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METHODS FOR SCREENING OF NOVEL L-ASPARAGINASE FROM MICROORGANISMS

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Abstract

L-asparaginase is a hydrolase able to mediate the cleavage reaction of the amide bond from asparagine to ammonia and aspartate. This hydrolysis reaction plays a very important role in the medicine, bringing the solution to diseases considered incurable such as acute lymphoblastic leukemia (ALL), malignant diseases of the lymphoid system, different lymphomas, being a treatment used in chemotherapy schemes. Despite the adverse effects that it can trigger in the body, L-asparaginase remains the main treatment for such diseases. It is well known that microorganisms, mainly bacteria, are able to produce important quantities of L-asparaginase. However, the level of this enzyme need to be improved in order to reduce the costs, and this could be realized both by identification of new microbial producers or optimization of the production technologies. Moreover, the new sources of L-asparaginase must be free of glutaminase and urease activity, these enzymes being involved in the side effects of enzymatic treatment of ALL. For this reason, the identification of new sources of L-asparaginase, free of glutaminase as well as urease, remains an important goal for researches in domain. The present review aims to discuss the microbial sources of L-asparaginase, the methods used for screening new microbial strains isolated from different sources able to produce large quantities of L-asparaginase, the molecular aspects of the main enzyme producers, as well as the characteristics of enzymes and their applications.

Key words*: different sources, L-asparaginase, molecular aspects, new microbial, screening.*

INTRODUCTION

There are more than 50 years from the first demonstration of the anti-tumour activity of *Escherichia coli* L-asparaginase II (Broome, 1961) and during the time, several applications (including commercial) in food and therapeutics have been described. The importance of this enzyme is proved by the high global demand for it, 380 million USD being used in 2017 for its commercialization. It is estimated that the total request of L-asparaginase to increase to up 420 million USD by 2025 (Alam et al., 2019, Muneer et al., 2020).

The L-asparaginases are widespread in nature, being found in plants, microorganisms (Gram-positive and Gram-negative bacteria, filamentous fungi, yeasts, algae) or animal tissues (pancreas, liver, brain, ovaries or testes, kidneys, spleen and lungs from fish, mammals or birds).

The applications in medicine, in the treatment of Acute Lymphoblastic Leukemia (ALL) are based on the effect of L-asparaginase that diminishes the supply of asparagine to cancer cells, leading to apoptosis. However, the clinical data proved that the depletion of glutamine due to the dual glutaminase and L-asparaginase activity of some of the commercial enzymes may lead to secondary effects like pancreatitis, central nervous system dysfunction, haemostasis abnormalities and immunological reactions due to antibody production (Ashok et al., 2019). Moreover, in some commercial preparations was detected the presence of urease activity, which can reduce the efficacy of enzymatic treatment of ALL. For this reason, as well as for industrial production of enzymes, new sources of L-asparaginase, that is free of glutaminase and urease activity, both prokaryotic and eukaryotic origin, remains the main objective of many scientists and are essential so to overcome the disadvantages associated with actual commercial products (*Escherichia coli* and *Erwinia chrysanthemi* asparaginases, and their derivative.) However, based on the main characteristics as producers (easy cultivation in non-expensive media, high biomass accumulation or increased secretion of the extracellular enzymes) microorganisms remain the main sources of the L-asparaginases.

CHARACTERISTICS OF L-ASPARAGINASE

L-asparaginase is an amidohydrolase characterized by the ability to catalyse L-asparagine to L-aspartate and ammonia (Shi et al., 2017).

The mechanism of action of L-asparaginases involves two steps with an intermediate - beta-acyl-enzyme (Figure 1). The first step of hydrolysis produced by L-asparaginase involves the activation of the nucleophilic residue of the enzyme by NH_2 and the production of the intermediate product beta-acyl-enzyme from L-asparagine. The second step of the process presume the hydrolysis of this intermediate to L-aspartic acid, with liberation of NH_3 (Shakambari et al., 2019).

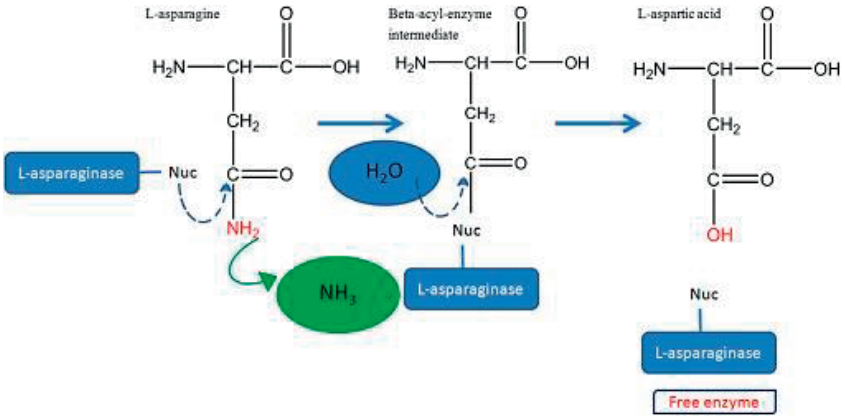

Figure 1. Mechanism of action of L-asparaginase (according Shakambari et al., 2019)

Normal cells as well as leukemic cells require L-asparagine for protein synthesis, but unlike malignant cells, normal cells are capable of synthesizing asparagine by using metabolic pathways that involve transaminase enzyme and asparagine synthetase. The transaminase converts oxaloacetate into an intermediate aspartate, which later on transfers an amino group from glutamate to oxaloacetate producing α-ketoglutarate and aspartate; then, aspartate is converted to asparagine by asparagine synthetase (Batool et al., 2016). In neoplastic cells, asparagine synthetase in not produced and exogenous asparagine is essential for protein synthesis. The presence of L-asparaginase in tumour tissues determines the hydrolysis of all exogenous asparagine, leading to the death of the cancer cells.

Based on the studies performed with bacterial L-asparaginases it was shown that the enzyme exists as a tetramer but hexameric, dimeric, and monomeric forms were detected in enzymes isolated from various sources (Batool et al., 2016). It was shown that the L-asparaginase is active as a homotetramer, with monomers of about 330 amino acid residues (Qeshmi et al., 2018). Bacterial L-asparaginases have many common biochemical properties (optimum pH, optimum temperature etc) with similar tertiary and quaternary structures. The results obtained demonstrated that most of the enzymes work optimally at a temperature of 40°C with a maximum range of pH 6.0-10, but thermostable L-asparaginases which work at 80-85°C were identified (Muneer et al., 2020). The properties of the L-asparaginases during biochemical processes as well as the kinetic parameters are vital in order to use enzymes efficiently in different industrial processes and, for these reasons, intensive studies are performed not only in identification of novel sources of enzymes but in characterization them.

SOURCES OF L-ASPARAGINASE

Occurrence of L-asparaginases in fungi, yeasts, bacteria, algae, plant cells, and animal cells, and their antitumor effects were reviewed by various scientists (Zuo et al., 2015; Shrivastava et al., 2016; Queshni et al., 2018; Shakambari et al., 2019; Muneer et al., 2020). The most intense studies were performed with microorganisms, both prokaryotic and eukaryotic. The microbial L-asparaginase studied until now can differ in their cellular location and properties, namely periplasmic asparaginase, extracellular asparaginase, intracellular asparaginase, and

glutaminase-asparaginase, and plays a role in basic metabolism (Zuo et al., 2015). Based on the studies performed with *E.coli* L-asparaginases, two types of enzymes that differ in their affinity for L-asparagine were described: type I of L-asparaginases are cytoplasmic enzymes that have a lower affinity to asparagine; the type II is found in the periplasmic space and has a high affinity to the substrate (L-asparagine), explaining their anti-leukemic activity (Souza et al., 2017; Cornea et al., 2002).

BACTERIAL L-ASPARAGINASE

Commercial asparaginases are produced using *Escherichia coli* and *Erwinia chrysanthemi* but a large number of Gram-positive and Gram-negative bacteria were identified as producers of L-asparaginase (Table 1). Bacterial L-asparaginases are differentiated by different affinity, different stability and different pH, the production rate that may be affected by the concentration of C and N sources, temperature, aeration rate, pH, time and inoculation (Ali et al., 2017).

The kinetic properties and biochemistry of the L-asparaginase vary depending on the microbial source. For example, L-asparaginase produced by *Erwinia* spp. is considered less toxic compared to the enzyme produced by *E.coli* that could cause allergic reactions. But the enzyme produced by *Erwinia* has a shorter half-life comparing with the *E. coli* enzyme (Prakasham et al., 2007).

Gram-negative bacteria (like *E. coli*) produce two types of L-asparaginase: L-asparaginase I (cytosolic enzyme that show relatively low affinity to L-asparagine and relatively high specific activity towards L-glutamine) and L-asparaginase II (periplasmic enzyme that displays low to negligible activity against L-glutamine in addition to high specific activity against L-asparagine) (Qeshmi et al., 2018).

In other Gram-negative bacteria, like *Rhizobium etli* were identified two L-asparaginases: a thermostable and constitutive L-asparaginase I, and a thermolabile, inducible (by asparagine) L-asparaginase II, repressible by the carbon source (Moreno-Enriquez et al., 2012). It was shown, according to the nucleotide sequence of the gene and the amino acid sequence of the protein, that *R. etli* asparaginase II are different from *E. coli* and *E. chrysanthemi* L-asparaginases. The potential application in chemotherapy of the L-asparaginase II from *R. etli* is related to the glutaminase-free property (Moreno-Enriquez et al., 2012).

In the genus *Bacillus* two L-asparaginases were also identified; they are encoded by specific genes: *ansZ* gene (encodes a functional L-asparaginase with 59% similarity to *E. chrysanthemi* L-asparaginase and 53% identity to the L-asparaginase II from *E. coli*) and *ansA* gene (encodes type I L-asparaginase) (Yano et al., 2008).

The production of L-asparaginase has been reported in different Actinobacteria, mainly in *Streptomyces* genus, isolated from various sources (soil, plant material, rivers, marine ecosystems etc) (Arévalo-Tristancho et al., 2019). These enzymes belong to the L-asparaginase II family, as it was demonstrated by phylogenetic tree analysis of *ansA* gene sequence, and the amino acid sequences of the enzymes are conserved among the similar proteins from different *Streptomyces* species (Mena et al., 2015). The advantages of L-asparaginase from streptomycetes are: production in extracellular manner, minimal or no L-glutaminase activity, and high activity at physiological conditions.

Relative recent studies demonstrated that several extremophiles (thermophiles and extreme halophilic bacteria) (*Pyrococcus, Thermus, Salinococcus, Thermococcus* etc) (Table 1) are able to produce thermostable L-asparaginase, efficient in a large range of pH (6.0-10).

Bacterial endophytes are now considered by many scientists as potential sources of L-asparaginase (Chow and Ting, 2015). According to Nongkhlaw and Joshi (2015), a number of endophytic bacteria isolated from ethnomedicinal plants identified as *Serratia marcescens* cenA, *Bacillus subtilis* cenB, *B. methylotrophicus* PotA and *B. siamensis* C53 are able to produce important quantities of L-asparaginase. Other authors (Krishnapura and Belur, 2015), reported high level of enzyme in 31 endophytic bacterial strains isolated from medicinal plants of the Family Zingiberaceae.

The marine environment is considered a good source of new bacterial strains capable of producing various bioactive compounds, including L-asparaginase. Recent studies

reported increased level of L-asparaginase produced by marine *Bacillus velezensis* (Mostafa et al., 2019), strains of *B. licehniformis* isolated from Red Sea (Alrumman et al., 2019), *Pseudonocardia endophytic* isolated from mangrove ecosystem (Muneer et al., 2020), actinomycetes (Dhevagi and Poorani, 2005) etc.

Table 1. L-asparaginase producing bacteria (adapted from Shakambari et al., 2019 and Muneer et al., 2020)

Bacteria	Species	References
Gram-negative	*E. coli*	Netrval, 1977, Cachumba et al., 2016
	Acinetobacter calcoaceticus	Joner et al., 1973 (cited by Shakambari et al., 2019)
	Erwinia cartovora	Maladkar et al., 1993 (cited by Shakambari et al., 2019)
	Acinetobacter soli	Jiao et al., 2020
	Pseudomonas stutzeri	Hosamani and Kaliwal, 2011 (cited by Muneer et al., 2020)
	Serratia marcescens	Agarwal et al., 2011(cited by Muneer et al., 2020)
	Rhizobium etli	Angélica et al., 2012 (cited by Muneer et al., 2020)
	Salmonella typhimurium	Kullas et al., 2012 (cited by Muneer et al., 2020)
	Thermus thermophilus	Pritsa and Kyriakidis, 2001 (cited by Muneer et al., 2020)
	Aeromonas sp.	Muslim, 2014
	Pseudomonas proteolytica	Shukla et al., 2014 (cited by Muneer et al., 2020)
	Pseudomonas aeruginosa	Kamble et al., 2012 (cited by Muneer et al., 2020)
	Pseudomonas fluorescens,	Sindhu and Manonmani, 2018
	Pectobacterium carotovorum	Jetti et al*.,* 2018
	Enterobacter aerogenes	Baskar et al., 2013 (cited by Muneer et al., 2020)
	Acinetobacter baumannii	Muslim, 2014
	Erwinia aroideae *Hydrogenomonas eutropha*	Ali et. al., 2017
	Aquabacterium sp.	Sun et al., 2016
Gram-positive	*Streptomyces gulbargensis,* *Streptomyces venezuelae*	Naveena et al., 2012 (cited by Muneer et al., 2020)
	Bacillus pseudomycoides *Paenibacillus denitriformis*	Joshi and Kulkarni (2016) Muneer
	Bacillus licheniformis, B. circulans	Gulati et al. (1997), Alrumman et al. (2019)
	Bacillus firmus (AVP 18)	Rudrapati and Audipudi (2017) (cited by Muneer et al., 2020)
	Streptomyces alkaliphilus, *Staphylococcus roseus*	Jha et al. (2012) (cited by Muneer et al., 2020)
	Staphylococcus capitis	Paglla et al. (2013) (cited by Muneer et al., 2020)
	Bacillus subtilis (hsw x 88)	Feng et al. (2017)
	Bacillus amyloliquefaciens	Yim and Kim (2019)
	Paenibacillus barengoltzii	Shi et al. (2017)
	Corynebacterium glutamicum	Ahmad et al. (2012)(cited by Muneer et al., 2020)
	Anoxybacillus flavithermus	Maqsood et al., 2020
	Lactobacillus reuteri (DSM 20016)	Aishwarya et al. (2017)
	Salinicoccus sp.	Jha et al. (2012) (cited by Muneer et al., 2020)
	Bacillus spp.	Safary ey al (2019)
Archaea	*Pyrococcus furiosus*	Jayam and Kannan (2014) (cited by Muneer et al., 2020)
	Pyrococcus yayanosii (CH1)	Li et al. (2018) (cited by Muneer et al., 2020)
	Thermococcus kodakaraensis	Chohan and Rashid (2013) (cited by Muneer et al., 2020)
	Thermococcus gammatolerans	Xue et al. (2014) (cited by Muneer et al., 2020)

FUNGAL ASPARAGINASES

There are many studies that demonstrated that eukaryotic microorganisms such as yeasts and filamentous fungi have potential for the production of L-asparaginase.

The advantages of the fungal enzymes are related to several characteristics: are stable, are excreted in the extracellular environment, are easy to extract and process downstream, and could be obtained in high quantities in relative cheap culture conditions (Dias et al., 2017; da Cunha et al., 2019). More than 85% of fungal L-asparaginases are produced by ascomycetes, and only 11% by species of basidiomycetes.

The most studied fungal L-asparaginase are originated from strains of *Aspergillus, Penicillium, Fusarium, Cladosporium* are studied for their ability to produce L-asparaginase (Table 2).

Table 2. Fungal producers of L-asparaginases

Fungal genus/group	Species	References
Aspergillus	*Aspergillus alliaceus,*	Gulati et al. (1997)
	Aspergillus tubingensis,	Gonçalves et al. (2017)
	Aspergillus oryzae (CCT 3940)	Dias et al. (2016)
	Aspergillus tamari, Aspergillus terreus	Sarquis et al. (2004)
	Aspergillus niger, Aspergillus nidulans,	Baskar and Renganathan (2011) (cited by Muneer et al., 2020)
	Aspergillus fumigatus	Benchamin et al. (2019) (cited by Muneer et al., 2020)
	Aspergillus sydowii	Ali et al. (2017)
Trichoderma	*Trichoderma viride*	Chow and Ting (2015)
	Trichoderma sp.	Muneer et al., 2020
Fusarium	*Fusarium culmorum* (ASP-87), *Fusarium equiseti*	Meghavarnam and Janakiraman (2018)
	Fusarium solani	Gulati et al. (1997)
	Fusarium oxysporum	Patro and Gupta (2012) (cited by Muneer et al., 2020)
	Fusarium roseum	Shakambari et al., 2019
	Fusarium incarnatum	Ali et al., 2017
	Fusarium tricinctum	Doriya and Kumar, 2016
	Fusarium verticillioides, Fusarium proliferatum	Chow and Ting, 2015
Penicillium	*Penicillium notatum*	Eisele et al. (2011)
	Penicillium sp.	Gulati et al. (1997)
	Penicillium simplicissimum	Chow and Ting, 2015
	Penicillium digitatum	Shrivastava et al., 2016
	Penicillium brevicompactum	Cachumba et al., 2016
Colletotrichum	*Colletotrichum gloeosporioides, Colletotrichum siamense*	Chow and Ting, 2015
Alternaria	*Alternaria* sp.	Doriya and Kumar, 2016
	Alternaria alternata	Shakambari et al., 2019
Cladosporium	*Cladosporium* sp.	Chow and Ting, 2015, Mohan et al., 2013
	Cladosporium cladosporioides	Ali et al., 2017
Beauveria	*Beauveria bassiana*	Nageshwara et al. (2014) (cited by Muneer et al., 2020)
Trichosporon	*Trichosporon asahii*	Ashok et al. (2019)
Yeasts	*Pichia polymorpha, Saccharomyces cerevisiae*	Jha et al. (2012) (cited by Muneer et al., 2020)
	Candida utilis	Shakambari et al., 2019
	Yarrowia lipolytica	Darvishi et al., 2019
Basidiomycetes	*Flammulina velutipes*	Eisele et al. (2011)

Generally fungal L-asparaginases are obtained by submerged fermentation (SmF) but the method has several disadvantages: it is too expensive, low concentration of the enzyme, large amount of effluents.

For these reasons, solid state fermentation (SSF) is preferred, as the yield of the product is higher (Mohan et al., 2013; Meghavarnam and Janakiraman, 2018).

Studies performed with strains of *Fusarium culmorum* cultivated on SSF media containing soybean meal and wheat bran in SSF medium reported 8.2 fold higher L-asparaginase production than in SmF (Meghavarnam and Janakiraman, 2018). Similar results were obtained by Souza et al., (2017) with strains of the genus *Aspergillus* in both solid and submerged fermentation.

Due to their properties, yeasts are considered as important producers of proteins useful for many applications. Relative recent data demonstrated the ability of strains of *Saccharomyces cerevisiae, Pichia* sp., *Yarrowia lipolytica* or *Candida utilis* to produce important quantities of L-asparaginase in presence of organic sources of nitrogen (2% proline, for example) (Sarquis et al., 2004, Darvishi et al., 2019; Shakambari et al., 2019, Muneer et al., 2020).

Despite the increased interest for compounds produced by macromycetes, the synthesis of L–asparaginase was detected only in strains of *Flammulina velutipes* (Eisele et al., 2011).

L-ASPARAGINASE FROM PLANTS

It was demonstrated that in higher plants, asparagine is catabolized by two metabolic routes: by transamination of the amino nitrogen by the asparagine-oxo-acid aminotransferase (as reported for soybean, pea, and lupine) or by L-asparaginase, releasing aspartate and ammonia (as in temperate legumes) (Qeshmi et al., 2018). The highest levels of L-asparaginase activity were detected mainly in leaves or roots of various plant species (Michalska et al., 2006). Two forms of L-asparaginase are described in higher plants: they are immunologically distinct, based on their dependence/independence of potassium and on the affinity for asparagine (Michalska and Jaskolski, 2006).

In seeds of *Lupinus* two types of enzymes have been also identified: a potassium independent form found in *L. arboreus* and *L. polyphyllus* and a potassium dependent form found in other *Lupinus* species and in *Pisum sativum* (Mohamed et al., 2015).

Significant amount of L-asparaginase was detected in green chillies (*Capsicum annum* L.) and tamarind (*Tamarindus indica* but the potential applicability in chemotherapy is reduced due to the low affinity to asparagine the simultaneous activity on glutamine (glutaminase activity) and urea (urease activity) (Bano and Sivaramakrishnan, 1980).

METHODS FOR SCREENING AND QUANTIFICATION L-ASPARAGINASE

The screening of microbial producers of L-asparaginase is based on the increase of pH as a consequence of ammonia liberation by enzymatic activity on a specific substrate (asparagine, glutamine, urea or $NaNO_3$). This property was used for development of rapid plate detection techniques using a pH indicator dyes incorporated in culture media that contain asparagine as sole nitrogen source (Gulati et al., 1997). Generally, two types of dyes are used for detection of pH modification: phenol red and bromothymol blue. Phenol red (PR) indicator is yellow under acidic condition and turns pink under alkaline condition due to increase in pH (Figure 2).

Bromothymol blue (BTB) is another pH indicator, coloured yellow at acidic pH, with a transient green color at neutral pH, 7.0 and dark blue at higher pH 8.0-9.0, useful for screening L-asparaginase activity, with an increased sensitivity and precision than the previous method (Mahajan et al., 2013) (Figure 3).

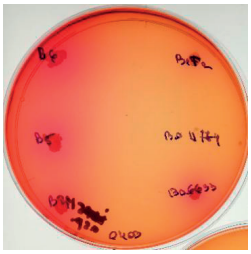

Figure 2. Screening of L-asparaginase producing bacteria in media containing asparagine and phenol red (original)

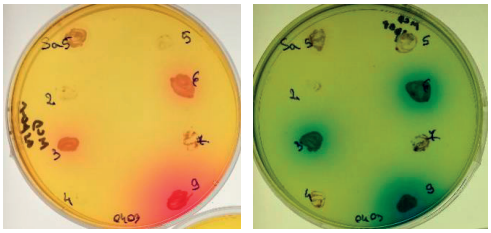

Figure 3. Screening of L-asparaginase producing yeasts in media containing asparagine and PR (left) or BTB (right) (original)

The level of enzymatic activity of L-asparaginase is very important in order to evaluate the efficiency and the practical approaches of the enzyme. The majority of the methods determine the amount of ammonia or aspartic acid released during the reaction or after the degradation of the asparagine (Batool et al., 2015). The method involves the enzymatic source (the supernatant, crude microbial lysate or purified enzyme) and Nessler reagent which leads to the appearance of a colour due to the liberation of ammonia during the enzymatic reaction (Shakambari et. al., 2019). Such methods allow the study of the influence of environmental factors on the enzymatic, and to optimize the pH and temperature values of the process (Goswami et al., 2019).

APPLICATIONS OF L-ASPARAGINASE

It is well documented that microbial L-asparaginase could be used in medicine to treat lymphoblastic leukemia and lymphosarcoma. Bacteria like *E. coli, E. carotovora, Bacillus* sp., *Enterobacter aerogenes, Corynebacterium glutamicum, P. stutzeri* and actinomycetes are described as good sources for asparaginase

(Narayana et al., 2008). More recent, fungal asparaginase has been shown to have acrylamide reduction effects in bakery products, chips, products that have a high starch component and which lead to high temperatures turn into carcinogenic products (Meghavarnam et al., 2018) (Figure 4).

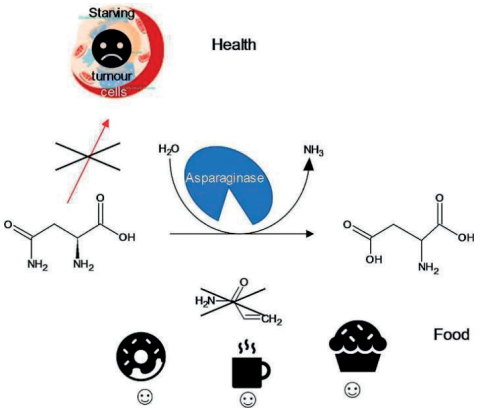

Figure 4. L-asparaginase hydrolyzes asparagine to aspartic acid, thus preventing feeding of asparagine to tumor cells and formation of acrylamide in heat-processed foods (Qeshmi et al., 2018)

The optimization of culture condition of the potential microbial producers allows the synthesis and the recovery of large amounts of the enzyme (Table 3).

ROLE OF L-ASPARAGINASE IN MEDICINE

For more than 30 years, L-asparaginase has been widely used in the treatment of acute lymphoblastic leukemia (ALL), the therapy being based on the intravenous injection of the enzyme resulting from *E. coli* and *E. chrysanthemi,* in combination with other drugs or radiotherapy (Qeshmi et al., 2018).

Table 3. Microbial strains producing L-asparaginase and their yield (adapted from Cachumba et al., 2016)

Name of the L-asparaginase producer	L-asparaginase activity
Pectobacterium carotovorum MTCC	35.24 U mg^{-1}
Bacillus licheniformis RAM-8	697.1 U mg^{-1}
Bacillus subtilis hswx88	23.8 U ml^{-1}
Bacillus aryabhattai ITBHU02	680.5 U mg^{-1}
Bacillus licheniformis MTCC 429	597.8 U mg^{-1}
Penicillium brevicompactum 829	574.24 U mg^{-1}
Fusarium solani	121 U/ml
Cladosporium sp.	83.3 U mg^{-1}

The amino acid L-asparagine is essential for the growth of tumor cells. This amino acid is not required for normal cell growth because they can synthesize the amount necessary for normal protein synthesis. Therefore, the L-asparaginase deprives the exogenous L-asparagine tumor cells so their growth is stopped (Han et al., 2014).

Bacterial L-asparaginase (from *E. coli* an *Erwinia chrysanthemy*) successfully treated acute lymphoblastic leukemia and other lymphoid malignancies. Several factors are involved in the mechanism of anti-leukemic action of L-asparaginase, for example: the hydrolysis capacity of L-asparaginase, pharmacological factors of serum clearance of the enzyme, growth of tumor cells resistant to asparaginase, activation of the immune system by producing anti-asparaginase antibodies (Shrivastava et al., 2016).

Three form of L-asparaginase are currently available for clinical applications: native L-asparaginase from *E. coli*, a PEG ylated (PEG: polyethylene glycol) form of L-asparaginase (PEG-asparaginase), and L-asparaginase from *Erwinia chrysanthemi* (*Erwinia* asparaginase). In treatment protocols, *E. coli* asparaginase or PEG asparaginase is used as a first-line treatment of childhood ALL, while *Erwinia* asparaginase has been adopted in European and US protocols for second - or third-line treatments (Shakambari et al., 2019).

Besides the advantages, bacterial L-asparaginases presents a number of problems: they can trigger allergic reactions, cause high toxicity including hepatotoxicity, acute pancreatitis, thrombosis and hyperglycemia, leading in some situations to the resistance of the disease to asparaginase (Shrivastava et al., 2016).

Hypersensitivity reactions resulting from the production of anti-asparaginase antibodies have been observed in 60% of patients at one time during *E. coli* asparaginase therapy. Symptoms include: anaphylaxis, pain, edema, Quincke edema, urticaria, erythema, rash, pruritis and major skin lesions (Pieters et al., 2010).

APPLICATIONS IN FOOD INDUSTRY

Foods containing starch, when subjected to high temperatures, may undergo changes, forming harmful compounds for the health of the consumer. One of these harmful compounds is

represented by acrylamide, formed in foods cooked at high temperatures. In fried potatoes, acrylamide is made up of reducing sugars and asparagine, being compounds of the Maillard reaction (Bethke and Bussan, 2013).

Acrylamide is generated as a by-product and usually occurs at temperatures above 100ºC and is noted for changing color and flavor in fried or baked foods containing starch. A significant amount was detected in fries, radishes, crispy chips, toast, cakes, biscuits, cereals and coffee (Alam et al., 2018). Recent studies demonstrated that the application of fungal L-asparaginase (isolated from *Aspergillus oryzae*) during potato frying significantly reduces the level of acrylamide (Dias el al., 2017). Similar aspects were observed with L-asparaginase isolated from *Fusarium culmorum*: the application of different concentration of this enzyme during the cooking of potatoes and bread reduce with 90% the formation of acrylamide (Meghavarnam and Janakiraman, 2018).

Many other L-asparaginases from diverse sources, such as *A. niger, B. licheniformis*, *B. subtilis, Cladosporium* sp., *Paenibacillus barengoltzii* or *Thermococcus zilligii* have been tested: the use of the enzyme allowed for a decrease from 34 to 97% of the acrylamide level in diverse food goods, namely biscuits, crisp bread, French fries, and sliced potato chips (Qeshmi et al., 2018).

The reduction of acrylamide level is based on the consumption of the asparagine precursor (Figure 4), without negative impact on organoleptic properties of the processed foods.

To mitigate the acrylamide formed in food, there are currently two commercial fungal asparaginases recognized by the FAO as a safe food additive: Prevent ASeTM 202 from a strains of *A. oryzae,* and Acrylaway® from a genetically modified *A. oryzae* (Xu et al., 2016).

CONCLUSIONS

L-asparaginase enzyme is a very important enzyme both for chemotherapy and for food industry. Bacterial L-asparaginase are currently used in the treatment of ALL, as first-line drug. Fungal asparaginases are useful in food industry, as food additive, for reduction the level of acrylamide produced during cooking at high temperatures.

The identification of new sources of L-asparaginase, free of glutaminase and urease activities represents the main objective of studies and are essential to overcome the disadvantages associated with actual commercial products.

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