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MINI-REVIEW

L-Amino acid oxidases from microbial sources: types, properties, functions, and applications

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Abstract L-Amino acid oxidases (LAAOs), which catalyze the stereospecific oxidative deamination of L-amino acids to α-keto acids and ammonia, are flavin adenine dinucleotide-containing homodimeric proteins. L-Amino acid oxidases are widely distributed in diverse organisms and have a range of properties. Because expressing LAAOs as recombinant proteins in heterologous hosts is difficult, their biotechnological applications have not been thoroughly advanced. LAAOs are thought to contribute to amino acid catabolism, enhance iron acquisition, display antimicrobial activity, and catalyze keto acid production, among other roles. Here, we review the types, properties, structures, biological functions, heterologous expression, and applications of LAAOs obtained from microbial sources. We expect this review to increase interest in LAAO studies.

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Introduction

L-Amino acid oxidases or deaminases (LAAOs; EC 1.4.3.2) are widely distributed in mammals (Puiffe et al. 2013; Nakano and Danowski 1966), snake venom (Du and Clemetson 2002), insect venoms (Sakurai et al. 2001), sea hare (Yang et al. 2005), fungi (Davis et al. 2005), bacteria (Huang et al. 2011), and algae (Vallon et al. 1993). As a result, LAAOs form a family of proteins with various enzymatic properties, similar overall structure, and a wide range of biological functions and applications. First described by Zeller and Maritz in (1944), LAAOs are predominantly dimeric flavoproteins with some monomeric types (Yang et al. 2012). They contain non-covalently bound flavin adenine dinucleotide (FAD) as a cofactor (Pawelek et al. 2000). LAAOs catalyze the stereospecific oxidative deamination of L-amino acids to α-keto acids that produces ammonia and H_2O_2 via an imino acid intermediate (Macheroux et al. 2001). Other LAAOs produce water instead of H_2O_2 and are associated with a respiratory electron transport chain, donating electrons to the quinone pool that are then transferred to a terminal cytochrome oxidase to reduce molecular oxygen to H_2O. Specifically, membrane-bound amino acid oxidases are associated with the respiratory electron transport chain (Anraku and Gennis 1987; Franklin and Venables 1976; Jones and Venables 1983; Olsiewski et al. 1980; Yu and DeVoe 1981). The membrane-associated LAAOs in *Proteus mirabilis* (Pelmont et al. 1972) and *Proteus rettgeri* (Duerre and Chakrabarty 1975) are suggested to interact with the respiratory electron transport chain.

Until recently, LAAOs in snake venom had been the most thoroughly investigated member of this enzyme family with respect to toxicology, biochemistry, physiology, and medicine. LAAOs are attracting increasing attention owing to their key biological roles, such as keto acid production, anti-tumor and antimicrobial activity, amino acid consumption, and anti-insect properties. Some of the biological roles of LAAOs are associated with the H_2O_2 produced (Ehara et al. 2002).

In this review, we provide an overview of the types of LAAOs, their structural, enzymatic, and functional properties, and recent advances in microbial LAAO production and application. Future research directions for the development of cost-effective fermentation processes using LAAOs on an industrial scale are also discussed.

Types of LAAOs from microbial sources

From a biochemical point of view, LAAOs from various sources are distinguished by substrate specificity, molecular mass, regulation, and post-translational modification. This assortment suggests that LAAOs have undergone enormous evolutionary alterations since their split-up from a putative ancestral protein (Macheroux et al. 2001). To date, different types of LAAOs have been reported from various microbes, including bacteria and fungi. Bacteria such as *Streptomyces* sp. X-119-6, *Rhodococcus opacus*, lactic acid bacteria, *Proteus mirabilis*, *Proteus vulgaris*, *Proteus rettgeri*, *Pseudomonas* sp. P-501, *Pseudoalteromonas tunicata*, *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas flavipulchra*, *Marinomonas mediterranea*, *Rheinheimera* sp., *Streptococcus oligofermentans*, *Corynebacterium*, *Cellulomonas cellulans*, *Bacillus carotarum*, *Providencia*, *Lechevalieria aerocolonigenes*, and *Synechocystis* sp. PCC 6803 produce different types of LAAOs. The fungal LAAOs from *Trichoderma viride*, *Trichoderma harzianum* (Cheng et al. 2011), *Aspergillus oryzae*, *Aspergillus flavipes*, *Aspergillus nidulans*, *Aspergillus fumigatus* (Singh et al. 2009), *Neurospora crassa* (Sikora and Marzluf 1982), *Hebeloma cylindrosporum* (Nuutinen et al. 2012), and *Laccaria bicolor* (Nuutinen and Timoneni 2008) produce various types of oxidases.

Functional, enzymatic, structural properties, and cellular localization of LAAOs

Some functional properties and cellular localization of the different types of LAAOs are shown in Tables 1 and 2, respectively. One of the main biological functions of LAAOs is antimicrobial activity. The *Pseudoalteromonas flavipulchra* LAAO has broad substrate specificity against methicillin-resistant *Staphylococcus aureus* that occurs during the stationary phase of the strain growth curve (Chen et al. 2010). The antimicrobial activity of LAAO is likely associated with H_2O_2 formation generated by enzyme activity because its antimicrobial effect is significantly decreased in the presence of H_2O_2 scavengers such as catalase and peroxidase. LAAOs can also be intracellular, membrane bound, or extracellular (Table 2) which depend on their sources as well as their corresponding functions.

Table 1 Sources of different microbial L-amino acid oxidases and their main functions

Microbial sources	Major functions	References
Bacillus subtilis	Oxidation of L-glycine	Nishiya and Imanaka 1998
Escherichia coli	Oxidation of L-aspartate	Bossi et al. 2002
Rhodococcus opacus	Oxidation of L-amino acid	Geueke and Hummel 2003
Streptomyces sp. X-119-6	Oxidation of L-glutamic acid	Arima et al. 2003
Proteus mirabilis	Deamination of L-phenylalanine, L-histidine, L-glutamic acid	Massad et al. 1995; Baek et al. 2011; Liu et al. 2013
Proteus vulgaris	Deamination of L-methionine	Takahashi et al. 1999
Providencia alcalifaciens	Oxidation of L-lysine	Hanson et al. 1992
Aspergillus nidulans	Oxidation of L-amino acid	Davis et al. 2005
Trichoderma viride	Anti-tumor, oxidation of L-lysine	Kusakabe et al. 1980
Trichoderma harzianum ETS 323	Biocontrol agent, oxidation of L-phenylalanine	Cheng et al. 2011
Pseudomonas putida	Oxidation of L-aspartic acid	Leese et al. 2012
Pseudomonas sp. P501	L-phenylalanine deamination	Koyama 1982
Marinomonas mediterranea	Antimicrobial, oxidation of L-lysine	Lucas-Elio et al. 2006
Corynebacterium A20	Oxidation of L-lysine	Coudert and Vandecasteele 1975
Neisseria meningitidis	Oxidation of L-cysteine	Yu and DeVoe 1981

Table 2 Cellular localization of different microbial L-amino acid oxidases

Cellular localization	Sources	References
Intracellular		
L-amino acid oxidase	*Rhodococcus opacus*	Geueke and Hummel 2003
L-glutamate oxidase	*Streptomyces sp*. X-119-6	Arima et al. 2003
L-aspartate oxidase	*Pseudomonas putida*	Leese et al. 2012
L-aspartate oxidase	*Escherichia coli*	Bossi et al. 2002
Membrane bound		
L-amino acid deaminase (*pma*)	*Proteus mirabilis*	Baek et al. 2008
L-amino acid deaminase (*pm1*)	*Proteus mirabilis*	Baek et al. 2011
L-amino acid deaminase	*Proteus vulgaris*	Takahashi et al. 1999
Extracellular		
L-amino acid oxidase	*Cellulomonas cellulans* AM8	Braun et al. 1992
L-amino acid oxidase	*Trichoderma harzianum* ETS 323	Yang et al. 2011

LAAO of *Streptococcus oligofermentans* seems to have a role in competition between species (Tong et al. 2008). A function of LAAO from the cyanobacteria *Aspergillus nidulans* is in electron transfer on the thylakoid membrane (Pistorius and Voss 1982). The LAAOs found in insects are cytotoxic, killing tumor cells via H_2O_2 generation (Ahn et al. 2000). Similarly, the L-tryptophan oxidase isolated from *Chromobacterium violaceum* is implicated in the biosynthesis of violacein, which is believed to have bactericidal and tumoricidal activities (Genet et al. 1995). As an immunosuppressive enzyme expressed in a series of 315 human malignancies, L-phenylalanine oxidase could be involved in a general mechanism for the relationship between tumor cells and the immune system (Carbonnelle-Puscian et al. 2009). Moreover, α-keto acids, which are the products of LAAO reactions, have roles as siderophores, as seen in *Proteus mirabilis* (Massad et al. 1995).

The enzymatic properties of LAAOs depend on their microbial sources, and these properties are usually dissimilar. Generally, the optimum pH of LAAOs depends on substrate, and enzyme activity is relatively stable within the range from 25 to 45 °C (Arinbasarova et al. 2012; Leese et al. 2012; Liu et al. 2013). LAAOs can be inactivated by decreasing the pH and vice versa (Wellner 1966), and inactivation by freezing is more pronounced between −20 and −30 °C (Curti et al. 1968). These characteristics suggest that enzyme inactivation is likely due to conformational changes in the protein structure (Curti et al. 1968). Most LAAOs exhibit a broad range of substrate spectra. They display a noticeable preference for hydrophobic amino acid substrates such as phenylalanine, leucine, tyrosine, and tryptophan (Du and Clemetson 2002; Mandal and Bhattacharyya 2008). This substrate partiality perhaps comes from the binding of hydrophobic side chains of amino acids with the enzyme. The catalysis is proposed to follow one of two diverse mechanisms: in the first pathway, the proton is transferred to the FAD cofactor from the α-carbon atom of the substrate, leaving a negative charge followed by a two-electron transfer which is also called a carbanion pathway; and in the second mechanism, an α hydrogen atom is transferred as a hydride ion carrying two electrons simultaneously by a hydride transfer pathway (Fitzpatrick 2004).

The LAAO isolated from *Rhodococcus opacus* oxidizes 39 L-amino acids, including not only all of the 20 L-amino acids but also some derivatives (Geueke and Hummel 2002). In addition, LAAOs of *Proteus rettgeri* (Duerre and Chakrabarty 1975), *Mytilus edulis* (Blaschko and Hope 1956), *Proteus vulgaris* (Stumpf and Green 1944), *Cellulomonas cellulans* (Braun et al. 1992), and *Corynebacterium* (Coudert and Vandecasteele 1975) exhibit broad substrate specificity. Although a few LAAOs accept D-amino acids as substrates, the reaction rates are relatively low compared with those using L-amino acids. The LAAO from *B. carotarum* 2Pfa accepts 10 L-amino acids and 7 D-amino acids as substrates (Brearley et al. 1994), among which, 3 aromatic L-amino acids phenyl alanine, tyrosine, and tryptophan are preferred. Some LAAOs, such as those from the fresh water cyanobacteria *Synechococcus elongatus* PCC 6301, *Synechococcus elongatus* PCC 7942, *Synechococcus cedrorum* PCC 6908 (Gau et al. 2007), and *Anacystis nidulans* (Pistorius and Voss 1980), have relatively narrower substrate specificities, with high preference for only basic L-amino acids. By contrast, several LAAOs have very firm preferences for specific substrates and are named according to their favored substrate—for example, L-glycine oxidase from *Bacillus subtilis* (Nishiya and Imanaka 1998), L-cysteine oxidase from *Neisseria meningitidis* (Yu and DeVoe 1981), L-glutamate oxidases from *Streptomyces endus* (Böhmer et al. 1989) and *Streptomyces* sp. (Arima et al. 2009), and L-lysine oxidases from *T. viride* (Kusakabe et al. 1980),

Pseudoalteromonas tunicata (James et al. 1996), and *Marinomonas mediterranea* (Lucas-Elio et al. 2005).

Structural knowledge about microbial LAAOs is extremely limited. LAAO from *Rhodococcus opacus* has been crystallized and examined with preliminary x-ray analysis (Faust et al. 2006, 2007). LAAOs have a range of isoelectric points varying widely from pH 4.0 to 9.4 and molecular masses between approximately 50 and 300 kDa. In general, LAAOs are first synthesized as precursors carrying a signal peptide and then form mature proteins via post-translational modification through limited proteolysis, possibly owing to their potential toxicity (Ahn et al. 2000). The LAAO activity that catalyzes the stereospecific oxidation of L-amino acids along with the release of ammonia and H_2O_2 is speculated to be toxic to or have negative effects on the growth of host cells. Therefore, LAAO exists in cells as a precursor with low initial activity and then is secreted with high activity from cells after digestion by an endopeptidase. For example, the LAAO from *Neurospora crassa* consists of 695 amino acids as a precursor, a length of 129 amino acids longer than that of the mature enzyme (Neidermann and Lerch 1990). Similarly, the mature form of L-phenylalanine oxidase from *Pseudomonas* sp. P-501 is produced and triggered by the proteolytic cleavage of a noncatalytic proenzyme (Ida et al. 2008). The *Rhodococcus opacus* LAAO gene shows an open reading frame coding for 534 amino acids, including a signal peptide of 45 amino acids (Geueke and Hummel 2002). Along with these structures, L-glutamate oxidase (LGOX) from *Rhodococcus opacus*, LAAO from *Streptomyces* sp. X-119-6 (Arima et al. 2009), L-aspartate oxidase from *Escherichia coli* (Bossi et al. 2002), and L-phenyl alanine oxidase from *Pseudomonas* sp. P501 (Ida et al. 2008; 2011) have been reported. A topological view of those structures shows that each LAAO subunit consists of three domains: one for FAD binding, one for substrate binding, and a helical domain. For example, the structure of L-aspartate oxidase includes a FAD-binding domain with the classic dinucleotide-binding fold observed in many flavoproteins, a capping domain with an irregular $\alpha + \beta$ topology, and a helical C-terminal domain. Although the structures of these enzymes are similar to a certain extent, they have several differences that make them unique. In the LGOX structure, two funnel-shaped entrances with distinct functions lead from the surface to the active site (Arima et al. 2009). The structure of L-phenyl alanine oxidase suggests that the pro-sequence peptide occupies the funnel of the substrate amino acid from the outside of the protein to the interior flavin ring (Ida et al. 2008). However, no funnel orientating and directing the substrates to the active site is present in the LAAO from *Rhodococcus opacus*. This funnel conformation and the structure of the enzyme are probably associated with substrate specificity, and the absence of a funnel may explain the broader substrate specificity of the *Rhodococcus opacus* LAAO (Faust et al. 2006). Moreover, LGOX has a hexameric structure, $\alpha_2\beta_2\gamma_2$, and by recombinant expression, it has been shown to have a homodimeric structure of its precursor (Arima et al. 2003).

Gene cloning and heterologous expression of LAAOs

The first bacterial heterologous expression of an LAAO of *Rhodococcus opacus* was reported by Geueke and Hummel (2003). The *lao* gene encoding LAAO was cloned into *E. coli*, *B. subtilis*, and *Streptomyces lividans* expression vectors. Expression in *E. coli* results in the accumulation of insoluble protein, but *Streptomyces lividans* is a relatively suitable host for the heterologous production of LAAO (Geueke and Hummel 2003; Liu et al. 2013). Comparison of native and recombinant LAAOs has shown the same specific activities (Liu et al. 2013), similar spectral properties, and the same molecular mass. The LAAO isolated from sea hare, which is not a microbial source, was first functionally expressed in *E. coli*, but the expression level for soluble recombinant LAAO is relatively low—approximately 0.2 mg/L culture medium (Yang et al. 2005). This phenomenon has several explanations: (1) much of the LAAO is present in insoluble inclusion bodies, and (2) LAAO inhibits the growth of *E. coli* and other bacteria at doses below 1 mg/L, which also likely inhibits the level of bacterial expression. Other LAAO genes from *Streptococcus oligofermentans* and *Proteus mirabilis* have been successfully cloned and overexpressed in *E. coli* BL21(DE3) strain (Liu et al. 2013; Tong et al. 2008). The functional expression of LAAO in heterologous hosts remains an enormous challenge.

To date, a wide variety of LAAO-encoding sequences have been published, revealing that LAAO family members have flavin as a common coenzyme, with the exception of the lysine oxidase of *Marinomonas mediterranea* (Lucas-Elio et al. 2006) and two characteristic sequence motifs. One is a dinucleotide-binding motif comprising a β-strand/α-helix/β-strand of the secondary structure; the other is a GG motif (x-G-G-R-x-x) positioning shortly after the dinucleotide-binding motif (Vallon 2000). In 1990, the *Neurospora crassa* LAAO gene was cloned and sequenced (Neidermann and Lerch 1990). The *Synechococcus* PCC6301 LAAO nucleotide sequence (Bockholt et al. 1995) infers that the protein consists of 355 amino acid residues showing no homology with the LAAO of *Neurospora crassa* (Neidermann and Lerch 1990), in which the expression of LAAO can be induced via addition of L-amino acids to nitrogen-starved cultures as well as addition of protein synthesis inhibitors or D-amino acids (DeBusk and Ogilvie 1984a, 1984b; Neidermann and Lerch 1991; Sikora and Marzluf 1982). Its gene expression is controlled by NIT2 and the *nmr* gene product, and the LAAO gene is regulated at the transcriptional level. Likely because of the

post-translational modification requirement of LAAO, only some heterologous expression systems have been reported.

Methods for measuring LAAO activity and high-throughput screening

A variety of methods can be used to measure LAAO activity, including capacity of production of compounds such as ammonia (Timmer et al. 2005), α-keto acid (Singh et al. 2009), and H_2O_2 (Okubo et al. 2012); measurement of oxygen consumption using a classic Warburg manometer or an oxygen-sensitive electrode (Ralph et al. 2006); and measurement of amino acid substrate change (Roth 1971). All of these methods have both benefits and drawbacks. Although detection methods for the measurement of H_2O_2 production have been most widely used for LAAOs assays in which horseradish peroxidase (HRP) acts as an H_2O_2-sensitive probe, in-gel detection of L-amino acid oxidases based on the visualization of H_2O_2 production is a reasonable and reproducible method (Rau and Fischer 2011; Yu et al. 2013). However, most HRP substrates, including 2,2′-azinobis (3-ethylbenzthiazoline-6) sulfonic acid, o-phenylenediamine, and o-dianisidine are tremendously toxic or carcinogenic, and even HRP is easily inactivated and costly. Moreover, high-throughput screening (HTS) is essential for the production of mutant LAAOs with improved activity and specificity for certain L-amino acids. Although mutagenesis techniques such as directed evolution and gene mosaics have become prevalent, the sensitivity and selectivity of direct enzyme assay has not advanced. In some cases, a peroxidase/o-dianisidine-based assay has been used to screen the library of mutant clones on microplates, but the method is hazardous, labor-intensive, time-consuming, and requires multiple handling of each clone—namely, cultivation in microplate wells, cell transfer to assay microplates, cell lysis, cell separation, and activity determination. Therefore, a suitable method must be developed for the HTS assay of LAAO that can detect micro-quantities of the enzyme in biological fluids and intact cell colonies and is also fast and cost-effective.

Applications of LAAOs

LAAOs have important potential biotechnological applications as components of biosensors (Mutaguchi et al. 2011; Pollegioni and Molla 2011). The fast and precise determination of amino acid concentrations has become increasingly important for a varied range of applications including medical, biological, and food technological analyses. For example, in case of phenylketonuria (PKU), which is a disorder of phenylalanine metabolism (Huang et al. 1998), abnormal levels of phenylalanine can be used to diagnose. In case of bioprocess optimization, amino acid concentrations can be used to monitor and control the state of fermentation processes (Varadi et al. 1999). In case of animal nutrition, concentrations of essential amino acids have a vital influence on animal weight gain. Although, amino acids can be measured by liquid- or gas-chromatography and spectrometric and colorimetric methods (Sarkar et al. 1999), the complex laboratory procedures, expensive equipment, and a reasonable time for the measurements are drawbacks for all of those methods (Pei and Li 2000). These problems can be overcome by using a simple, rapid and reliable amino acid biosensor based on the LAAOs. L-amino acids can be determined using an LAAO/platinum biosensor (Chauhan et al. 2013). L-Amino acid concentrations have been measured through amperometric measurement of oxygen consumed by LAAO using specific potentials and electrode systems (Kacaniklic et al. 1994; Simonian et al. 1994) or hydrogen peroxide produced (Preuschoff et al. 1993). For rapid determination of the concentration of L-amino acids, a unique amperometric biosensor has been developed using LAAO by immobilizing with poly(carbamoyl) sulfonate hydrogel (Kwan et al. 2002). LAAOs have also been used as biosensors in the food industry to determine the quality of many products by their L-amino acid content (Lee and Huh 1999; Varadi et al. 1999), in particular, L-lys in maize, rice, and wheat. Although, there are some drawbacks of LAAO sensors due to their various sensitivities to different substrates and the variations in their kinetically limited sensitivity to particular amino acids (Scheller and Schubert 1989), their applicability is much simpler and cost-effective compared to other measuring methods. In food processing, LAAOs can be applied in the enzymatic treatment of dough to reduce viscosity and provide better workability (Christiansen and Budolfsen 2002).

LAAOs can be used in textile bleaching and it has been observed that the bleaching effect of hydrogen peroxide, which is produced enzymatically by LAAOs in typical washing, the bleaching and cleaning process can be considerably improved, even in the absence of any activators. A novel LAAO of *T. harzianum* in combination with its substrate has been used for in situ generation of hydrogen peroxide in the textile bleaching and may be favorably incorporated into detergent compositions that include a peroxidase for preventing the relocation of dye from dyed fabric to other fabrics during washing (Schneider et al. 1998). It also can be mixed with toothpaste or used for cosmetic preservation. Moreover, the enzyme has been mentioned in applications for wastewater treatment, pulp bleaching in the paper industry, water treatment in pulp production, and lignin improvement for particle board production (Schneider et al. 1998).

LAAOs have important potential biotechnological applications as catalysts in biotransformations of L-amino acids (Mutaguchi et al. 2011; Pollegioni and Molla 2011). LAAO from *Proteus mirabilis* can be used in the production of α-

ketoglutarate from L-glutamic acid (Hossain et al. 2013; Liu et al. 2013). The LAAO of *Rhodococcus* sp. is used in bioconversion to synthesize amino adipic derivatives that are precursors for β-lactam antibiotics (Isobe et al. 2008) and for the biotransformation of L-DOPA to its α-keto acid (Findrik et al. 2006). LAAOs from *Providencia alcalifaciens* and *T. viride* have been applied as a catalyst in the biotransformation of Nε-carboxy(CBZ)- L-Lys into the corresponding keto acid (Hanson et al. 1992).

Since optically pure D-amino acids have industrial significance as chiral building blocks for the synthesis of pharmaceuticals, food ingredients, and drug intermediates, LAAOs can be used to separate enantiomers from racemic mixtures of D-/L-amino acids (Jang et al. 2012; Qi et al. 2009; Singh et al. 2009). LAAOs can also be useful as anti-cancer and anti-tumor drugs. For example, a *Trichoderma*/L-lysine oxidase has been tested in vivo on several tumors and cancers and presented encouraging data on the promising application of this LAAO in clinical oncology for patients with colorectal cancer (Pokrovsky et al. 2013; Treshalina et al. 2000). Studies have also proved the efficacy of sequential pretreatment with LAAO and LAAO antiserum in the modulation of melphalan activity against intracranial glioma (Moynihan et al. 1997).

LAAOs can be used as biofertilizers for nitrogen acquisition and as biocontrol agents. LAAOs that show the capability to release nitrogen from amino acids (Nuutinen and Timoneni 2008) are present in many soil microorganisms with a variety of lifestyles. Therefore, LAAO-producing microorganisms are potential candidates for biological control or biofertilizers. Most LAAOs from microorganisms have broad substrate spectra, making them ideal molecular mechanisms for the acquisition of nitrogen from diverse amino acid sources. For example, an LAAO of *Neurospora crassa* can be induced by L-Arg and L-Phe in the absence of readily metabolizable nitrogenous compounds (DeBusk and Ogilvie 1984c). Another LAAO of *Chlamydomonas reinhardtii* is also inducible if no primary nitrogen source is available to produce ammonia (Muñoz-Blanco et al. 1990). In 2005, Davis et al. found that the LAAO of *Anacystis nidulans* is the primary route of catabolism for amino acids such as L-cysteine, L-histidine, L-leucine, L-lysine, L-methionine, L-α-amino butyrate, and L-citrulline for growth (Davis et al. 2005). LAAOs expressed in *Hebeloma* spp. and *Laccaria bicolor* can also catalyze the mineralization of amino acid nitrogen to NH_4^+ (Nuutinen and Timoneni 2008). The released inorganic nitrogen might then be re-assimilated and distributed to organic compounds, which complements the previously identified amino acid catabolic machinery, e.g., glutamate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase. In general, bacterial LAAOs are assumed to be necessary in nitrogen and L-amino acid metabolism for the plants. The necessary steps and research perspectives to develop the LAAOs for the future applications are summarized in Fig. 1.

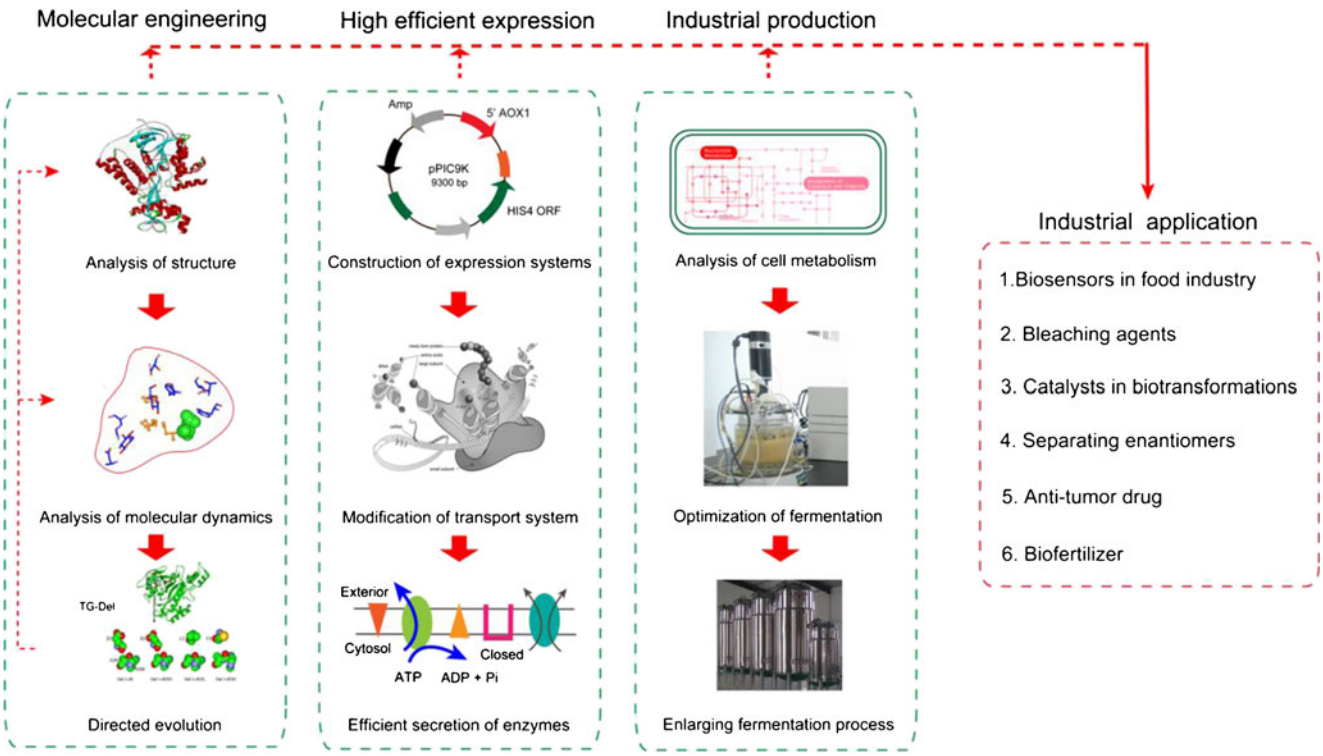

Fig. 1 The research perspectives of LAAOs: from molecular engineering to high-level expression to industrial production and applications

Conclusions and perspectives

The microbial production and application of LAAOs is gaining increasing attention, and noteworthy progress has been made to date. In future research, several key topics might be considered. First, a crucial factor for the successful development of biocatalytic processes is the possibility of using an inexpensive production enzyme with suitable properties (i.e., high activity, stability, and selectivity) on a large-scale. Developing whole-cell biocatalysts with evolvable properties is a potentially effective strategy to achieve this objective in industrial processes (Hossain et al. 2013). With whole-cell biocatalysts, the stability of the dimeric LAAOs could be increased, and downstream enzyme processing costs could be decreased significantly. Second, although the production of LAAOs is low in recombinant *E. coli*, the gram-positive bacteria *B. subtilis* and *Streptomyces lividans* may be promising overproducers of LAAOs for industrial use. Finally, to achieve superior LAAO utilization in biotechnological production, structural and protein engineering with mutagenesis techniques should be applied efficiently.

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