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Industrial Production and Clinical Application of L-Asparaginase: A Chemotherapeutic Agent

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***Abstract*—**This article comprises detail information about L-asparaginase, encompassing topic such as various sources of L-asparaginase, mechanism and properties of L-asparaginase. Also describe the production, cultivation and purification of L-asparaginase along with information about the application of L-asparaginase. L-asparaginase catalyzes the conversion reaction to convert asparagine to aspartic acid and ammonia. Asparagine is a nutritional requirement for both normal and tumor cell. Present scenario has found that L-asparaginase has been found to be a best anti tumor or antileukemic agent. In the recent years this enzyme gained application in the field of clinical research pharmacologic and food industry. It has been characterized based on the enzyme assay principle hydrolyzing L-asparagine into L-aspartic acid and ammonia. It has been observed that eukaryotic microorganisms such as yeast and filamentous fungi have a potential for L-asparaginase production. L-asparaginase has been and is still one of the most lengthily studied therapeutic enzymes by scientist and researchers worldwide.

***Keywords*—**L-asparaginase, antitumor, solid state fermentation, chemotherapeutic.

I. Introduction

IN recent years enzymes have gained great importance in clinical research. L-asparaginase is one of them which are widely present in nature. L-asparaginase (EC3.5.1.1) catalyzes the hydrolysis of L-asparagine into aspartic acid and ammonia. L-asparaginase has been a clinically satisfactory antitumor agent for the valuable treatment of acute lymphoblastic leukemia (ALL) and lymph sarcoma [75]. L-asparagine is an essential amino acid for the production of protein in tumor cells whereas the growth of normal cell is independent of its requirement. L-asparaginase can be produced within the cells by an enzyme called asparagine synthase are can be absorbed from the outside. Lymphatic tumor cell required huge amount of asparagine to keep up their rapid malignant growth. In the presence of L-asparaginase tumor cell get deprivated and cannot survive [4], [13], [20]. This fact suggests that L-asparaginase enzyme used as anti tumor or anti leukamatic drug.

L-asparaginase is widely distributed among the microorganism, animals, and plant. The microorganisms are a better source of L-asparaginase because they can be cultured easily [46], [74], [96], [24]-[26]. *Erwinia caratovira*, *Corynebacterium glutamicum*, *Bacillus* sp, *Psudomonas stutzeri,* and *E. coli* are most commonly used microorganisms for the production of L-asparaginase [89], [5]. L-asparaginase from *E. chrysanthemi* is pharmacologically active and that from *E. coli* is also having anti tumor effect. Since these two L-asparaginases possess different immunological specification and the availability to provide an important alternative therapy. Unlike other chemotherapy agents, it can be given as intramuscular, intravenous or subcutaneous injections without fear of any side effect or tissue irritation [45].

The exact mechanism of L-asparaginase is still unknown although hydrolysis proceeds in two steps via beta-acyl-enzyme intermediates [42]. L-asparaginase also plays important role in biosynthesis of aspartic acid family of amino acids. Different types of L-asparaginase can be used for different pharmacological and industrial application. L-asparaginase is used to reduce the formation of acrylamide [32]. The main side effect is hypersensitivity or allergic reactions; anaphylaxis is a possibility [4], [11], [72]. Additionally it can also be associated with a coagulopathy as it decrees protein synthesis, including synthesis of anti coagulant factor, leading to bleeding or thrombolytic events such as stroke [76], [41].

II. Source

Wide range of bacteria yeast fungi algae, actinomycetes and higher plant such as *Withnaria somnifera* [103], *Sphagnum fallax* [18] [19], *Lupine araboreuse* and *Lupin amgustplius* [17], [12] are used as source of L-asparaginase. L-asparaginase is also found in the soil of root of *Pinus pinaster* and *Pinus radiata* due to ectomycorrhizal fungi [83]. L-asparaginase is generally found in *E. coli* and other gram negative bacteria such as *achromobacteriaceae* [101], [59]. L-asparaginase production has been reported in *Pseudomonas fluoresces* [71]. *Mycobacterium phlai* [80] and various numbers of nitrobacteria the production of a homodymer L-asparaginase from *Rhodosprium toruloides* [33] and *Rhodotorula* sp [87]. *Aspergillus nidulanus* and *A. terreus* are also cable to produce L-asparaginase [73]. L-asparaginase is the first such enzyme to be purified form a marine microalgae *Chlamydomonas* sp. [65]. Actinomycetes are also a good source of L-asparaginase. Streptomyces, actinomycetes are capable to producing detectable amount of L- asparaginase [66], [88] (Table I).

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III. Mechanism of Action

In normal cells, the asparaginase used for protein synthesis is generated from aspartate by asparagine synthase. Outside the cell asparagines is converted into aspertate by L-asparaginase. L- asparaginase causes selective toxicity for tumor cell because they lack L- asparaginase synthase [1] [2]. L- asparaginase catalyses the hydrolysis reaction to convert L-asparaginase into L aspertate and ammonia [16] (Fig. 1). Asparagene is required for cell survival and DNA synthesis; however, most of the cells are capable to synthesizing asparaginase from glutamine [82], [37]. Acute lymphoblastic leukemia cells lack adequate level of the asparagines synthase and cannot survive in asparagine depletion. Asparginase is cycle specific for the G1 of cell cycle [47].

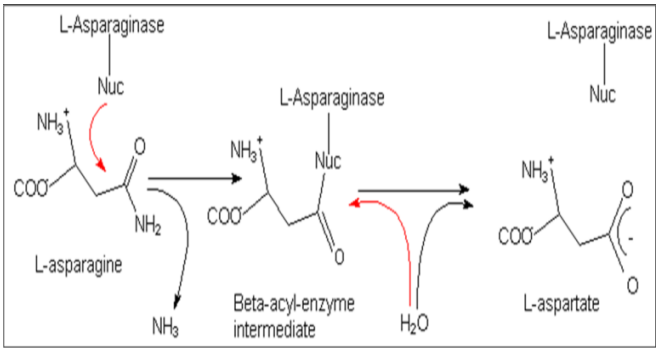

Fig. 1 Map Schematic illustration of the reaction mechanism of L-asparaginases. The proposed covalent intermediate is produced in the course of nucleophilic attack by the enzyme

IV. Properties

L-asparaginase catalyses the deamination reaction to produce L- aspartic acid and ammonia. L-asparaginases are mainly tetrameric in nature. In some harsh condition like high PH and freeze drying changed the tetramer structure of the enzyme in to monomer. [43]-[50]. For enzyme activity ionization and deionization of the functional group of the active center are responsible. L-asparginase has anticancer and antitumor property. It is used as anticancer agent because it is biodegradable and non-toxic [49].

V. Production

Wide range of bacterial yeast, fungi, actinomycetes and algae are very effective procedure of L- asparginase. There have been many reports about the production of asparaginase under different condition by different microorganism and plant. S. cerevacea synthesize two forms of asparaginase, L-asparaginase I and L- asparaginase II. They are genetically and chemical different [36]. The synthesis of L- asparaginase in *E. coli* is almost completely suppressed if glucose is added at a concentration of 0.5% to the growth medium glucose causes catabolic repression and catabolite inhibition and locates stimulated L-asparginase synthesis [36]. In lupin arboreas plant part such as root tips, leaves, flower bud and developing seeds is the main source of asparaginase. Cell growth and enzyme formation were studied in batch fermentation for the production of therapeutic L- asparaginase form *Erwinia aroidae* [30] [31]. Yeast extract was important for the cell was formation and L-asparaginase synthesis, but high concentration of L- asparaginase production was inhibited. L- asparaginase I is constitute and L- asparaginase II is secreted in the nitrogen starvation condition bacteria growing in the ample nitrogen condition having high L-asparaginase activity [73], [38]. Keiselguhr composite and CM sepharose is and for the large scale production of L-asparaginase from *Erwinia chrysanthemi* [79]. In the medium optimum lactase concentration was l0 g/lit and addition of L-asparaginase production. Yeast extract was important source for the cell mass formation and L- asparaginase synthesis, put in high concentration L- asparaginase secretion was inhibited [81].

Production of staphyloecoccal L- asparaginase shows that carbon such as maltose, sucrose, lactose, mannitol, galactose and mannose inhibited while exogenous cAMP in pressure of carbon sources [3]. Stimulated cheese why supplementation with tryptophan (0.3%) and asparagine (0.5%) was used for the production of L- asparaginase enzyme [39]. An acrobicactinomycete, *Nacardia asterodies*, was grown in three different medium, normally sabour and dextrose broth tryptic soya broth and synthetic medium as a shake culture at 37°C for days. The sabour and dextrose broth shows maximum cell biomass growth and maximum L- asparaginase production [99].

6% n-dodecane compound increased cell concentration by 12.7% and production of L- asparaginase by 21% and give 60.81 V/ml in the fermentation medium of *E. coli* [99]. The optimum pH was 9.2 and the Km for L- asparaginase was 2.8 mM. At native state the enzyme is a hexamer and does not hydrolyze L-glutamine. High L- asparaginase activity was found in cells cultured on D galactose L-fructose, sucrose or maltose and in cell cultured on L- asparaginase as a sole nitrogen source. A new *Erwinia* sp has been reported a good source of L- asparaginase production [90].

A pH of 7.9 corn steep liquor (3.6) and casein hydrolysate (3.11%) were important factor for enzyme production process [87]. L-asparaginase production from *E. coli* cell with aqueous two phase micelle or system by using tritonx-100 and K_2HPO_4 [60].

VI. Effect of Temperature

The optimum temperature and stability of enzyme to temperature was determined by gaffer protocol. The optimum temperature for L-asparaginase activity is 37°C L-asparaginase active at a wide range of temperature condition from 30 to 75°C [58]. Beyond this temperature the enzyme becomes unstable. This property of enzyme plays important role for complete elimination of asparagine from the patient body when they treated with L- asparaginase in vivo. The residual activity is 100% at 70°C for 30 and 60 minutes At 77°C it retain 100% activity [34] [35].

VII. Effect of pH on Enzyme Activity

The L-asparaginase activity below pH 8 would not be expected to be effective for the treatment of the tumor patient. The membrane bound L- asparaginase from *T. pyriformis* acts optimally at pH9.6. The enzyme activity is slightly lowered at pH value of 7.5 to 8.0 [44]. *E. carotovora* L- asparaginase is evidently more stable than *E. coli* enzyme in the alkaline pH region. The lily enzyme preparation is completely dissociated in to the 1.85 subunits within 30 minutes of adjusting the pH to 11.8 [34].

VIII. Effect of Agitation

Agitation is another factor which affects enzyme production. Aeration and agitation were most significant at interactive level for L-asparaginase production by isolated Staphylococcus sp. - 6A [93].

IX. Effect of Inducer

L-asparaginase synthesis was increased by the addition of L-aspartic acid, L-glutamic acid, L-asparagines and L glutamine by using *Serratia marcescense*. The addition of L glutamic acid or L-aspartic acid to the medium containing sodium fumarate and corn streep liquor slightly enhance enzyme production but these amino acid may not be conceded specific inducer for enzyme synthesis [8].

X. Impact of Carbon and Nitrogen Source

The effect of carbon and nitrogen sources on growth and enzyme production was studied using various concentration of yeast extract. Microbes are capable of utilizing a verity of carbon and nitrogen sources [91]. Central composite rotatable design was applied to optimize the level of nitrogen and carbon sources of the medium in shake flask. Experiment the organism grown in the define medium contain 1% (w/v) different carbon sources and 0.1% w/v yeast extract as well as nitrogen sources. Glucose, maltose, fructose, Raffanose, mannose and lacks are used as carbon sources for the production of L- asparaginase amino acid and like theremins, praline, valine. Aspartic acid, glutamic acid and yeast extract were used in the medium along with L- asparaginase. The best carbon sources for K. pneumonia growth were sorbitol, melibose, maltose, mannitol and sucrose used for L-asparaginase production. Asparagine was used as a nitrogen sources in synthetic media to stimulated more enzyme production. At pH 8-5 starch (1.0%) carbon and asparagine (0.8%) as nitrogen sources were optimum for enzyme production [3].

XI. Cultivation Method

A. Solid State Fermentation

L- asparaginase is an important antitumor agent used for the treatment of a verity of lymphoid proliferative disorders, various microorganism and plants are produce L-asparaginase. In recent years, the production of enzyme based on the solid state fermentation (SSF) [6]. SSF is suitable for the production of enzyme by using natural substrate because they mimic the condition under which the microbe grows natured. The solid state fermentation has several advantages over submerged fermentation including superior productivity, low capital investment, simple technique, low energy requirement less water output and better product recovery [23]. Solid state fermentation holds wonderful potential for the production of secondary metabolites and has been increasing application in recent years. Rice bran served as a most appropriate substrate compared to other existing starchy materials, for solid stats cultivation of *Serratia marcescens* SBOB for L-asparaginase production. Solid-state fermentation is a very useful technique as the yield of the product is many times higher in comparison of submerged fermentation (SF). Submerged fermentation of has many disadvantage such as the low concentration production reduction and disposal of large value of water during the downstream processing. L-asparaginase also produced form *P. aerceginase* 50071 under solid state fermentation [7].

TABLE I
List of Various Sources for Production of L- Asparaginase

Microorganisms	References
Bacteria	
Acinetobacter colcoaeticus	[48]
Bacillus sp.	[63]
B.mesentericus	[94]
B.polymyxa	[69]
Citrobacter sp.	[10]
Corynebacterium glutamicum	[60], [56]
Escherichia coli	[70], [50]
E. cloaceae	[68]
Enterobacter aerogenes	[67]
E. carotovoro	[95]
Helicobacter pylori	[58]
Klebsiella pneumonia	[64]
Mycobacterium phlei	[71]
P. stutzeri	[57]
Pseudomonas ovalis	[9]
Serratia marcescens	[85]
Staphylococcus sp.	[61]
S. aureus	[84]
Staphylococcus albus	[81]
Tetrahymena pyriformis	[98]
Thermus thermophilus	[77]
T. aquaticus	[22], [21]
Vibrio succinogenes	[28]
Yeast	
Candida utilis	[52]
C . gulliermondii	[92]
Pichia polymorpha	[33]
Saccharomyces cerevisiae	[15]
Actinomycetes	
Streptomyces karnatakensis	[65]
S. venezuelae	[65]
S. collinus	[65]
Thermoactinomyces vulgaris	[66]
Fungi	
Aspergillus nidulans	[29]
A . terreus	[4], [40]
Cylidrocapron obtulans	[78]
Mucor sp .	[62]
Algae	
Chlamydomonas sp.	[73]
Plant	
Sphagnum fallax	[41]
Lupin araboreus	[18]
Lupin angustiplius	[17]

B. Submerged Fermentation

Production of L- asparaginase highly influenced by fermentation media composition and culture condition such as pH, temperature, agitation rate inoculums size, incubation time [54]. Submerged fermentation in values the nurturing of microorganism in high oxygen concentrated liquid median viscosity of the nutrient median is the major problem associated with fungal submerged fermentation [14], [55]. The submerged batch fermentation of *Aspergillus terrcuse* for L-asparaginase production was conducted in 250 ml flask with 50 ml of modified czapek-dox medium. L- asparaginase is produced throughout the world wide by submerged fermentation. This technique has many disadvantages such as the low concentration of product, reduction and disposal of large volume of water during downstream processes and consequent handling. L- asparaginase can be produced by *Penicillium* sp. with 3.75 U/ml enzyme activities by submerged fermentation [40]. Extracellular L- asparaginase produced under submerged fermentation from sponge associated *Streptomyces noursei* MTCC 10469. Tryptophan glucose yeast extract was used for the production of L-asparaginase. Submerged fermentation gives reproducible yield of L- asparaginase from *Serratia marcesens* for the production of L- asparaginase. AYE (4%) medium was used for the optimum enzyme production [23].

XII. Purification

The enzyme L-asparaginase from Erwin carotovory was purification by fractionation with ammonium sulphate, sephadex G100, GM cellulose and DEAE sephadex chromatography. Enzyme activity was studied in presence of thiol protecting agent like 2 merceptoethanol dithiothreitol and glutathionl to increase the activity and enzyme was inhibited by the lodoacetamide and p-chloromer curybenzoate [102]. The enzyme was purified from Mycobacterium phlei using ammonium sulfate precipitation absorption of contaminating protein on Ca-P gel and sephadex G-150 and DEAE cellulose chromatography [80]. The measured Km for L- asparaginase was 7mM and the energy of activation was 9800 cal/mol. L-asparaginase was purified and characterized from Candida lilies by acetone and by column A-50, DEAE and sephadex G-200. At optimum pH6 the enzyme was stable for 10 minutes at 5 °C. Chelating agent, metal ions and -SH inhibitor not show any effect on the enzyme [86].

Two form of L- asparaginase I and L- asparaginase II form Sphagnum fallax was purified by anion–exchange chromatography [97]. Triton X 100 $NaClO_4$ and KSCN have been and for the solublization of enzyme purified form T. pyriformis. The molecular weight of the enzyme was 126,000 and optimum pH was 8.2. Extracellular L- asparaginase was isolated from soil *Bacillus* sp which can further be purified by using ammonium sulphate chromatography optimum pH was 7 at 37°C activated by $MgCl_2$ and inhibited by DEAE [42]. Thermos thermopiles derived L-asparaginase has a dual L-asparaginase and kinase activity. It was purified and its observed molecular weight by SDS-PAGE was found to be 33 KDa. Sephadex G-100 gel filtration and SDS-PAGE analysis of the protein was performed for the purification of enzyme from *P. aerugunosa* [3], [77].

XIII. Clinical Application

A. As Antitumor Agent

The enzyme L-asparaginase has been a clinical acceptable antitumor agent for the cure of lymph sarcoma and acute lymphoblastic leukemia (ALL) [100]. L-asparaginase catalyzes the hydrolysis of L- asparaginase into L- aspartic acid and ammonia. L-asparaginase acts as an essential amino acid for the growth of tumor cells. It can be produced within the cell by an enzyme called asparagines synthesizes or can be absorbed from the outside [51]. When L- asparaginase is provided to the tumor cells it cause the deprivation of the cells and the cells cannot survive any more lymphatic tumor cells required huge amount of asparagines to keep up with their malignant growth. Thus the L- asparaginase from the diet as well as what can be mandatory themselves is utilized by them to specify their large asparagines demand [56]. Therefore L-asparaginase is a very essential amino acid for the growth of tumor cells where as the growth of normal cell is not dependent on its requirement. The present of L- asparaginase enzyme derives tumor cells of a significant growth factor and they not succeed to survive. These act as a potent antitumor or antileukemic drug [75].

XIV. Future Aspect

L-asparaginase enzyme has been a major research area for many research word wide Acute lymphoblastic leukemia and lymph sarcoma has been one of the major eminent disease of modern times [53]. L-asparaginase having chemotherapeutic potential for treating ALL. A novel L- asparaginase, GLIAP present in rat brain atrocities and involved in astrological production of the retroactive amino acid [27]. Thus L-asparaginase enzyme and the research being carried out on it may only be the tip of the iceberg. This paper show that L-asparaginase has a great potential application in clinical research and diagnose. It appears that there is still a long way to go in exploring this enzyme.

XV. Conclusion

L- asparaginase is a clinical acceptable antitumor agent for the effective treatment of lymphosarcoma and lymphoblastic leukemia (ALL). L-asparagines (L-asparagine amino hydrolase) catalyses the hydrolysis of L- asparagine into aspartic acid and ammonia. L- asaraginase is isolated from various sources such as bacteria, yeast, fungi and plant cell. L-asparagines produced by different cultivation process namely solid state fermentation and submerged fermentation. Production of L- asparaginase affected by various physical and chemical parameters such as C and N concentration, pH, temperature. Many purification techniques used for the purification of L- asparaginase. Among the number of treatments acute leukemia such as steroids, intensive

combined treatments, radiation therapy, including stem cell transplants or bone marrow chemotherapy is most preferable.

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