of the processing routes such as microbial strains, typical biomass kinds, titers, productivity, yields, and the main advantages, are listed in detail in Table 1. The "bio-based manufacturing" possibility using Lys as a feedstock to expand the Lys industry beyond the traditional usage is discussed in the latter part of this review, including biological and chemical hybrid routes, to guarantee a bigger profit and less environmental pollution. These two strategies might together help to expand the Lys industry in the fierce international competition (Fig. 2).

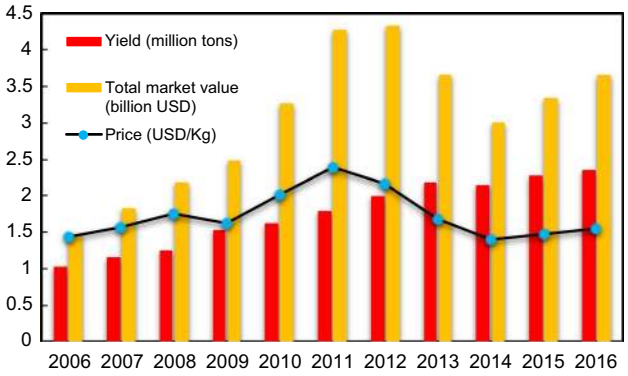

Fig. 1 Production of lysine increase over the past decade, the fluctuation of the lysine price, and the total market value

Lys production often employs mutant strains or engineered strains, like *Corynebacterium glutamicum* and engineered *Escherichia coli* to produce Lys through fermentation. Recently, accompanying the development of emerging technology like metabolic engineering and synthetic biology [21,

Table 1 Characteristics of the lysine microbial processing routes are listed in detail about the microbial species, typical biomass kinds and titers, production titers, yields, and the main advantages

The kind of strain	The characteristic of the strain	The yield achieved by this process (g/g)	The titer achieved by this process (g/L)	The biomass kinds	The biomass titers (g/L)	The main advantages	References
E. coli	A threonine and methionine double auxotrophic, with *ppc*, *pntB*, *aspA* overexpression	0.45	134.9	Glucose	300	Increasing the yield	[98]
C. glutamicum	*IolT1* and *iolT2* overexpression	0.24	9.30	Glucose	36.36	Enhancing the carbon utilization	[27, 43]
C. glutamicum	12 defined genome-based changes in genes encoding central metabolic enzymes	0.55	120	Glucose	700	Increasing the yield	[4]
C. glutamicum	MurE mutation	0.36	7.56	Glucose	25.4	Increasing the yield	[5, 6]
C. glutamicum	A *S. mutans*-type glycolic pathway reconstruction	0.19	9.55	Glucose	50	Increasing the yield	[85]
C. glutamicum	Replacement of the NADH biosynthesis enzyme	0.47	130.99	Glucose	280	Increasing the productivity	[96]

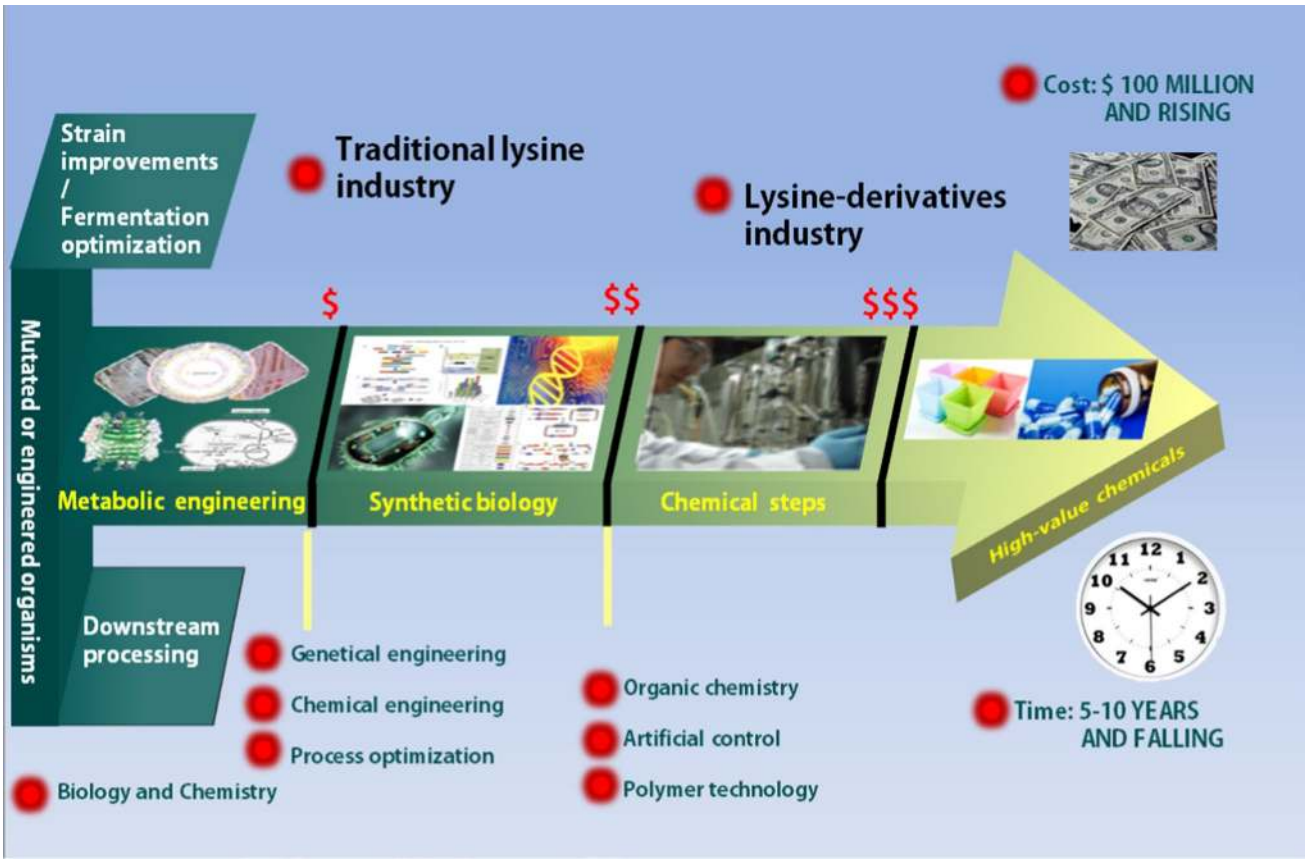

Fig. 2 Two strategies help to expand the lysine industry. The first is the great efforts for low-cost biomanufacturing of lysine. The second is the "bio-based manufacturing" combined with chemical steps possibility using lysine as a feedstock

23], non-natural metabolic pathways could be constructed beyond the natural networks. Thus, chemicals traditionally produced from oil refining are now included in the list of the bio-based chemical derivatives. This review will focus on chemicals that are mass-produced from fossils, and on special Lys derivatives that might have economical potential in the future. To the best of our knowledge, the microbial synthesis of commodity derivatives from Lys has never been reviewed before. However, this field has rapid development and great potential in special chemical industry, agricultural application, medical treatment, and biopolymer production. This review has discussed the efforts now being made for low-cost biomanufacturing of Lys, while the latter part has been organized along the sequence of the fields the novel derivatives belong, which are summarized in Table 2 and Fig. 3. Chemicals used as functional materials have been first presented, and then the pharmaceutical products. However, some chemicals are used in both these two areas.

Recent efforts for low-cost biomanufacturing of Lys

To expand the Lys industry, one strategy is to further decrease the production cost and the market price, which might result in the requirement of more supplementation. Recent efforts for low-cost biomanufacturing of Lys include strain improvement, fermentation optimization, and downstream processing as reported. The natural metabolic pathways for Lys synthesis, together with the related enzymes and genes, have been omitted here, since such fundamental information is easily available in books and articles.

Table 2 Summary of some of the novel lysine derivatives produced through biomanufacturing strategy

The derivatives from lysine	The concentrations achieved by enzyme catalysis (g/L)	The yields achieved by enzyme catalysis (g/g)	The concentrations achieved by whole-cell catalysis (g/L)	The yields achieved by whole-cell catalysis (g/g)	The concentrations achieved by fermentation (g/L)	The yields achieved by fermentation (g/g)	The preferred pathway for the derivatives production	The economic potential of the strategy nowadays[a]	References
L-Pipecolic acid	27	0.79	45.1	0.87	17.25	0.69	Whole-cell catalysis	+	[56, 86, 99]
6-Aminocaproic acid	0.031	0.012	–	–	0.16	0.32	Fermentative process	–	[70, 89, 106]
cadaverine	42.58	0.29	221	0.92	88	0.50	Whole-cell catalysis	+	[36, 41, 59, 61]
5-Aminovalerate	20.8	0.69	90.6	0.94	47.96	0.8	Whole-cell catalysis	+	[46, 64, 68]
δ-Valerolactam	–	–	54.35	0.60	0.705	0.071	Chemo-enzymatic synthesis	–	[8, 64, 101, 102]
ε-Caprolactam	–	–	–	–	0.00215	0.001	Chemo-enzymatic synthesis	–	[8, 14, 102]

The table just lists the highest concentrations or yields of the derivatives nowadays achieved. More chemicals might be included due to the development of the bio-catalysis strategy

[a]+ means that the bio-transformation strategy nowadays has economic potential; – means that the bio-transformation strategy nowadays has not economic potential nowadays

Strain improvement

Lys fermentation is the No. 2 oldest amino acid fermentation processes next to microbial glutamate synthesis, which is first industrialized by the Kyowa Hakko Bio Co., Ltd. [29]. Besides the famous strain *C. glutamicum*, *E. coli* has recently also been enrolled in the Lys production.

Escherichia coli

In an era of metabolic engineering and synthetic biology, Lys-producing *E. coli* has been constructed by rational design, and reached a yield capacity almost as higher as that of *C. glutamicum* industrial strains.

An engineered *E. coli* strain was reported to reach 130 g/L titer, with a yield of 0.45 g/g glucose. Systematic metabolic engineering was adopted, with *ppc* (phosphoenolpyruvate carboxylase), *aspA* (aspartase ammonia-lyase), and *pntB* (pyridine nucleotide transhydrogenase) overexpression [98]. Overexpression of the Lys exporter YbjE could also enhance the Lys production [65]. Recently, the ensemble modeling (EM) framework is carried out, and desensitization of aspartate kinase is found to be a rate-controlling step in the L-lysine (L-Lys) pathway [10]. A synthetic RNA device is used to screen the key enzymes involved in Lys biosynthesis pathway in *E. coli*. It is reported that a chimeric aspartate kinase shows 160% increase in vitro activity, and results in 0.674 g/L Lys production when introduced in vivo in engineered strains [92].

Corynebacterium glutamicum

Based on the developmental history of *C. glutamicum*, three stages are clearly shown. The first stage was related to the traditional cognition known as "metabolic regulatory fermentation". Rational design was then executed, and the strain improvement strode forward into the second stage, which was called "metabolic engineering". Recently, the third stage is achieved, which is characterized by "genome breeding" and "synthetic biology" [94, 95]. Recent improvements focus on carbon utilization, metabolic precursor enhancement, genome reduction, as well as new screening method to further reduce the cost.

The uptake of glucose could be activated by the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS), as recognized until decades ago. However, recently, myo-inositol transporters, especially proteins encoding by *iolT1* and *iolT2*, have been identified. Overexpression of these two genes could accelerate the glucose uptake rate [27, 43]. The *bglF*-specified EII permease and glucokinase form the third non-PTS glucose transporter system, which has first been acknowledged in *C. glutamicum* ATCC 31833, could contribute to further enhance the carbon utilization while increasing their activities in vivo [28]. The spectrum

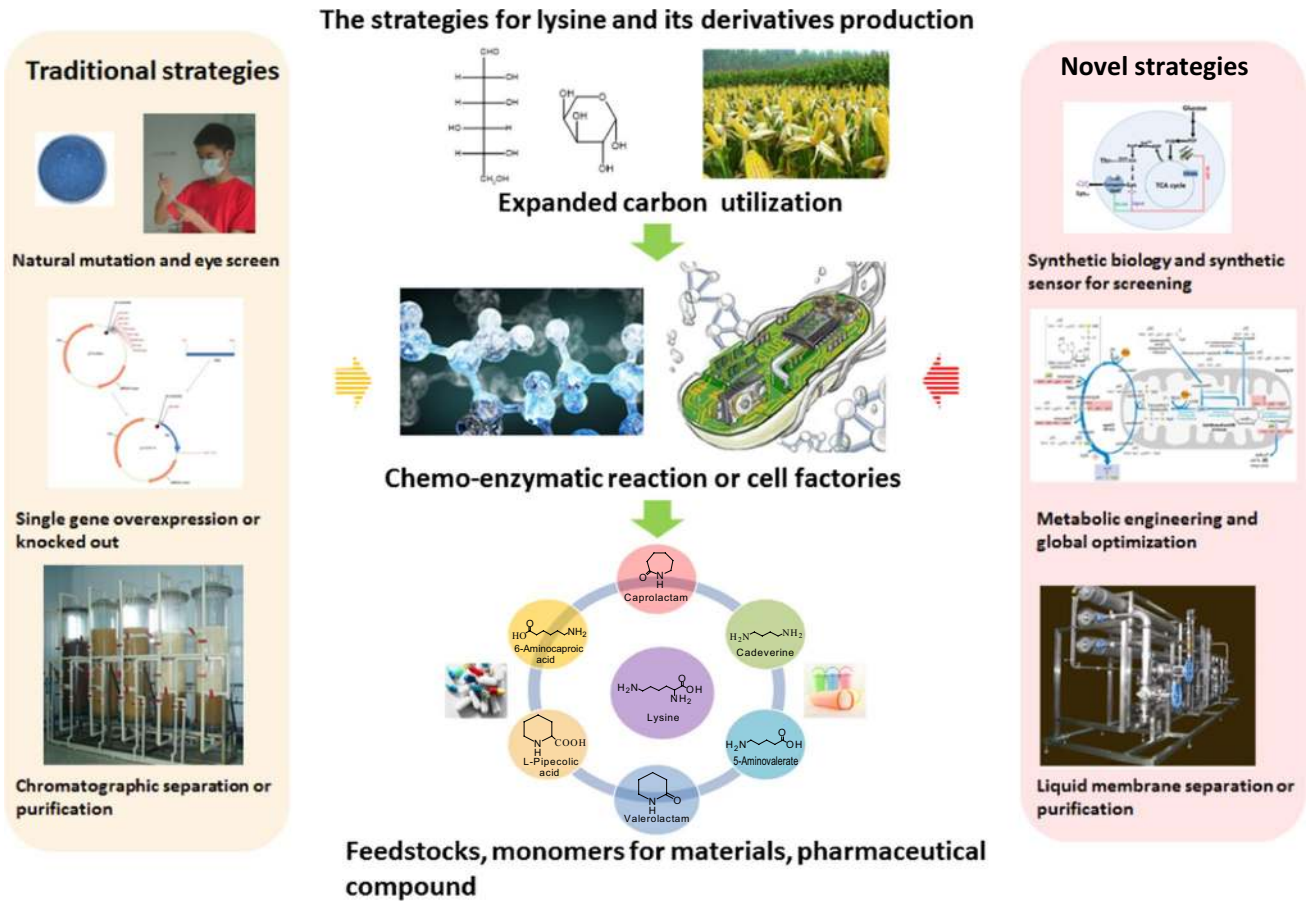

Fig. 3 Production of lysine and novel lysine derivatives from biomass, traditional technologies, and novel strategies

of carbon source of the engineered strains could be expanded by heterologous pathway reconstituted. Arabinose, ethanol, and acetic acid, even the industrial molasses [97], and the lignocellulosic hydrolysate could support the good growth and productivity of *C. glutamicum* [77, 100]. With the introduction of the heterogeneous *araBAD* operon and the *xylA* gene, the recombinant could produce 42 mM Lys on rice straw hydrolysate and wheat bran hydrolysate [20]. Since the expenditure on carbon source occupies a majority of the fermentation cost, expanding the substrate utilization has a significant effect on the low-cost biomanufacturing of the Lys.

Recently, rational design and metabolic engineering has developed extensively that novel engineered strains have been constructed and the supplement of the precursor has been enhanced in vivo. It was found that a one-step dehydrogenase reaction could replace the original four-step succinylase reaction [4]. Kind reported that the supplementation of succinyl-CoA could be enhanced with the deletion of the succinyl-CoA synthetase within the tricarboxylic acid cycle, and a Lys yield of 0.17 mol/mol glucose was achieved [40, 41]. To avoid the transformation of D,L-diaminopimelate to peptidoglycan, overexpression of lysA could increase the Lys production with the enhanced decarboxylation reaction [4]. In addition, recently, introduction of a mutation MurE, which was a UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate:meso-diaminopimelate ligase utilizing D,L-diaminopimelate as the substrate, could result in increased Lys concentration [5, 6].

In the history of strain improvement, classically generated strains and rational designed strains are combined together as repertoire for the selection of production strains. Recently, new screening methods are used, which expedites strain development. Based on the tandem ligation of gene *lysE* encoding the transcriptional activator LysG and eYFP, a Lys sensor was constructed [5]. The analysis of large amounts of single cells by fluorescence-activated cell sorting (FACS) strategy could facilitate the selection of new Lys producers. In Binder's study, a mutation *murE* (G81E) as an efficient D,L-diaminopimelate utilizing ligase was identified and overexpressed for Lys production enhancement [6].

Synthetic biology, especially the riboswitch, could help us to dynamically control metabolic pathways, and thus be applied for Lys production. A recombinant strain *C.*

glutamicum LPECRS was constructed to dynamic control of the enzyme citrate synthase and *lysE*, with two artificial riboswitches and additional deregulated aspartokinase. The strains achieve a 21% increase in the yield compared to the control strain [107]. System engineering under the guidance of in silico modeling helped the genetic engineering of the Lys strains [4]. In this work, 12 changes in genes encoding central metabolic key enzymes pushed major carbon fluxes towards the Lys synthesis route, and finally resulted in a titer of 120 g/L of Lys. Recently, introduction of *S. mutans*-type glycolic pathway in *C. glutamicum* has led to a fabulous result in L-Lys production [85]. Another experiment, which replaced the NADH biosynthesis enzyme with NADP-dependent GADPH, could also increase the cofactor supplementation. The final L-Lys productivity achieved 2.73 g/L h for *C. glutamicum* Lys5–8 [96]. However, there are advantages of using a deleted host. Therefore, many studies following similar strategies focus on the dissection of cellular networks and construction of strains with a reduced genome [15, 104]. Different combinatory deletions of all irrelevant gene clusters were investigated, which decreased the size of the native genome [90]. The strains with irrelevant clusters deletion could grow on defined medium".

Other microorganisms

Although *C. glutamicum* and *E. coli* was considered as the most powerful industrial strains, some other organisms are undergo constructing to obtain significant Lys production ability.

Since industrial condition is often high temperature, thermotolerant bacterial organisms such as *B. methanolicus* and *C. efficiens* have been focused on being developed as promising Lys producers [58]. *C. efficiens* is phylogenetically close to *C. glutamicum*, while the former could grow at approximately 10 °C higher than the latter [60]. *B. methanolicus* could grow on methanol, an alternative carbon source which does not compete with human food, while grow at 35–60 °C [45].

Fermentation optimization

Based on the engineered or mutated strains, fermentation optimization is executed to achieve high concentration of Lys to facilitate the downstream purification. In different countries, different industrials, with different equipment and different substrates, fermentation optimization usually needs to start from scratch, although the same strain is used.

The carbon source accounts for the majority of the fermentation cost, so its utilization should be paid much attention. Except for the strain improvement in the utilization of the low value carbon source as indicated in the last section, the optimization of the kinds of the substrate, the substrate initial concentration, and the substrate fed module also contributes to the decreasing of the fermentation cost. In China, starch hydrolysate, i.e., corn syrup, is the substrate usually used for Lys fermentation, while soybean hydrolysate is usually used in America. South America and Europe give preference to beet molasses and cane, respectively, due to the cost and availability of the substances. A titer of 130 g/L with a yield of 0.45 g/g glucose and 120 g/L could be reached in *E. coli* and *C. glutamicum*, respectively.

Industrial fermentation is executed in large-scale tank fermenters with a volume of 500 kL or above and often adopts fed-batch fermentation process to accumulate high titer of Lys in the final process. Another fermentation style is called continuous fermentation. In this style, the fed-batch fermentation process could be extended by sucking out part of the broth one or several times intermittently and supplement the fermenter with fresh broth or concentrated nutrition at a specific rate, correlating to the total substrate concentration, or the pH, or the specific growth rate of the strain, etc. This fermentation strategy could often result in higher productivity and final concentration, while it gives full play to the production capacity of mature strains by prolonging the synthesis period.

Downstream processing

In the Lys whole production process, downstream expenditure often occupies 60–80% of the total cost, depending on the purity of the final product and the intended use. In the past, the animal-feed Lys from the broth has mainly been recovered based on the developed chromatographic separation [13]. However, this strategy often results in lower product concentrations and increased cost of waste-water treatment. Recently, novel promising technologies are emerged and explored for Lys purification as industrial applications.

Traditional production process produces Lys sulfate and/or Lys hydrochloride, using sulfate and/or chloride as counter anion(s) to main pH. However, a new process, which employs hydrocarbonate and carbonate ions to produce Lys carbonate, is developed [42]. On the one hand, sequestration of the CO_2 is advocated by the government; on the other hand, decreasing the amounts of ammonium chloride and/or ammonium sulfate supplemented into the broth result in significant reduce of the fermentation cost and the environmental loads. Recently, retracting of Lys was investigated with *sec*-octylphenoxy acetic acid in sulfonated kerosene or supported liquid membrane (SLM) on a flat sheet. A mixture of mono-(2-ethylhexyl) ester of phosphoric acid (M2EHPA) and bis-(2-ethylhexyl) ester of phosphoric acid (D2EHPA) is used as carriers, while kerosene adds as diluent. All the studies reveal new mechanisms and give high extraction efficiency [50, 105].

Spray drying has many advantages such as relative ease in operation, easy industrialized production, cost-effectiveness, and ready availability of suitable equipment [17, 18, 25]. Spray-dried fermentation broth is also a commercially available lysine preparation on the market [34]; for example, Evonik and Global Bio-chem Technology Group Company Limited all used this strategy. Henke et al. have described the metabolic engineering of *C. glutamicum* for the combined production of lysine and cell-bound value-added compounds [24]. A proof of principle was proposed, and 48 g/L Lys and 10 mg/L astaxanthin were coproduced by fed-batch fermentation. Moreover, this strategy could be applied to secreted Lys production with the cell-bound carotenoids decaprenoxanthin, zeaxanthin, canthaxanthin, and lycopene [29].

Development of new Lys-derived products

Except the recent efforts to further reduce the Lys production cost, to maintain the price of the original Lys and the profit of the amino acid industry in the international market, the best way is to extend the industry chain, especially to develop the new Lys-derived products.

Lys derivatives for bio-based materials

The potential usage of Lys as precursor for polymer materials relates to the functional group it gains, which includes the amino group and the carboxyl group. Under special conditions with catalysts, the derivatives of Lys might retain the functional groups to form products with structural similarity, which could be used as monomers for polymer materials. The monomers for polyamides are now produced from fuel refineries, but biomanufacturing could be a potential strategy to replace the traditional production. Here, we will show that the monomers of nylon family, like cadaverine, 5AVA, valerolactam, caprolactam, etc., could also be obtained through bio-transformation.

Cadaverine

Also named 1,5-diaminopentane, cadaverine has various applications in industry and agriculture [3]. Most importantly, bio-based cadaverine could be used as an important platform chemical to generate various bio-based polyamides such as PA 54 [54], PA 510 [41], and PA 512 [36]. Cadaverine could be generated by microbial fermentation or whole-cell bio-catalysis from L-Lys via microorganisms, which includes recombinant *E. coli* strains [38, 81, 84] and *C. glutamicum* [37, 39, 61, 87].

For microbial fermentation, Qian et al. reported that while the cadaverine utilization and degradation pathways were inactivated [57, 69], the production of cadaverine could be enhanced in the engineered strain. The maximum cadaverine yield of 9.61 g/L from glucose was achieved in *E. coli* [69]. A *C. glutamicum* strain DAP-16 was engineered by Kind et al., in which L-Lys decarboxylase (LDC) encoded by LDC gene (cadA) and a major facilitator permease were overexpressed. The cadaverine yield of 88 g/L could be accumulated in *C. glutamicum* [41, 59]. Cadaverine production from xylooligosaccharides using engineered strains displaying xylosidase on the cell surface was also investigated [30]. Finally, the engineered strains enabled production of 1.18 g/L cadaverine from 13 g/L of consumed xylooligosaccharides. Furthermore, Tween 40 could enhance the cadaverine production in engineering strains [52]. One of the reason might be the increase in fluxes for the anaplerotic reactions; another possibility might be an increase in membrane permeability with Tween 40 addition.

At present, the whole-cell bioconversion is another promising method explored for efficient cadaverine synthesis from L-Lys. The PelB signal sequence had an important effect on the whole-cell bioconversion of L-Lys to cadaverine. Cadaverine antiporter (CadB) was expressed with the PelB signal sequence, which could generate cadaverine of 221 g/L, increasing the production of cadaverine by 12% [48]. The pyridoxal 5-phosphate pathway was introduced into the engineered *E. coli* strain BL-CadA, which could produce cadaverine of 168 g/L [49]. The effects of buffering conditions, substrate concentrations, substrate pH, and biocatalyst concentrations were optimized, and a final concentration of 133.7 g/L was obtained with a molar yield of 99.9% [61].

5-Aminovalerate

5-Aminovalerate (5AVA) is a potential feedstock for the manufacture of nylon 5 and nylon 6,5, and also a valuable C5 platform chemical used to produce glutarate, valerolactam, 1,5-pentanediol, and 5-hydroxyvalerate [46]. Due to the importance of 5AVA, biotechnological production of 5AVA has attracted considerable interest.

5AVA is naturally produced through the 5AVA pathway by *Pseudomonas putida* [72–74]. 5AVA synthesis is sequentially mediated by Lys 2-monooxygenase (DavB) and δ-aminovaleramidase (DavA) as follows: in the first step, L-Lys is converted to 5-aminovaleramide by DavB; in the second step, 5-aminovaleramide is hydrolyzed to 5AVA by DavA (Fig. 4a). Park et al. found that while DavB and DavA were overexpressed in the recombinant *E. coli* WL3110, the engineered strain could generate 3.6 g/L 5AVA with L-Lys as the substrate [63]. DavB and DavA were purified and coupled to form 5AVA by enzymatic method. Under this strategy, 20.8 g/L of 5AVA could be achieved from 30 g/L Lys [46]. High cell density fermentation and whole-cell catalysis were carried

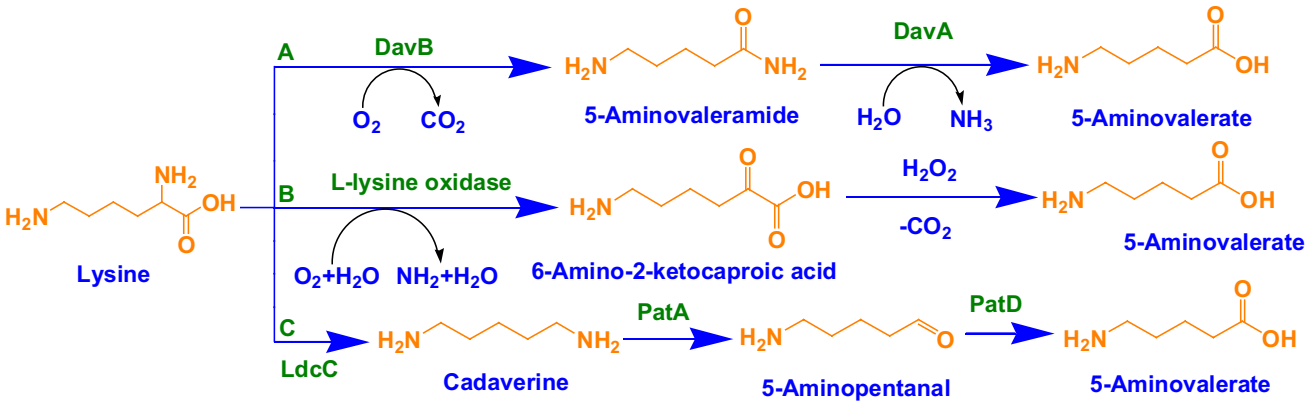

Fig. 4 Scheme for the production of 5-aminovalerate from L-lysine. The enzymes included in those routes are: A: lysine 2-monooxygenase (*DavB*), delta-aminovaleramidase (*DavA*); B: L-lysine α-oxidase (*rAIP*); C: L-lysine decarboxylase (*LdcC*), putrescine transaminase (*PatA*), and γ-aminobutyraldehyde dehydrogenase (*PatD*)

out for the production of 5AVA with recombinant *E. coli* strain W3110/DavAB. The highest yield of 90.59 g/L 5AVA was achieved consuming a substrate of 120 g/L of L-Lys [64]. Various *C. glutamicum* strains including different origins of replication and promoters were engineered by Shin et al.. The superior strain could generate 33.1 g/L 5AVA from 250 g/L glucose [78]. Recently, Jorge et al. proposed a new route to produce 5AVA from Lys via cadaverine as intermediate (Fig. 4c) [31]. The pathway involved LDC, putrescine transaminase (PatA), and γ-aminobutyraldehyde dehydrogenase (PatD). A final concentration of 5.1 g/L 5AVA and a yield of 0.13 g/g could be achieved. Moreover, a de novo bio-based production process for the coupling production of two C5 platform chemicals 5AVA and glutarate was established [76]. The optimized strain could generate 28 g/L 5AVA with a maximal productivity of 0.9 g/L h.

Kusakabe et al. first proposed another 5AVA synthesis pathway [44]. The α-carbon atom of Lys was oxidized into 6-amino-2-keto-caproic acid, NH_3 and H_2O_2 by L-Lys α-oxidase (LysOx) from *Trichoderma viride*, which then could be oxidatively decarboxylated to form 5AVA with no addition of catalase (Fig. 4b). The 5AVA was successfully produced by the immobilized LysOx in the absence of catalase [68]. A 13.4 g/L of 5AVA was achieved in aerobic incubation for 5 days at 37 °C.

δ-Valerolactam

δ-Valerolactam is usually obtained by the cyclization of 5AVA, which is dehydrated under vacuum conditions [64]. Nylon 5 is formed by the self-polymerization of δ-valerolactam. Nylon 6,5 is generated by the polymerization of δ-valerolactam and ε-caprolactam initiated by acetyl-caprolactam (acetyl-CL) and catalyzed by potassium *tert*-butoxide (PtB) [64]. Therefore based on the discussion of "5-Aminovalerate" in this paper, a hybrid route to obtain δ-valerolactam from bio-based Lys could be constructed. The production of δ-valerolactam and ε-caprolactam was demonstrated using recombinant *E. coli* strains with the overexpression of an acyl-CoA ligase ORF26 from *Streptomyces aizunensis* [102]. The recombinant stains could produce δ-valerolactam and ε-caprolactam when 5AVA and 6ACA were added to the culture medium. Moreover, an efficient platform metabolic pathway for the production of δ-valerolactam and ε-caprolactam was constructed [8]. ω-Amino acids were activated by β-alanine CoA transferase (Act) from *Clostridium propionicum* followed

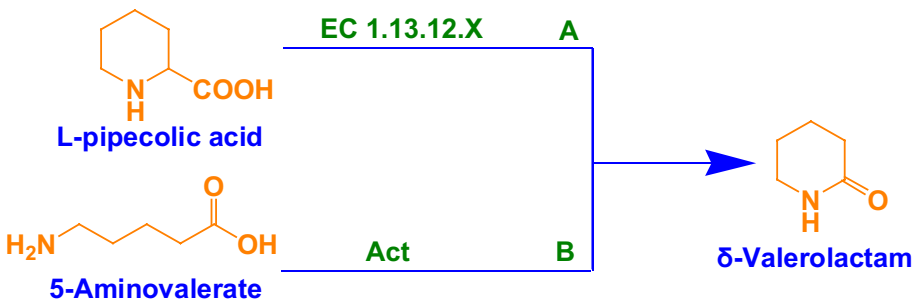

Fig. 5 Synthesis of δ-valerolactam from L-pipecolic acid or 5-aminovalerate. There are potential enzymes to do this work. A: like lysine 2-monooxygenase (DavB) (EC 1.13.12.2) and lactate 2- monooxygenase (EC 1.13.12.4); B: β-alanine CoA transferase (Act)

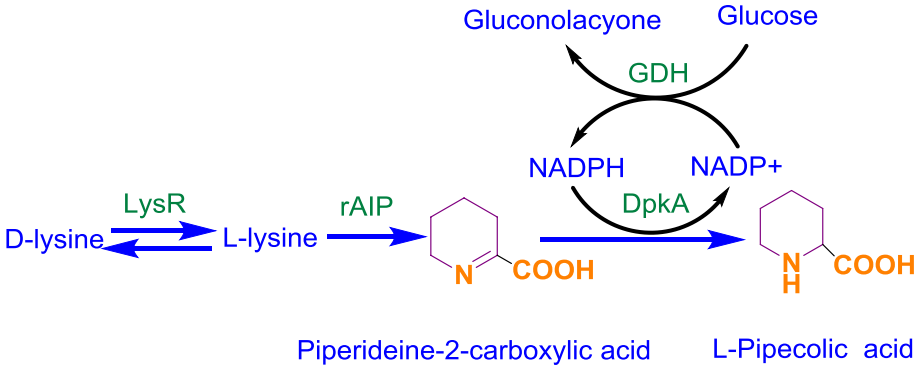

Fig. 6 One-pot bioconversion to L-pipecolic acid from DL-lysine. The enzymes included in these routes are: lysine racemase (*LysR*), L-lysine α-oxidase (*rAIP*), glucose dehydrogenase (*GDH*), and Δ^1-piperideine-2-carboxylase/reductase (*DpkA*)

by spontaneous cyclization (Fig. 5b). In addition, zhang et al. discovered a lactam biosensor based on the ChnR/Pb transcription factor-promoter pair, which could sense δ-valerolactam and ε-caprolactam in a dose-dependent manner [101]. This biosensor could potentially be applied for industrially high titer lactam biosynthesis.

To exclude the chemical synthesis, another novel route is proposed based on L-PA (Fig. 6). Converting D-Lys to L-PA could be found in D-Lys metabolic pathway [55]. Comparing the chemical structure of L-PA and δ-valerolactam, it could be deduced that an oxidative decarboxylase might catalyze the transformation of δ-valerolactam from L-PA (Fig. 5a). There are potential enzymes to do this work, like DavB (EC 1.13.12.2), lactate 2-monooxygenase (EC 1.13.12.4), etc. However, the natural substrates of these enzymes are not L-PA, so rational design and evolution of enzymes might be required.

ε-Caprolactam

As it has been discussed in the above sections, ε-caprolactam is the starting material for nylon 6,5 synthesis and the bulk building block chemicals for nylon 6 nowadays in the worldwide. Frost et al. developed a route for the production of ε-caprolactam, where biomass-derived lysine was chemically converted into ε-caprolactam [14]. The process was as follows: in optimized conditions, a cyclization reaction was initiated after neutralization of lysine hydrochloride with sodium hydroxide (NaOH). The resulting α-amino-ε-caprolactam could then be transformed to ε-caprolactam with the subsequently addition of KOH and hydroxylamine-*O*-sulphonic acid under − 20 °C.

A microbial 6ACA synthesis pathway from L-Lys is supposed by our lab and is shown in Fig. 8. If this hypothesis pathway could be confirmed by further experiments, ε-caprolactam could be obtained by the chemical cyclization or bio-cyclization of purified bio-6-ACA. The chemo-enzymatic catalysis strategy might replace the nowadays production of ε-caprolactam from oil.

Lys derivatives for active pharmaceutical ingredients or drugs

Bio-transformation of Lys to the medical intermediate or final therapeutically drugs could expand the Lys industry and result in increasing profits of these enterprises. Since Lys naturally has chiral structure, the derivatives obtained from Lys through enzyme catalysis or whole-cell catalysis often keep this characteristic, and could be used as a chiral compound with biological function. This section will focus on the production of two kinds of medical intermediate L-PA and 6ACA.

L-Pipecolic acid

L-Pipecolic acid (L-PA) is a crucial non-proteinogenic amino acid. It is a very important intermediate of many pharmaceutically and biologically compounds, such as the anticancer agents swainsonine, VX710, and sandramycin [22], the immunosuppressive agents FK506 and rapamycin [16, 35], the anaesthetic analogue ropivacaine and bupivacaine [1, 62], and antibiotics meridamycin, demethoxyrapamycin, Cyl-2, virginiamycin, apicidin, and trapoxins [80, 93] could also be synthesized from L-PA. Currently, synthesis of L-PA involves biosynthesis [53] and chemical synthesis, such as enantioselective reduction [19, 75], diastereoselective synthesis [9], and stereoselective transformation [12]. However, these chemical synthesis processes are difficult to provide an economic and acceptable manner for the production of chiral L-PA on large scale due to the low yield, the cumbersome procedures, and other disadvantages. The current market price of the L-PA produced by chemical synthesis reaches up to 15,000–30,000 $ per ton. Therefore, the biosynthesis of the L-PA has been attracted great attention from the chemical company worldwide.

It has been known that there are four synthetic routes of L-PA. The Δ^1-piperideine-2-carboxylase (P2C) pathway and the Lys cyclodeamination pathway are both via the loss of the α-nitrogen of Lys and the condensation of the ε-nitrogen to produce L-PA. The Δ^1-piperideine-6-carboxylase (P6C)

pathway and the α-aminoadipic acid pathway are both through the loss of the ε-NH_2 and the incorporation of α-NH_2 into L-PA [22]. Lys cyclodeaminase (LCD) alone could catalyze the direct formation of L-PA from L-Lys. Tsotsou and Barbirato revealed further the presence of iron(II) and glycerol could obviously improve the LCD activity [88]. Byun et al. investigated the optimal reaction parameters for LCD from *Streptomyces pristinaespiralis* to the production of L-PA, such as temperature, pH, buffer condition, and carbon length of substrate [7]. The purification and the first characterization in vitro of LCD encoded by the *rapL* gene were studied, and the mechanism of the LCD reaction was revealed [22]. Ying et al. used an engineered *E. coli* strain harboring another LCD encoded by *pipA* from *Streptomyces hygroscopicus*. This whole-cell conversion process with NAD^+ supplement could reach a relative higher L-PA production of 17.25 from 25 g/L Lys [99].

Muramatsu et al. utilized an enzyme-coupled system for the production of L-PA from L-Lys based on a combination of LysOx, Pip2C reductase, and glucose dehydrogenase (GDH). This system could produce 27 g/L of L-PA [56]. Tani et al. constructed a one-pot synthesis of L-PA by overexpression of LysOx from *Scomber japonicus*, GDH from *Bacillus subtilis*, Lys racemase (LysR), and Pip2C reductase from *Pseudomonas putida* in *E. coli* strains. 45.1 g/L of L-PA could be obtained after 46 h in this one-pot process, which was by far the highest yield of L-PA with substrate flexibility for industrial application [86] (Fig. 6).

In our lab, LysOx, Pip2C reductase, Lys permease (LysP), and GDH are overexpressed in a mutated *E. coli* strain ML04 with *cadA* knocked out, in which ribosome-binding sites and promoters for each gene are optimized. This strain could produce 46.3 g/L L-PA at the end of the 48 h fed-batch fermentation, which is the highest level reported in the world (unpublished data). Furthermore, the Lys 6-dehydrogenase gene and pyrroline 5-carboxylate reductase gene were overexpressed to produce L-PA, and the final yield of 0.09 g/g could be achieved [66]. Furthermore, fermentative production of L-PA from glucose glycerol, starch, glucosamine, and xylose was investigated [67]. L-PA production from these alternative carbon sources was established by expressing the heterologous genes *glpF* (glycerol facilitator), *glpK* (glycerol kinase), and *glpD* (glycerol-3-phosphate dehydrogenase), etc.

6-Aminocaproic acid

6-Aminocaproic acid (6ACA) is a non-natural amino acid, which could potentially inhibit the activity of some enzymes, such as plasmin, elastase, and pepsin. Therefore, it could be potent in treatment of some bleeding disorders [47, 83]. 6ACA is also the building block of the PA 6. Therefore, production of 6ACA from bioresources, especially Lys, could significantly reduce the emissions of the fuel refineries and be more biosafety preferred.

Raemakers-Franken et al. previously showed that L-Lys could be converted to 6ACA by a series of chemical and biological methods. The L-Lys is successively converted to 6-amino-2-hydroxyhexanoate, 6-aminohex-2-enoate, 6-aminohexanoate, and 6ACA sequentially [70]. The direct production of 6ACA from 5-formyl valeric acid was investigated [71]. Turk et al. first proposed two complete biosynthetic pathways for the fermentative production of 6ACA [89] (Fig. 7). The adipoyl-CoA route starts with the condensation of acetyl-CoA and succinyl-CoA. Another important pathway is the α-ketopimelate route, which starts with acetyl-CoA and 2-oxoglutarate using biosynthetic pathway enzymes for coenzyme B [26]). This pathway could directly produce 160 mg/L 6ACA from glucose after 120 h. If there are some unknown enzymes that could catalyze the alpha aminoadipic acid to form ketoglutaric acid or succinyl-CoA, 6ACA could be potentially formed from Lys, as depicted in Fig. 7. A strategy to simultaneously vary genetic and process factors was first reported by Zhou et al., which was then applied to 6ACA production and increased the final titer from 9 to 48 mg/L [106].

Here, a new 6ACA synthesis pathway engineered in *E. coli* using the leucine pathway enzymes LeuABCD together with the LysOx, 2-keto-acid decarboxylase (KIVD), and phenylacetaldehyde dehydrogenase (PadA) (Fig. 8) is proposed [51, 82, 103]. LysOx is the first step to transform Lys into 6-amino-2-keto-caproic acid, and then, LeuABCD could perform the C1-elongation to produce 2-keto-7-aminoheptanoate, which could be decarboxylased by KIVD and oxidized to 6-ACA by PadA. However, this engineered pathway has not yet been confirmed by any experiments until now.

Concluding remarks

Transformation of high-value-added chemicals from renewable biomass, especially from the overcapacity of fermentative Lys, will be successful if the process cost is competitive compared with the traditional fuel refineries and derivative industry chain and, however, supports from government, and the taxpayers are welcomed to transform this green manufacturing manner from lab to industrial scale. Moreover, life-cycle analysis should be done to confirm the feasibility of the new mode of manufacturing.

In this review, several kinds of chemicals could be produced from Lys, which includes L-PA, cadaverine, 5AVA, δ-valerolactam, 6ACA, and ε-caprolactam, but there might be some other chemicals could be converted from Lys and not discussed here. Except for L-PA and 6ACA, which could be used as pharmaceutical intermediate, the other chemicals discussed are all related to the nylon polymer

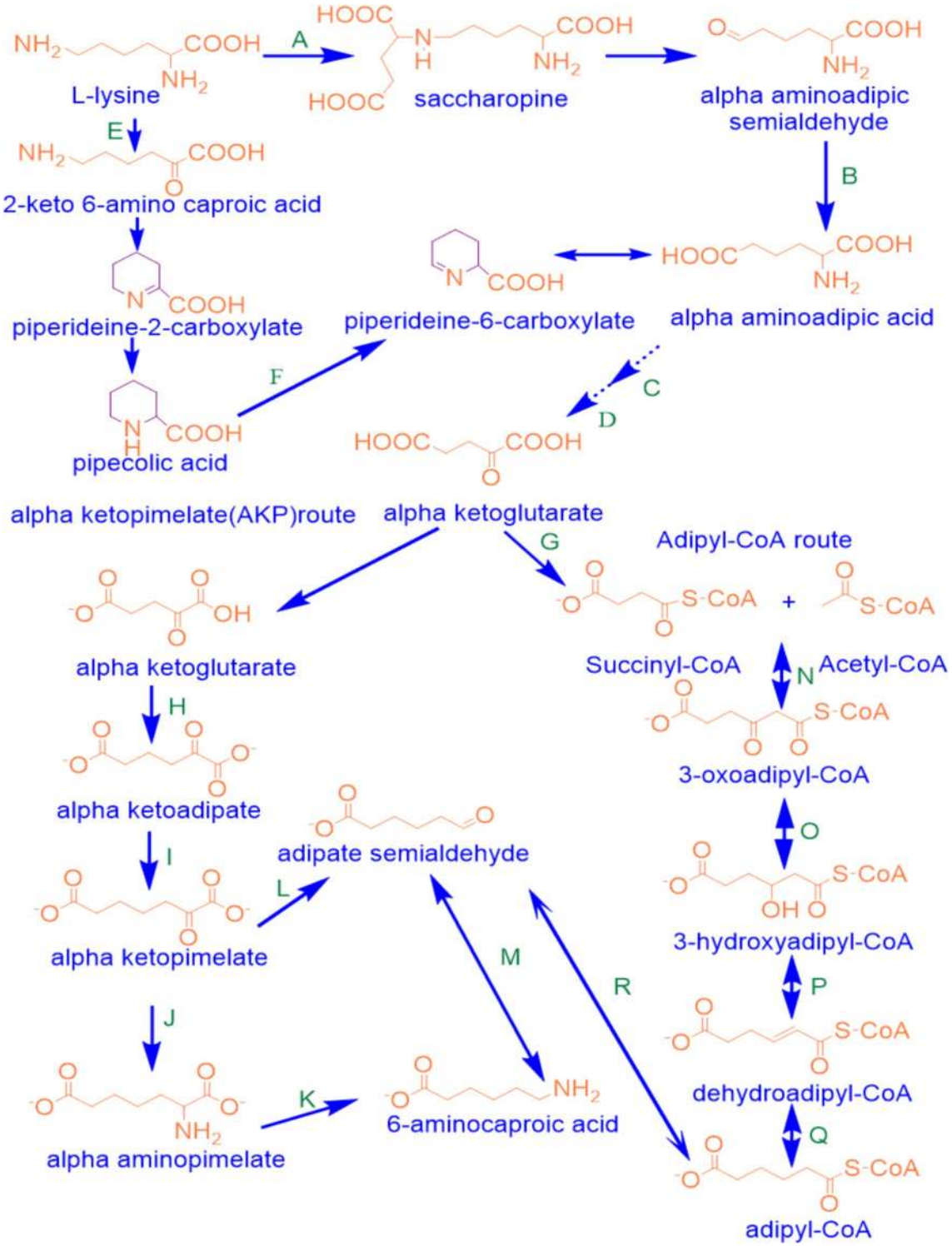

Fig. 7 Schematic representation of the AKP and adipoyl-CoA route for fermentative production of 6-ACA from lysine, partly is confirmed by experiments and partly is deduced from the references. The most important enzymatic activities of these routes are or might be: aminoadipic semialdehyde synthase (AASS) (A), aminoadipic semialdehyde dehydrogenase (B), unknown enzymes (C, D), deaminase (E), pipecolic acid oxidase (F), unknown enzyme (G), (R)-homocitrate synthase, (R)-homocitrate dehydratase, cis-homoaconitate dehydratase and threo-isohomocitrate dehydrogenase (H), (homo)2citrate synthase, dihomocitrate dehydratase, cis-(homo)2aconitate dehydratase and threoiso(homo)2citrate dehydrogenase (I), Aminotransferase (J), 2-aminopimelate decarboxylase (K), α-ketopimelate decarboxylase (L), aminotransferase (M), 3-oxoadipyl CoA thiolase (N), 3-hydroxyadipyl-CoA dehydrogenase (O), enoyl-CoA hydratase (P), hexenoyl-CoA-reductase (Q) (acetylating), and aldehyde dehydrogenase (R)

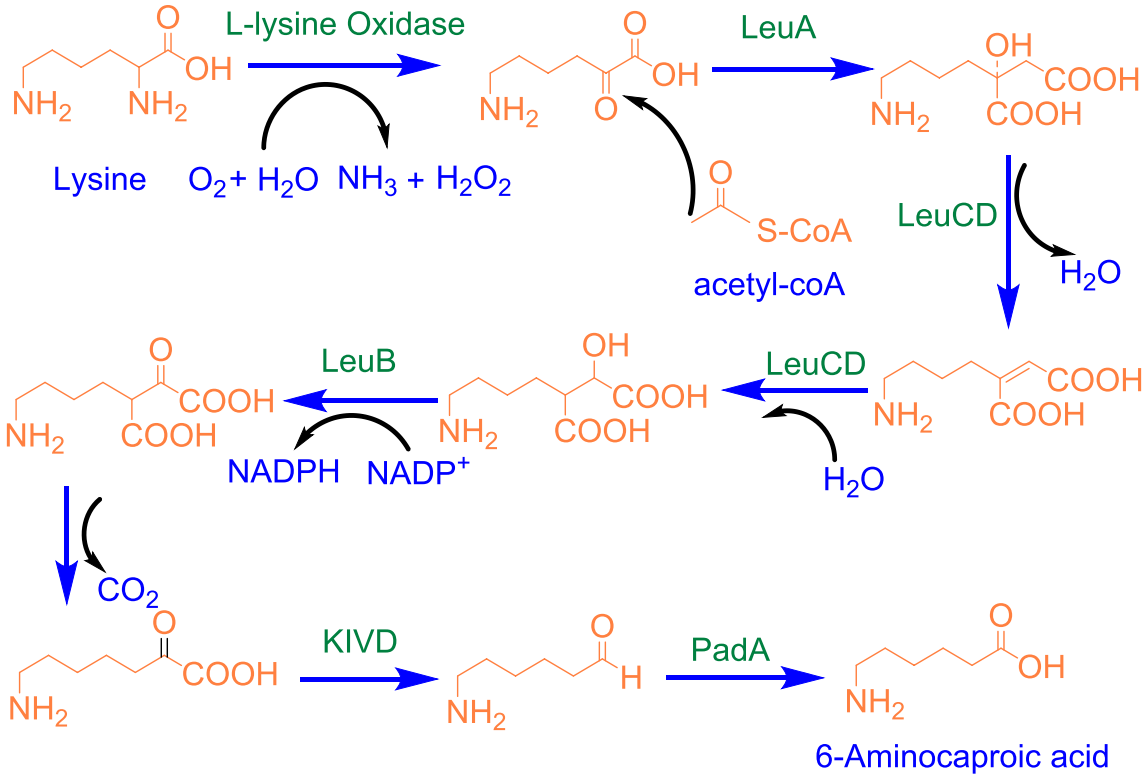

Fig. 8 Proposed engineered pathway for the biosynthesis of 6-aminocaproic acid. The most important enzymatic activities of these routes are: lysine oxidase, 2-isopropylmalate synthase (*LeuA*), isopropylmalate isomerase complex (*LeuC*, *LeuD*) and 3-isopropylmalate dehydrogenase (*LeuB*), 2-keto-acid decarboxylase (*KIVD*), and aldehyde dehydrogenases (*PadA*)

production. Since nylon is well known for their durability and strength, it is indispensable for modern life and the amount demanded are arising year by year. Considering the decreasing oil reserves and increasing environmental pollution, it is important to build biochemical routes to develop a renewable and clean bio-based nylon production to satisfy the need of the people and society.

In the traditional Lys production industry, studies are undergoing to further decrease the production cost and the market price, which include strain improvement, fermentation optimization, and downstream processing as reported. With great efforts for low-cost biomanufacturing of Lys and the possibility of using "bio-based manufacturing" Lys as a feedstock, the Lys industry might be expanded in the next decades and enterprises still could maintain profits amidst fierce competition.

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