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# L-Amino Acid Oxidases-Microbial and Snake Venom

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## Abstract

L-amino acid oxidases (EC 1.4.3.2, L-aao) are flavoenzymes that catalyse the stereospecific deamination of an L-amino acid to their corresponding α-keto acid with the production of hydrogen peroxide and ammonia. These enzymes are widely distributed across diverse phyla from bacteria, fungi to mammals and many venomous snakes. Although they are mainly involved in cellular amino acid catabolism, many other physiological functions are attributed to L-aao including their antibacterial property and ability to protect from infection. L-aao has also been correlated with penicillin production, violacein synthesis and biofilm development and cell dispersal. Snake venom L-aaos are studied extensively for their ability to induce apoptosis, aggregate platelets, induce haemorrhage, edema and many other toxic effects. L-aaos have been characterized biochemically and found to differ in terms of biochemical parameters not only among different species but also among members of the same species. L-aao act on L-amino acids, preferentially basic, aromatic and aliphatic L-amino acids. The snake venom enzyme shows broad oxidizing activity towards aromatic and hydrophobic L- amino acids such as leucine, phenylalanine and isoleucine.

L-aaos have practical value in biochemical and chemical investigations as they have been used to destroy L-isomer of a racemic DL-amino acid and thus yield an optically pure preparation of the D- isomer. As such, L-aao finds numerous applications as catalysts in biotransformation and for production of keto acids. L-aao has also been used for the determination of L-amino acids as part of biosensors. L-amino acids are reported to be found in physiological fluids of patients with certain diseases and disorders. In addition, the content of certain amino acids essentially controls the nutritional quality of the food. L-aao is useful in this aspect by development of biosensors to detect the L-amino acids.

Snake venom L-aaos are known to induce apoptosis and antibacterial effects mediated by the hydrogen peroxide produced during the L-aao reaction. The hydrogen peroxide induces oxidative stress which in turn activates the heat shock proteins and initiates an array of functions ultimately leading to apoptosis and cell death. In this aspect, L-aao can be greatly useful for the development of efficient therapeutics and drugs to control tumor cells, bacterial, leishmanicidal, viral and protozoal infections. L-aao is also reported to display dose dependent inhibition on HIV-1 infection and replication and as such can be studied for development of anti HIV medicine.

**Keywords:** L-amino acid oxidases; Function; Characterization; Substrate specificity; Structure; Applications

## Introduction

Enzymes that catalyse the oxidation of amino acids have been known for many years. L-amino acid oxidases (EC 1.4.3.2) (L-aaos) are flavoenzymes that catalyse the stereospecific deamination of an L-amino acid substrate to their corresponding α-keto acid with the production of hydrogen peroxide and ammonia via an imino acid intermediate. These enzymes are widely distributed across diverse phyla from bacteria to mammals and many venomous snakes. L-aaos in microorganisms are involved in the utilization of nitrogen sources and those in animals have been characterized as having distinct biological and physiological functions.

Much attention has been given on the snake venom L-aaos which have become an interesting subject for pharmacological as well as structural and molecular biology studies. Snake venom L-aao has been characterized extensively in terms of their molecular mass, substrate preference, apoptosis, cytotoxicity, bactericidal activities etc.

In the present review emphasis will be given on the relatively recent advances in my knowledge of the flavoprotein L-aaos of organisms other than snakes and also to some extent on the snake venom L-aaos.

## Physiological role of L-aao

L-aao are reported to be extracellular enzymes in *Aplysia californica* [1], and *Myoxocephalus polyacanthocephalus* [2] while the enzyme was found to be localized in the cell envelope of *Proteus mirabilis* [3] and cytoplasmic membrane of *Proteus* sps [4]. In *Myoxocephalus polyacanthocephalus*, the skin mucus isozyme MPLAO3 contains a signal peptide, comprising residues Met1-Ala26. *Synechococcus elongatus* L-aao was found in the membrane where it helps in photosynthesis as part of photosystem II particles [5]. Different localization of L-aao was reported in *Meleagris gallopavo* [6] in mitochondria, while *Chlamydomonas reinhardtii* [7] and *Synechococcus elongatus* L-aao were found in the periplasm [8]. Soluble L-aao was also reported from *Corynebacterium* sp [9] and *Neurospora crassa* [10].

Fungal L-aao is involved in the utilization of amino acids as nitrogen sources. *Neurospora crassa* expresses an L-aao whose synthesis is induced in nitrogen starved cultures by amino acid addition [11]. Depending on the amino acid used as nitrogen source, the catabolic pathway using a broad range L-aao can coexist with alternative pathways in *Aspergillus nidulans* [12]. An L-aao having antitumour

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activity has been identified in the fungus *Trichoderma viride*. This enzyme is highly specific and is an L-lysine oxidase which also has promising antibacterial and cytotoxic properties [13].

Lysyl oxidase is a different type of L-aao found in mammalian tissues which catalyse the Ɛ-oxidative deamination of lysyl residues in mammalian sclerotic proteins, especially collagen and elastin to yield allysyl residues that rapidly crosslink those proteins during the formation of the extracellular matrix and therefore play an important role in the development, elasticity and extensibility of the connective tissue [14].

Mouse milk is enriched in various nutrients like proteins, carbohydrates, lipids, minerals and vitamins together with many bioactive substances and hence bears a great risk for bacterial infection and proliferation. But milk also contains antibacterial factors that protect the mother and the offspring. This antibacterial capacity of milk can be attributed to some extent on the presence of L-aao [15]. It displays antibacterial effect through the production of hydrogen peroxide from the oxidative deamination of free amino acids [16].

An interesting observation was made by Knight [17], on the correlation between the penicillin producing ability and the L-aao content of the penicillin producing molds. The highest penicillin producers (*P. chrysogenum*, *P. notatum*) are more active and have more L-aao content as compared to the low penicillin producres like *P. expansum*, *P. sanguineum*, *Aspergillus niger* etc.

*Hebeloma* sp and *Laccaria* bicolour contain L-aao that is involved in cellular amino acid catabolism. They are potential candidates for causing nitrogen mineralization from amino acids at the ecosystem level [18].

Since the first finding of antibacterial activity in an L-aao from snake (*Crotalus adamanteus*) venom [19], antibacterial L-aaos have been reported from various snake venoms of *Pseudochis australis* [20], *Trimeresurus jerdonii* [21] and *Bothrops alternatus* [22], the body surface of the giant African snail, *Achatina fulica Ferussac* (termed as achacin) [23], the albumen gland of the sea hare, *Aplysia kurodai* (termed as aplysianin A) [24] and the ink of the sea hare *Aplysia californica* (termed as escapin) [1]. Antibacterial LAO isozymes may be involved in the innate immunity which was demonstrated in the rockfish *Sebastes schlegelii* and the sculpin *Myoxocephalus polyacanthocephalus* skin. The skin secretion of the rockfish *Sebastes schlegeli* produces an L-aao which is a potent antibacterial protein with strict selectivity against Gram negative bacteria like *Aeromonas hydrophila*, *A. salmonicida*, *Photobacterium damselae* ssp piscida and *Vibrio parahaemolyticus*, but not against enteric bacteria such as *Escherichia coli* and *Salmonella typhimurium* suggesting the importance of the antibacterial protein as a primary innate immunity strategy in the rockfish skin. The antibacterial action is elicited by hydrogen peroxide generated from the enzyme reaction [25]. The skin mucus of *Myoxocephalus polycanthocephalus* contain an L-aao which has antibacterial activity against Gram positive and Gram negative bacteria and is most active against *Aeromonas salmonicida*. This enzyme containing the antibacterial activity help in the innate immunity of the sculpin skin [2].

Yang et al. [1] described a monomeric antibacterial protein from the purple ink of sea hare *Aplysia californica* which was named as escapin because of its potential role in sea hare defence. The escapin is released when the sea hare is attacked by predators and it has cytotoxic effects against a predatory sea anemone. This protein which is an L-aao has a wide spectrum of antimicrobial activities including that against bacteria, yeasts and fungi but with variable efficacies. The hydrogen peroxide generated from the enzyme reaction plays a prominent role in the bacteriostatic effect and a weak role in bactericidal effect.

The physiological role of L-aao in bacteria is greatly unknown. A tryptophan oxidase from *Chromobacterium violaceum* is involved in violacein synthesis [26]. In *Marinomonas mediterranea* Lod A shows lysine oxidase activity and it is described as a novel antimicrobial protein [27].

*Pseudoalteromonas tunicata* also expresses a protein similar to Lod A that has important role in biofilm development and cell dispersal [28,29].

The L-aao produced from *Pseudoalteromonas luteoviolacea* is also a broad range L-aao which has antimicrobial activity [30].

Snake venom is useful sources of bioactive substances showing a wide range of pharmacological activities. This complex cocktail of both toxic and nontoxic components includes several peptide and enzymes such as L-aaos which may represent 1-9 % of the total venom proteins. Although the exact biological function of snake venom L-aaos is still unknown, these enzymes are postulated to be toxins that may be involved in the allergic inflammatory response and specifically associated with mammalian endothelial cell damage [31,32], cytotoxic activities [33], induction of apoptosis, platelet aggregation, hemorrhage, edema and other toxic effects [34-36].

## Enzymatic Properties of L-aaos

L-aaos differ in the parameters of enzymatic properties greatly, not only among different species but also among the same species. A comparison of various L-aaos and their properties is given in Table 1.

In terms of substrate preference, L-aao show great variations. The *Pseudoalteromonas* L-aao has broad substrate specificity, oxidizing several amino acids but it shows some preference for L-glutamine [30].

Mouse milk L-aao accepts a broad substrate range i.e., phenylalanine, methionine, tyrosine, leucine, lysine and histidine but they do not oxidize isoleucine [15].

L-aao produced from *Marinomonas mediterranea* has high affinity for L-lys. α-N-acetyl-L-lys is a good substrate indicating that the amino group in position α is not oxidized by this enzyme. On the contrary, ε-N-acetyl-L-lys is not a substrate which points out that the modification of the ε-group abolished the enzyme activity. Other substrates with lower affinity are L-orn, D-lys and 5-hydroxy-L-lys. This shows the importance of the appropriate distance between the amino and the carboxyl groups in L-lys, the stereospecificity and the negative effects of the introduction of a hydroxyl group adjacent to the ε-amino group on the side chain of lysine. Other compounds with structural similarity to L-lysine, such as the tetrapeptide LSKL, amino acids such as arginine and p-amino-L-phenylalanine are not substrates of this enzyme [37].

The mold (*Penicillium chrysogenum*) enzyme deaminates simple short chain amino acids like L-alanine, L-methionine, L-cysteine more rapidly than longer, branched or substituted amino acids [17] while the substrate specificity of *N. crassa* enzyme is broad with the best utilized including L-histidine, L-α amino butyrate, L-canavaine and L-tyrosine [38].

Basic, aromatic and aliphatic L-amino acids are generally good substrates for *Rhodococcus opacus* L-aao. Threonine, proline and

| Species | Metal and ions | Inhibitors | pI value | pH optima | Temp. optima | Mol.wt. and subunits | Reference |
|---|---|---|---|---|---|---|---|
| *Anacystis nidulans* | NI | 2-napthol, $Ba^{2+}$, $Ca^{2+}$, $Cd^{2+}$, $Co^{2+}$,chloropromazine, $Cu^{2+}$,$La^{3+}$,$Mg^{2+}$,$Mn^{2+}$,$Na^{+}$, $Ni^{2+}$,o-phenanthroline,$NaN_3$, $Sr^{2+}$, $Zn^{2+}$ | NI | NI | NI | 98 kDa (dimer of 49 kDa) | [5] |
| *Rhodococcus opacus* | NI | Glycine (competitive inhibition) | 4.8 | 8 | 30°C | 99-104 kDa(dimer) | [39] |
| *Proteus sp.* | NI | Atebrine, HgCl, KCN, quinine sulphate, $NaN_3$ | NI | 7.0-7.6 | NI | NI | [4] |
| *Proteus vulgaris* | NI | Caprylic alcohol, Ag, Cu, Hg, 0.01 M HCN (88% inhibition) under aerobic condition | NI | 6.8 | 50°C | NI | [43] |
| *Proteus rettgeri* | NI | KCN, α,α'-dipyridyl,salicylaldoxime,1, 10-phenanthroline, 8- hydroxyquinoline | NI | 7.4-7.8 | NI | NI | [42] |
| *Meleagris gallopavo* | $Mn^{2+}$ (activator) | Mg, Fe and molybdene | NI | NI | NI | NI | [6] |
| *Chlamydomonas reinhardti* | $CaCl_2$ (2 mM, 50% activation) | EDTA (10 mM, 90% inhibition), hydroxylamine (5mM,75% inhibition) KCN (10mM,complete inhibition) NaF ( 10mM,85%inhibition) | NI | 9 | NI | 900-1300 kDa (oligomer) | [7] |
| *Aplysia californica* | NI | NI | NI | 7 | 37°C | 60 kDa (monomer) | [1] |
| *Neurospora Crassa* | NI | NI | NI | 9.5 | 49°C | 300 kDa | [10] |
| *P. luteoviolacea* | NI | NI | NI | NI | NI | 110 kDa, oligomeric | [30] |
| *Lechevalieria aerocolonigenes* | NI | NI | NI | NI | NI | 101 kDa (dimer of 55 kDa) | [68] |
| *Marinomonas mediterranea* | NI | B-APN, cadaverine, 6-aminocaproic acid (strong inhibitors) 5-aminovaleric acid, amiloride aminoguanidine, (weak inhibitors) | NI | NI | NI | 140 kDa | [27] |
| *Penicillium chrysogenum* | NI | Capryl alcohol, $CuSO_4$, $(NH_4)2SO_4$, 2-4-dinitrophenol, benzoic acid, iodoacetic acid | NI | 8-8.5 | 50-55ºC | NI | [17] |
| *Streptomyces endus* | NI | $Ag^{+}$ and $Hg^{2+}$ ions | 6.2-6.3 | 6.5-8.0 | 30-45ºC | 90 kDa, dimeric | [41] |
| *Bombyx mori* | NI | $CuSO_4$, $HgCl_2$ ($10^{-3}$M), KCN, EDTA ($10^{-2}$ M), riboflavin, isoriboflavin. | NI | 7.2 | 55ºC | NI | [81] |
| *Calloselasma rhodostoma* | NI | Anthranilate (competitive) | NI | 9 | NI | 132 kDa (dimer of 66 kDa) | [58,32] |
| *Crotalus durissus cascavella* | NI | Aspirin, indomethacin | 5.4 | 6.5 | NI | 68 kDa | [82] |
| *Trimeresurus mucrosquamatus* | NI | Benzoic acid, $CdCl_2$, HgCl, Iodoacetamide, KCN, $MnCl_2$, p-aminobenzoic acid, $ZnCl_2$ p-chloromercuribenzoate | 5.6 | 9 (L-trp), 8(L-his), 7(L-leu,phe,tyr) | NI | 140 kDa (dimer of 70 kDa) | [59] |
| *Crotalus adamanteus* | $Cl^{-}$ (activator) | Aromatic carboxylates, benzoate, iodoacetic acid, orthanilic acid, vinylglycine, mandelate | NI | 7.5 | NI | Dimeric with 58.7 kDa subunit | [83,84] |
| *Agkistrodon piscivorus piscivorus* | NI | Benzoic acid, Iodoacetic acid, $NH4^{+}$ | NI | 7.2-7.5 | NI | 150 kDa | [56] |
| *Rattus norvegicus* | NI | Benzenearsonic acid, Benzoic acid, $CuSO_4$, iodoacetic acid, p-chloromercuribenzoate, $NH^{4+}$ | NI | 8.8-9.2 | NI | 138 kDa | [56] |
| *Bothrops pirajai* | NI | NI | 4.9 | 6.0-7.4 | 37ºC | 130 kDa (dimer of 66 kDa) | [50] |

NI = No information

**Table 1:** Comparison between different L-aao in terms of some enzymatic properties.

glycine are the only proteogenic amino acids that are not accepted by this broad range enzyme. Among the aliphatic amino acids, alanine showed the highest activity followed by leucine, valine and isoleucine. Phenylalanine, tyrosine and tryptophan showed similar activity among the aromatic amino acids. Among the basic amino acids, asparagine and arginine are good substrates. The S-containing amino acids like serine, methionine and cysteine are also good substrates for this enzyme [39].

The *Saccharomyces cerevisiae* has a specific lysine oxidase activity; however it also accepts L-arginine, L-asparagine and other L-amino acids like alanine, leucine, glutamic acid and tryptophan as substrates [40].

*Streptomyces endus* is a specific L-glutamate oxidase which oxidizes only L-glutamate. L-aspartate, even in high concentrations is not converted to any extent. However the L-glutamic acid 4-benzyl ester is oxidized by the enzyme but the diester is not. The 4-substituted L-glutamic acid derivatives, L-glutamine and glutathione as well as peptide derivatives are also not substrates for this enzyme [41].

*Proteus rettgeri* contains two separable L-aaos that differ in their substrate specificities. One of them catalyses the oxidative deamination of aromatic, monoaminomonocarboxylic, sulphur containing, imino and β-hydroxy L-amino acids with no affinity for the basic amino acid, L-citrulline. The other oxidase catalyses the oxidative deamination of L-arginine, histidine, ornitnine, citrulline and lysine only [42] while the *Proteus vulgaris* enzyme oxidizes the unsubstituted monocarboxylicmonoamino, primary amino acids like nor-leucine, phenylalanine, leucine, tryptophan, methionine with the exceptions of alanine and valine [43].

*A. fumigatus* L-aao also shows certain degree of substrate preference. The enzyme has a greater specificity towards hydrophobic aromatic amino acids namely tyrosine and phenylalanine. D-amino acids are not attacked. The enzyme does not act on basic amino acids [44] and the best substrates for this enzyme are the L-isomers of phenylalanine, tyrosine, leucine and isoleucine.

L-aaos from various organisms exhibit different substrate specificity. For e.g. leukocyte L-aao (IL411) prefers aromatic L-amino acids such as phenylalanine [45] while the enzyme from ink of sea hare is most active to positively charged L-amino acids like arginine and lysine [1,46].

The snake venom enzyme shows broad oxidizing activity towards aromatic and hydrophobic L-amino acids such as leucine, phenylalanine and isoleucine [47,48]. In general, the best substrates for this enzyme are the L-isomers of phenylalanine, tyrosine, leucine, isoleucine, methionine and tryptophan [49-51].

*Bungarus fasciatus* L-aao (BF L-aao) substrate specificity is similar to that of L-aao from *Calloselasma rhodostoma*, *Naja naja kaouthia*, *Agkistrodon blomhoffii ussurensis*, *Bothrops jararaca*, *Daboia russellii siamensis*, *Vipera lebetina* in that these enzymes have affinity towards hydrophobic amino acids including phenylalanine, tryptophan, tyrosine and leucine. However, BF L-aao is also active towards acetic L-amino acids aspartic acid and glutamic acid [33,52,53]. The *Agkistrodon contortrix laticinctus* enzyme shows greater activity against hydrophobic L-amino acids but a significant activity was also present for the basic L-amino acids arginine and histidine but not detected for lysine [49].

FAD acts as a cofactor for these enzymes. L-aao family members possess in common, flavin as a coenzyme and two motifs, a dinucleotide binding motif comprising of β-strand/ α-helix/ β-strand of the secondary structure and a GG motif (R-X-G-G-x-x-T/S) shortly after the dinucleotide binding motif [54]. In *Anacystis nidulans* [5], 1 mol of FAD is bound per mol of enzyme while in *Bacillus carotarum* [55], *Rhodococcus opacus* [39] and most other organisms, the enzyme is a homodimer complex containing 2 FAD molecules.

Snake venom L-aao of *Agkistrodon piscivorous piscivorous*, *Crotalus adamanteus* [56], *Ophiophagus hannah* [57] binds to 2 FAD molecules per enzyme, while *Calloselasma rhodostoma* [58], *Trimeresurus jerdonni* [21], and *T. mucrosquamatus* [59] contain 2 mol of FMN per mol of enzyme.

An exception to this observation is that, the enzyme from *Marinomonas mediterranea* is an unusual amine oxidase. This melanogenic marine bacterium synthesizes marinocine, which is a type of lysine oxidase that has antibacterial activity. It does not depend on flavin as a cofactor, and is copper enzyme, requiring tyrosine derived quinine as a cofactor [37].

Although L-aao are flavoprotein enzymes, glycoprotein L-aao are reported in many organisms like *Aplysia californica* where the L-aao sequence contains one potential glycosylation site but glycosylation is not essential for its antimicrobial activity [1], *Chlamydomonas reinhardtii* [7] and *Sebastes schlegelii* where the protein contains N-linked glycochains [25]. Most snake venom L-aao are reported to be glycoproteins [22,60].

## Structural properties of L-aao

Primary structures of snake venom L-aao were determined for *Crotalus adamanteus* [61], *A. contortrix laticinctus* [49], *Crotalus atrox* [62], *Agkistrodon halys blomhoffi* [63], *Trimeresurus stejneggeri* [64] and *Bothrops* sp [65]. Phylogeny analysis shows that *Bothrops* and *Crotalus adamanteus* L-aao form a cluster and are more closely related to each other.

The complete nucleotide sequence of *Rhodococcus opacus* L-aao gene was determined and its primary structure was deduced [39]. The nucleotide sequence revealed that the L-aao is synthesized as a precursor carrying a signal peptide of 45 amino acids, which is processed after translation. The proteolytic cleavage site of the precursor protein does not agree with the predicted cleavage site. The nucleotide sequence of *Neurospora crassa* L-aao gene was obtained from a partial c-DNA and a complete genomic DNA clone [66]. The gene encodes a protein consisting of 695 amino acids and unlike most of the cloned genes of *N. crassa*, the L-aao gene is devoid of introns. The enzyme is synthesised as a precursor exceeding the mature form (566 amino acids) by 129 amino acids.

Various workers report the cloning of the L-aao gene into vectors for their expression in large quantities. Geueke and Hummel [39] report the expression of *Rhodococcus opacus* L-aao in *E. coli* but, this yields only inclusion bodies, while the expression in a *Streptomyces lividans* strain yields both soluble and active enzyme, but at low yields. The *Calloselasma rhodostoma* L-aao (CRL-aao) was cloned in a yeast expression system (*Pichia pastoris*) after the α-MF-signal sequence that promotes secretion. The expression was repressed when glycerol was used as the sole carbon source while switching the carbon source to methanol leads to the secretion of recombinant CRL-aao with good yields [67]. The L-aao of *Aplysia californica* (escapin) was cloned, sequenced and functionally expressed in *E. coli* [1]. The bioactive recombinant escapin level was relatively low because much of the escapin is present in the form of insoluble inclusion bodies. Also the escapin inhibits growth of *E. coli* which likely inhibits the level of bacterial expression. *Lechevalieria aerocolonigenes* produces an L-aao (rebeccamycin) (RebO) which was overexpressed in *E. coli* using an expression vector (pDHS5514). A KLAAALEHHHHHH amino acid sequence was engineered onto the C-terminus of RebO to facilitate purification by a Ni-nitrilotriacetic acid strategy. The co-expressed plasmid was used to facilitate protein folding and to prevent aggregation and degradation of RebO in *E. coli*. Thus, the recombinant RebO protein was found to be >95% pure [68]. Nagashima et al. [2] report the c-DNA cloning of *Myoxocephalus polycanthocephalus* L-aao (MPL-aao) which show that the full length of c-DNA was 2659 bp and it encodes the signal peptide (Met1-Ala26) and the mature protein (Val28-Phe520). MPL-aao shares 74% sequence identity with the antibacterial L-aao from skin mucus of the rockfish *Sebastes schlegeli*.

## Applications of L-aao

L-amino acid oxidases have practical value in biochemical and chemical investigations. The usefulness of these enzymes arises from the fact that they exhibit absolute antipodal specificity. It is therefore possible to detect as little as one part of a susceptible amino acid isomer in the presence of ten thousand times the concentration of its enantiomorph. The kinetic resolution of race mates is a highly successful strategy for the synthesis of enantiomerically pure chiral compounds and has found widespread usage in industry. The amino acid oxidases have been used to destroy one isomer of a racemic amino acid and thus yield an optically pure preparation of the other isomer [69]. We demonstrated the racemic resolution of DL-

amino acids to yield optically pure D-amino acids. DL-tyrosine, DL-phenylalanine and DL-alanine were successfully resolved with the help of *Aspergillus fumigatus* L-aao to yield D-tyrosine, D-phenylalnine and D-alanine respectively [70]. Further applications of this enzyme were demonstrated as catalysts in biotransformation [71] and could also be used for production of keto acids, which can function as siderophores [72].

The determination of amino acids is important for several purposes. Determination of certain amino acids in tissue or physiological fluids may be a useful indicator of certain diseases and disorders. Also the content of certain amino acids in food essentially controls the nutritional quality of the food. L-aao can be useful in this aspect by development of amperometric biosensors to detect the amino acids. Amperometric biosensors based on screen printed electrodes have been developed by Sarkar et al. [73]. An amperometric microbial biosensor based on *Saccharomyces cerevisiae* cells was developed for selective and rapid determination of L-lysine [40]. In addition, L-aao was physically immobilized on diamond paste to construct an amperometric biosensor that detects L-leucine by measuring the hydrogen peroxide formed when L-aao catalyses the conversion of L-leucine to their keto acids and $H_2O_2$ [74]. L-aao was immobilized on a preactivated nylon membrane by using glutaraldehyde to develop an enzyme sensor for L-amino acids that can detect ammonia [75].

Snake venom L-aaos are known to induce apoptosis and antibacterial effects mediated by the hydrogen peroxide produced during the L-aao reaction. The hydrogen peroxide is a strong inductor of apoptosis in promastigote forms of *Leishmania* ssp. The hydrogen peroxide induces oxidative stress which in turn activates the heat shock proteins and initiates cell membrane/ cytoplasmic disorganization, DNA fragmentation, apoptosis and therefore cell death [76]. In this aspect, L-aao can be greatly useful for the development of efficient therapeutics and drugs to control tumor cells, bacterial and leishmanicidal infections. Also the *Trimeresurus stejnegeri* L-aao displays dose dependent inhibition on HIV-1 infection and replication [64].

Antiviral (against Dengue virus) and antiprotozoal (trypanocidal and leishmanicidal) activities have been reported from *Bothrops jararaca* L-aao (LAAO-I) [77]. The *B. jararaca* L-aao was found to significantly inhibit Ehrlich ascites tumour growth and induce an influx of polymorphonuclear cells, as well as spontaneous liberation of $H_2O_2$ from peritoneal macrophages. Later, LAAO-I induce mononuclear influx and peritoneal macrophage spreading. Animals treated with LAAO-I show higher survival time [78] and thus, the application of these enzymes in tumour inhibition was implicated.

In *Deinagkistrodon acutus*, L-aao (ACTX-6) demonstrates cytotoxicity in vitro and inhibits tumour growth in vivo and can markedly increase accumulation of sub-G1 phase, which suggests that this enzyme can induce apoptosis. ACTX-6 is a potential substance to develop into an antitumor drug [79] since it induces apoptosis in Hela cervical cancer cells in a concentration- and time-dependent manner. Caspase activation and PARP cleavage are involved in ACTX-8-induced apoptosis. ACTX-8 activates a mitochondrial pathway of apoptosis, which is regulated by Bcl-2 family members. Reactive oxygen species generated by ACTX-8 are involved in apoptosis [80].

In *Viridovipera stejnegeri* the L-aao enzyme displays dose dependent inhibition on HIV-1 infection and replication [64] and as such can be studied for development of anti HIV medicine. Thus L-aaos are useful enzymes due to their biotechnological potential as model of therapeutic drugs and medicine.

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**References**

1. Yang H, Johnson PM, Ko KC, Kamio M, Germann MW, et al. (2005) Cloning, characterization and expression of escapin, a broadly antimicrobial FAD-containing L-amino acid oxidase from ink of the sea hare *Aplysia californica*. J Exp Biol 208: 3609-3622.
2. Nagashima Y, Tsukamoto C, Kitani Y, Ishizaki S, Nagai H, et al. (2009) Isolation and cDNA cloning of an antibacterial L-amino acid oxidase from the skin mucus of the great sculpin *Myoxocephalus polyacanthocephalus.* Comp Biochem Physiol B Biochem Mol Biol 154: 55-61.
3. Pelmont J, Arlaud G, Rossat AM (1972) [L-amino acid oxidases of *Proteus mirabilis*: general properties]. Biochimie 54: 1359-1374.
4. Cioacă C, Ivanof A (1974) Bacterial amino acid oxidases. I. L-amino acid oxidase and its distribution in bacteria. Arch Roum Pathol Exp Microbiol 33: 211-222.
5. Pistorius EK, Voss H (1982) Presence of an amino acid oxidase in photosystem II of *Anacystis nidulans*. Eur J Biochem 126: 203-209.
6. Mizon J, Biserte G, Boulanger P (1970) [Properties of turkey (*Meleagris gallopavo L.*) liver L-amino acid oxidase]. Biochim Biophys Acta 212: 33-42.
7. Vallon O, Bulté L, Kuras R, Olive J, Wollman FA (1993) Extensive accumulation of an extracellular L-amino-acid oxidase during gametogenesis of *Chlamydomonas reinhardtii.* Eur J Biochem 215: 351-360.
8. Gau AE, Heindl A, Nodop A, Kahmann U, Pistorius EK (2007) L-amino acid oxidases with specificity for basic L-amino acids in cyanobacteria. Z Naturforsch C 62: 273-284.
9. Coudert M (1975) Charcterization and physiological function of a soluble L-amino acid oxidase in Corynebacterium. Arch Microbiol 102: 151-153.
10. Aurich H, Luppa D, Schücker G (1972) [Purification and properties of l-amino acid oxidase from neurospora]. Acta Biol Med Ger 28: 209-220.
11. Sikora L, Marzluf GA (1982) Regulation of L-amino acid oxidase and of D-amino acid oxidase in *Neurospora crassa*. Mol Gen Genet 186: 33-39.
12. Davis MA, Askin MC, Hynes MJ (2005) Amino acid catabolism by an areA-regulated gene encoding an L-amino acid oxidase with broad substrate specificity in *Aspergillus nidulans*. Appl Environ Microbiol 71: 3551-3555.
13. Kusakabe H, Kodama K, Kuninaka A, Yoshino H, Misono H, et al. (1980) A new antitumor enzyme, L-lysine alpha-oxidase from *Trichoderma viride*. Purification and enzymological properties. J Biol Chem 255: 976-981.
14. Palamakumbura AH, Jeay S, Guo Y, Pischon N, Sommer P, et al. (2004) The propeptide domain of lysyl oxidase induces phenotypic reversion of ras-transformed cells. J Biol Chem 279: 40593-40600.
15. Sun Y, Nonobe E, Kobayashi Y, Kuraishi T, Aoki F, et al. (2002) Characterization and expression of L-amino acid oxidase of mouse milk. J Biol Chem 277: 19080-19086.
16. Nagaoka K, Aoki F, Hayashi M, Muroi Y, Sakurai T, et al. (2009) L-amino acid oxidase plays a crucial role in host defense in the mammary glands. FASEB J 23: 2514-2520.
17. Knight SG (1948) The l-Amino Acid Oxidase of Molds. J Bacteriol 55: 401–407.
18. Nuutinen JT, Timonen S (2008) Identification of nitrogen mineralization enzymes, L-amino acid oxidases, from the ectomycorrhizal fungi *Hebeloma* spp. and *Laccaria* bicolor. Mycol Res 112: 1453-1464.
19. Skarnes RC (1970) L-amino-acid oxidase, a bactericidal system. Nature 225: 1072-1073.
20. Stiles BG, Sexton FW, Weinstein SA (1991) Antibacterial effects of different snake venoms: purification and characterization of antibacterial proteins from *Pseudechis australis* (Australian king brown or mulga snake) venom. Toxicon 29: 1129-1141.
21. Lu QM, Wei Q, Jin Y, Wei JF, Wang WY, Xiong YL (2002) L-amino acid oxidase

from *Trimeresurus jerdonii* snake venom: purification, characterization, platelet aggregation-inducing and antibacterial effects. J Nat Toxins 11: 345–352

22. Stábeli RG1, Marcussi S, Carlos GB, Pietro RC, Selistre-de-Araújo HS, et al. (2004) Platelet aggregation and antibacterial effects of an l-amino acid oxidase purified from *Bothrops alternatus* snake venom. Bioorg Med Chem 12: 2881-2886.

23. Ehara T, Kitajima S, Kanzawa N, Tamiya T, Tsuchiya T (2002) Antimicrobial action of achacin is mediated by L-amino acid oxidase activity. FEBS Lett 531: 509-512.

24. Jimbo M, Nakanishi F, Sakai R, Muramoto K, Kamiya H (2003) Characterization of L-amino acid oxidase and antimicrobial activity of aplysianin a, a sea hare-derived anitumor- antimicrobial protein. Fish Sci 69: 1240-1246.

25. Kitani Y, Tsukamoto C, Zhang G, Nagai H, Ishida M, et al. (2007) Identification of an antibacterial protein as L-amino acid oxidase in the skin mucus of rockfish *Sebastes schlegeli*. FEBS J 274: 125-136.

26. Genet R, Bénetti PH, Hammadi A, Ménez A (1995) L-tryptophan 2',3'-oxidase from *Chromobacterium violaceum*. Substrate specificity and mechanistic implications. J Biol Chem 270: 23540-23545.

27. Lucas-Elío P1, Gómez D, Solano F, Sanchez-Amat A (2006) The antimicrobial activity of marinocine, synthesized by *Marinomonas mediterranea*, is due to hydrogen peroxide generated by its lysine oxidase activity. J Bacteriol 188: 2493-2501.

28. James SG, Holmström C, Kjelleberg S (1996) Purification and characterization of a novel antibacterial protein from the marine bacterium D2. Appl Environ Microbiol 62: 2783-2788.

29. Mai-Prochnow A, Webb JS, Ferrari BC, Kjelleberg S (2006) Ecological advantages of autolysis during the development and dispersal of *Pseudoalteromonas tunicata* biofilms. Appl Environ Microbiol 72: 5414-5420.

30. Gómez D, Espinosa E, Bertazzo M, Lucas-Elío P, Solano F, et al. (2008) The macromolecule with antimicrobial activity synthesized by *Pseudoalteromonas luteoviolacea* strains is an L-amino acid oxidase. Appl Microbiol Biotechnol 79: 925-930.

31. Suhr SM, Kim DS (1999) Comparison of the apoptotic pathways induced by L-amino acid oxidase and hydrogen peroxide. J Biochem 125: 305-309.

32. MacHeroux P, Seth O, Bollschweiler C, Schwarz M, Kurfürst M, et al. (2001) L-amino-acid oxidase from the Malayan pit viper *Calloselasma rhodostoma*. Comparative sequence analysis and characterization of active and inactive forms of the enzyme. Eur J Biochem 268: 1679-1686.

33. Wei JF, Yang HW, Wei XL, Qiao LY, Wang WY, et al. (2009) Purification, characterization and biological activities of the L-amino acid oxidase from *Bungarus fasciatus* snake venom. Toxicon 54: 262-271.

34. Masuda S, Araki S, Yamamoto T, Kaji K, Hayashi H (1997) Purification of a vascular apoptosis-inducing factor from hemorrhagic snake venom. Biochem Biophys Res Commun 235: 59-63.

35. Torii S, Naito M, Tsuruo T (1997) Apoxin I, a novel apoptosis-inducing factor with L-amino acid oxidase activity purified from Western diamondback rattlesnake venom. J Biol Chem 272: 9539-9542.

36. Li ZY, Yu TF, Lian EC (1994) Purification and characterization of L-amino acid oxidase from king cobra (*Ophiophagus hannah*) venom and its effects on human platelet aggregation. Toxicon 32: 1349-1358.

37. Gómez D, Lucas-Elío P, Sanchez-Amat A, Solano F (2006) A novel type of lysine oxidase: L-lysine-epsilon-oxidase. Biochim Biophys Acta 1764: 1577-1585.

38. Thayer PS, Horowitz NH (1951) The L-amino acid oxidase of Neurospora. J Biol Chem 192: 755-767.

39. Geueke B, Hummel W (2002) A new bacterial L-amino acid oxidase with a broad substrate specificity: Purification and characterization. Enzyme and microbial technology 31: 77-87.

40. Akyilmaz E, Erdoğan A, Oztürk R, Yaşa I (2007) Sensitive determination of L-lysine with a new amperometric microbial biosensor based on *Saccharomyces cerevisiae* yeast cells. Biosens Bioelectron 22: 1055-1060.

41. Böhmer A, Müller A, Passarge M, Liebs P, Honeck H, et al. (1989) A novel L-glutamate oxidase from *Streptomyces endus*. Purification and properties. Eur J Biochem 182: 327-332.

42. Duerre JA, Chakrabarty S (1975) l-amino acid oxidases of *Proteus rettgeri*. J Bacteriol 121: 656-663.

43. Stumpf PK, Green DE (1944) L-Amino Acid Oxidase of *Proteus vulgaris*. Journal of Biological chemistry 153: 387-399.

44. Singh S, Gogoi BK, Bezbaruah RL (2009) Optimization of medium and cultivation conditions for L-amino acid oxidase production by *Aspergillus fumigatus*. Can J Microbiol 55: 1096-1102.

45. Mason JM, Naidu MD, Barcia M, Porti D, Chavan SS, et al. (2004) IL-4-induced gene-1 is a leukocyte L-amino acid oxidase with an unusual acidic pH preference and lysosomal localization. J Immunol 173: 4561-4567.

46. Johnson PM, Kicklighter CE, Schmidt M, Kamio M, Yang H, et al. (2006) Packaging of chemicals in the defensive secretory glands of the sea hare *Aplysia californica*. J Exp Biol 209: 78-88.

47. Tõnismägi K, Samel M, Trummal K, Rönnholm G, Siigur J, et al. (2006) L-amino acid oxidase from *Vipera lebetina* venom: isolation, characterization, effects on platelets and bacteria. Toxicon 48: 227-237.

48. Pessatti M, Fontana JD, Furtado MF, Guimãraes MF, Zanette LR, et al. (1995) Screening of Bothrops snake venoms for L-amino acid oxidase activity. Appl Biochem Biotechnol 51-52: 197-210.

49. Souza DH, Eugenio LM, Fletcher JE, Jiang MS, Garratt RC, et al. (1999) Isolation and structural characterization of a cytotoxic L-amino acid oxidase from *Agkistrodon contortrix laticinctus* snake venom: preliminary crystallographic data. Arch Biochem Biophys 368: 285-290.

50. Izidoro LF, Ribeiro MC, Souza GR, Sant'Ana CD, Hamaguchi A, et al. (2006) Biochemical and functional characterization of an L-amino acid oxidase isolated from *Bothrops pirajai* snake venom. Bioorg Med Chem 14: 7034-7043.

51. Wei XL, Wei JF, Li T, Qiao LY, Liu YL, et al. (2007) Purification, characterization and potent lung lesion activity of an L-amino acid oxidase from *Agkistrodon blomhoffii ussurensis* snake venom. Toxicon 50: 1126-1139.

52. Ciscotto P, Machado de Avila RA, Coelho EA, Oliveira J, Diniz CG, et al. (2009) Antigenic, microbicidal and antiparasitic properties of an l-amino acid oxidase isolated from *Bothrops jararaca* snake venom. Toxicon 53: 330-341.

53. Zhong SR, Jin Y, Wu JB, Jia YH, Xu GL, et al. (2009) Purification and characterization of a new L-amino acid oxidase from *Daboia russellii siamensis* venom. Toxicon 54: 763-771.

54. Vallon O (2000) New sequence motifs in flavoproteins: evidence for common ancestry and tools to predict structure. Proteins 38: 95-114.

55. Brearley GM, Price CP, Atkinson T, Hammond PM (1994) Isolation, identification and characterization of a soil bacterium producing an enzyme with L-phenylalanine oxidase activity. Arch Microbiol 41: 670-676.

56. Meister A, Wellner D (1963) The Enzymes. 2nd Ed 7: 609-648.

57. Tan NH, Saifuddin MN (1989) Isolation and characterization of an unusual form of L-amino acid oxidase from King cobra (*Ophiophagus hannah*) venom. Biochem Int 19: 937-944.

58. Ponnudurai G, Chung MC, Tan NH (1994) Purification and properties of the L-amino acid oxidase from Malayan pit viper (*Calloselasma rhodostoma*) venom. Arch Biochem Biophys 313: 373-378.

59. Ueda M, Chang CC, Ohno M (1988) Purification and characterization of L-amino acid oxidase from the venom of *Trimeresurus mucrosquamatus* (Taiwan habu snake). Toxicon 26: 695-706.

60. Geyer A, Fitzpatrick TB, Pawelek PD, Kitzing K, Vrielink A, et al. (2001) Structure and characterization of the glycan moiety of L-amino-acid oxidase from the Malayan pit viper *Calloselasma rhodostoma*. Eur J Biochem 268: 4044-4053.

61. Raibekas AA, Massey V (1998) Primary structure of the snake venom L-amino acid oxidase shows high homology with the mouse B cell interleukin 4-induced Fig1 protein. Biochem Biophys Res Commun 248: 476-478.

62. Torii S, Yamane K, Mashima T, Haga N, Yamamoto K, et al. (2000) Molecular cloning and functional analysis of apoxin I, a snake venom-derived apoptosis-inducing factor with L-amino acid oxidase activity. Biochemistry 39: 3197-3205.

63. Takatsuka H, Sakurai Y, Yoshioka A, Kokubo T, Usami Y, et al. (2001) Molecular characterization of L-amino acid oxidase from *Agkistrodon halys blomhoffii* with special reference to platelet aggregation. Biochim Biophys Acta 1544: 267-277.

64. Zhang YJ, Wang JH, Lee WH, Wang Q, Liu H, et al. (2003) Molecular characterization of *Trimeresurus stejnegeri* venom L-amino acid oxidase with potential anti-HIV activity. Biochem Biophys Res Commun 309: 598-604.

65. França SC, Kashima S, Roberto PG, Marins M, Ticli FK, et al. (2007) Molecular approaches for structural characterization of Bothrops L-amino acid oxidases with antiprotozoal activity: cDNA cloning, comparative sequence analysis, and molecular modeling. Biochem Biophys Res Commun 355: 302-306.

66. Niedermann DM, Lerch K (1990) Molecular cloning of the L-amino-acid oxidase gene from *Neurospora crassa*. J Biol Chem 265: 17246-17251.

67. Kommoju PR, Macheroux P, Ghisla S (2007) Molecular cloning, expression and purification of L-amino acid oxidase from the Malayan pit viper *Calloselasma rhodostoma*. Protein Expr Purif 52: 89-95.

68. Nishizawa T, Aldrich CC, Sherman DH (2005) Molecular analysis of the rebeccamycin L-amino acid oxidase from *Lechevalieria aerocolonigenes* ATCC 39243. J Bacteriol 187: 2084-2092.

69. Meister A (1957) Biochemistry of the amino acids. Academic Press Inc. Publishers, New York.

70. Singh S, Gogoi BK, Bezbaruah RL (2011) Racemic resolution of some DL-amino acids using *Aspergillus fumigatus* L-amino acid oxidase. Curr Microbiol 63: 94-99.

71. Takahashi E, Furui M, Seko H, Shibatani T (1997) D-lysine production from L-lysine by successive chemical racemization and microbial asymmetric degradation. Appl Microbiol Biotechnol 47: 347-351.

72. Drechsel H, Thieken A, Reissbrodt R, Jung G, Winkelmann G (1993) Alpha-keto acids are novel siderophores in the genera Proteus, Providencia, and Morganella and are produced by amino acid deaminases. J Bacteriol 175: 2727-2733.

73. Sarkar P, Tothill IE, Setford SJ, Turner AP (1999) Screen-printed amperometric biosensors for the rapid measurement of L- and D-amino acids. Analyst 124: 865-870.

74. Staden R-I S-v, Muvhulawa LS (2006) Determination of L- and D- enantiomers of Leucine using amperometric biosensors based on diamond paste. Instrumentation Science & Technology 34: 475–481.

75. Lee YC, Huh MH (1999) Development of a biosensor with immobilized, l-amino acid oxidase for determination of , l-amino acids, Journal of Food Bio Chemistry 23: 173 – 185.

76. Tempone AG, Andrade HF Jr, Spencer PJ, Lourenço CO, Rogero JR, et al. (2001) *Bothrops moojeni* venom kills *Leishmania* spp. with hydrogen peroxide generated by its L-amino acid oxidase. Biochem Biophys Res Commun 280: 620-624.

77. Sant'Ana CD, Menaldo DL, Costa TR, Godoy H, Muller VD, et al. (2008) Antiviral and antiparasite properties of an L-amino acid oxidase from the snake *Bothrops jararaca*: cloning and identification of a complete cDNA sequence. Biochem Pharmacol 76: 279-288.

78. de Vieira Santos MM, Sant'Ana CD, Giglio JR, da Silva RJ, Sampaio SV, et al. (2008) Antitumoural effect of an L-amino acid oxidase isolated from *Bothrops jararaca* snake venom. Basic Clin Pharmacol Toxicol 102: 533-542.

79. Zhang L, Wu WT (2008) Isolation and characterization of ACTX-6: a cytotoxic L-amino acid oxidase from *Agkistrodon acutus* snake venom. Nat Prod Res 22: 554-563.

80. Zhang L, Wei LJ (2007) ACTX-8, a cytotoxic L-amino acid oxidase isolated from *Agkistrodon acutus* snake venom, induces apoptosis in Hela cervical cancer cells. Life Sci 80: 1189-1197.

81. Kotaka S (1963) The L-amino acid oxidase from silkworm eggs (*Bombyx mori* L.). J Gen Physiol 46: 1087-1094.

82. Toyama MH, Toyama Dde O, Passero LF, Laurenti MD, Corbett CE, et al. (2006) Isolation of a new L-amino acid oxidase from *Crotalus durissus cascavella* venom. Toxicon 47: 47-57.

83. Marcotte P, Walsh C (1976) Vinylglycine and proparglyglycine: complementary suicide substrates for L-amino acid oxidase and D-amino acid oxidase. Biochemistry 15: 3070-3076.

84. Wellner D (1971) [218b] l-Amino acid oxidase (snake venom) Methods Enzymol. 17B: 597-600.