Archives of Microbiology
https://doi.org/10.1007/s00203-020-01814-1

MINI-REVIEW

Microbial L-asparaginase: purification, characterization and applications

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Received: 6 November 2019 / Revised: 2 January 2020 / Accepted: 21 January 2020

Abstract

L-asparaginase (E.C.3.5.1.1) is an important enzyme that has been purified and characterized for over decades to study and evaluate its anti-carcinogenic activity against different lymphoproliferative disorders such as acute lymphoblastic leukemia (ALL) and Hodgkin's lymphoma. The ability of the enzyme to convert L-asparagine into aspartic acid and ammonia is the reason behind its anti-cancerous activity. Apart from its medicinal uses, it is widely used in food industry to tackle acrylamide, a probable human carcinogen and, production in carbohydrate-rich foods cooked at high temperatures. There are variety of organisms including microorganisms such as bacteria, fungi, algae, and plants that produce L-asparaginase. The enzyme obtained from different microbial and plant sources have different physiochemical properties and kinetic parameters. L-asparaginases have an optimum pH range between 6 and 10 and an optimum temperature between 37 and 85 °C. This article has reviewed the lowest molecular mass for L-asparaginase in *Yersinia pseudotuberculosis* Q66CJ2 which is 36.27 kDa, while the highest for *Pseudomonas otitidis* which has a molecular mass of 205 ± 3 kDa. This review is an attempt to summarize most of the available sources, their phylogenetic relationships, purification methods, data regarding different physiochemical and kinetic properties of L-asparaginase.

Keywords Acrylamide · Chromatography · Lymphoblastic leukemia · Hodgkin's lymphoma · Asparagine · Biosensor · Phylogenetic tree · Millard reaction · Biosynthesis

Introduction

Enzyme technology has enabled scientists to use, modify and enhance the efficiency of enzymes in such a way that maximum functionality and enhanced efficacy is obtained (Offman et al. 2010; Savitri et al. 2003). Microbial L-asparaginase (L-asparagine amidohydrolase, E.C.3.5.1.1) has been widely used and studied for its potential to act as an oncological agent (Benchamin et al. 2019). L-asparaginase has numerous applications in pharmaceutical, health and food industry (Ahmad et al. 2012; Cachumba et al. 2016).

A number of studies have shown that L-asparaginase acts as an anti-carcinogenic agent due to its potential and chemical efficiency to convert asparagine (present beyond its normal concentrations in cancerous cells) in to ammonia and aspartic acid (Xu et al. 2016; Ray et al. 2019). L-asparaginase is, therefore, an inevitable tool for the treatment of acute malignancies of lymphoid systems and melanosarcoma (Dias et al. 2016; Narta et al. 2007). L-asparaginase inhibits the growth of cancerous cells by depleting the L-asparagine (a non-essential amino acid) which is a vital nutrient of malignant or cancerous cells. L-asparaginase is mostly produced by a number of microbes dwelling a wide range of ecosystem (Shukla et al. 2014). Tomar et al. (2014) have confirmed that depending upon the localization of the L-asparaginase in the cell, it is divided into two isoforms, L-asparaginase-I and -II, these two forms were genetically differentiated from one another and L-asparaginase derived from different microorganisms show different properties.

In nature, L-asparaginase exists in different structural forms such as dimers, tetramers, and hexamers.

Communicated by Erko Stackebrandt.

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Published online: 12 February 2020

L-asparaginase can actively degrade acrylamide (C_3H_5NO), a small hydrophilic molecule which is a potential human carcinogen. Acrylamide was declared as carcinogenic in 1994, by the international agency for research on cancer (IARC). In 2000, the WHO (World Health Organization) endorsed the decision taken by IARC in declaring acrylamide as a carcinogenic molecule (Krishnakumar and Visvanathan 2015; FAO/WHO 2002; Keramat et al. 2011). Acrylamide is present in carbohydrate-rich foods which are prepared on high temperature (FAO/WHO 2002). Acrylamide is formed by the Millard reaction that occurs at extreme temperature between L-asparagine and carbonyl compounds (Krishnakumar and Visvanathan 2015). The temperature for acrylamide production is usually above 100 °C (Alam et al. 2019). Acrylamide is present in baked, fried and starchy foods because of L-asparagine which is the main source of acrylamide formation (Xu et al. 2016). L-asparaginase has shown to counter the acrylamide production (Rani et al. 2012; Xu et al. 2016).

There are several sources of L-asparaginase isolation and purification but for the commercial purposes, it is currently being isolated from two main bacterial sources first from *E. coli* (mainly from its recombinant and genetically modified versions) and second, from *Erwinia chrysanthemi* (Rodrigues et al. 2019). L-asparaginase is present in most of the bacteria, fungi, plant tissues, algae and in some animals. Rodents have L-asparaginase in their blood serum, however, humans lack this vital enzyme (Cachumba et al. 2016; Deshpande et al. 2014). L-asparaginase enzyme is believed to be the most important due to its role in lymphoblastic therapy and for the inhibition of acrylamide production as a side reaction during the industrial processing of food (Narta et al. 2007). The importance of this enzyme is also evident from its high global demand which was 380 million USD in 2017 and is estimated to rise up to 420 million USD by 2025 (Alam et al. 2019). Due to such vital importance of the enzyme in health and industry, L-asparaginase is currently being investigated for its efficient role against different types of cancers including breast cancer (Benchamin et al. 2019). The stunning properties of the enzyme in health, pharmaceuticals and food industry have a demand that this enzyme must be studied thoroughly. This review has discussed thoroughly its diverse sources most importantly the microbial one with special emphasize on its purification methods and characterization.

Sources of L-asparaginase

L-asparaginase is abundantly found in nature from prokaryotic microorganisms to vertebrates (Eisele et al. 2011). It is commonly present in microbes and in some important plants and a few animals have the potential to produce this enzyme. Some rodents have this enzyme in their blood serum, but humans lack this important anti-carcinogenic agent (Cachumba et al. 2016). This review will only discuss the microbial sources of L-asparaginase.

Microbial sources

Microbes (bacteria, fungi, and microalgae, etc.) are the best sources for L-asparaginase isolation and purification (Cachumba et al. 2016). More than 364 isolates of a tropical soil fungi were screened for L-asparaginase production, one hundred and thirty five (135) of the total isolates showed positive reaction (Meghavarnam and Janakiraman 2015). It confirms that there is an abundant environment for the isolation and screening of a wide range of bacterial strains to produce L-asparaginase.

On industrial scale, L-asparaginase can be easily purified from different types of microorganisms (Borah et al. 2012). Different bacterial and fungal strains have been studied for the production and purification of L-asparaginase. Some of the common bacterial sources are *Serratia marcescens* and *Enterobacter aerogenes* (Amena et al. 2010). *E. coli* and *Erwinia carotovora* yield better concentrations of the enzyme and are, therefore, being used for the treatment of acute lymphoblastic leukemia. A large number of filamentous fungi, yeast and bacteria are also considered as important sources of this enzyme (Verma et al. 2007a, b). Some *Penicillium* sp. are also important for the production and synthesis of the enzyme under submerged fermentation conditions (Patro and Gupta 2012).

Since 1970s, many microbial strains have been reported to show the potential for L-asparaginase production. Some of these strains include *Erwinia aroideae, Pseudomonas aeruginosa*, *Aspergillus tamari*, *Vibro succinogenes, Aspergillus terreus*, *Pseudomonas stutzeri*, and *Staphylococcus* sp. From a commercial point of view, the main sources of L-asparaginase production are *E-coli*, and *Erwinia carotovora* (Kataria et al. 2015). L-asparaginase purified from *E. coli* exists in two forms, L-asparaginase -I and -II, the isoform-II is more active against lymphoma than isoform-I. Asparaginase-I is usually located in the cytosol, while asparaginase-II is present in the periplasmic region of the microbial cell. Both the isoforms have conserved regions at molecular level which are known as motifs, however, they vary in their quaternary structure.

Salinicoccus sp. have been identified for the commercial production of L-asparaginase most of its strains can be isolated from soil and high salted lakes. Jha et al. (2012) have reported some important species of *Salinicoccus* which have shown good potential to produce L-asparaginase some of these are *S. albus, S. iranensis, S. salsiraiae, S. alkaliphilus,* and *S. roseus,*. *Helicobacter pyroli* isolated from hot springs has shown good potential for the production of

L-asparaginase (Pradhan et al. 2013). ELSPAR, ONCASPAR, ERWINASE and KIDROLASE are commercially available forms of L-asparaginase which are being used as medicinal drugs and obtained from *E. coli* and *Erwinia carotovora* (Kataria et al. 2015). Some of the most commonly used L-asparaginase producer bacterial, fungal and algal strains are summarized in Tables 1, 2 and 3, respectively.

Phylogenetic analysis

To understand the phylogenetic relation among different bacterial and fungal L-asparaginase producers (Tables 1 and 2), phylogenetic trees were built using MEGA 7 software. The protein sequences for L-asparaginase were retrieved from NCBI and aligned using clustalW in MEGA 7. The

Table 1 Bacterial sources of L-asparaginase

Bacterial strain(s)	Reference(s)	Bacterial strain(s)	Reference(s)
Erwinia caratovora	Deokar et al. (2010)	*Serratia marcescens*	Agarwal et al. (2011)
Streptomyces gulbargensis, Streptomyces venezuelae	Naveena et al. (2012)	*Escherichia coli, Acinetobacter glutaminasifican*	Cachumba et al. (2016)
Escherichia coli (MTCC 739)	Vidya et al. (2011)	*Pseudomonas stutzeri*	Hosamani and Kaliwal (2011)
Thermus thermophilus	Pritsa and Kyriakidis (2001)	*Bacillus licheniformis, Bacillus circulans*	Gulati et al. (1997)
Paenibacillus barengoltzii, Bacillus (PG03), *Bacillus* (PG04), *Bacillus pumilus*	Qeshmi et al. (2018); Rahimzadeh et al. (2016)	*Bacillus subtilis* (hswx88)	Pradhan et al. (2013)
Bacillus pseudomycoides Paenibacillus denitriformis	Joshi and Kulkarni (2016)	*Bacillus firmus* (AVP 18)	Rudrapati and Audipudi (2017)
Pseudomonas aeruginosa	Kamble et al. (2012)	*Pyrococcus furiosus*	Jayam and Kannan (2014)
Acinetobacter baumannii	Muslim (2014)	*Staphylococcus capitis*	Paglla et al. (2013)
Enterobacter aerogenes	Baskar et al. (2013)	*Thermococcus kodakaraensis*	Chohan and Rashid (2013)
Pseudomonas proteolytica (S13D)	Shukla et al. (2014)	*Thermococcus gammatolerans*	Xue et al. (2014)
Streptomyces ginsengisoli	Deshpande et al. (2014)	*Pyrococcus yayanosii* (CH1)	Li et al. (2018)
Rhizobium etli	Angélica et al. (2012)	*Salmonella typhimurium*	Kullas et al. (2012)
Streptomyces iranensis	Dharmaraj (2011)	*Corynebacterium glutamicum*	Ahmad et al. (2012)
Aeromonas sp.	Muslim (2014)	*Streptomyces alkaliphilus, Staphylococcus roseus*	Jha et al. (2012)

Table 2 Fungal sources of L-asparaginase

Fungal strain(s)	Reference(s)	Fungal Strain(s)	Reference(s)
Aspergillus alliaceus, Penicillium sp., *Fusarium solani*	Gulati et al. (1997)	*Pichia polymorpha, Saccharomyces cerevisiae*	Jha et al. (2012)
Aspergillus tamari, Aspergillus terreus	Sanjotha and Manawadi (2017; Sarquis et al. (2004)	*Aspergillus niger, Aspergillus nidulans, Bipolaris* sp.	Baskar and Renganathan (2011)
Aspergillus tubingensis, Fusarium equiseti	Gonçalves et al. (2017)	*Aspergillus fumigatus*	Benchamin et al. (2019)
Aspergillus sp.	Ahmed et al. (2015)	*Fusarium culmorum* (ASP-87), *Fusarium equiseti*	Meghavarnam and Janakiraman (2016); Hosamani and Kaliwal (2011)
Cladosporium sp. *Macrophoma* sp., *Taxomyces andrenae Trichoderma viride*	Chow and Ting (2015)	*Penicillium notatum, Flammulina velutipes*	Eisele et al. (2011)
Aspergillus oryzae (CCT 3940)	Dias et al. (2016)	*Helminthosporium* (RF3)	Gupta et al. (2009)
Beauveria bassiana	Nageshwara et al. (2014)	*Fusarium oxysporum*	Patro and Gupta (2012)

Table 3 Algae sources of L-asparaginase

Algae strain(s)	Reference(s)	Algae strain(s)	Reference(s)
Spirulina maxima	Baky (2016)	*Chlorella vulgaris*	Ebrahiminezhad et al. (2014)
Chlamydomonas sp.	Paul (1982)	*Vaucheria uncinata*	Batool et al. (2016)

trees obtained during the analysis can be seen in Figs. 1a, b and 2. The phylogenetic tree (Fig. 1a) was developed for the Gram-positive bacterial strains. 13 g positive strains were used to develop the tree. It can be seen from the protein homology sequence for L-asparaginase enzyme that some of these Gram-positive bacterial strains are more closely related as compare to the other. *Salinicoccus roseus*, *S. luteus, S. halodurans S. albus* and *S. kekensis* have a more conserved protein sequence for asparaginase as compare to the other strains in the tree. The conserved motif in the protein sequences (L-asparaginase enzyme) is given in front of closely related strains in the tree.

Figure 1b shows the phylogenetic tree for the Gram-negative bacterial strains that had shown the potential for

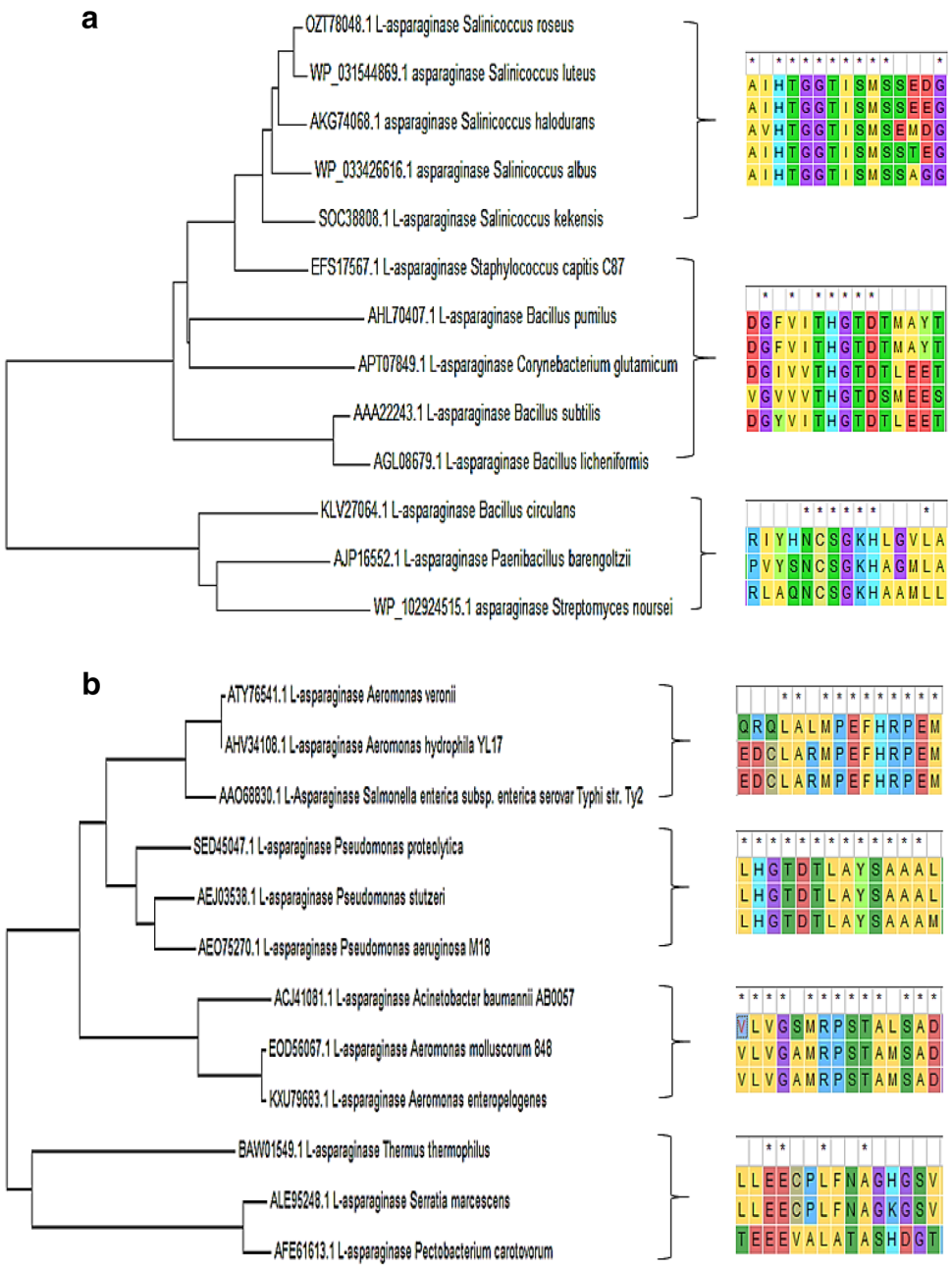

Fig. 1 **a** Phylogenetic tree for L-asparaginase producing Gram-positive bacterial strains. Neighbor-joining method was used to infer the evolutionary history. The branch length of the optimal tree is 6.77011222. Poisson correction method was used to compute the evolutionary distances which are in the units of the number of amino acid substitutions per site. **b** Phylogenetic tree for L-asparaginase producing Gram-negative bacterial strains. Neighbor-joining method was used to infer the evolutionary history. The branch length of the optimal tree is 6.00775136. Poisson correction method was used to compute the evolutionary distances which are in units of the number of amino acid substitutions per site

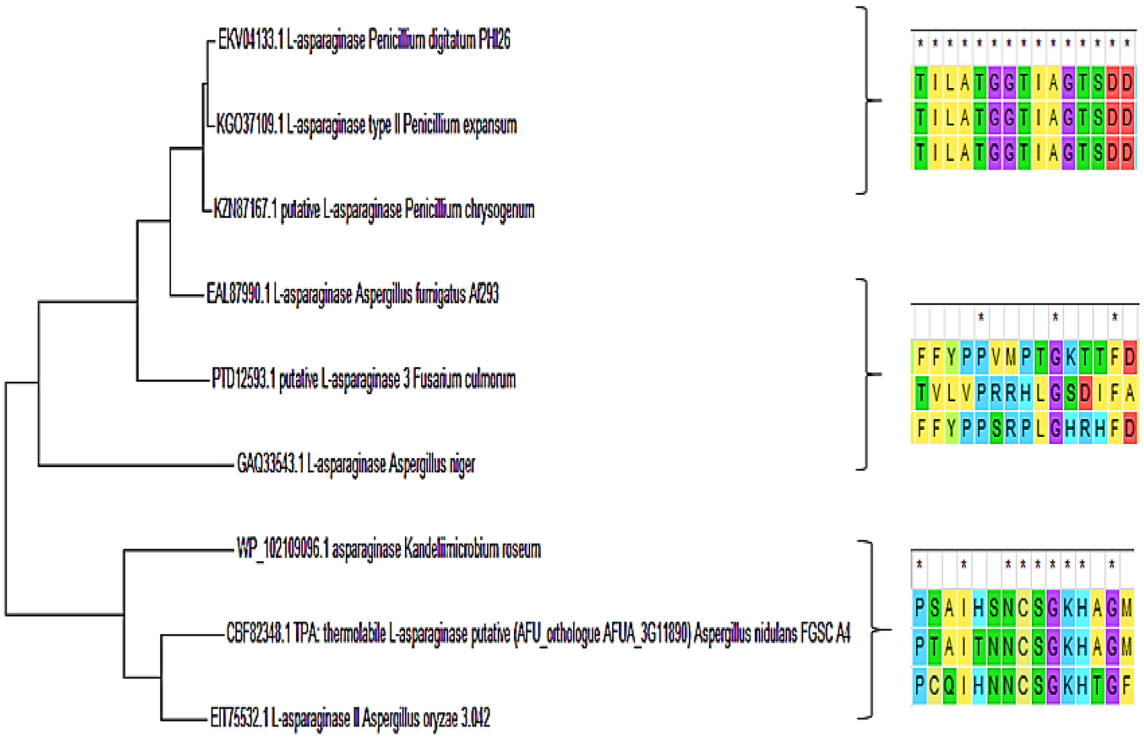

Fig. 2 Phylogenetic tree for L-asparaginase producing fungal strains. Neighbor-joining method was used to infer the evolutionary history. The sum of the branch length of the optimal tree is 4.79093222. Poisson correction method was used to compute the evolutionary distances which are in the units of the number of amino acid substitution per site

L-asparaginase production. There are 12 g negative strains whose protein sequence was inferred from the NCBI and then used to develop the phylogenetic tree. It can be seen from Fig. 1b that some of the bacterial strains are more close in their evolution with a more conserved L-asparaginase sequence as compare to those which are evolutionary more distant hence, keeping the same fact in mind, we can say that the L-asparaginase protein sequence in *Aeromonas veroni* and *Aeromonas hydrophila* shows more homology as compare to other strains in the tree.

There are a number of fungal strains that have been reported in the literature for the production of L-asparaginase, however, only nine most studied fungal strains were selected for phylogenetic analysis. The phylogenetic tree for the fungal strains can be seen in Fig. 2. We can clearly see that the phylogenetic tree for fungal strains shows that *Penicillium digitum*, *Penicillium expansum* and *Penicillium chrysogenum* are not only close in term of their evolutionary distance in the tree but also show maximum homology in their protein sequence for L-asparaginase.

Purification of microbial L-asparaginase

A purified and concentrated enzyme is required to determine its physiological activity and stability. Purification of the enzyme must be at homogeneity level for the determination of its structure and conformation (Nadeem et al. 2015a, 2009). Kinetic and thermodynamic properties including the reaction mechanisms can easily be studied once the enzymes are in purified form. Purified L-asparaginase is important to study its anti-carcinogenic activity and other medicinal properties (Ahmad et al. 2012). It is important for an enzyme to be purified to derive important formulations for industrial and medicinal uses (Javed et al. 2018; Nadeem et al. 2015b).

L-asparaginase can be purified from crude microbial extract with minor differences depending upon the microbe being used i.e., bacteria or fungi. L-asparaginase can be purified in different steps according to its biological and physiochemical properties for therapeutic purposes.

Extra-cellular L-asparaginase is considered more efficient and is preferred over intracellular L-asparaginase, because it gives good yield under normal parameters. It has been studied that different microbial conditions require slightly varying conditions of its isolation and purification. For example, in the case of *Salinicoccus* species, a pH of 7 at 37 °C gives high yield when incubated for 30 min (Amena et al. 2010). Monovalent ions such as K^+ and divalent ions such as Mg^{2+} and Ca^{2+} act as activators. On the other hand, Hg^{2+} and EDTA act as inhibitor for the enzyme (Emmanuel et al. 2015). There is no well-defined medium of fixed composition for the production of L-asparaginase from different microbial sources, however, nitrogen and carbon sources have shown to play an important role for the microbial growth and production of the enzyme. Different microbes need variable growth conditions and parameters to show optimum production of L-asparaginase, for example *Enterobacter aerogenes,* requires sodium citrate (1%) and diammonium hydrogen phosphate (0.16%) as their carbon and nitrogen sources, respectively (Mukherjee et al. 2000). As there are a number of bacterial and fungal strains for L-asparaginase production with different requirements and limitations, a summary of all these is given in Table 4.

Characterization of L-asparaginase

Microbial L-asparaginase is characterized on the basis of its different physiochemical and kinetic properties.

Physiochemical properties of L-asparaginases

Physiochemical properties of some recently studied (from 2006 to 18) microbial L-asparaginases are summarized in Table 5. It can be noted that most of the microbial strains have optimum temperature range of 37–40 °C but the strains such as *Thermococcus kodaka* TK1656 and *Thermococcus gammatolerans* EJ3 produce thermostable L-asparaginase with an optimum temperature of 85 °C (Chohan and Rashid 2013; Xue et al. 2014). L-asparaginases from different sources vary in optimum temperature and pH for maximum activity. L-asparaginase from *E. coli* works at pH 6, the lowest pH in the list which shows that the enzyme works in a slightly acidic medium (Borah et al. 2012). Almost all L-asparaginases isolated and purified from microbial sources work in a basic medium that ranges from a pH of 8.0 to 10. L-asparaginase obtained from *Bacillus licheniformis* work efficiently from slightly acidic to strongly basic conditions, i.e., 6.0–10 pH (Mahajan et al. 2014). The data for the molecular mass of the enzymes show that the lowest molecular mass is for *Yersinia pseudotuberculosis* Q66CJ2 L-asparaginase i.e., 36.27 kDa (Pokrovskaya et al. 2012), while the enzyme with maximum molecular mass of 205 ± 3 kDa on native-PAGE was produced from *Pseudomonas otitidis* (Husain et al. 2016).

Graphical representation of optimum temperature and pH of microbial L-asparaginases

A graphical interpretation (Fig. 3) explains the variation in optimum temperature and pH of microbial L-asparaginases. It is evident from the graph that most of the enzymes work optimally at a temperature of 40 °C with a maximum range of pH 6.0–10. The graph also shows that there are only three strains that give thermostable L-asparaginase which works at 80–85 °C, such strains can be used to produce L-asparaginase for reactions that take place at high temperatures. L-asparaginase obtained from *Thermococcus kodaka* TK1656 (Chohan and Rashid 2013) and *Thermococcus gammatolerans* EJ3 (Xue et al. 2014) have the highest optimum temperature of 85 °C. It can be seen clearly from the graph that most of the enzymes required a basic pH for optimum activity, while only a few such as *E. coli* L-asparaginase had a pH of 6 (acidic) (Borah et al. 2012). L-asparaginase from *Staphylococcus* sp. OJ82 and *Pectobacterium carotovorum* MTCC 1428 show the maximum activity in a highly basic environment (pH 10), while *Thermococcus kodaka* TK1656 and *Thermococcus gammatolerans* EJ3 have the highest optimum temperature of 85 °C (Chohan and Rashid 2013; Xue et al. 2014).

Kinetic properties of microbial L-asparaginase

To understand the rate and specificity of most of the biological processes, it is indispensable to know the kinetic parameters of the enzyme (Bar-Even et al. 2011). Apart from understanding different properties of the enzyme during a biochemical process, kinetic parameters are vital to be understood to use enzymes efficiently in different industrial processes (Choi et al. 2017). Table 6 shows the kinetics of substrate hydrolysis of L-asparaginase for different substrates. The affinity of the substrate hydrolysis for the enzyme is calculated by Michaelis constant (K_m). V_{max} is defined as the maximum rate at which an enzyme is fully saturated with its substrate concentration (Javed et al. 2018). Some of the bacterial and fungal L-asparaginases are summarized in Table 6 along with the kinetic parameters. Kinetic properties depend upon the factors such as pH, temperature, type, and concentration of the substrate.

Applications of L-asparaginase

L-asparaginase is an excellent source and a potential drug to fight for acute lymphoblastic leukemia (ALL) (Krishnapura et al. 2016) and non-hodgkin lymphoma (NHL) (Kaminsky

Table 4 Purification of microbial L-asparaginase

Strain(s)	Purification steps	Purification Yield(%)/Fold(s)	Reference(s)
Acinetobacter baumannii (R7)	Isopropanol (1:2) and CM-Sephadex C-50 chromatography	77/93	Muslim (2014)
Aspergillus niger	Ammonium sulphate (80%) DEAE cellulose	72.05/1.44	Mishra (2006)
Aspergillus oryzae (CCT 3940)	Ammonium sulphate precipitation	28.6 fold	Dias et al. (2016)
Aspergillus sp.	Ammonium sulphate precipitation, Sephadex G-200	82/1.1 43.6 / 8.3	Qeshmi et al. (2018)
Bacillus sp.	Ammonium sulphate precipitation	100/1	Moorthy et al. (2010)
Bacillus licheniformis	Acetone precipitation, DEAE cellulose chromatography, Gel filtration	100/1	Mahajan et al. (2014)
Bacillus aryabhattai (ITBHU02)	Ammonium sulphate precipitation	73.6/1	Singh et al. (2013)
Bacillus pumilus	Ammonium sulphate precipitation, DEAE cellulose	0.05/1.5	Qeshmi et al. (2018)
Bacillus megaterium (H-1)	Ni-IDA (iminodiacetic acid) affinity chromatography	42.4/3.3	Zhang et al. (2015)
Cladosporium sp.	DEAE cellulose column, Methanol precipitation	190 fold	Kumar and Manonmani (2013)
Cornyebacterium glutamicum	Protamine sulphate precipitation, Sephacryl S-200 gel filtration	98 fold	Kumar and Sobha (2012)
Escherichia coli	Ammonium sulphate precipitation, Ion exchange chromatography	N.R	Borah et al. (2012)
Fusarium culmorum (ASP-87)	Ammonium sulphate precipitation, Gel filtration chromatography	2.6/14.0	Meghavarnam and Janakirman (2016)
Mucor hiemalis	Acetone precipitation, Affinity chromatography	18.5/4.6	Thakur et al. (2014)
Penicillium sp.	Ammonium sulphate precipitation Sephadex G-100–120 gel filtration	36.2%	Patro and Gupta (2012)
Pseudomonas otitidis	Ammonium sulphate precipitation, DEAE cellulose chromatography	38.9/151.88	Husain et al. (2016)
Pectobacterium carotovorum (MTCC 1428)	Ammonium sulphate precipitation, DEAE cellulose chromatography	72.12/42.05	Kumar et al. (2011)
Pseudonocardia endophytica (vuk-10)	Ammonium sulphate precipitation, Sephadex G-100 gel filtration,	61/96	Mangamuri et al. (2016)
Paenibacillus barengoltzii (CAU904)	Ni-IDA (iminodiacetic acid) Affinity chromatography using	61.7/2.0	Qeshmi et al. (2018)
Pseudomonas fluorescens	Ni–NTA (nitrilotriacetic acid) affinity chromatography	85.4/4.1	Sindhu and Manonmani (2018)
Proteus vulgaris	Ammonium sulphate precipitation, Sephadex G-100 gel filtration,	N.R	Kumar and Sobha (2012)
Rhizobium etli	Hi-Trap-Ni	97/18	Angélica et al. (2012)
Rhizomucor miehei	Ni-IDA (iminodiacetic acid) affinity chromatography	48.8/2.6	Huang et al. (2014)
Streptomyces fradiae (NEAE-82)	Ion exchange chromatography using DEAE Sepharose C L-6B	4.9/3.3	Naggar et al. (2016)
Staphylococcus sp. (OJ82)	Ni–NTA (nitrilotriacetic acid) affinity chromatography	98.8%	Han et al. (2015)
Streptomyces gulbargensis	Ammonium sulphate precipitation Sephacryl S-200 gel filtration	50.6/1.8 37.8/26.88	Amena et al. (2010)
Streptomyces noursei (MTCC 10469)	Ammonium sulphate precipitation, Sephacryl S-200 gel filtration	100/1.0	Qeshmi et al. (2018)
Thermococcus kodaka (TK1656)	Ammonium sulphate precipitation, Ion exchange column, Size exclusion column	23/5.5	Chohan and Rashid (2013)

Table 4 (continued)

Strain(s)	Purification steps	Purification Yield(%)/Fold(s)	Reference(s)
Thermococcus gammatolerans (EJ3)	SDS-PAGE and gel filtration chromatography	N.R	Xue et al. (2014)
Thermus Thermophilus	Ammonium sulphate precipitation, DEAE Phenyl sepharose	43/60	Pritsa and Kyriakidis (2001)
Yersinia pseudotuberculosis (Q66CJ2)	DEAE-Toy pearl pooled peak	82%	Pokrovskaya et al. (2012)

N.R not reported

Table 5 Physiochemical properties of microbial L-asparaginases

Strain(s)	Mass (kDa)	pH opt	Temp. opt. (°C)	References
Acinetobacter baumannii (R7)	160	7.2	37	Muslim (2014)
Aspergillus niger	N.R	6.5	40	Mishra (2006)
Aspergillus oryzae (CCT 3940)	115	7.0–8.0	30–40	Dias et al. (2016)
Aspergillus sp.	56	6.0	47	Qeshmi et al. (2018)
Bacillus pumilus	71	7.0	45	
Streptomyces noursei (MTCC 10469)	N.R	8.0	50	
Streptomyces fradiae (NEAE-82)	53	8.5	40	Naggar et al. (2016)
Bacillus sp.	45	7.0–8.0	37	Moorthy et al. (2010)
Bacillus licheniformis	135	6.0–10	40	Mahajan et al. (2014)
Bacillus aryabhattai (ITBHU02)	155	8.5	40	Singh et al. (2013)
Bacillus megaterium (H-1)	40	7.0	40	Zhang et al. (2015)
Bacillus firmus (AVP 18)	N.R	9.0	37	Rudrapati and Audipudi (2017)
Cornyebacterium glutamicum	80	7.0	40	Kumar and Sobha (2012)
Cladosporium sp.	121	6.3	30	Kumar and Manonmani (2013)
Escherichia coli	153	6.0	55	Borah et al. (2012)
Fusarium culmorum (ASP-87)	90	8.0	40	Meghavarnam and Janakirman (2016)
Mucor hiemalis	96	7.0	37	Thakur et al. (2014)
Pseudomonas otitidis	205 ± 3 (native-PAGE), 34 ± 1 (SDS-PAGE)	7.5	40	Husain et al. (2016)
Pectobacterium carotovorum (MTCC 1428)	144	8.0–10	40	Kumar et al. (2011)
Pseudonocardia endophytica (vuk-10)	120	N.R	N.R	Mangamuri et al. (2016)
Paenibacillus barengoltzii (CAU904)	41	8.5	45	Qeshmi et al. (2018)
Pseudomonas fluorescens	140	7.5	37	Sindhu and Manonmani, (2018)
Proteus vulgaris	N.R	7.0–8.0	57	Kumar and Sobha (2012)
Penicillium sp.	66	7.0	37	Patro and Gupta (2012)
Rhizobium etli	47	9.0	37	Angélica et al. (2012)
Rhizomucor miehei	133.7	7.0	45	Huang et al. (2014)
Staphylococcus sp. (OJ82)	37.5	9.0–10	37	Han et al. (2015)
Streptomyces gulbargensis	N.R	9.0	40	Amena et al. (2010)
Thermococcus kodaka (TK1656)	71	9.5	85	Chohan and Rashid (2013)
Thermococcus gammatolerans (EJ3)	36.5	8.5	85	Xue et al. (2014)
Thermus thermophilus	33	9.2	77	Pritsa and Kyriakidis (2001)
Yersinia pseudotuberculosis (Q66CJ2)	36	8.0	60	Pokrovskaya et al. (2012)

N.R not reported

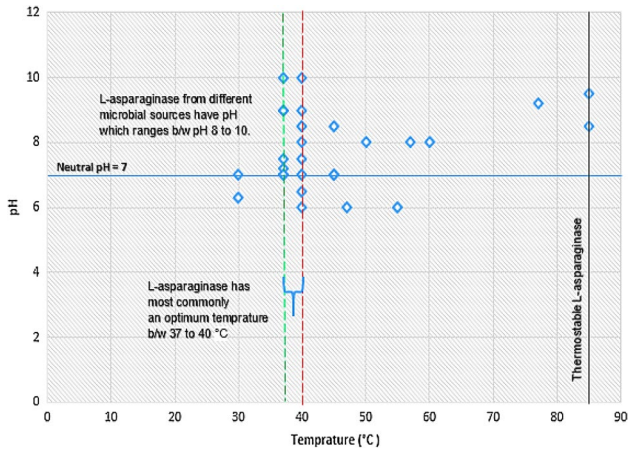

Fig. 3 Graph showing the variation in temperature and pH of microbial L-asparaginase

2017). Apart from cancer, L-asparaginase has also shown medicinal importance in the treatment of infectious and autoimmune diseases. L-asparaginase has also helped the food industry to tackle the problems that are generated due to the unwanted production of acrylamide, a carcinogenic agent in certain food products such as potato chips, etc. (Vimal and Kumar 2017). Some of the important applications of L-asparaginase in health, pharmaceuticals and food industry are discussed briefly.

Health and pharmaceuticals

L-asparaginase is a potent anti-cancer drug that has been widely studied in health and pharmaceutical research circles. It is enlisted as essential medicine, to be one of the safest and effective medicines by World Health Organization (WHO 2015). The anti-proliferative effect of L-asparaginase on leukemic cells was first studied during the 1970s (Saeed et al. 2017). The anti-cancerous activity of L-asparaginase

Table 6 Kinetic properties of L-asparaginase

Strains	Substrate	*V*max	*K*m (mM)	Specific activity (U/mg)	References
Streptomyces fradiae	N.R	51 IU/mL	10.07	30.6	El-aggar et al. (2016)
Bacillus megaterium (H-1)	L-asparagine	1.58 IU/µg	0.8	45	Zhang et al. (2015)
Bacillus (PG03)	N.R	0.067 µmol/min	23.8	N.R	Rahimzadeh et al. (2016)
Bacillus (PG04)	N.R	0.024 µmol/min	3.3	N.R	
Bacillus licheniformis	L-asparagine	4.03 IU	0.014	0.22	Mahajan et al. (2014)
Bacillus coagulance	N.R	N.R	4.7	10.9	Han et al. (2015)
Bacillus aryabhattai ITBHU02	N.R	1.537 IU/µg	0.257	680.47	Singh et al. (2013)
Bacillus subtilis B11—06	L-asparagine	77.51 µmol/min	0.43	9.98	Jia et al. (2008)
Cladosporium sp.	L-asparagine	4.44 µmol/mL/min	0.100	83.3	Kumar and Manonmani (2013)
Escherichia coli (DE3)	L-glutamine L-asparagine	4.032 mol/min N.R	50 N.R	1.2 8.4	Sindhu and Manonmani (2018)
Fusarium culmorum (ASP-87)	N.R	0.5 µmol/mL/min	3.57	3.125	Meghavarnam and Janakirman (2016)
Fusarium sp.	N.R	40 IU	444	2.9	Asha and Pallavi (2012)
Mucor hiemalis	L-asparagine	625 U/mL	4.3	69.43	Thakur et al. (2014)
Penicillium sp.	L-asparagine	N.R	4.00	13.97	Patro and Gupta (2012)
Pseudomonas fluorescens	L-glutamine L-asparagine	4.032 mol/min N.R	50 N.R	0.1 6.4	Sindhu and Manonmani (2018)
Pectobacterium carotovorum (MTCC 1428)	N.R	4.45 IU / µg	0.657	N.R	Kumar et al. (2011)
Rhizobium etli	L-asparagine	128 ± 2.8 U/mg	8.9 ± 0.967	1.1	Angélica et al. (2012)
Rhizomucor miehei	L-asparagine	3,380.0 ± 133.4 µmol/min/mg	25.30 ± 2.4	1985	Huang et al. (2014)
Staphylococcus sp. (OJ82)	N.R	61.4 U/mL	2.2	86	Han et al. (2015; Guo et al. (2017)
Thermococcus kodakaraensis	L-asparagine	N.R	5.5	3300	
Thermococcus kodakaraensis (TK1656)	L-asparagine	3300 µmol/mg /min	5.5	2350	Chohan and Rashid (2013)
Thermococcus gammatolerans (EJ3)	L-asparagine	N.R	10	N.R	Xue et al. (2014)
Yersinia pseudotuberculosis	L-asparagine	N.R	0.017 ± 0.9	N.R	Pokrovskaya et al. (2012)

N.R not reported

is due to its ability to hydrolyze asparagine into aspartic acid and ammonia (Krishnapura et al. 2016). It has been studied that monitoring the asparagine depletion and anti-asparaginase antibody (Abs) is vital to evaluate the efficacy of L-asparaginase therapy (Paillassa et al. 2018). Injecting or inoculating purified L-asparaginase into the blood stream rapidly counters and depletes L-asparagine concentrations in the blood plasma as a result of which the tumor cells are suppressed due to unavailability of asparagine, which is the highly required amino acid for the fast-growing tumor cells (Krishnapura et al. 2016). *E. coli* derived L-asparaginase is used to treat acute lymphoblastic leukemia but due to the clinical hypersensitivity these enzymes are inactivated and the desired results are not obtained. To avoid all these limitations, clinically modified versions of the enzyme are being used and L-asparaginase from different sources is being investigated for less cytotoxic effects (Mohamed et al. 2016). Furthermore, protein engineering modifications in the purified L-asparaginase has eliminated completely or partially the immunogenicity of the enzyme that has enabled it to act as an antineoplastic agent (Savitri et al. 2003). Although there are a number of therapeutic L-asparaginase products present in the market, current studies have shown that the L-asparaginase from *Erwinia carotovora* (ERWINASE) might be more efficient with fewer side effects. Other brands approved by the FDA include ELSPAR, KIDROLASE, and ONCASPAR which are currently being used against acute lymphoblastic leukemia and lymphosarcoma (Varalakshmi 2013). The need for new and scientifically modified versions of therapeutic enzymes is of great interest in both biotechnology and medicine (Ahmad et al. 2012). Although L-asparaginase has been used against different types of cancers but it is not without the side effects as it is associated with different allergic reactions and toxicity which demands bio-better (new drugs designed from existing peptides or protein-based biopharmaceuticals by enhancing and improving their properties such as affinity, selectivity, and stability against degradation) L-asparaginase in the market (Brumano et al. 2019).

Food industry

Food is the basic human need, and with the advent of industrialization, it is now available in different forms, packaging, and with highly preservative treatments. The food industry is now one of the most important and giant industries of the global village. Apart from the great efforts of the food industry to provide well-treated and preserved food items, there are certain problem that may arise due to large-scale production of the food products, for example when potato chips are made, potato is cooked at high temperature which results in the production of an unwanted carcinogenic agent, i.e. acrylamide (Adebo et al. 2017). This process is not only limited to delicious potato chips production but also to all the carbohydrate-rich foods that are cooked at high temperatures, a brief account is given in Table 7.

Table 7 Acrylamide contents of different food products

Food product	Acrylamide content (ug/kg)	References
Fried potato chips	375–7024	Adebo et al. (2017)
Cakes	13–50	
Fries	3300	
Chocolate	750	
Potato chips	90–802	
Infant cereals (ready to eat)	11–52	
Bread	90–802	
Roasted coffee	45–9359	Krishnakumar and Visvanathan (2015)
Dairy products	< 10–130	
Bakery products and biscuits	18–3324	

There are serious risks and huge concerns of acrylamide exposure to millions of individuals consuming these products daily (Adebo et al. 2017). Studies have confirmed that formation of acrylamide is due to the Millard reaction which occurs at high temperature between L-asparagine and carbonyl compounds present in carbohydrate-rich food sources (Krishnakumar and Visvanathan 2015). Asparaginase hydrolyzes asparagine into aspartic acid and ammonia (Borda and Alexe 2011) and successfully reduces acrylamide levels in potato and a number of different bakery products. Treatment of different food products with pure asparaginase like dough of ginger bread resulted in nearly 70–75% decrease in free asparagine and a reduction of 50–55% in acrylamide level in the baked products. Currently, two products are available commercially in the market: Acrylaway® (Novozymes, Denmark) and PreventAse™ (DSM Food Specialties, Denmark), synthesized from *Aspergillus oryzae* and *Aspergillus niger*, respectively (Adebo et al. 2017). FDA has suggested that the recombinant enzyme from some microbial sources such as *B. subtilis* is considered safe for food processing (Baskar et al. 2019). This is how L-asparaginase has proved to be an excellent agent to fight carcinogenic chemicals like acrylamide in the food industry and is saving hundreds of thousands of food lovers around the globe.

It must be noted that highly thermostable L-asparaginase is required in the food industry as the raw food is treated at high temperature (Li et al. 2018). L-asparaginase conjugated magnetic nanoparticles have efficiently minimized the production of acrylamide contents in food and furthermore, it confirmed that immobilizing the enzyme on the surface of magnetic nanoparticles through covalent bonds increased its reusability, catalytic efficiency, and thermal stability (Alam et al. 2018). Enzyme technology can help researchers and enzyme engineers to design highly thermostable versions

of the enzyme which can be used in different industrial processes with greater ease.

Development of biosensors

L-asparaginase-based biosensors have been developed to diagnose leukemia in leukemic patients. Levels of asparagine can be measured using these biosensors. Different enzyme immobilization techniques are used to develop plant-based L-asparaginase biosensors. Such biosensors have shown positive results by effectively measuring the asparagine levels in normal and leukemic blood serum samples. The range of detection was between 10^{-10} and 10^{-1} M (Kumar et al. 2013). Verma et al. (2007a) showed that *E. coli* K-12-derived asparaginase-based biosensors can detect asparagine levels of 10^{-9} M. Phenol red indicator and L-asparaginase can be co-immobilized together on beads made up of silicone gel and calcium alginate to produce a diagnostic biosensor for leukemia by color visualization. It is found that plant-based biosensors are more efficient as compare to microbial based, because later give more response time as compared to the former one (Kumar et al. 2013). Verma and Kumar (2012) developed a whole-cell-based fiber optic biosensor by co-immobilizing L-asparaginase producing microbes and phenol red indicator with tetramethyl orthosilicate gel, which finds application in monitoring L-asparagine content in food samples. *Solanum nigrum* is an excellent plant source for the development of biosensor to monitor L-asparagine in different samples. L-asparaginase is co-immobilized with phenol red in TEOS-chitosan disc, while spectrophotometer fiber optic is used as a transducer. The response time of the biosensor was 5 min with 10^{-1} to 10^{-10} M linear range of detection (Kataria et al. 2015).

Biosynthesis of amino acids

L-asparaginase plays an important and vital role in the biosynthesis of amino acids mostly those derived from aspartate, these include threonine, lysine, methionine and isoleucine. Aspartate being the precursor of these amino acids is formed from asparagine through its catalyzed hydrolysis by asparaginase. Krebs cycle also produces aspartate. L-asparaginase is of great importance for the biosynthesis of these amino acids (Qeshmi et al. 2018).

Modification of L-asparaginase

L-asparaginase has a short half-life with high immunogenicity due to glutaminase activity. Moreover, it is studied that tumorous cells start producing L-asparagine intracellularly, which make them resistant to L-asparaginase action. Apart from this, the induction of some antibodies in patients' body undergoing asparaginase therapy can render L-asparaginase ineffective due to its accelerated cleavage (Varalakshmi 2013). The enzyme is also susceptible to thermal and proteolytic digestion, while sometimes, it is denatured due to exposure to organic solvents (Krishnapura et al. 2016). To counter all these limitations during the enzymatic action of L-asparaginase, modified versions of the enzyme are highly needed. Some such modifications in the enzyme that are done to enhance its effectiveness are summarized in Table 8.

Conclusion and future prospects

L-asparaginase obtained from different sources is of great importance because of its therapeutic effects against cancerous and tumorous cells. Apart from pharmaceutical uses, it is being used in the food industry to efficiently hinder the acrylamide production in carbohydrate-rich foods. L-asparaginase obtained from different microbial sources slightly differ in physical and biochemical properties with different kinetic parameters. The variation in the physiochemical and kinetic properties of the enzyme have provided us the opportunity to search and characterize this enzyme according to the requirements. Some sources provide L-asparaginase that works efficiently at 37 °C which is the normal human body temperature, hence L-asparaginase from these sources can be purified and used in the pharmaceutical industry to make drugs for human, while the highly thermostable version of this enzyme can be applied in industrial processes where high temperature is usually required. Despite the fact that L-asparaginase is being widely used as an anti-cancerous drug and in the industrial process for food treatment, it has some limitations which affect its stability and activity, therefore, there is a need of new modifications in it at the molecular level through enzyme engineering technology to obtain highly versatile versions of the enzyme. Future perspectives of the studies on L-asparaginase aims to enhance L-asparaginase activity and eliminate the glutamine and immunosuppressive side activity. Highly thermostable versions of the enzyme are being genetically engineered for a large number of industrial applications.

Table 8 Modification/engineering of L-asparaginase

Modified/engineered property	Modification	Result	References
Drug immunogenicity	Enzymes modified using polyethylene glycol (PEG) which resulted in the production of PEGylated asparaginase	Low immunogenicity obtained as compared to its native enzyme	Savitri et al. (2003)
Thermal stability, protolytic digestion, half-life	The enzyme is coupled with dextran	High retention, catalytic activity, protease stability and prolonged half-life obtained as compared to the native enzyme	Varalakshmi (2013)
Proteolytic cleavage	Lactosylation: reductively coupling L-asparaginase and lactose	Becomes thermostable and resistant to proteolytic cleavage	Marsh et al. (1977)
Stability and activity	L-asparaginase immobilized with polyaniline nanofibers	Enhanced stability and activity obtained	Ghosh et al. (2012)
Immunogenicity	Non-digestible fructan also known as inulin is oxidized with periodate which is then conjugated to L-asparaginase enzyme	Decreased immunogenicity	Tabandeh and Aminlari (2009)
Stability and kinetic properties	Levan (glycoprotein) from bacteria *Zymomonas mobilis* and was covalently coupled to *Erwinia carotovora* L-asparaginase	Improved kinetic properties, with decreased electrophoretic mobility	Vina et al. (2001)
Biological activity and stability	Site-directed mutagenesis to replace destabilizing amino acids with stabilizing residues	Enhanced biological activity and stability	Vidya et al. (2013)
Substrate affinity	Microparticles of sericin (a globular protein) and glutaraldehyde were used to covalently immobilize L-asparaginase	Higher substrate affinity	Zhang et al. (2004)
Quaternary structure stabilization	L-asparaginase immobilized on activated agarose-glutaraldehyde gel, and chemically cross-linked with aldehyde-dextran	The fully stabilized multimeric structure was obtained that prevented enzyme inactivation by subunit dissociation	Balcão et al. (2001)

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