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Production, Purification and Advance Applications of L-Asparaginase (Review)

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ABSTRACT

L-Asparaginase, an enzyme (E.C.3.5.1.1) that is responsible for breakdown of L-asparagine into aspartic acid and ammonia, has significant applications in the therapeutics and food technology. Presently L-Asparaginase is mostly produced by using microbial sources including bacteria, fungi and actinomycetes. In therapeutics L-Asparaginase is used as antileukemic agent to treat acute lymphoblastic leukemia (ALL), a malignancy mostly present in children. In food industry this enzyme is used to diminish the quantity of acrylamide that is present in fried and baked starchy foods and carcinogenic in nature. L-Asparaginase done deamination of asparagine thus reduced acrylamide concentration. Another application is its use in biosensors. Both at industrial scale and at laboratory level production of L-Asparaginase can be done by submerged fermentation (SmF) or by solid-state fermentation (SSF). Comparison of both methods revealed that SSF is preferable over SmF because it is inexpensive, environment-friendly and it provides high yield of L-Asparaginase enzyme. SSF method consumes both industrial and agricultural wastes as their substrate. The contamination is significantly decreased in SSF because of presence of low moisture content. Purification of enzyme can be done by using different techniques i.e. precipitation of sample, dialysis and various chromatographic techniques are used in different combinations. A wise combination of steps increase the purification and yield.

Keywords: E.C.3.5.1.1, ALL, SSF, L-Asparaginase enzyme, pH

I. INTRODUCTION

L-Asparaginase (E.C. 3.5.1.1) is an amidohydrolase that is found in a wide range of organisms i.e. plants, microbes and animals that catalyzes the hydrolysis aspargine (amino acid) into aspartic acid and ammonia. It is difficult to extract it from plants and animals therefore microorganisms are assessed as potential sources for the production L-Asparaginase enzyme (Arima *et al*., 1972). This enzyme has significant applications in the therapeutics and food industry. In therapeutics L asparaginase act as anti-tumor agent and show tumor inhibitory properties. Acute lymphoblastic leukemia (ALL) is mainly treated by Asparaginsae. Normal body cells can produce L-asparagine by using asparagine synthetase enzyme, while certain malignant cells are not able to synthesize aspargine and require an external source of L-asparagine for their growth. In ALL treatment with L-Asparaginase, in the body of the patient all the circulating asparagine is hydrolyzed into ammonia and aspartic acid as a result of which absorption of asparagine by tumor cells is prevented and hence divesting the tumor cells of their extracellular L-asparagine source (Broome, 1963). The cancerous cells cannot carry out the de novo asparagine synthesis (Killander *et al*., 1976). Anaphylaxis, thrombosis, pancreatitis, coagulation abnormality, liver dysfunction, hyperglycemia, and cerebral dysfunction are side effects that are associated with L-Asparaginase therapy. L asapaginase can evoke immune response in which antiasparaginase antibodies are formed in the body. The other cause of side effects is L-glutaminase activity of L-Asparaginase enzyme used (Haskell *et al*., 1969). L-Glutaminase activity of enzyme leads to a reduction of plasma L-glutamine level (Avramis *et al*., 2002). These side effects can be life threatening like anaphylactic shock (Soares *et al*., 2002). The same enzyme also plays

vital role in food industry to stop the production of a carcinogen named as acrylamide at high temperatures such as frying or baking of starchy food items.

J.G. Kidd, in 1953 presented that transplanted lymphomas of mice and rat are inhibited in vivo when mice repeatedly injected with guinea pig serum. He found some active component in pig serum that plays an important role in selective necrosis of lymphoma cells (Kidd, 1953). In 1961 Broome revealed that the enzyme that is responsible for inhibitory action of lymphoma and present in guinea pig serum is L-Asperaginase (Broom, 1961). A major development resulted when two researchers Mashburn and Wriston in 1964 stated that *Escherichia coli* asparaginase can also inhibit the growth of tumors (Mashburn & Wriston, 1964). Lymphatic tumor cells need high quantity of L-asparagine to ensure their rapid malignant growth while L-Asparaginase enzyme is used for the reduction of asparagine. Then this mechanism was acknowledged as a favorable method for the reduction of tumors. Now a days a number of microbial species are identified and studied that can produce L-Asparaginase for medical trials of Acute Lymphoblastic Leukemia chemotherapy. (Hill *et al*., 1967).

Mechanism of Action

L-asparagine is a nonessential amino acid that is produced in human cells by an enzyme that is called L-asparagine synthetase. Tumor cells require huge amount of asparagine as an essential factor for their survival and growth. During the treatment of patients with ALL, L-Asparaginase is injected into the body and start hydrolysis of L-asparagine into aspartate and ammonia as result of which asparagine starvation occur and cancerous cells die (Broome, 1963). The mechanism of action of L-Asparaginase occurs in two steps. First step includes formation of beta-acyl-enzyme intermediate, a covalent intermediate occur by L-Asparaginase nucleophilic attack. In the second step, a water molecule attacks the acyl-enzyme intermediate to produce L-aspartate and ammonia (Michalska & Jaskolski, 2006). Quantification of release of ammonia or quantification of aspartic acid are used to examine hydrolysis reaction. Release of ammonia is measured by Nessler's reagent. Mechanism of catalytic activity of L-Asparaginase is alike to serine protease. Exact mechanism of action of L-Asparaginase is still unknown because it has two types of activities i.e glutaminase and asparaginase activities (Gulati *et al*., 1997).

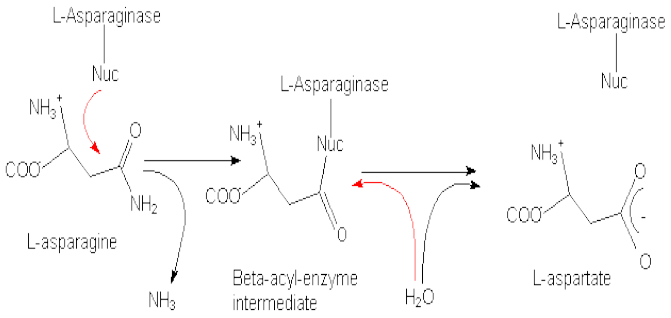

Figure 1: Illustrate the mechanism of action of L-Asaparaginase (Kumar & Sobha, 2012).

L-Asparaginase is also use glutamine as substrate but it have less affinity for glutamine than L-Asparagine. Glutamine donates an amino group to L-asparagine synthetase for de novo pathway for L-asparagine biosynthesis, therefore L-Asparaginase decreases the level of glutamine and also help in supporting the reduced level of asparagine and so contributes the chemotherapic effect of L-Asparaginase (Zeidan *et al*., 2009).

Microbial Sources

(a) Bacterial Asparaginase:

A number of bacterial sources are widely used for the production of L Asapaginase therefore they are studied extensively. Easy manipulation of bacteria also evoke its extensive use in enzyme production. For the production of L-Asparaginase For the chemotherapy of ALL bacterial L-Asparaginases are mostly use. Especially *E. chrysanthem*i and *E. coli* are medically approved to treat ALL. Depending upon different bacterial sources L-Asparaginase show differences in stability, pH, and affinity (Imada *et al*., 1973). Microbial sources that can reduce the allergic reactions and also decrease the toxic effects of bacterial L-Asparaginase are tried to produce by using different methods.

(b) Fungal Asparaginase:

Due to the hypersensitive reactions like anaphylactic shock by bacterial L-Asparaginase, filamentous fungal L-Asparaginase from *Aspergillus terreus* exhibited a better anti-tumor effect on static tumor (De-Angeli *et al*., 1970). Presence of amidase activity was first observed in

fungal strains by Imada *et al Penicillium clavgorme and P. expansum* (Imada *et al*., 1973). Later extracellularly produced L-Asparaginase purified from *A. terreus* was attached with polyethylene glycol (PEG) and estimated for its antiproliferation activity. Results showed that *A. terreus* L-Asparaginase is effective against proliferation of two leukemic cell lines, with a molecular weight similar to that of *E. coli* asparaginase. These L-Asparginases does not show any glutaminase activity (Loureiro *et al*., 2012). Fungal L-Asparaginase is a significant alternative of bacterial L-Asparaginase to decrease the toxic effects of bacterial asparaginases. Fungi are eukaryotic and are evolutionarily closer to human, chances of immunological reactions against fungal L-Asparaginase are less (Shrivastava *et al*., 2012). Colletotrichum, Eupenicillium, Fusarium, Penicillium, Talaromyces, *Aspergillus niger, Aspergillus terreus, Aspergillus tamarii,* are fungal sources for L-Asparaginase (Theantana et al., 2009; Patro & Gupta, 2012).

(c) Actinomycetes L-Asparaginase:

In recent times, marine microbes are consider to use for the production of bioactive compounds. A potential source of actinomycetes is marine environment from where these microorganisms can be isolated and used for antibiotics and bioactive compounds production. Marine actinomycetes also synthsize L-Asparaginase that can kill malignant cells and show cytotoxic effects on myelogenous leukemia and acute T-cell leukemia (Dhevagi & Poorani, 2006). Six strains of actinomycetes *streptomyces aureofasciculus, Streptomyces chattanoogenesis, Streptomyces hawaiiensis, Streptomyces orientalis, Streptomyces canus, and Streptomyces olivoviridis* were screened in marine fish (sahu *et al*., 2007). In 2011 Dharmaraj reported L-Asparaginase of marine actinomycetes where purified L-Asparaginase exhibited 78.88 IU/mg specific activity at pH 8. L-Asparaginase formation from various actinomycetes like Streptomyces ABR2 and *Streptomyces albidoflavus* have been explored (Sudhir *et al*., 2012).

Table 1. Different microbial sources of L-Asparaginase.

Fungal Sources	Bacterial Sources	Actinomycetes
Aspergillus niger (Patro & Gupta, 2012).	*Erwinia aroideae* (Peterson & Ciegler, 1969a, 1972)	*Streptomyces albidoflavus* (Narayana *et al*., 2008).
Aspergillus terreus (De-Angeli *et al*., 1970).	*Hydrogenomonas eutropha* (Peterson & Ciegler, 1969a, 1972).	*Streptomyces ABR2* (Sudhir *et al*., 2012).
Aspergillus tamari (Patro & Gupta, 2012).	*E. chrysanthem*i (Imada *et al*., 1973 & Verma *et al*., 2012).	*S. gulbargensis* (Amena *et al*., 2010).
Penicillium expansum (Imada *et al*., 1973)	*E. coli* (Imada *et al*., 1973 & Verma *et al*., 2012).	*streptomyces aureofasciculus* (sahu *et al*., 2007)
Fusarium tricinctum (Scheetz *et al., 1971).*	*Serratia marcescens* (Heinemann and Howard., 1969).	*Streptomyces orientalis* (sahu *et al*., 2007)
Penicillium clavgorme (Imada *et al*., 1973).	*B. subtilus* (Fisher *et al*., 2002).	*Streptomyces olivoviridis* (sahu *et al*., 2007)

Production of Asparaginase

(A) Solid State Fermentation: (SSF)

L-Asparaginase is generally produced by using SmF method. But different disadvantages like high cost, low product yield, and the production of huge quantities of waste water effluent are associated with SmF. Solid State Fermentation (SSF) is widely used in many processes like in biodetoxification of different harmful compounds, production of various therapeutic enzymes, bioremediation, production of secondary metabolites, and as an operative alternate of SmF. a process in which the growth of microbes takes place on a solid surface (substrate) in the absence or presence of minute quantity of water or with least moisture content is known as SSF. In this approach solid substrate act as the only source for the microbes. In 2002 Watanabe *et al* reported that SSF uses agricultural trashes and industrial remains that are inexpensive and freely available material as source of growth for microbes for the production of different

products. (Watanabe *et al*., 2002). Marine actinomycetes L-Asparaginase was produced by SmF and SSF and compared for enzyme activity, results showed that L-Asparaginase isolated from SSF process have greater activity (Basha *et al*., 2009). Substrates includes wheat bran, soy bean meal, black sesame oil cake, coconut oil cake, and sugar cane bagasse are usually used for the production of enzyme by SSF. In 2006 Mishra reported optimum production of L-Asparaginase by *A. niger* when Glycine max used as substrate. Several statistical systems have been described for the optimization of the process of extraction of L-Asparaginase from microorganisms. Estimation of nutritional and environmental factors should be estimated for the production of enzyme.

Production of L-Asparaginase by Cladosporium specie was studied with five different experimentally designed variables that are temperature, pH, moisture content, inoculum concentration, and time, while substrate was wheat bran on SSF, indicated an enzyme yield of 3.74 U (Mohan Kumar & Manonmani, 2013). Production of L-Asparaginase on sesame oil cake by using *A. niger* C4 as a source of enzyme in a SSF media was optimized for three parameters that are temperature, aeration rate and bed thickness, showed maximum L-Asparaginase yield of 310 U/g (Uppuluri *et al*., 2013). This enzyme was produced by *Pseudomonas aeruginosa* 50071 on SSF media and purified 106 fold using ammonium sulfate precepitation followed by Sephadex gel filtration (El-Bessoumy *et al*., 2004). There are different types of bioreactors are present for SSF such as tray, packed bed, rotating drum, and fluidized bed. SSF cannot be used in large-scale extraction and purification of the enzyme because the scaling up of SSF bioreactor is tough due to problems of heat and mass transfer in heterogeneous system.

(B) Submerged Fermentation: (SmF)

Submerged fermentation (SmF) is a process in which the growth of microbes takes place in liquid broth media at where which is optimization of essential nutrients is done to enhance cultivation of microbes. In this process selected microbes grow carefully in a closed reactor that have fermentation media and a high oxygen concentration. SmF has well-established equipment that makes use of the existing microorganisms. Among different microbes Bacteria are usually used as a source in this process because they need high content of moisture. Fungal species such as *A. tamari, Aspergillus niger, A. terreus,* Fusarium, and Penicillium are reported for L-Asparaginase production by SmF (Patro & Gupta, 2012), and studies on optimization of culture media and environment conditions in both batch and continuous SmF have been reported. Production of L-Asparaginase depends upon the type of the microorganism as well as on different factors that are nutrient composition, temperature, pH of medium, inoculation concentration, dissolved oxygen concentration and fermentation time (Kumar & Sobha, 2012).

Table 2. Types of fermentation processes by using microbes.

Organism Name	Type of fermentation	Reference
Pseudomonas aeruginosa 50071	SSF	(El-Bessoumy *et al*., 2004)
Aspergillus niger	SSF	(Mishra, 2006; zia *et al*., 2013)
B. circulans (MTCC 8574)	SSF	
Aspergillus terreus, A. tamarii	SMF	(Sarquis *et al*., 2004)
P. carotovorum	SSF	(Kumar *et al*., 2010)
Fusarium equiseti	SSF	
Cladosporium sp.	SSF	(Mohan Kumar *et al*., 2013)
Serratia marcescens	SSF/SMF	
Erwinia carotovora	SMF	(Kamble *et al*., 2006)
Bacillus sp.	SMF	(Moorthy *et al.*, 2010)
Enterobacter aerogenes	SMF	
Thermus thermophilus	SMF	(Pritsa & Kyriakidis, 2001)
Streptomyces gulbargensis	SMF	(Amena *et al*., 2010)

Penicillium sp.	SMF	(Patro & gupta, 2012)
Penicillium digitatum	SMF	(Shrivastava *et al*., 2012)
Cladosporium sp.	SSF	(Mohan Kumar & Manonmani, 2013).

Presence of L-Asparaginase from marine bacteria was separated and found as a good choice (Mohapatra *et al*., 1995). Fungal L-Asparaginase Production was examined in *A. tamarii* and *A. terreus and* showed that *A. terreus* exhibited maximum L-Asparaginase activity of 58 U/L in a 2% proline medium (Sarquis *et al*., 2004). Use of agricultural waste like corn steep liquor for the production of L-Asparaginase was studied as cheap source (Zia *et al*., 2013). To overcome or minimize the side effects of immunological reactions from Bacterial L-Asparaginase ASP3 gene from *Saccharomyces cerevisiae* was cloned and expressed in *Pichia pastoris* then this recombinant strain used in SmF for the production of enzyme as a result of which 800 U/g per dry cell mass can be yielded. (Ferrara *et al*., 2006). SmF is extensively used for large-scale production of L-Asparaginase. A comparison of SSF and SmF shows that SSF provides high yield and constitutes an eco-friendly process that avoids environmental pollution.

Purification

Purification of L-Asparaginase mainly consists of alkaline lysis, ammonium sulfate precipitation, ion-exchange, gel filtration, dialysis, and crystallization. L-Asparaginase of Erwinia specie purified firstly by using cation-exchange chromatography technique then affinity chromatography, a highly specific chromatography technique was applied and at the end dialysis was done. Different important enzymes purification was achieved by cation-exchange chromatography technique because enzymes of bacteria are acidic in nature (Lee *et al*., 1986). Side effects of L-asparginase that are caused by glutaminase activity of this enzyme can be decreased by producing glutaminase free L-Asparaginase. L-Asparaginase free of glutaminase was purified by using ammonium sulfate precipitation followed by Sephadex G-100 chromatography and Diethylaminoethyl (DEAE) cellulose column chromatography. Kinetic factors, Km and Vmax of purified L-Asparaginase from *P. carotovorum* MTCC 1428, were 0.657 mM and 4.45 U/μg (Kumar *et al*., 2011). Usually, microbial L-Asparaginase shows activity at pH 7–9 but enzyme derived from *T. thermophilus* shows optimal activity at pH 9.2 and a Km value of 2.8 mM (Pritsa & Kyriakidis, 2001). The production and purification of L-Asparaginase from bacteria are studied and worked a lot while the yield of the enzymes of bacteria is low. Mohan Kumar *et al* cultivated fungal strain Cladosporium specie on wheat bran by SSF and purified with Vmax of 4.44 μmol/mL/min and Km of 0.1 M representing greater affinity of L-Asparginase for L-Asparagine (Mohan Kumar *et al*., 2013).

(A) Protein precipitation:

Separation by precipitation is a classical technique that is used for the recovery and purification of biomolecules from a liquid solution (Golunski *et al*., 2011). Process of precipitation reversibly disrupt secondary and tertiary structures of protein that cause precipitation of a protein. It is an easy method for scale up, with simple requirements of equipment, cheap. Additionally, the precipitant agent can be recycled in the final process by distillation, evaporation or heat drying, thus decreasing the environmental influence related to its removal. Recovery of Asparginase from different sources usually done by precipitation. A number of researchers have purified L-Asparginase up to apparent homogeneity by ammonium sulphate precipitation (Basha *et al*., 2009; Amena *et al*., 2010; Moorthy *et al*., 2010; Gervais *et al*., 2013). When ammonium sulphate added to crude sample extract that have enzyme at its saturation precipitation of Asparaginase occur and crude sample partially purified. Different yields depending on the source of Asparginase can be obtained by using 35% to 100% ranges of salts concentrations. In 2010 about 96.2% Asparaginase was recovered from Bacillus specie of bacterial source that was grown on glucose and after precipitation purified 10.9 fold (Moorthy *et al*., 2010). Salt precipitation is usually followed by centrifugation and column filtration to increase the purification yield. *Streptomyces gulbargensis* Asparaginase was yielded 50.6%, with 1.8 fold purification after production and purification. Purification of the same enzyme was improved and approaches up to 26.9-fold after centrifugation of precipitate then precipitant dialyzed against buffer

followed by Sepharcryl S-200 column filtration (Amena *et al*., 2010).

In 2004 El-Bessoumy *et al*., employed comparable strategy to synthesize, separate and purify Asparaginase from *P. aeruginosa* after SSF. After ammonium sulphate precipitation 5.2-fold Purification was obtained and with the use of Sephadex G-100 gel filtration technique it was increased about five times. For the purpose of concentration of crude sample ethanol can also be used and act as pre purifier or precipitant. In Brazil production of ethanol is very extensive. Ethanol can be reused after the process of precipitation and as a result decreases the environmental effect (Golunski *et al*., 2011). Precipitation is one of the first steps in the downstream process and it is usually combined with traditional techniques to enhance biomolecules purification fold and process yield. However, advanced techniques should be explored for a speedier and cost effective purification methods.

(B) Liquid–liquid extraction:

Liquid– liquid extraction (LLE) is one of the exciting alternate that can be used for the purpose of purification or extraction of different proteins or enzymes by using two-phase aqueous systems. Removal of a solute from a liquid mixture after the interaction with another non-soluble or partially soluble liquid mixture at which solute is differently soluble is known as liquid-liquid extraction. Affinity ligands can be used to enhance the partition in LLE (Lam *et al*., 2004). Aqueous two-phase systems can be achieved after combining the mixtures of water-soluble polymers with a different polymer or with some inorganic salts at or above critical concentrations. Different physicochemical properties effect partition of protein in two-phase systems, like surface hydrophobicity, molar mass, isoelectric point and components of system and some other factors that influence partitioning are concentration of polymer or surfactant, salt addition and pH. In 2003 Qin and Zhao reported a collective approach to release and isolate Asparaginase from *E. coli* ATCC 11303 cells by using micellar aqueous two-phase systems. When cells were treated with Triton X-100 (15%, w/v) and 9.4% (w/v) potassium hydrogen phosphate for 15-20 hours at 25 °*C about* 80% enzyme was released from cells of *E. coli*. As L-Asparaginase is present in periplasmic space, the authors suggested that Triton X-110 micelles can interrupt the outer membrane of *E. coli* release the L-Asparaginase enzyme from the periplasmic space of bacterial cells. For surfactant/phosphate/ water system, phase parting into top phase that is micelle-rich and bottom phase (salt-rich phase) was detected above concentration of critical micelle. In phosphate-rich (salt rich), bottom phase abundance of released L-asparaginase was obtained. Although aqeuous two-phase micellar systems have a potential for the release or purification of asparaginase but there is still no data on purification factor.

In 2007 Jian-Hang *et al*., described another approach in which cells are disrupted by high-pressure homogenization and the enzymes are taken by aqueous two-phase micellar system for of intracellular asparaginase extraction from *E. coli*. Those researchers employed polypropylene oxide (PPO) and polyethylene oxide (PEO) as copolymers in triblock like PEO–PPO–PEO, to form main aqueous two-phase systems for purification of L-asparaginase. Asparaginase purification via this in-situ process resulted in increased enzyme yield by in-situ process (from 52% to 73%) and specific activity (from 78.60 to 94.80 U/mg) compared with the conventional process including cell disruption, centrifugal clarification and subsequent aqueous two-phase micellar system.

For the commercial purification of Asparaginase, aqueous two-phase system still requires more exploration for its implementation. Additionally, we believe that there is a requirement to further study aqueous two phase system that are made up of ionic liquids for the purification of Asparaginase which appears to be a predominantly promising substitute (Souza *et al*., 2015).

(C) Chromatography:

A high level of purity is needed for circulatory enzymes preparations like Asparaginase that is why a defined system of purification method is required. In 2008 Gra¨slund *et al* reported that an elevated degree of purity of enzyme is also significant from the perspective of process control, but steps that are required for the process of purification, In common, enzyme activity loss

and increased expenses are resulted. Asparaginase from *P. aeruginosa* 50071 attained by SSF was purified by ammounium sulphate precepitation(NH4)2SO4, gel filtration (Sephadex G-100) and ionic exchange (CM-Sephadex C50) then the specific activity was increased up to 1900 IU/mg in final product from 17.90 in crude extract (El-Bessoumy *et al*., 2004). A similar method was used by in 2013 Singh *et al*. used a similar process for the purification of extracellular Asparaginase from protease-deficient *Bacillus aryabhattai* ITBHU02 strain. After ammounium suphate precipitation and DEAE-Sepharose fast flow chromatography and Seralose column chromatography, Asparaginase was purified with specific activity of 680.47 U/mg up to 68.9-fold yield. who extracted and purified Asparaginase from *E. carotovora* was extracted and purified by (NH4)2SO4 precipitation, followed by CM cellulose, Sephadex G-100 and DEAE Sephadex chromatographies specific activity was increased from 1.36 to 1034 IU/mg, with a least mass loss and an ultimate recovery yeilded 36.5% (Warangkar & Khobragade, 2010). *Penicillium digitatum* was another used source to synthesize extracellular Asparaginase. After the process of protein precipitation desalting was done, chromatography technique i.e gel filtration was used with Sephadex G-25 then followed by Sephadex G-100 as a result of which 60.9-fold purification was obtained. purified Asparaginase was purified from Aspergillus specie by ion exchange chromatography (DEAE Sepharose) after which gel filtration (Sephacryl S-200HR) chromatography was done at different flows results showed 12% ending yield and 7.72 purification factor (Loureiro *et al*., 2012).

When an extra purification phase was applied with the mutual fraction to the similar Sephacryl S-200HR chromatography column resulted in purification factor of 10.7 with a lower product yield (7.28%). Recently asparaginase has been taken from the fungal genus Cladosporium. Precipitation followed by DEAE cellulose ion exchange and subsequent size exclusion chromatography ensured a final specific activity of 83.3 U/mg (Kumar & Manonmani, 2013). The summary tells that protocols for the purification of Asparaginase from various sources lead to different purification results. However gel filtration and ion exchange chromatography are the mostly used purification steps. They are often preceded by precipitation with (NH4)2SO4 used as the pre-chromatographic procedure that can effectively be replaced by aqueous two-phase system.

II. Applications

i. Role of L-Asparaginase in Amino Acid Metabolism:

For the production of different amino acids like lysine, threonine, and methionine are biosynthesized by L-Asparaginase. These amino acids are known as aspartic family amino acids. In addition to Kreb's cycle aspartic acid is synthesized by L-asparaginase. This aspartic acid is further used as a direct precursor of threonine and lysine (Sinha *et al*., 2013)

ii. Role of L-Asparaginase in Food industry:

In food processing L-asparaginase is also extensively used enzyme. Current advances in food technology revealed that a crystalline solid that is colorless and odorless in nature is produced by Millard reaction, is known as acrylamide (IUPAC name is 2-propenamide). Millard reaction occurs when starchy foods are fried or baked at 120 °C (Lingert *et al*., 2002**)**. Acrylamide is act as neurotoxin and considered as carcinogenic agent and therefore it is harmful for human (Gokmen *et al*., 2008). Millard reaction occur when α-amino group of free asparagine and carbonyl groups of reducing sugars like glucose react with each other during the process of frying and baking. High temperature and pH induce the production of acrylamide (Friedman, 2003). L-asparaginase has an ability to convert asparagine amino acid into aspartate because of this reason L-Asparaginase can be used in food industry to reduce acrylamide formation in starchy foods. To avoid acrylamide production pre-treatment of starchy foods such as potato and bread dough is done with L-Asparaginase (Pedreschi *et al*., 2008). Though, acrylamide cannot completely removed because of presence of asparagineindependent formation of acrylamide (Dhanam & Kannan, 2013). Fungal sources such as *Aspergillus oryzae* and *Aspergillus niger* produce L-Asparaginase that are used in food processing.

In 2006 Ciesarova, Kiss, & Boegl described that use of L-Asparaginase (2 U/g) effectively reduced acrylamide concentration up to 90% in potato products that have high concentration of asparagine. Recombinant asparaginase from *Aspergillus oryzae* was tested on

various food items like ginger biscuits, semisweet biscuits, sliced potato chips, French fries and crisp bread and the results showed 34–92% reduction in acrylamide content (Hendriksen *et al*., 2009). In another study it was confirmed that the use of L-Asparaginase could decrease acrylamide level up to 99%, when used before baking and frying of starchy food (Zyzak *et al*., 2003). In past, some other researches have dealt with this application of L-Asparaginase that can decrease the side effects of acrylamide having foods without damage their features.

iii. Anticancer Drug:

L-Asparaginase plays a vital role in treatment of Acute Lymphoblastic Leukemia (ALL) with vincristine and a glucocorticoid (Szymanska *et al*., 2012). L-Asparaginase have anti-melignant properties that is why it has been considered as a therapeutically significant antitumor drug. Now L-asparaginase is a famous chemotherapeutic agent which is used in combination with other drugs to treat certain malignancies such as ALL (mostly in childs), acute myelocytic leukemia, Hodgkin's disease, chronic lymphocytic leukemia, acute myelomonocytic leukemia, reticulosarcoma, melanosarcoma and lymphosarcoma, (Kidd, 1953; Broome, 1961).

L-asparagine, act as an important amino acid required for synthesis of protein, for various tumor cells and cell growth, whereas L-asparaginase converts L-asparagine to aspartate that is why presence of L-Asparaginase destruct the growth factors of malignant cells as a result of which depletion of asparagine occur and malignant and ultimately tumor cells die (Salzer *et al*., 2014). L-asparaginase is present in plants, animals, and microbes, but only selected L-asparaginase from *E. chrysanthemi* and *E. coli* was permitted for chemotherapy for ALL treatment (Verma *et al*., 2012). However latest research shows that autophagy is associated with L-asparaginase treatment, so it is recommended to use anti-autophagy drug in combination with L-asparaginase for the treatment of ALL. Inhibition of autophagy enhances L-asparaginase-induced cytotoxicity and overcomes the acquired resistance to L-asparaginase in ALL cells (Takahashi *et al*., 2017).

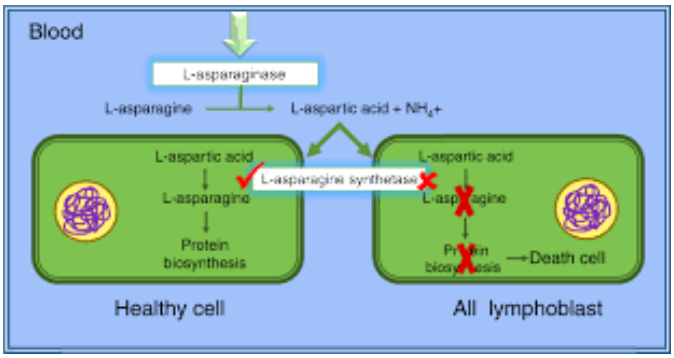

Figure 2: shows anti-leukemic action of L-Asparaginase (Van den Berg, 2011).

iv. Role of L-Asparaginase in Biosensor:

To study the levels of asparagine in leukemic patients and food industry biosensors of L-Asparaginase are developed (Verma *et al*., 2012). A number of spectroscopy techniques like XPS, XRD, TEM, and SEM are recently used for the analysis of L-asparagine, but the main disadvantage is their high cost (Zubavichus *et al*., 2004). As compare to these expensive techniques, biosensor technology can be a reliable, cheap, and user-friendly approach. The mechanism of action of the biosensor is based on 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).

III. CONCLUSION

In conclusion, L-Asparaginase from different microbial sources shows such propertites which make it an important enzyme in both pharmaceutical and food industries. Further studies should be conducted for reducing the cost of enzyme production. This could be done by increasing the yield of L-asparaginase through optimization of the production process or by strain improvement. Although bacterial L-Asparaginase is clinically used for treatment of ALL, it also induces certain adverse reactions. Novel producers of L-asparaginases should be explored which may have less side effects while clinical use.

IV. REFERENCES

[1]. Amena, S., N. Vishalakshi,M.Prabhaka,A. Dayanand and K.Lingappa.2010. Production purification and characterization of L-asparaginase from Streptomyces gulbargensis.BJM., 41: 173–178.

[2]. Arima, K.,T.Sakamoto,C.Araki and G.Tamura.1972. Production of extracellular L-Asparaginases by microorganisms. Agric Biol Chem., 36(3): 356–361.

[3]. Avramis,I.,S. Sencer,P. Antonia,P.Sather,B.Bostrom, L.Cohen, A.Ettinger, L.Ettinger, J.Franklin,P.Gaynon, J.Hilden, B.Lange, F.Majlessipour, P.Mathew, M.Needle, J.Neglia, G.Reaman and J.Holcenberg.2002. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood., 99 (6):1986–1994.

[4]. Basha,S.,R.Rekha,M.Komala and S.Ruby. 2009. Production of extracellular antileukaemic enzyme L-asparaginase from marine actinomycetes by solid- state and submerged fermentation: Purification and characterisation. TJPR., 8: 353–360.

[5]. Broome,D.1961. Evidence that the L-asparaginase activity of guinea pig serum is responsible for its antilymphoma effects. Nature., 191(4793): 1114–1115.

[6]. Broome, D. 1963. Evidence that the L-Asparaginase of guinea pig serum is responsible for its antilymphoma effects I. Properties of the L-Asparaginase of guinea pig serum in relation to those of the antilymphoma substance. J. Exp. Med., 118(1): 99–120.

[7]. Ciesarova´, Z., E.Kiss and Boegl. 2006. Impact of L-Asparaginase on acrylamide content in potato products. J FOOD NUTR RES., 45(4): 141–146.

[8]. De-Angeli,C.,F.Pocchiari,S.Russi, A.Tonolo,E. Zurita and E.Ciaranfi. 1970. Effect of L-asparaginase from Aspergillus terreus on ascites sarcoma in the rat. Nature., 225(7): 549–550.

[9]. Dhanam,G. and S.Kannan.2013. L-asparaginase: types perspectives and applications. Adv Bio Technol., 13: 1–5.

[10]. Dharmaraj, S.2011. Study of L-Asparaginase production by Streptomyces noursei MTCC 10469, isolated from marine sponge Callyspongia diffusa.IJB., 9(2): 102–108.

[11]. Dhevagi, P. and E.Poorani. 2006. Isolation and characterization of L-asparaginase from marine actinomycetes. IJBT., 5: 514–520.

[12]. El-Bessoumy, A.,M. Sarhan and J.Mansour.2004. Production, isolation, and purification of L-Asparaginase from Pseudomonas aeruginosa 50071 using solid-state fermentation. IJBMB., 37(4): 387–393.

[13]. Ferrara, A., N.Severino, J.Mansure,S.Martins,M.Oliveire and C.Siani.2006. Asparaginase production by a recombinant Pichia pastoris strain harbouring Saccharomyces cerevisiae ASP3 gene. Enzyme Microb Technol., 39(7): 1457–1463.

[14]. Friedman, M. 2003. Chemistry biochemistry and safety of acrylamide. J. Agric. Food Chem., 51: 4504–4526.

[15]. Gervais, D.,N.Allison,A.Jennings,S.Jones and T.Marks. 2013. Validation of a 30-yearold process for the manufacture of L-asparaginase from Erwinia chrysanthemi. Bioprocess Biosyst Eng., 36: 453–60.

[16]. Gokmen, V. and K.Palazoglu.2008. Acrylamide formation in foods during thermal processing with a focus on frying. Food Bioprocess Tech., 1(168): 35–42.

[17]. Golunski, S.,V.Astolfi,N.Carniel,D.Oliveira,M.Di Luccio,A.Mazutti and H.Treichel.2011. Ethanol precipitation and ultrafiltration of inulinases from Kluyveromyces marxianus. Sep Purif Technol., 78: 261–265.

[18]. Gräslund,S.,P.Nordlund,J.Weigelt,J.Bray,O.Gileadi ,S.Knapp,U.Oppermann,C.Arrowsmith, R.Hui,J.Ming and W.Park. 2008. Protein production and purification. Nat Methods., 5: 135–146.

[19]. Gulati, R.,K.Saxena and R.Gupta.1997. A rapid plate assay for screening L-Asparaginase producing micro-organisms. Lett Appl Microbiol., 24(1): 23–26.

[20]. Hendriksen,V.,B.Kornbrust,R.Ostergaard and M.Stringer.2009. Evaluating the potential for enzymatic acrylamide mitigation in a range of food

products using an asparaginase from Aspergillus oryzae. J. Agri Food Chem., 57(10): 4168–4176.

[21]. Hill, M.,J.Roberts,E.Loeb,A.Khan,A.MacLellan and W.Hill. 1967. LAsparaginase therapy for leukemia and other malignant neoplasms. Remission in human leukemia. JAMA., 202(9): 882–888.

[22]. Imada, A.,S.Igarasi,K.Nakahama and M.Isono.1973. Asparaginase and glutaminase activities of micro-organisms. J Gen Microbiol., 76(1): 85–99.

[23]. Jain, R.,U. Zaidi, Y.Verma and P.Saxena.2012. L-asparaginase: a promising enzyme for treatment of ALL. People's J Sci Res., 5: 29–35.

[24]. Kidd, G. 1953. Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum I. course of transplanted cancers of various kinds in mice and rats given guinea pig serum, horse serum, or rabbit serum. J. Exp. Med., 98(6): 565–582.

[25]. Killander, D.,A. Dohlwitz,L.Engstedt,S.Franzen,G.Gahrton,B.Gullbring,G.Holm,A. Holmgren,S.Höglund,A,Killander and D.Lockner. 1976. Hypersensitive reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. Cancer., 37 (1): 220–228.

[26]. Kumar, S., V.Dasu and K.Pakshirajan.2011. Purification and characterization of glutaminase-free L-Asparaginase from Pectobacterium carotovorum MTCC 1428. Bioresour Technol., 102(2): 2077–2082.

[27]. Kumar, S. and K.Sobha.2012. L-Asparaginase from microbes: A comprehensive review. Adv. Biores., 3(4): 137–157.

[28]. Kumar,M. and K.Manonmani. 2013. Purification, characterization and kinetic properties of extracellular L-asparaginase produced by Cladosporium sp. World J Microbiol Biotechnol., 29: 577–587.

[29]. Kumar, K.,M. Kataria and N. Verma. 2013. Plant asparaginase-based asparagine biosensor for leukemia. Artif Cells Nanomed Biotechnol., 41: 184–188.

[30]. Lam ,H.,M. Kavoosi and A.Haynes . 2004. Affinity-enhanced protein partitioning in decyl b-D-glucopyranoside two-phase aqueous micellar systems. Biotechnol Bioeng., 89: 381–392.

[31]. Lee, S.,T. Ross,E.Gustafson,H.Wroble and M.Muschik.1986. Large-scale recovery and purification of L-Asparaginase from Eriwinia carotovora. Appl Biochem Biotech., 12: 229–247.

[32]. Lingnert,H.,S.Grivas, M.Jagerstad, K.Skog, M.Tornqvist and P.Aman. 2002. Acrylamide in food— mechanisms of formation and influencing factors during heating of foods. Scand J Food Nutr., 46: 159–172.

[33]. Loureiro,B.,S. Borges, F.Andrade,G.Tone and S.Said. 2012. Purification and biochemical characterization of native and pegylated form of L-asparaginase from Aspergillus terreus and evaluation of its antiproliferative activity. Adv Microbiol., 2: 138–45.

[34]. Mashburn,T. and C.Wriston.1964. Tumor inhibitory effect of L-Asparaginase from Escherichia coli. Arch Biochem and Biophy., 105: 450–452.

[35]. Michalska, K. and M.Jaskolski. 2006. Structural aspects of L-Asparaginases, their friends and relations. Acta Biochimica Polonica., 53(4): 627.

[36]. Mishra, A. 2006. Production of L-Asparaginase, an anticancer agent, from Aspergillus niger using agricultural waste in solid state fermentation. Appl Biochem Biotech., 135(1): 33–42.

[37]. Mohan, S. and K.Manonmani.2013. Purification, characterization and kinetic properties of extracellular L-Asparaginase produced by Cladosporium sp. IUMS., 29(4): 577–587.

[38]. Mohapatra,R., K.Sani and C. Banerjee.1995. Characterization of L-asparaginase from Bacillus sp. isolated from an intertidal marine alga (Sargassum sp.). Lett Appl Microbiol., 21(6): 380–383.

[39]. Moorthy, V.,A. Ramalingam,A.Sumantha and T.Shankaranaya.2010. Production, purification and characterisation of extracellular L-Asparaginase from a soil isolate of Bacillus sp. Afr. J. Microbiol. Re., 4(18): 1862–1867.

[40]. Patro, R. and N. Gupta. 2012. Extraction, purification and characterization of L-As paraginase from Penicillium sp. by submerged fermentation. IJBM Res., 3(3): 30–34.

[41]. Pedreschi, F.,K. Kaack and K. Granby. 2008. The effect of asparaginase on acrylamide formation in French fries. Food Chem., 109: 386–392.

[42]. Pritsa, A. and A. Kyriakidis. 2001. L-Asparaginase of Thermus thermophilus: Purification, properties and identification of essential amino acids for its catalytic activity. Mol Cell Biochem., 216: 93–101.

[43]. Qin ,M. and F. Zhao. 2003. L-asparaginase release from Escherichia coli cells with aqueous two-phase micellar systems. Appl Biochem Biotechnol., 110: 11–21.

[44]. Sahu,K.,K.Sivakumar,E.Poorani,T.Thangaradjou,L. Kannan.2007. Studies on L-asparaginase enzyme of actinomycetes isolated from estuarine fishes. J Environ Biol., 28:465–474.

[45]. Salzer,L.,L. Asselin,V.Plourde,T.Corn and S.Hunger.2014. Development of asparaginase Erwinia chrysanthemi for the treatment of acute lymphoblastic leukemia. Ann. N. Y. Acad. Sci.,1329: 81–92.

[46]. Sarquis, M.,M. Oliveira,S.Santos and L.Costa.2004. Production of L-Asparaginase by filamentous fungi. Mem Inst Oswaldo Cruz., 99(5): 489–492.

[47]. Shrivastava, A.,A.Khan,A.Shrivastav, K.Jain and K.Singhal.2012. Kinetic studies of L-Asparaginase from Penicillium digitatum. Prepara Biochem Biotech., 42(6): 574–581.

[48]. Sinha, R.,R. Singh and K.Jha.2013. L-Asparaginase: present and future prospective. IJIRSET., 2: 7031–7051.

[49]. Soares,L., M.Guimarães,B.Polakiewicz,N.Pitombo and J.Neto.2002. Effects of polyethylene glycol attachment on physicochemical and biological stability of E. coli l-asparaginase. Int J Pharm., 237: 163–70.

[50]. Souza,L., P.Ventura,M.Soares,A.Coutinho and A.Lima.2015. Lipase purification using ionic liquids as adjuvants in aqueous two-phase systems. Green Chem., 17: 3026–34.

[51]. Sudhir,P.,R.Dave, K.Trivedi and B. Subramanian. 2012. Production and amplification of an L-Asparaginase gene from actinomycete isolate Streptomyces ABR2. Ann Microbiol., 62(4): 1609–1614.

[52]. Szymanska, B., U.Wilczynska-Kalak,H.Kang,L. Liem,H.Carol and B.Lock.2012. Pharmacokinetic modelling of an induction regimen for in vivo combined testing of novel drugs against pediatric acute lymphoblastic leukemia xenografts. PloS One., 7(3): 33894–33994.

[53]. Takahashi,H., J. Inoue,K .Sakaguchi, M. Takagi, S. Mizutani and J. Inazawa.2017. Autophagy is required for cell survival under L-asparaginase-induced metabolic stress in acute lymphoblastic leukemia cells.Oncogene.,59:1-2.

[54]. Theantana, T., D.Hyde and S. Lumyong. 2009. Asparaginase production by endophytic fungi from Thai medicinal plants: cytotoxicity properties. Int J Integr Biol., 7: 1–8.

[55]. Uppuluri,B.,R. Dasari, V.Sajja,S. Jacob and D.Reddy. 2013. Optimization of L-Asparaginase production by Isolated Aspergillus niger C4 from sesame (black) oil cake under SSF using Box–Behnken design in column bioreactor.INT J CHEM REACT ENG., 11(1): 1–7.

[56]. Van, H. 2011. Asparaginase revisited. Leuk Lymphoma., 52(2): 168–178.

[57]. Verma, N.,M.Bansal and S.Kumar.2012. Whole cell based miniaturized fiber optic biosensor to monitor L-asparagine. J. Appl Sci Res., 3: 809–814.

[58]. Warangkar,C. and N.Khobragade. 2010. Purification, characterization, and effect of thiol compounds on activity of the Erwinia carotovora L-asparaginase. Enzyme Res., 1: 1–10.

[59]. Watanabe,I., N.Miyata, A.Ando, R.Shiroma, K.Tokuyasua and T.Nakamura.2002. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Biochem Eng Jour., 93(1): 90–95.

[60]. Zeidan, A.,S.Wang and M.Wetzler.2009. Pegasparaginase: where do we stand? Expert Opin Biol Ther., 9 (1): 111–119.

[61]. Zia,A.,R.Bashir,I.Ahmed and T.Iftikhar.2013. Production of L-Asparaginase from Aspergillus niger using agro wastes by-products in submerged fermentation process. Jurnal Teknologi., 62(2): 47–51.

[62]. Zyzak,V.,R. Sanders, M.Stojanovic, H.Tallmadge, L. Eberhart ,K.Ewald, C.Gruber, R.Morsch, A.Strothers, P.Rizzi and D.Villagran. 2003. Acrylamide formation mechanism in heated foods. J Agric Food Chem., 51(16):4782–4787.