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L-ASPARAGINASE AND L-GLUTAMINASE: SOURCES, PRODUCTION, AND APPLICATIONS IN MEDICINE AND INDUSTRY

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ABSTRACT

Amidases (L-asparaginase and L-glutaminase) catalyze the deamination process of L-asparagine and L-glutamine to their corresponding acidic form with ammonia releasing. Both enzymes are considered one of the most biomedical and biotechnologically important groups of enzymes, besides their international contributing as an important commercial products. L-asparaginase and L-glutaminase have been receiving more attention as antileukemic agent for treatment of acute lymphoblastic leukemia (ALL) and other types of cancer. On the other hand, these enzymes also used in food manufacture for their hydrolysis effect and is a possible way to decrease the amount of free L-asparagine in the preliminary ingredients of food making, thus minimize the imminent risk of causing neurotoxic and carcinogenic acrylamide compound which formed when food heated above 120 °C. Glutamic and aspartic acid are important amino acids in food processing achieve a delicious, fine, sour and umami taste beside their nutritional important to food. A recent review discusses the mode of action of L-asparaginase and L-glutaminase. Also, this review lists the sources of L-asparaginase and L-glutaminase, production optimization of enzymes, and uses of the two enzymes in cancer therapy and other industrial purposes.

Keywords: L-asparaginase, L-glutaminase, Sources, Fermentation, Applications

INTRODUCTION

L-asparaginase and L-glutaminase are members of homologous amidohydrolase enzymes, which hydrolyze asparagine and glutamine into their acids and ammonia. Enzymes are very specific and selective catalytic proteins created by living cells to control, regulate and accelerate the biochemical process in the body **(Sabu *et al*., 2000)**. Microorganisms characterize as a major source of many therapeutic enzymes due to their susceptibility to genetic manipulation and their extensive biochemical variety. Nowadays microbial enzymes have an important role in the biochemical investigation, diagnosis, curing, and monitoring of many dreaded diseases. L-asparaginase and L-glutaminase (L-asparagine amidohydrolase E.C. 3.5.1.1; L-glutamine amidohydrolase EC 3.5.1.2) have been proved to be particularly promising enzymes in the treatment of acute lymphocytic leukemia (ALL) mainly in children **(Vidhya *et al*. 2010; Unissa *et al*., 2014)**. L-asparaginase also has a therapeutic effect in the treatment of acute myelomonocytic leukemia, Hodgkin disease, acute myelocytic leukemia, melanosarcoma lymphosarcoma treatment, reticulosarbom and chronic lymphocytic leukemia **(Wetzler *et al*., 2007)** and L-glutaminase has an antiviral effect **(Kumar and Chandrasekaran 2003)**. The first observation of the two enzymes as anticancer agent; asparaginase began in 1922 when **Calimanti** observed enzyme at high activity in serum of guinea pig. **Kidd** in **1953**, when he applied serum of guinea pig for transplanted rat leukemia, suppression occurred. In the time between 1953 and 1972, many observations confirm these results. **Yalin and Wriston** in **1966** purified the two isoforms of L-asparaginase from the serum of genie pig and also from microorganisms. In the same side, the first observation for L-glutaminase as antileukaemia agent was in **1964** by **Greenberg *et al***. Followed by **El-Asmar and Greenberg** in **1966** when they indicated that L-glutaminase from *Pseudomonas* sp. had an inhibition effect on rat carcinomas but it had little effect on the survival time of experimental animals. At **1970 Roberts *et al***. proved that L-glutaminase from Gram-negative rod-shaped bacterium suppressed Ehrlich ascites carcinoma. L-asparaginase also used in food technology, to reduce the amount of free asparagine in the beginning materials due to its ability to hydrolysis of asparagine to aspartate and ammonia, so that the risk of making a potentially carcinogenic and neurotoxic acrylamide in food product decreased **(Nanda *et al*., 2003)**. Glutaminase also used as flavor enhancing agent to the presence of glutamate **(Wakayama *et al*., 2005)**. L-glutaminase and L-asparaginase used in food processing for their hydrolysis activities and the l-glutamine and L-asparagine produced considered important amino acids in food manufacturing for a delicious and fine taste, Sour and Umami taste and nutritional important to the food products **(Nanda *et al*., 2003). Sinha *et al*. (2013)** reported L-asparaginases and L-glutaminases play a vital role in the biosynthesis of fine-chemicals. The researcher also used L-glutaminase as biosensors to monitoring L-glutamine level in mammalian and hybridoma cell lines **(Huang *et al*., 2006)**. Two related families of asparaginase are designated type I and type II according to the terminology in *Escherichia coli*, which has both: L-asparaginase I is a low-affinity enzyme found in the cytoplasm, while L-asparaginase II is a high-affinity secreted enzyme synthesized with a cleavable signal sequence. Archaeal putative asparaginases are involved in type I but have an extra ~80 residues in a conserved N-terminal region **(Vidya and Vasudevan, 2011)**. On the other hand, L-glutaminases members tend to be designated as glutaminase A (glsA), where B (glsB) is unknown and may not be homologous (as in *Rhizobium etli*) some species have two isozymes that may both be designated A (GlsA1 and GlsA2) **(Botman *et al*., 2014)**.

BIOLOGICAL ROLE AND MODE OF ACTION OF L-ASPARAGINASE AND L-GLUTAMINASE

Cancer treatment by enzyme therapy could be provided with either the use of enzyme prodrug therapy or the use of antineoplastic enzyme therapy. Enzyme prodrug therapy uses antibody-conjugated enzymes, converting prodrug into cytotoxic drug at tumor cells and thereby killing tumor cells. A technique using amino acid deprivation methodology for anti-cancer therapy where depletion, thereby the inductions of starvation of amino acids are attained in tumor cells which are auxotrophic to particular amino acids. This often reduces tumor proliferation. In both normal cells and tumor cells cannot synthesize L-asparagine and L-glutamine although they need them in large amount for cell growth. Asparagine and glutamine are non-essential amino acids used by immature lymphocytes for their proliferation and run as substrate for respiration, nitrogen for the production of hexosamines, proteins, and macromolecules **(Unissa *et al*., 2014)**. Therefore, they are considered one of the key molecules in cancer

metabolism through cell proliferation. In a healthy cell, asparagine and glutamine synthetases convert aspartate to asparagine and glutamate to glutamine, respectively by using ATP as a source of energy (Figures 1 a or b and 2 a or b). While in cancer cells, they need an extraordinarily high amount for the amino acid asparagine and glutamine and cannot synthesize adequate endogenous of these amino acids due to deficiency in levels of L-asparagine and L-glutamine synthetase and consequently are reliant on serum levels for their proliferation and survival **(Miller *et al*., 1969)** or the failure of these cells to increase L-asparagine and L-glutamine synthetase level (limited) **(Gaffar and Shethna, 1977)**. Hence, administration of L-asparaginase and L-glutaminase eradicates reliant on the extracellular source of L-asparagine and L-glutamine and lead to apoptosis **(Miki *et al*., 2005)**. Still, healthy cells unaffected by way of them are gifted by synthesizing asparagine and glutamine de novo with the aid of the enzymes L-asparagine and L-glutamine synthetase **(Kumar and Sobha, 2012; Unissa *et al*., 2014)**.

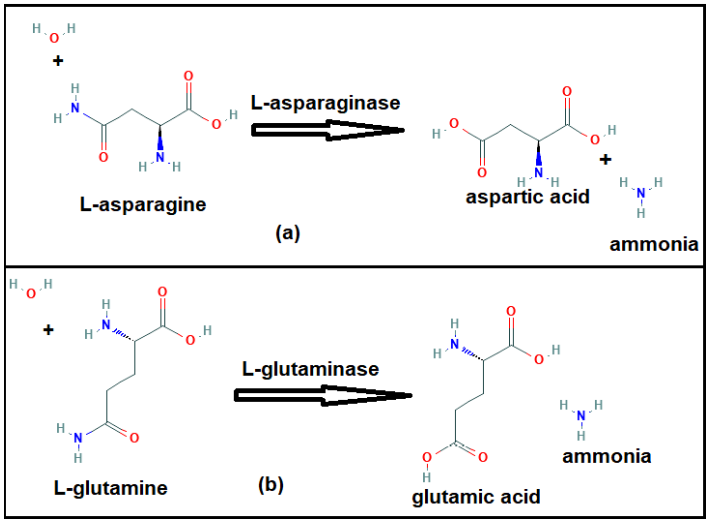

Figure 1 Schematic representation of the mode of action of a) L-asparaginase, b) L-glutaminase.

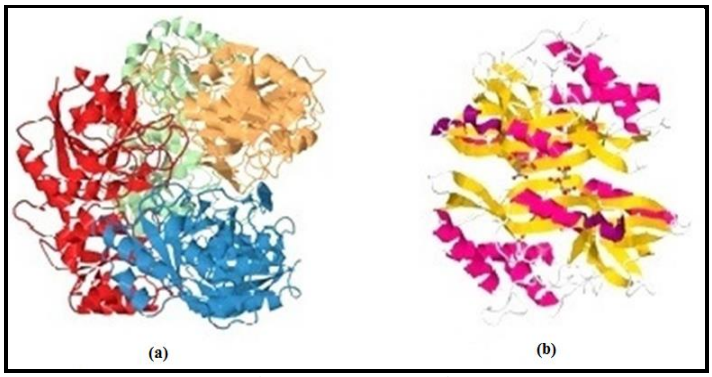

Figure 2 Crystal structure of a) L-asparaginase of *Escherichia coli* (PDB code 3ECA) **(Swain *et al*., 1993)**, b) dimeric protein-glutaminase from *Chryseobacterium proteolyticum* (PDB: 3A56) **(Hashizume *et al*., 2011)**.

SOURCES OF L-ASPARAGINASE AND L-GLUTAMINASE

BACTERIAL SOURCES

The most potent enzymes used for the treatment of leukemia for more than 40 years are bacterial sources which are mostly produced from *Erwinia carotovora* and *E. coli* **(Huang *et al*., 2006)**. Most of *Enterobacteriaceae* family members produce only L-asparaginase, while *Pseudomonas* species produce both L-asparaginase and L-glutaminase **(Imada *et al*., 1973, Dutta *et al*., 2015)**., *Serratia marcescens* SB08**,** *E. coli*, *P. pseudoalcaligenes JHS-71*, and *E. carotovora* produce L-asparaginase intracellularly **(Venil *et al*., 2009; Sajitha *et al*., 2015; Badoei-Dalfard 2016; Faret *et al*., 2018)**, while *P. aeruginosa* 50071 and *B. subtilis* WB600 produce L-asparaginase extracellularly **(El-Bessoumy *et al*., 2004; Yue *et al*., 2017)**. In other hand, L-glutaminase produced extracellularly by *Vibrio azureus* JK-79**,** and *P. otitidis* **(Kiruthika and Nachimuthu, 2013; Husain *et al*., 2016)**. Not all bacterial amidases have anticancer activities; the anticancer effect of these enzymes varies according to their affinity for substrates and their clearance rate **(El-Ghonemy, 2014)**.

FUNGAL SOURCES

Different fungal isolates from varies environments and origins show high L-asparaginase and L- glutaminase activities. Fusarium, aspergillus, and penicillium are the most reported and common fungi used for both enzymes production **(Curran *et al*., 1985; Elshafei *et al*., 2014)**. L-asparaginase with anti-oxidant properties produced by *Aspergillus* sp. and *Aspergillus oryzae* **(Soniyamby *et al*., 2011; Sudarkodi and Sunda, 2018)**. In case of *Aspergillus sojae* and *Beauveria sp*. have extracellular L-glutaminase activity and also *Trichoderma koningii* **(Sabu *et al*., 2000; Pallem *et al*., 2010; Ito *et al*., 2013)**. While intracellular L-glutaminase free L-asparaginase is obtained from *Penicillium brevicompactum* NRC 829 **(Elshafei *et al*., 2012)**.
The preproduction of L-asparaginase and L-glutaminase are also produced from yeast by *Pichia polymorpha* **(Foda *et al*., 1980)**. *Rhodotorula rosa* and *Cndida utils* produce L-asparaginase extracellularly, whereas *Saccharomyces cervisiae* produce it intracellularly **(Arima *et al*., 1972; Bon *et al*., 1997; Costa *et al*., 2016)**. *Cryptococcus albidus*, *Hansenula jadini*, *Candida scottii*, *Zygosaccharomyces rouxii* and *Rhodotorula rubra* have L-glutaminase activity **(Kashyap *et al*., 2002, Iyer and Singhal, 2009; Unissa *et al*., 2014)**.

ACTINOMYCETES

Actinomycetes are widely distributed in terrestrial and marine habitats. They have commercial significance due to their capability to form novel metabolites. Actinomycetes are considered as comparatively less explored source for L-asparaginase and L-glutaminase production and therefore act as candidates for the production of these enzymes. Actinomycetes like *S. griseoluteus* (Kumar *et al*., 2011), *Nocardia levis* and *Streptomyces ginsengisoli* (Deshpande *et al*., 2014) were reported to be potential producers of L-asparaginase. *Sterptomyces* sp. especially act as a source for L-asparaginase and L-glutaminase. *S. griseus* **(Dejong, 1972)**, *S. karnatakensis*, *S. venezuelae* **(Mostafa, 1979)**, *Nocardia asteroids* **(Gunasekaran *et al*., 1995)**, *S. albidoflavus* **(Narayana *et al*., 2008),** *S. gulbargensis* **(Amena *et al*., 2010)** and *S. parvus* NEAE-95 **(El-Naggar, 2015)** all have L-asparaginase activity. In addtion, *S. cyaneus*, *S. exfoliates* and *S. phaeochromogenes* were found to be potential candidates for production of L-asparaginase (**Saxena *et al*., 2015**). While few actinomycetes like *S. rimosus*, *S. olivochromogenes***,** and *S. pratensis* NRC 10 have L-glutaminase activity **(Balagurunathan *et al*., 2010; Tork *et al*., 2018)**. Abdallah *et al*. (2012) reported that both *S. avermitilis* and *S. Labedae* strains possess remarkable ability to produce L-glutaminase. Also, Abd-Alla *et al*. (2013) reported that *S. variabilis* that isolated from Rhizosphere of *Triticum Vulgaris* has the capicity to produce L-.glutaminase.

PLANTS

Several plants such as tamarind, chillies and tomato contain appreciable quantities of L-asparaginase and L-glutaminase; onions, potatoes and lemons have trace quantities, whereas both enzymes could not be identified in ginger and drumsticks (Bano and Sivaramakrishnan, 1980). Plants consider natural sources of both enzymes and they distinguish with their availability and safety as compared with microbes (Barbaree and Harless, 1995). Therefore, extraction of important enzymes such as L-asparaginase and L-glutaminase from plant sources are considered more safe and easier. There are many studies reported that neumerous plant species can produce L-asparaginase and L- glutaminase like *Tamarindus indica*, *Capsicum annum* (green and red chillies) and narrowleaf lupin (*Lupinus angustifoliuos*) **(Bano and Sivaramakrishnan, 1980; Kiran *et al*., 2011)**. Ashwagandha or winter cherry (*Withania somnifera*), pole beans (*Phaseolus vulgaris*) and soybean root nodules have high specificity of enzyme L-asparaginase **(Oza *et al*., 2009; Al Zobaidy *et al*., 2016; Liu *et al*., 2019).** In addition, L-asparaginase was successfuly extracted from *Phaseolus vulgaris* seeds (Mohamed et al. 2015), Vigna unguiculata (Ali, 2009), Lupinus polyphyllus (Lea et al., 1984) and pea leaves (Sieciechowicz and Ireland, 1989).

MICROALGAE

L-asparaginase from blue-green microalgae is receiving more attraction, to its high cost-effectiveness, no seasonal variation, low cost of production nutrient contents, and to its high operative producers, can easily cultivate and harvested at large scale **(Prihanto and Wakayama, 2016)**. L-asparaginase is the first such enzyme to be extracted form a marine microalgae *Chlamydomonas* sp. (Paul, 1982). *Chlorella vulgaris***,** *Spirulina maxima*, and *Phormidium formosum* **(Ebrahiminezhad *et al*., 2014; Abd El-Baky and El-Baroty, 2016; Elkomy and Farag, 2018)** considered a novel microalgal source for L-asparaginase production. Also, cyanobacterium *Oscillatoria Terebriformis* can provide a rich source of L-asparaginase producing candidate (Elkomy, 2018).

ENTRAPMENT IN ERYTHROCYTES

Red cells using as micro-bioreactor; asparagine can enter in red cells by reversible osmotic lysis from surrounding medium **(Young *et al*., 2009)**. Effect of

anti-L-asparaginase antibodies can be overcome to the protection given by erythrocytes membrane to L-asparaginase so reduction of hypersensitivity and half-life increased. Also, human glycosyl asparaginase studied to its potentiality to hydrolysis of L-asparagine to L-aspartic and ammonia without L-glutaminase activity as L-asparaginase produced from bacteria so reduce associated side effect **(Kelo *et al.*, 2009; Young *et al*., 2009)**.

PRODUCTION OF L-ASPARAGINASE AND L-GLUTAMINASE

Several methods designed for the production and optimization of L-asparaginase and L-glutaminase from various microorganisms in solid state fermentation (SSF) and submerged fermentation (SmF) also in batch and continuous fermentation. Most of the microbial amidases examined are intracellular in nature while few are extracellular. Purification of intracellular asparaginases is tiresome (hard) as compared to extracellular enzymes. Production and optimization conditions differ from one organism to another, and L-asparaginase and L-glutaminase can be produced constitutively or after induction **(Ahmad *et al.*, 2012)**. Production of L-asparaginase and L-glutaminase depends on various parameters like the concentration of carbon and nitrogen sources, pH of culture medium, temperature, fermentation time and oxygen transfer rate also these parameters differentiate from one organism to another **(Vidhya *et al*. 2010)**. L-asparaginase and L-glutaminase are mostly obtained by SmF. Many researchers have studied amidases production and purification and try to reduce the impurities that cause allergenic reactions **(Ahmad *et al.*, 2012; Ebrahiminezhad *et al.*, 2014; Sinha and Nigam, 2016)**.

THE NUTRITIONAL REQUIREMENTS AND CULTURE CONDITIONS

The nutritional requirements and culture conditions for biosynthesis of amidases differ from one microorganism to another (Table 1, 2). It was observed that maximum L-asparaginase activity by *Serratia marcescens* ATCC 60 at 4% (w/v) of Autolyzed Yeast Extract (AYE) medium compared with a complete dehydrated medium, corn steep liquor, and protein hydrolysate. Different carbon sources at 0.4% (w/v) added to the basal medium was studied and the enzyme production for each one was compared and depression effect was to lower pH of fermentation of carbohydrate **(Vidhya *et al.*, 2010).** Also, in media containing 0.05% (w/v) yeast extract, no yield was observed. It was found that, complete inhibition of the growth of cells and enzyme production when 3% (w/v) yeast extract was used **(Liu and Zajic, 1973)**. Glucose gives maximum enzyme activity rather than maltose in case of *Bacillus* sp **(Vidhya *et al.*, 2010)**. In another study, glucose found to have an inhibition effect on the synthesis of L-asparaginase in *Serratia marscences*, *Erwinia carotovora*, *Escherichia coli*, *Erwinia aroideae* to catabolic suppression **(Peterson and Ciegler, 1972; Vidhya *et al*., 2010)**. Other studies also showed that, a significant reduction in asparaginase activity when glucose was added to 3% nutrient broth and 1% (w/v) of monosodium glutamic acid **(Barnes *et al*., 1978)**. Recently addition of 0.1% (w/v) glucose stimulates L-asparaginase activity compared to glucose-free medium and 1% (w/v) glucose had a complete inhibition effect **(Geckil and Gencer, 2004)**. Yeast extract and lactose also have a critical role in enzyme activity not only for growth. It's observed that yeast extract 1.5% (w/v), 1.0% (w/v) lactose have maximum enzyme production **(Liu and Zajic, 1973)**. Also, it was demonstrated that 0.16% (w/v) of di-ammonium hydrogen phosphate and 1.0% (w/v) sodium citrate have the maximum L-asparaginase activity in *Enterobacter aerogenes* and there was no intracellular asparaginase activity with sodium citrate **(Mukherjee *et al*., 2000)**. Addition of 1.0% tryptone has maximum L-asparaginase activity. Similarly, supplementing asparagine, as the sole source of nitrogen, *E. coli* was able to grow and produce an enzyme **(Cedar and Schwartz, 1967)**. It has shown a high yield of L-asparaginase from actinomycetes, *Streptomyces griseus* ATCC 10137 when growing on medium yeast malt glucose, 4.0% peptone without glucose and synthetic glucose-asparagine **(Peter, 1972)**.

Bacterial asparaginases in long term of use cause hypersensitivity, anaphylaxis, and allergic reactions. So attention going to eukaryotic microorganisms producing L-asparaginase with fewer side effects especially filamentous fungi and yeast **(Sarquis *et al*., 2004)**. *Saccharomyces cerevisiae* was reported as nitrogen-regulated **(Oliveira *et al*., 2003)**. Several studies were achieved by changing different nitrogen sources in media compassion for L-asparaginase production from *Aspergillus terreus* and *Aspergillus tamari* **(Sarquis *et al*., 2004)**. The medium used for the fungal and soil bacteria is modified Czapeks medium which include glucose 0.2% (w/v) and 1.0% L-asparagine (w/v) for fungi while 0.5% (w/v) for bacteria **(Gulati *et al*., 1997)**. Several researchers showed the production of recombinant L-asparaginase and give maximum enzyme activity from maltose, yeast extracts peptone and beef extract as a sole source of carbon and nitrogen **(Maria *et al*., 2006)**. Many of microorganisms can utilize L-glutamine as carbon and nitrogen sources and produce L-glutaminase, it was reported that addition of glucose enhanced the enzyme production in *Candida nodaensis* **(Sato *et al*., 1999),** *Beauveria* sp. **(Sabu *et al.*, 2000)**, *Pseudomonas* sp. **(Kumar and Chandrasekaran, 2003)**, *Trichoderma koningii* **(El-Sayed, 2009)** and *Providencia* sp. **(Iyer and Singhal, 2009)**. While glucose addition can suppress production of L-glutaminase production from *Achromobacteraceae* **(Roberts *et al*., 1972)** and *Stenotrophomonas maltophilia* NYW-81 **(Wakayama *et al*., 2005)**. Addition of sorbitol for *Beauveria bassiana* BTMF S10 and sucrose for *Zygosaccharomyces rouxii* plus glucose enhance L-glutaminase production **(Keerthi *et al*., 1999)**. The maximum enzyme production enhanced by glucose followed by lactose and maltose when using different carbon sources like glucose, lactose, sucrose, soluble starch, maltose, and fructose at 1.0% (w/v) in the medium of *A. oryzae*. Organic nitrogen sources more preferable than inorganic sources in production media for L-glutaminase producing microorganisms. It was noticed that *S. rimosus* **(Wakayama *et al.*, 2005)** utilize malt extract and give maximum L-glutaminase production while *C. nodaensis* **(Sato *et al*., 1999)**, *B. bassiana* BTMF S10 **(Keerthi *et al*., 1999)** and *Z. rouxii* **(Iyer and Singhal, 2010)** can utilize yeast extract and give high yield. *Providencia* sp. and Achromobacteraceae use urea and ammonium sulfate, respectively, improved the enzyme production **(Roberts *et al*., 1972)**. The L-glutaminase production was improved by a seawater-based medium supplemented with L-glutamine (0.25%) **(Sabu *et al*., 2000)**. For the determination of amidases activities by semi-quantity plate assay pH play a major role **(Peter, 1972)**. In this assay phenol red as a pH indicator which is yellow at the acidic condition and turns to Pink at the alkaline condition. L-asparagine or L-glutamine used as a sole nitrogen source and pH 5.5 to 7.0 **(Gulati *et al*., 1997)**. Temperature is one of the main process parameters for the enzymes production. It was shown that *Erwinia aroideae* **(Liu and Zajic, 1973),** *Citrobacter* sp. and *Serratia marcescens* ATCC 60 **(Vidhya *et al*., 2010)** needs optimum temperature for production L-asparaginase ranges between 25 °C and 37 °C and pH 5.0 while for *vibrio* pH 8.0. Maximum enzyme production is obtained after 24h from *Erwinia aroideae* NRRL B-138 **(Peterson and Ciegler, 1972)** and 48h in shake flask from *Serratia marcescens* ATCC **(Geckil and Gencer, 2004)**. The purified enzyme tested from *Serratia marcescens* and *E.coli* has shown low response when compared with *Erwinia aroideae* NRRL B-138 **(Peterson and Ciegler, 1969)**. It has been observed that, the maximum enzyme production from *Thermus thermophilus* HB8 at 70 °C and pH 7.0 **(Prista and Kyridio, 2001). Erva (2018)** reported the maximum temperature and pH for L-asparaginase production from *Bacillus subtilis* was 49.9 °C and 8.3 while in another study by **Jia *et al.* (2013)** the maximum production from *B. subtilis* B11−06 at 40 °C and 7.5, respectively. While **Vidhya *et al*. (2010)** reported that optimum pH was 7.0 and temperature was 37 °C from *Bacillus* sp**.** The optimum incubation temperature and pH values for L-glutaminase production were reported by **Sinha and Nigam (2016)** from *Bacillus* sp. at 35 °C and 7.0**.** In the case of recombinant L-asparaginase production, the optimum temperature and pH are 28-30 °C and pH 6.0-7.0, respectively **(Maria *et al*., 2006)**. In order to minimize hypersensitivity produced by bacterial L-asparaginase, the study of eukaryotic microorganisms for L-asparaginase with fewer side effects were focused **(Sarquis *et al*., 2004)**. Many researchers studied the production of L-asparaginase from *Saccharomyces cerevisiae* **(Oliveira *et al*., 2003)**. Maximum enzyme activity of *Aspergillus tamari* and *Aspergillus terreus* 10C217 observed at an optimum pH 6.2 at 30 °C for 48 h **(Sarquis *et al*., 2004)**. Few studies are available on the actinomycetes species, *Streptomyces* sp. named S3 shown optimum enzyme activity at fermentation conditions of pH 7.5 at 50 ℃ **(Saleem *et al*., 2009)**. L-glutaminase production also affected by pH and temperature and each organism has optimum pH and temperature. It was reported that *Pseudomonas* sp. has a maximum activity for L-glutaminase at 37 ℃ and pH 7.0 **(Roberts, 1976)**. Whereas, L-glutaminases produced from marine *Micrococcus luteus* have maximum activity at high-temperature 50℃ and at alkaline pH 8.0 to 8.5 **(Moriguchi *et al*., 1994)**. Also, it was reported that glutaminase from *Aspergillus oryzae* has pH optima of 8.0 to 9.0 while optimum temperature from 37 ℃ to 45 ℃ **(Koibuchi *et al*., 2000)**. L-glutaminase produced from *Penicillium brevicompactum* NRC 829 exhibited its maximal activity when incubated at 50 ℃ and at pH 8.0 **(Elshafei *et al*., 2014)**.

EFFECT OF METAL IONS, ACTIVATORS, INHIBITORS, AND SALT TOLERANCE

L-asparaginase and L-glutaminase activity differed in the existence of enhancers or enzyme inhibitors. To study the synergistic effect on the amidases production it was found that metal ions as Fe^{3+}, Ni^{2+} and Fe^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+}, and Hg^{2+} had inhibitory effect on enzyme activity **(Saleem *et al*., 2009)** and also activity inhibited in the presence of thiol-group-blocking reagents such as iodoacetamide and p-chloromercuribenzoic acid (PCMB). Activity was enhanced by the addition of amino acids as L-histidine and L-cysteine, EDTA and some reducing agents like dithiothreitol (DTT), 2-mercaptoethanol (2-ME), and reduced glutathione (GSH) **(Vidhya *et al*., 2010)**. All of these results specified that L-asparaginase is not a metalloprotein. Therefore, the sulfhydryl group has an important role in the catalytic activity of L-asparaginase **(Gaffar and Shethna, 1977)**.

L-asparaginase from *Azotobacter vinelandii* has high sensitivity to heavy-metal ions N-ethylmaleimide and iodoacetate also demonstrated the reliance of the activity of the enzyme upon sulfhydryl group **(Gaffar and Shethna, 1977)**. Marine *Bacillus* sp. have maximum enzyme production by addition of 2.0 % (w/v) NaCl which has comparatively high ability compared to other concentrations **(Mohapatra *et al*., 1995)**, while, *E.coli* has better salt tolerance up to 5.0 % (w/v) but has no effect on enzyme production **(Cedar and Schwartz,**

1967). Many researchers have shown that bacterial L-glutaminase was stimulated by certain divalent ions and inhibited by monovalent anions and by some competitive inhibitors like 6-diazo 5-oxo L-nor leucine, L-glutamate, and NH_3 **(Soda *et al*., 1972)**. In the case of fungal L-glutaminase produced from *Aspergillus oryzae* and *P. brevicompactum* was inhibited by Hg^{2+}, $Cr^{+2,}$ and Fe^{+2} but were not affected by sulphydroxyl reagents while Na^{+} or K^{+} act as enhancers **(Kumar and Chandrasekaran, 2003; Elshafei *et al*., 2014)**. It has been found that EDTA has no effect on enzyme activity which indicates that L-glutaminase might not be a metalloenzyme also not affected by thiol-blocking group, reducing agents as 2-ME and GSH so no indication for the participation of SH group(s) in the catalytic site of this enzyme **(Elshafei *et al*., 2014)**. Sodium chloride was found to influence the activity of microbial glutaminase. L-glutaminase from *A. sojae, P. fluorescence, Cryptococcus albidus* and *E.coli* in presence of 18% NaCl showed only 6, 75, 65 and 65% respectively of their original activity **(Yokotsuka *et al*., 1987)**. On the other hand, L-glutaminases may be inhibited by high salt concentrations **(Sabu *et al*., 2000)**.

RECOMBINANT PRODUCTION OF L-ASPARAGINASE AND L-GLUTAMINASE

Although several native L-asparaginase and L-glutaminase were produced from bacteria, fungi, actinomycetes, and plants, few studies on the heterologous expression of recombinant L-asparaginase and L glutaminase (**Wakayama et al., 2005; Shakambari et al., 2019**). **Vidya and Vasudevan (2011), Shakambari et al. (2019)** reported that *E. coli* has two types of L-asparaginases by notably different properties, known as L-asparaginase I and II. Type I is cytoplasmic and has a low affinity for L-asparagine and produced constitutively. While the type II is periplasmic and has a high-affinity for L-glutaminase and its expression is positively regulated by different inducers as the cyclic AMP receptor protein and anaerobiosis (Fumarate and Nitrate Reductase FNR protein) so attract great importance in anticancer treatment. When studying the L-asparaginase genetics, revealed that the sequences of coding genes are different, and ansA encodes for type I while gene ansB encoding type II. Also, efforts to cloning ansB and overexpression of L-asparaginase successfully performed and resulted in the production of L-asparaginase II. On the other hand, L-glutaminases members tend to be designated as glutaminase A (glsA), where B (glsB) is unknown and may not be homologous (as in *Rhizobium etli*) some species have two isozymes that may both be designated A (glsA1 and glsA2) (**Botman et al., 2014**). **Fisher and Wray (2002)** reported L-asparaginase from *B. subtilis* regulated by two various controlled genes and their expression regulated by independent regulative factors. The ansZ gene encodes a functional L-asparaginase which expression activated by the TnrA transcription factor during nitrogen-limited growth through binding to a DNA site that lies upstream of the ansZ promoter. And, the ansA gene encodes another L-asparaginase and its expression effected by L-asparagine. In an operon, ansA gene located with ansB gene encodes L-aspartase. The expression of the ansAB operon hindered by AnsR which activity monitored by either L-aspartate or L-asparagine. In another hand, for recombinant glutaminases isolated from *B. subtilis* (ylaM and ybgJ genes) and from *E. coli* (ybaS and yneH genes), tested the biochemical characterization of the four L-glutaminases and determined the crystal structures of Ybg and YbaS (Brown et al., 2008). Also, GlsA gen from *B. licheniformis* expressed in *E. coli*, under the effective control of the promoter Ptac (**Sinsuwan et al., 2012**). Another study in production recombinant glutaminases. **Calderon et al. (1999), Huerta-Saquero et al. (2001)** reported the sequencing gene codes of *Rhizobium etli* thermolabile glutaminase A (glsA) and expressed in the heterologous host *Sinorhizobium meliloti* and in expression vector pTrcHis. while L-glutaminase gen from *A. oryzae* RIB40 (AoglsA) expressed heterologously in *S. cerevisiae* and *E. coli* and the expressed enzyme showed glutaminase activity and was produced in a soluble protein in *E. coli* and a cell wall fraction of *S. cerevisiae* (**Masuo et al., 2004**). **Ito et al. (2011, 2012)** isolate and cloned novel glutaminases genes CagahA and CngahA and AsgahA from *Cryptococcus albidus* and *Cryptococcus nodaensis* and *A. sojae*. The expression of L-glutaminase activity was enhanced by the introduction of multiple copies of AsgahA into *A. oryzae* RIB40. The gene coded AsgahA secreted at the cell surface in submerged culture, and extracellularly in solid-state culture. **Jia et al. (2013)** reported cloning L-asparaginase ansZ gene from *B. subtilis* B11-06 a non-pathogenic strain and its overexpression and purification of the thermostable protein was performed. Also, cloning of the gene Tk1656 coding L-asparaginase of *Thermococcus kodakarensis* KOD1 achieved in *E. coli* BLR (DE3) (**Hong et al., 2014**). **El-Gendy et al. (2017)** studied cloning and protoplast fusion of filamentous fungi glutaminases gen such as *Cladosporium* sp. (gen 20) and *Trichoderma* sp. (gen 9) and screened for L-glutaminase production. The recombinant L-asparaginase and L-glutaminase fortunately over-expressed and purified. Thus recombinant DNA technologies have been applied successfully to yield many folds increased L-asparaginase and L-glutaminase production and to maintain enhanced properties of activity and stability (**Binod et al., 2017; Shakambari et al., 2019**). The list of organisms whose genes cloned for L-glutaminase and L-asparaginase overexpression listed in Table 3.

Table 1 Summary of fermentation conditions for the production of L-asparaginase and L-glutaminase by submerged fermentation (SmF)

Microorganisms	Enzyme produced	Nutrition requirements % (w/v)	Fermentation conditions	Activity	References
Serratia marcescens ATCC 60	LAase	AYE 4.0	pH 5.0, 26°C, 48 h	3.7 U/ml	**(Heinemann and Howard, 1969)**
*Enterobacter aerogenes*NCIM2340	LAase	Sodium citrate 1.0, di-ammonium hydrogen phosphate 0.16	pH 7.0,37 °C, 24 h	0.60 U/ml	**(Mukherjee *et al*., 2000)**
Erwinia aroideae NRRL B-138	LAase	Tryptone 0.05, Yeast 0.05, glucose 0.1	2.8L flasks: pH 7.0, 28 °C, 200 rpm, 8 h 20 L fermenter: pH 7.0, 28 °C, 24 h, 300 rpm.	1250 U/ml 960 U/ml	**(Peterson and Ciegler, 1969)**
Bacillus sp. DKMBT10	LAase	L-Asparagine 0.6, glucose/maltose 0.3	pH 7, 37°C, 200 rpm, 24 h	1.12 U/ml	**(Vidhya *et al*., 2010)**
Bacillus pumilus	LAase	Galactose 2.0, asparagine 0.1	28 – 30°C, 100 rpm, 48 h	75.73 U/ml	**(Sindhwad and Desai, 2015)**
Pseudomonas aeruginosa 50071	LAase	Soya bean meal 5.0g/l, Inducers- casein hydrolysate 3.11g/l, corn steep liquor 3.68g/l.	pH 7.4, 37 °C, 96 h	1.428 U/ml	**(Yasser and Olama, 2002)**
Pseudomonas sp BTMS-51	LGase	L-glutamine 2.0, D-glucose 1.0	pH 6, 30°C	21.07 U/ml	**(Kumar and Chandrasekaran, 2003)**
Vibrio SP.	LGase	M9	pH 7, 35°C	28.7 U/ml	**(Saravanan *et al*., 2014)**
Vibrio azureus JK-79	LGase	glutamine 2.0, soybean meal 2.0, maltose 1.5	pH 8, 37°C	321 U/ml	**(Kiruthika and Nachimuth, 2013)**
Providencia sp	LGase	Glucose 1.0 and urea 0.5	pH 8, 25°C	119.23U/l	**(Iyer and Singhal, 2009)**
*Stenotrophomonas maltophilia*NYW-81	LGase	L-Glutamine 1.0	pH 7, 30°C	3.25 U/mg	**(Wakayama *et al*., 2005)**
*Aspergillus terreus*IOC 217	LAase	Proline 2.0	pH 6.2, 30°C 48 h, 160 rpm.	0.058	**(Sarquis *et al*.,2004)**
Pichia pastoris	LAase	BSM2	(2L) flask: pH 5.0, 30°C.	85.6	**(Maria *et al*., 2006)**

Aspergillus terreus MTCC 1782	LAase	L-asparagine 1.0, glucose 0.4, yeast extract 1.0, peptone 0.6	pH 6.0, 30°C, 72 h, 160 rpm.	24.10	**(Baskar and Renganathan, 2011)**
Aspergillus oryzae	LAase	L-asparagine 0.5	pH 6.2, 37°C, 96 h, 250 rpm	0.14	**(Gulati *et al*., 1997)**
Aspergillus terreus	LAase	Dextrose, asparagine	pH 6.0, 35 °C	8.26 U/mg	**(Farag *et al*., 2015)**
Aspergillus sp.	LAase	Dextrose, ammoniumsulphate	pH 7.5, 35°C	185	**(Sanjotha, 2017)**
Zygosaccharomyces rouxii NRRL-Y 2547	LGase	Sucrose 1.78, glutamine 0.5, yeast extract 4.8.	pH 7, 37°C	458.68 U/ml	**(Iyer and Singhal, 2010)**
Aspergillus oryzae S 2	LGase	Dextrose 0.1, yeast extract 0.3	pH 5-35°C	217650	**(Sunil *et al*., 2014)**
Penicillium politans NRC 510	LGase	Modified Czapek Dox'	pH 7.5-8.5, 60°C	133U/mg	**(Thanaa, 2009)**
Cryptococcus nodaensis	LGase	D-Glucose 3.0, yeast extract 0.5	pH 6, 28°C	2060 U	**(Sato *et al*., 1999)**
Pectobacterium carotovorum MTCC 142	LAase	L-asparagine 0.52, glucose 0.2	Flask: pH 7.0, 30°C 12h, 180rpm (4 L) fermenter: 30°C, 12 h, 200 rpm, 1.5aeration.	14.71 15.39	**(Sanjay *et al*., 2009)**
Thermus thermophilus HB8	LAase	Tryptone 0.5, yeast extract 0.3, glucose 0.1	pH 9.2, 70°C	494	**(Prista and Kyridio, 2001)**
Streptomyces griseus ATCC10137	LAase	Peptone 4.0	pH 8.5, 30°C	0.01	**(Peter, 1972)**
Actinobacterial sp.	LAase	Asparagin	pH 8.0, 35°C	670.04 U/mg	**(Varma *et al*., 2016)**
Streptomyces sp. SBU1	LGase	NaCl 2.0, malt extract 1.0, glucose 1.0.	pH 9, 30°C	18.0	**(Krishnakumar *et al*., 2011)**
Streptomyces labedae	LGase	Glutamine,mineral salt, sodium citrate 0.1g/L, NaCl 25.0g/L, glucose 10.0g/L	pH 7-8, 30°C	12.23	**(Nagwa *et al*., 2012)**

Legend: Production L-asparaginase (LAase) and L-glutaminase (LGase) performed in SmF; U/ml, U/g; U/gds, U/mg International units (IU).

Table 2 Summary of fermentation conditions for the production of L-asparaginase and L-glutaminase by solid state fermentation (SSF)

Microorganisms	**Enzyme produced**	**Nutrition requirements % (w/v)**	**Fermentation conditions**	**Activity**	**References**
Pseudomonas aeruginosa 50071	LAase	Soya bean meal 5.0g/l, Inducers- casein hydrolysate 3.11g/l, corn steep liquor 3.68g/l.	pH 7.4, 37 °C, 96 h	1.428 U/ml	**(Yasser and Olama, 2002)**
Serratia marcescens	LAase	Sesame oil cake	pH 7.0 - 7.5, 37 °C, 48 h	110.795 U/ml	**(Wang *et al*., 2003)**
*Serratia marcescens*SB08	LAase	Rice bran 10.0g/l, Lasparagine 0.01, yeast extract 0.5	pH 7.0, 30 °C, 36 h.	79.84 U/ml	**(Lakshmanaperumalsamy 2009)**
Bacillus cereus MAB5	LAase	soyabean meal 6.2g/l, wood chips 1.383 8 g/l, aspargine 5.5 g/l, and NaCl 4.535 4 g/l	-	51.54 U/ml	**(Thenmozhi *et al*., 2011)**
Vibrio costicola ACMR 267	LGase	Glutamine, polystyrene beads as an inner support	pH 7, 35°C, 24 h	596.67 U/mg	**(Prabhu, 1997)**
Serratia marcescens	LGase	Rice bran	pH 7.1, 37°C	193.10 U/ml	**(Suresh *et al*., 2013)**
Pseudomonas stutzeri P	LGase	agro-industrial waste as green gram husk, Bengal gram husk, cattle feed, wheat bran, groundnut cake	pH 7.0, 37°C	3.7 952 U/ml	**(Athira *et al*., 2014)**
Fusarium equiseti	LAase	Glucose 0.5, Ammonium sulphate 0.5, Yeast extract 0.5.	pH 7.0, 37 °C, 48 h, 20%inoculum volume	8.51	**(Hosamani and Kaliwal, 2011)**
Aspergillus niger	LAase	Bran of glycine max	pH 6.5, 40 °C, 96 h, moisture content 70 %	17.52	**(Abha, 2006)**
Beauveria bassiana MSS18/41	LAase	Wheat bran	pH 9.0, 26ºC, 96h,50%moisture content	90U/gds	**(Nageswara *et al*., 2014)**
Aspergillus flavus	LGase	Tea dust	pH 4.0, 30°C, 5 D	42.37U/g	**(Nathiya *et al*., 2011)**
Trichoderma koningii	LGase	Wheat bran	pH 7.0, 30°C	45Ugds-1	**(Cook *et al*. 1981)**
Trichoderma koningii	LGase	Wheat bran, D-glucose 1.0, L-glutamine 2.0	pH 7.0, 30°C 7 D, 70% initial moisture content	23.2U/mg	**(El-Sayed ,2009)**
Zygosaccharomyces rouxii NRRL-Y 2547	LGase	Sesame oil cake	pH 7.0, 30°C	0.01161	**(Kashyap *et al*., 2002)**

Aspergillus sp. ALAA-2000	LGase	Soybean	pH 4 and 27°C.	21.89	**(Ahmed *et al*., 2016)**
Aspergillus wentii MTCC 1901	LGase	L-glutamine 3.0, sucrose 4.0, peptone 2.0, magnesium sulphate 1.0	pH 8.0, 32°C, 96 h, 40% initial moisture	259.32 U/gds	**(Revanth and Raju, 2013)**
Aspergillus Wentii MTCC 1901	LGase	Peptone	pH 6.8, 30°C,144 h, 45%moisture content	703.83 U/gds	**(Durgasi and Raju, 2016)**
Beauveria sp. BTMFS 10	LGase	L-glutamine 0.25, Polystyrene beads as inner support,D-glucose 0.5	pH 9.0, 27°C	49890.00	**(Sabu *et al*., 2000)**

Legend: Production L-asparaginase (LAase) and L-glutaminase (LGase) performed in SSF; U/ml, U/g; U/gds, U/mg International units (IU).

Table 3 Recombinant production of L-asparaginase and L-glutaminase from microbial origin

Recombinant enzyme	Microbial sources of the foreign gene	Host cell	Reference
RGase	*Rhizobium etli*	*E. coli*	**(Huerta-Saquero *et al*. ,2001)**
RAase	*A. oryzae*	*S. cerevisiae* and *E. coli*	**(Masuo *et al*. , 2004)**
RAase	*E. chrysanthemi*	*E. coli B*L21(DE3) pLysS	**(Kotzia and Labrou, 2007)**
RGase	*Cryptococcus* sp.	*S. cerevisiae*	**(Ito *et al*. , 2011)**
RAase	*S. thermoluteus* subsp. *fuscus N*BRC 14270	*S. lividans 1326*	**(Hatanaka *et al*., 2011)**
RGase	*B. licheniformis*	*E. coli*	**(Sinuswan *et al*., 2012)**
RAase	*E.* sp. NII	*E. coli* BL21(DE3)	**(Vidya and Pandey, 2012)**
RGase	*A. sojae*	*A. sojae*	**(Ito *et al*. , 2012)**
RAase	*B. subtilis* B11-06	*B. subtilis*	**(Jia *et al*., 2013)**
RAase	*V. cholera*	*E. coli* BL21 (DE3)	**(Radha *et al*., 2018)**

Legend: Production Recombinant L-asparaginase (RAase) and Recombinant L-glutaminase (RGase)

L-ASPARAGINASE AND L-GLUTAMINASE ASSAY METHODS

Several techniques developed and reported to assay L-asparaginase and L-glutaminase activities. L-asparaginase and L-glutaminase activities determined by estimating the amount of ammonia or acids liberated through the reaction due to their hydrolysis of asparagine and glutamine, respectively. The quantitative and qualitative techniques employed for the measuring of L-asparaginase and L-glutaminase activities. In the quantitative methods, rapid plate assay, a pH indicator incorporated medium containing L-asparagine or L-glutamine as sole nitrogen source with the addition of a pH indicator. The activities of L-asparaginase and L-glutaminase characterized by an increase in pH because of the liberation of ammonia **(Gulati *et al*., 1997)**. **Gulati *et al*. (1997)** stated phenol red indicator as pH indicator while **Mahajan *et al*. (2012)** used bromothymol blue (BTB) as pH indicator instead of phenol red in rapid plate assay detection. In the quantitative assay of L-asparaginase and L-glutaminase concern with the detection of ammonia liberated when the reaction catalyzed by L-asparaginase and L-glutaminase on their natural substrate L-asparagine and L-glutamine, respectively **(El-Naggar, 2015; Tork *et al*., 2018)**. Briefly, the Nesslerization assay includes the cell lysate (intracellular) or the crude enzyme supernatant (extracellular) incubated with the substrate for 10 mins, and after incubation, the reaction stopped by addition of trichloroacetic acid (TCA). After that, the liberated ammonia examined by addition of Nessler's reagent in the diluted enzyme substrate mixture and formation of the yellow color due to the presence of ammonia which used quantitatively for determining the enzyme activity. One unit of enzyme activity defined as the amount of enzyme that produces 1 µmol of ammonia per minute at slandered reaction condition (pH 8.6 and 37 °C). There are additional methods for enzymes detection but plat assay and Nesslerization method is the most common **(El-Sayed, 2009; Vidhya *et al*., 2010)**.

APPLICATION OF L-ASPARAGINASE AND L-GLUTAMINASE

IN MEDICINE

Regulation of cancer cells can be reached by inhibition of nucleic acid and protein biosynthesis by the absence of any component of these macromolecules The World Health Organization (WHO) and Food and Drug Administration (FDA) have agreed that L-asparaginase is the most effective cure of lymphosarcoma and acute lymphoblastic leukemia (ALL). L-asparaginase hydrolyzes asparagine from blood serum, causing tumor death by removing a vital factor for protein synthetases (p53-dependent apoptosis). While L-asparagine synthetase found in adequate amounts in healthy cells so it not affected **(Shakambari *et al*., 2019)**. L-asparaginase has been produced from variant sources but only from *Erwinia* and *E. coli* used on a manufacturing scale. While the two drugs have the same mode of action and toxicities, pharmacokinetic properties are altered and allergic to one drug are often resistant to the other **(Dhanam and Kannan, 2013)**. **Bhat and Marar (2015)** reported L-asparaginase from *Salinicoccus sp*. MKJ997975 inhibited the growth of both Jurkat and HeLa cell line. While **Shanmugaprakash *et al*. (2015)** reported L-asparaginase from *Capsicum annuum* L showed maximum activity in the KB cell line, the least activity was found in A549 and moderate activity in HeLa cell lines by using. **Moharib (2018)** showed L-asparaginase has higher efficiency in growth inhibition against Hep-G2 and HCT-116 but lower against HeLa and MCF-7 carcinoma cell lines. Recently, **Oza et al. (2010)** reported *Withania somnifera* L-asparaginase in vitro has antitumor activity using the MTT method (this research proposed as the first report of plant asparaginases).

The modified type of the enzyme is PEG-asparaginase. PEG-asparaginase produced by covalently conjugate L-asparaginase and monomethoxy polyethylene glycol (PEG). Patients with hypersensitivity to native *E. coli* asparaginase, PEG-asparaginase is considered more effective. PEG-asparaginase reduces the immunogenicity of the protein, increases its stability in plasma and is suitable for use in heavily pretreated patients **(Inada *et al*., 1995)**. Results showed that PEG-asparaginase successfully depleted the asparagine and acted as a part of an intensive multiagent healing system in adult acute lymphoblastic leukemia (**Wetzler *et al*., 2007)**. Combination of L-asparaginase and ABT-737 induced mitochondrial cytochrome-C release, stimulation of Bax, Bid, better mitochondrial depolarization, and finally apoptosis neither drug alone **(Kang *et al*., 2007)**. L-asparaginase brand names are KIDROLASE, ELSAPAR, ERWINASE, and ONCASPAR **(El-Ghonemy, 2014)**. The medical utility of L-asparaginase is frequently restricted by the side effects including pancreatitis and immunosuppression **(Wang *et al*., 2003)**, ten percent of patients suffer a relapse followed by occurrence of resistant tumors to further L-asparaginase therapy **(Woo *et al*., 2000)** and long term treatment with L-asparaginase increases the metastatic activity and develops the growth of resistant malignant tumor **(Asselin *et al*., 1999)**. Recently the small molecule ABT-737 which inhibits and binds the Bcl-2 family antiapoptotic proteins BclW, Bcl-2, and Bcl-XL *Erwinia* asparaginase also have been used in combination with other chemotherapeutic agents as a therapy to hypersensitivity patients of a drug derived from *E. coli* **(Dhanam and Kannan, 2013)**.

On the other hand, L-glutaminase inhibits uptake of glutamine (precursor for the nucleotide and protein synthesis) by cancer cells, so the growth stopped **(Sarada, 2013).** Many researches demonstrated L-glutaminase have an anticancer effect **(Roberts *et al*., 1970; Spiers and Wade, 1976; Elshafei *et al*., 2014)**. The main problems associated in L-glutaminase treatment are the intravenous introduction had to act at the cancer location within the short span of time it persisted in circulation before cleared at the kidneys and the induction of immune responses against the enzyme. Many investigations are done to avoid these problems and found that L-glutaminase used in the treatment of the definite types of cancers. **Spiers and Wade (1976)** explain the difficulties occurred in clinical treatments of L-glutaminase as anti-leukemia. It has been shown that enzyme optimal activity over a wide pH range was obtained when immobilization of the L-glutaminase in polyethylene glycol due to the matrix effect. The latter approach was the use of L-glutaminases in treatment Ehrlich ascites tumor (one type of breast carcinoma) **(Lobo *et al*., 2000)**. Using MTT assay, L-glutaminase from *Penicillium brevicompactum* NRC 829 and *Aspergillus niger* effect on the growth of four human tumor cell lines A549 (Human lung carcinoma), MCF-7 (Breast cancer cell line), Hep-G2 (Human hepatocellular carcinoma cell line) and HCT-116 (Colon cell line) revealed that L-glutaminase have antiproliferative action in

four cell lines growth **(Elshafei *et al*., 2014; Dutt *et al*., 2014)**. L-glutaminases also are used in the treatment of melanoma **(El-Ghonemy, 2014)**. By using MTT assay, *Streptomyces canarius* L-glutaminase tested against MCF-7, HepG-2, RAW264.7, HCT-116, and HeLa cells and the enzyme had a high cytotoxic effect against HeLa and HepG-2 cell lines, moderate HCT- 116 and RAW264.7 and no effect against MCF-7 cells (Reda, 2015). From that L-glutaminase concerning as a promising candidate for cancer therapy.

Some researchers showed that glutaminase–asparaginase enzyme from *Achromobacter* and *Aspergillus niger* have anticancer effects in patients with acute myeloid leukemia and acute lymphoblastic leukemia in a preliminary clinical experiment **(Spiers and Wade, 1976, Elzainy *et al*., 2006).** The most promising therapeutic applications for L-glutaminase is in the treatment of human immunodeficiency virus (HIV) where L-glutaminase from *Pseudomonas* sp. 7A is directed to inhibit HIV replication in infected cells **(Kumar and Chandrasekaran, 2003)**. **Sarada (2013)** reported L-glutaminase lowers L-glutamine levels in serum and tissues for prolonged periods through decreasing reduction of serum reverse transcriptase activity of HIV.

IN FOOD INDUSTRY

L-glutaminase and L-asparaginase are the most important enzymes used in food manufacture for their hydrolysis glutamine and asparagine to glutamic and aspartic acid. Glutamic and aspartic acid are two important amino acids in food processing for a delicious and fine taste, Sour and Umami taste and nutritional important to food **(Nanda *et al*., 2003)**. The palatable and pleasant flavors of oriental fermented foods as sufu, soy sauce and miso are due to the content of L-glutamic acid in it. In addition, aspartic acid decarboxylase catalyzes the conversion of aspartic acid into alanine, an influential amino acid constituent of soy sauce. Also, the additions of bacterial glutaminase or to the fermentation process increase the amount of glutamine so improve the umami taste of soy sauce (Kijima and Suzuki, 2007). Where there is increase about of glutamic acid in soy sauce made by the addition of glutaminase from *Cryptococcus albidus* ATCC 20293 **(Binod *et al*., 2017)**. L-glutaminase from *Koji* mold is highly active so used for increasing the L-glutamate concentration of soy sauce **(Yamamoto and Hirooka, 1974)**. In the Japan Tokko Koho Company, to improve the flavor of soy sauce they used peptide glutaminase of *B. circulans* **(Hamada and Mashall, 1988).** For improving Ushijima and Nakadai used protoplast fusion and mutation techniques for L-glutaminase production by *A. sojae* used in shoyu fermentation **(Kikuchi and Sakaguchi, 1973)**. Industrial processes of glutamine to glutamate in food preparations either immobilized L-glutaminase or whole cells of L-glutaminase require high salt environments as in the fermentation of soy sauce. L-glutaminases from *A. oryzae* are inhibited by high salt concentrations. Salt tolerant L-glutaminases are patent for use in manufacturing manners **(Sabu *et al*., 2000)**. Therefore, it is not unexpected that L-glutaminase is studied as a catalyst for the processing of fermented condiments as Japanese soy sauce **(Wakayama *et al*., 2005)**. Glutaminases from salt tolerant bacteria are most interesting, L-glutaminase from halophilic rather than halotolerant tolerating for the use of high salt concentrations **(Moriguchi *et al*., 1994)**. On the other hand the ability of L-asparaginases to hydrolyze asparagine into L-aspartate and ammonia is a possible way to decrease the amount of free L-asparagine in the preliminary ingredients of food making, thus minimize the imminent risk of causing neurotoxic and carcinogenic compound an odorless and colorless crystalline solid, acrylamide (2-propenamide) which formed from reducing sugars and L-asparagine in carbohydrate-containing foods when they are heated above 120°C as a result of Millard reaction **(Mottram *et al*., 2002)**. Acrylamide formation decreased about 96-99 % in the products when raw materials pre-treated by L-asparaginase before thermal treatment **(Lindsay and Jang, 2005; Morales *et al*., 2008)**. The brand names of L-asparaginase as a processing aid in the manufacture of food are Prevent ASe and Acrylaway **(EL-Ghonemy, 2014)**. Recombinant L-asparaginase Novozymes A/S Denmark (submitted by Novozymes Australia Pty Ltd) used as a food processing aid **(Pedreschi *et al*., 2008)**. **Hendriksen *et al*. (2009)** reported the reduction in acrylamide content was 34–92% when recombinant asparaginase from *Aspergillus oryzae* tested on different food samples (French fries, ginger biscuits, crisp bread, semisweet biscuits, and sliced potato chips).

ANALYTICAL APPLICATION

The biosensor technology can be a user-friendly approach, cheap, and reliable. L-asparaginase and L-glutaminase are used for the development of biosensor applications. Enzymatic determination of asparagine, aspartate, glutamine, and glutamate are more reliable and accurate compared to the older methods like Nesslerization followed by determination of liberated ammonia. The mechanism of action of the biosensor is based on L-asparaginase activity, ammonium ions produced from the hydrolysis of asparagine cause a change in pH resulting in the change of color and absorption **(Kumar *et al*., 2013)**. L-asparaginase is used to analyze asparagine levels either in the food industry or leukemia **(Verma *et al*., 2012)**. Many spectroscopy techniques such as Transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), and scanning electron microscope (SEM) are recently used for L-asparagine analysis, but tedious procedures and high cost make them less favorable **(Zubavichus *et al*., 2004)**. Nowadays analyses of levels of the body fluids as L-glutamine and glutamate are vital in health monitoring and clinical diagnostics. L-glutaminases biosensors are used immobilized on membranes or in free enzyme forms for monitoring glutamine and glutamate concentration **(Unissa *et al*., 2014; Binod *et al*., 2017)**. Currently, researches are going to search for the most stable forms for a longer period of using in biosensors, and plants are ongoing to manufacturing highly purified L-glutaminase enzyme definitely for that aim **(Binod *et al*., 2017)**. Although the main L-glutaminases clinically used came from mammalian sources with little exceptions, the Kikkoman Corporation (Japan) deal with L-glutaminase in clinical studies from microbial source especially *Bacillus* sp. and used it with conjunction with L-glutamate oxidase and peroxidase for determination of glutamine **(Sabu *et al*., 2000)**. L-glutaminase based biosensors have been used for investigation of glutamine and glutamate in pharmaceutical formulations, in hybridoma culture media by flow injection analysis and in the monitoring of glutamine and glutamate levels in mammalian cell culture media **(Huang *et al*., 2006; Sarada, 2013)**. On the other hand, free enzyme form has been used in the determination of glutamine in media of insect cell culture **(Meyerhoff *et al*., 1995)**. Important applications of L-glutaminase based biosensors are using in the online monitoring of fermentation **(Wang *et al*., 2010)**.

MANUFACTURE OF FINE-CHEMICALS

L-asparaginases and L-glutaminases play a vital role in the biosynthesis of fine-chemicals. L-asparaginase is incorporated in the production of the aspartic family of amino acids such as methionine, lysine, and threonine. Aspartic acid, that is considered as a precursor of lysine and threonine is formed by L-asparaginase enzyme hydrolysis of asparagine **(Sinha *et al*., 2013)**. One of the most vital submissions of L-glutaminase in the industry is using it in the production of γ – glutamyl alkamides. Theanine (γ-l-glutamyl ethylamide) is a product result from transfer γ-glutamyl from glutamine or glutathione to a glutamyl acceptor as glycylglycine, methylamine or ethylamine. Theanine is unique as a taste-enhancing amino acid in Japanese infused green tea. In recent times, more attention has been focused on the physiological characters of theanine, exclusively in a clinical part because their role as antihypertensive agents and its capability to suppress stimulation by caffeine, to increase effects of antitumor agents. In plants theanine synthetase (EC 6.3.1.6) is used to synthesize theanine. Combination reaction of baker's yeast was used with bacterial glutaminases to produce theanine from glutamate and ethylamine also produce γ-glutamylmethylamide by using L-glutaminase from *P. nitroreducens* in addition to threonine by using methylamine as an acceptor of γ-glutamyl **(Tachiki *et al*., 1998)**. Scientists at the Taiyo Kagaku Co., Ltd., Japan, designed a technique for continuous manufacture of threonine using immobilized *Pseudomonas nitroreducens* as a source of L-glutaminase **(Abelian *et al*., 1993)**. Unfortunately, L-glutaminase and L-asparaginase can cause an allergic response leading to silent inactivation or inactivation of the drug. Currently, no therapeutically appropriate asparaginases and glutaminases presented which can be manufactured cheaply and with little or no contamination by other substances as endotoxins of a host microorganism. Two probable mechanisms have been suggested for amidases especially L-asparaginase resistance **(Woo *et al*., 2000)**. The first mechanism appears to be neutralization of L-asparaginases impeding their enzymatic activity by the production of anti-asparaginase antibodies in the host cells **(Chakrabarti and Schuster, 1997)**. The second is related to an increase in asparagine synthase levels, which had been established in the blasts cells of patients with clinically unaffected to the drug **(Asselin *et al*., 1999)**. For glutaminases, a suitable enzyme is unavailable in an amount large enough to permit for wide-spread clinical trials. For the amidases to be ideally matched for using in antineoplastic therapy, it should fulfill a diversity of criteria. The selected organisms should be able to grow in large quantities on an inexpensive and simple medium and give a high yield of amidases. The purification of the enzyme must be simplified as possible and rapid, handing ultrapure enzyme in high yield. The refined enzymes stability should have a long term on storage, a Km for substrate below it's in the blood and maximal activity at a physiological pH. Besides, a full empathetic of the regulation of gene expression constructed on molecular methodologies and other factors would give great developing effective strategies for strain improvement which is critical for any industrially significant enzymes. Further researches and regulatory approvals will aid the introduction of new amidases drugs and other beneficial products with potential welfares **(El-Ghonemy, 2014)**.

CONCLUSION

L-aparaginase and L-glutaminase from various sources have properties that act as an antineoplastic agent and also in another industrial process to minimize the risk of acrylamide and as flavor enhancing agent. Even though bacterial L-Asparaginase and L-glutaminase are clinically applied for treatment of acute lymphoblastic leukemia and other types of cancer, it causes adverse reactions. For pharmaceutical applications and food industry, several studies to obtain L-Asparaginase and L-glutaminase from novel sources to obtain extracted enzymes with prolonged half-life and higher specificity towards their substrate. Nowadays

efforts going to the production of recombinant L-asparaginase and L-glutaminase by heterologous expression. Further studies on agro-industrial residues proved to be promising sources for the industrial production of these enzymes using SSF and controlling the factors during synthesis should improve the yield of the enzyme.

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