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A REVIEW ON L-ASPARAGINASE ENZYME

Praveen Kumar Dasari*, Kumar Raja Jayavarapu, E. Krishna Kumari, D. Prameela Rani, P. Pavan Kumar and P. Haritha

Associate Professor, Mother Teresa Pharmacy College, Sathupally, Khammam, Telangana, India.

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Corresponding Author: Praveen Kumar Dasari

Address: Associate Professor, Mother Teresa Pharmacy College, Sathupally, Khammam, Telangana, India.

ABSTRACT

L-asparaginase is a chemical agent that stimulus mostly the asparagine esterification in L-aspartic acid and ammonium. This enzyme is dispense in different organisms, such as microorganisms, vegetal, and some animals, including definite rodent's serum, but not reveal in humans. It can be used as important anti–metabolite agent for the treatment of a variegation of lymphoproliferative disorders and lymphomas (particularly acute lymphoblastic leukemia and Hodgkin's lymphoma), and has been a pivotal agent in chemotherapy agreement from around 30 years. Also, other important appeal is in food industry, by using the properties of this chemical agent to reduce acrylamide levels in sales fried foods, continue their characteristics (color, flavor, texture, security, etc.) Actually, L-asparaginase catalyzes the decarboxylation of L-asparagine, not allowing the reaction of reducing sugars with this aminoacid for the creation of acrylamide. ptresently, production of L-asparaginase is mainly based in biotechnological manufacture by using some bacteria. yet, industrial production also needs research work aiming to obtain better manufacture yields, as well as novel process by applying varying microorganisms to increase the range of appeal of the produced enzyme. Within this context, this mini-review accord L-asparaginase applications, production by different microorganisms and some limitations, current investigations, as well as some challenges to be achieved for successful industrial manufacture.

KEYWORDS: L –asparaginase, microbial production, industrial production, pharmaceutical applications.

INTRODUCTION

L-asparaginase is an enzyme found in a extensive range of organisms including plants, microbes, animals, and in the serum of definite mouse but not in human beings. L-Asparaginase is an amidohydrolase, that prompt the disintegrate of the amide group on the side chain of asparagine, an amino acid into aspartic acid and ammonia. This enzyme has applicable appeal in the pharmaceutical and food industry. So, L-asparaginase has been reported to have malignancy inhibitory properties and it is mainly used in the therapy of acute lymphoblastic leukemia. Normal cells can arrange L-asparagine with the help of the enzyme asparagine synthetase, whereas certain sensitive tumour cells cannot synthesize it by itself and require an external source of L-asparagine for optimal growth. During the treatment of ALL with L-Asparaginase, all the circulating asparagine in the body of the patient get dessintegrate to aspartic acid and ammonia preventing the absorption of asparagine by malignancy cells and hence depriving the dependent tumor cells of their extracellular source of L-asparagine. L-Asparaginase is commonly used as a mixture chemotherapy drug for the treatment of ALL in adults and children and non-Hodgkin's lymphoma in children. L-Asparaginase therapy may develop side effects such as anaphylaxis, coagulation abnormality, thrombosis, liver disorder, pancreatitis, hyperglycemia, and cerebral dysfunction. These side effects are progress either due to the production of antiasparaginase antibody in the body or due to L-glutaminase activity of L-Asparaginase enzyme used. This enzyme is also used in food constructing to prevent the formation of acrylamide at high temperatures like frying or oven of food items containing starch. Currently, new studies have been carried out aiming to enhance manufacturing process and establish new ways for enzyme synthesis. Thus, some of these regard are discussed, besides some concept concerning L-asparaginase appeal in pharmaceutical and food industries.

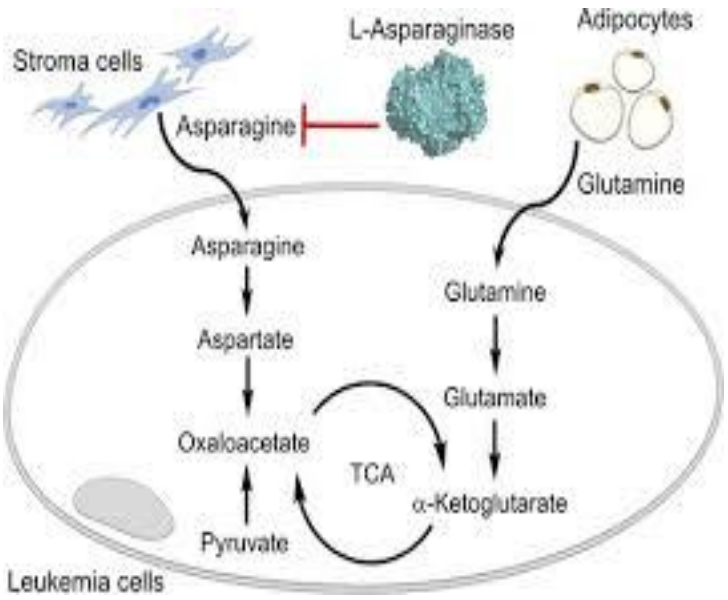


Fig. 01: L Asparaginase.

The enzyme is active in the basic pH range and at temperature 37 °C that makes it extremely valuable in the chemotherapeutic use. It has been proved a potent antineoplastic agent in animals and has given complete suspension in human leukemias. The reason of its favour use is its high biodegradability, non-toxicity and can be manage at the local site quite easily. Other agents are based quite painful when administered to the patient and are found on costly (Prema, Devi, & Alagumanikumaran, Citation. However, there are some repercussion associated with existing therapy like anaphylaxis, coagulation abnormality, thrombosis, liver dysfunction, pancreatitis, high blood sugar, and cerebral disorder. The production of anti-asparaginase antibody or the presence of L-glutaminase and urease activity causes such probhylactic response in the treated patient. The review describes the key issues related to L-asparaginase source, improved production strategy, and its multifarious appeal.

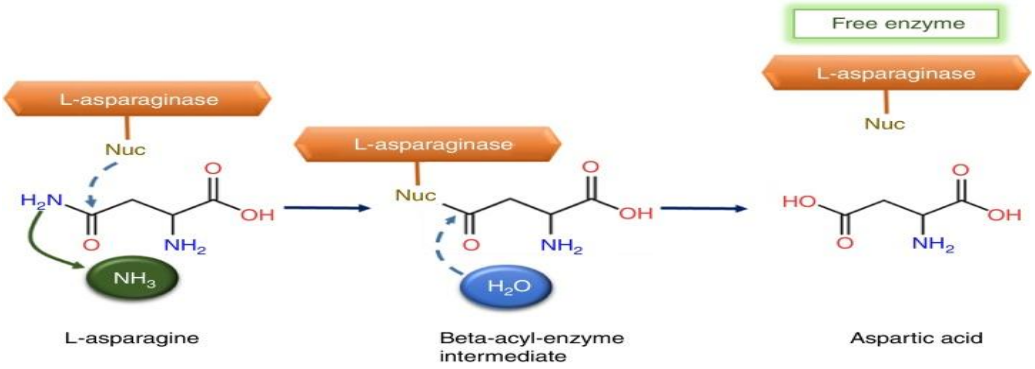


Fig. 02 a: Chemical reaction of asparaginase.

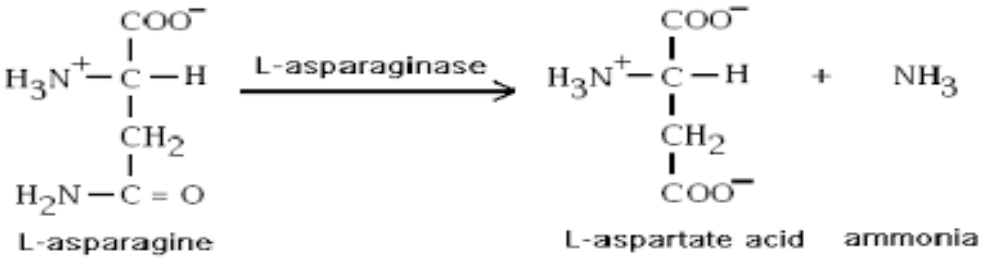


Fig. 02 b: Chemical reaction of asparaginase.

Mechanism of Action

Mechanism of action of Asparginase. Graphic of the mechanism of action of asparaginase as an anti-leukemic factor. The venture of asparaginase prime to the expending of extra-cellular asparagine and/or glutamine and ultimate cell death is counteracted by the intra-cellular manufacture of these amino acids through asparagine synthetase and glutamine synthetase, respectively. To be noted: the relative dissimilar in size between the Asparagine and Glutamine Pathways is required to reflect the magnitude of the outcome of Asparaginase on amino acide draining which is more prominenet in the context of the asparagine compared to glutamine

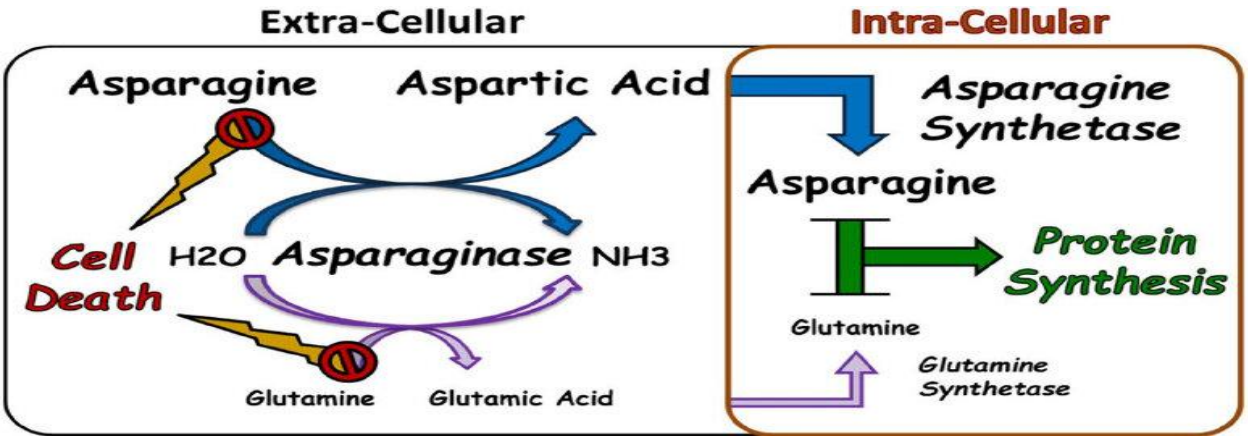


Fig. 03: Mechanism of Action of enzyme.

Production of asparaginase

L-asparaginase is produced from a variegation of microbial origin including fungi, yeast, bacteria and actinomycetes by the manufacture immerse fermentation. Presently, L-asparaginases obtained from two bacterial sources, Erwinia chrysanthemi and E. coli, are in separated use for the therapy.

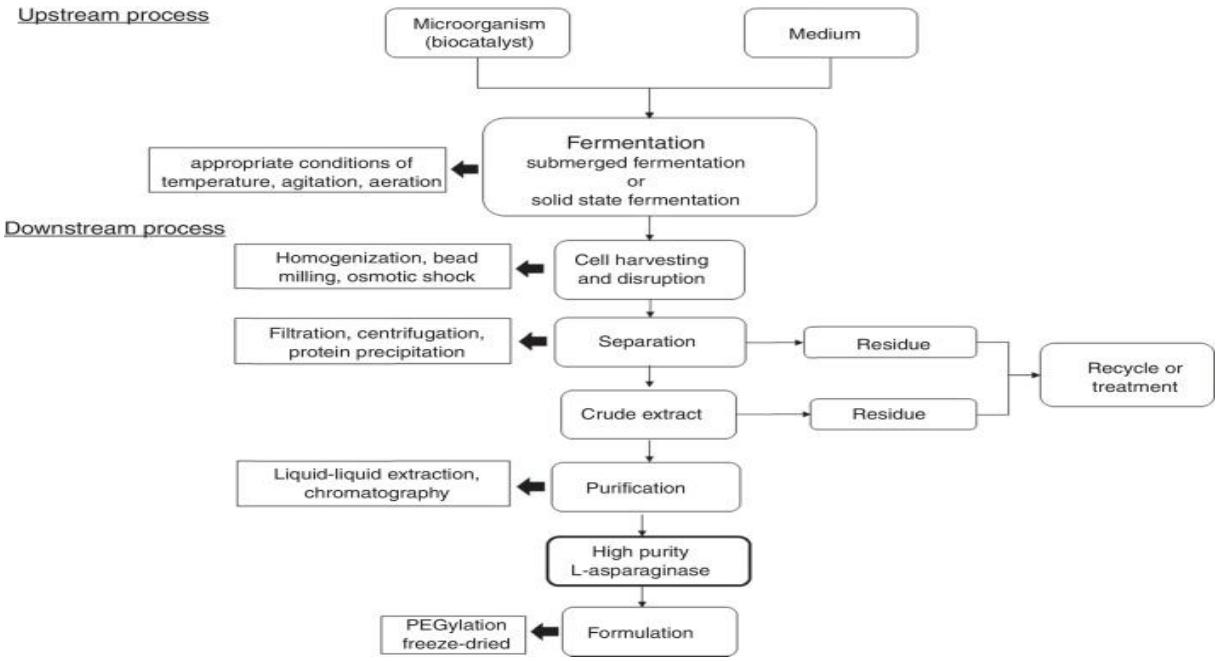


Fig. 04: Production of L-asparaginase from various sources.

Plant and other source

Plant origin are less inspect in terms L-asparaginase manufracture. In plants, asparagine is one of the major nitrous storehouse and carry compound. It collect during physiological processes like seed germination as well as during stress order like drought, mineral deficiencies, and pathogen attack. L-asparaginase releases ammonia from the stored asparagine mandatory for plant growing and development. Literature reports a lot of plant origin like *Tamarindus indica, Capsicum annum*, *Withania somnifera*, *Vicia faba*, *Lupinus angustifolius, Phaseoulus vulgaris* as L-asparaginase producers that are condense. Apart from bacteria, fungi, and plants, the enzyme is also manufacture by yeast, algae, and animals.

Bacterial sources

It is produced by several bacterial variety, but the enzymes (type-II) from *E. coli* and *Erwinia chrysanthemi* are produced on the industrial scale for the clinical approach. The drugs from both sources have similar mechanisms of action and toxicities, however, their pharmacokinetic properties are different, and patients that are sensitive to one source of the enzyme are frequently resistant to the other. Moreover, the enzyme from *E. coli* has a higher affinity (K_m = 18 ± 3 μM) than enzyme from *E. chrysanthemi* (K_m = 33 ± 6 μM) toward the substrate L-asparagine. Most of the disease L-asparaginase are intracellular in mother earth look for a few that are produce outside the cell. From industrial prospects, extracellularly secreted enzyme is more favourable as it could be produced abundantly in culture under

normal condition. Additionally, the purification process will be easier and profitable. Gram-positive bacteria do not have a periplasmic space and therefore they secrete several enzymes into nearby medium (exoenzymes) that normally would be periplasmic in gram-negative bacteria**.** This indirect that the screening of gram positive bacteria would be more advantageous in order to get extracellular enzyme. There are several describe bacterial sources of this enzyme.

Fungal sources

It is discussed earlier that industrial production of this enzyme is primarily done from bacterial origin for the treatment of ALL and Non-Hodgkin Lymphoma (NHL). However, the enzyme produced from the bacterial sources causes some pathological reaction like hypersensitivity, coagulation abnormality, pancreatitis, anaphylaxis, thrombosis, liver dysfunction, allergic reactions, high blood sugar, and cerebral dysfunction. For minimizing such immunological problems, an alternative source of the enzyme is required. The present situation commands a noble source with minimum or no cross-reactivity and that too with higher yield. As fungi are gradually closer to human beings (as compared to bacteria), it is approximate that the enzyme isolated from them will cause less immunogenicity. The various detail cases of L-asparaginase producing fungi.

Production strategies for L-asparaginase

They are two types of strategies

1. Solid state fermentation(SSF)
2. Submerged fermentation(SF)

Solid state fermentation

In past recent years, SSF is motivate for the production of enzymes especially where the crude foam product can be directly used as enzyme source. In most of the studies, it was found that L-asparaginase manufacture is higher in SSF as compared to SmF. The culture condition in SSF mimic natural growth condition of microbes encourage high enzyme growth. The process utilizes inexpensive agriculture waste such as rice bran, wheat bran, sesame oil cake, soya bean meal, corn cob, gram husk, orange peel, coconut oil peanut oil, and tea waste. The use of agricultural wastes makes the process cost effective as well as lesson environmental pollution. In this process, the steam level is low that means the low volume of medium per unit weight of the substrate. Hence, enzyme specific venture is usually very high. Another advantage is that the procedure involves less contaminate effluents and

requires a small quantity of anti-alkaline for product freezing since product application is high. The procedure also offers benefits like ease of downstream processing, low energy requirement (less or no mechanical agitation), and simple media constitution. The numerous cases where L-asparaginase was manufacture using SSF.

Submerged fermentation

This fermentation method is generally working for bacterial enzyme production. The L-asparaginase manufacture is mostly carried out by immerse fermentation throughout the world. The reason is that SmF is well established, manipulation of medium components is comparatively easier that leads to high relent along with quality. The benefit of SmF over SSF are (i) no pre-treatment of base required, (ii) easy control of procedure variable (heat, pH, etc.), (iii) supports the utilization of genetically change organisms to a greater extent than SSF, and (iv) salvation of result is easier. However, there are numerous defect related with SmF that inspire researchers to move toward SSF. The process scarcity are higher energy command(in agitation and aeration), higher risk of contamination, low relent, higher cost of production, and a huge amount of waste generation that is difficult to dispose of. At present, the market command of L-asparaginase is met by SmF using genetically change strains but the cost of production is very high that promotes the requirement for an alternative method like SSF. The fermentation condition for the production of L-asparaginase employing SmF method.

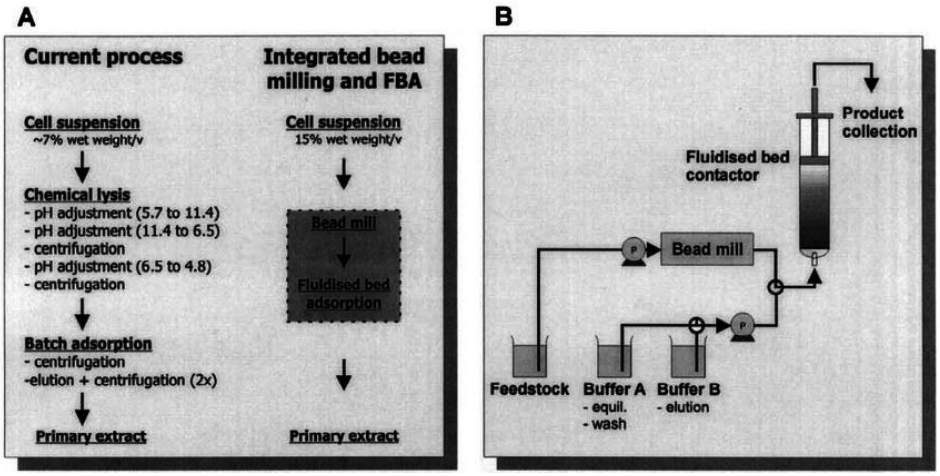


Fig 05: Fermentation Process

Assay of asparaginase

To determine the organic catalyst task, 5ml of the culture puree was withdrawn aseptically from the flasks at an mean time of every 24 h. The broth was filter using Whatman filter

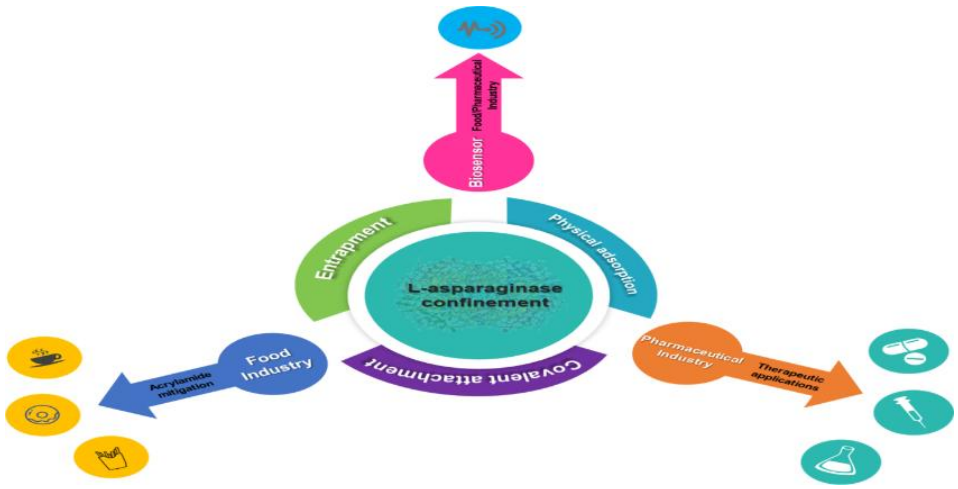
paper No.1 and then diffusive (Sigma 3K30) at 9,000 g for 8 min . The supernatant thus get was used as racy extract for L-asparaginase assay. Assay of organic catalyst was carried out as per Imada *et al*. The organic catalyst activity was expressed in IU. One IU of L-asparaginase is the amount of animate catalyst which discharge 1µmole of ammonia per ml per min (µmole/ml/min).

Purification of asparaginase

The enzyme was filter by the following steps at 0–4°C, unless otherwise introduce. Finely powdered ammonium sulfate was added to the crude fragment. The L-asparaginase intrest was linked with the tiny part bring about at 40–60% soak.

Purification steps

- ✓ Removal of insoluble material
- ✓ Product enrichment and concentration
- ✓ Recovery of biological products
- ✓ High resolution purification
- Ion exchange chromatography
- Gel- filtration chromatography
- Affinity chromatography
- Aqueous two phase miscellar systems



Therapeutic applications of asparaginase

- ❖ Cancer treatment
- ❖ Infectious disease
- ❖ Auto-immune disease

- Food industry
- Novel application
- Drug delivery
- Canine chemotherapy
- Anti-microbial agent

CONCLUSION

Asparaginase is a cornerstone of various agents chemotherapy for patients with all and has been a give factor to the improved affect in patients treated on pediatric-based agreement. Optimizing the treatment intensity and time of asparaginase therapy will potentially result in further improvements in effect. while the asparaginase activity level required for maximal effectiveness is unknown, the literal target and recent consensus recommendations support a manager level of ≥0.1 IU/mL. Future studies will be needed to define the optimal therapeutic asparaginase manager level.systemic monitoring of asparaginase venture levels allows practitioners to detect patients with silent discharge and switch these patients to asparaginase *E. chrysanthemi,* secure the continued depletion of asparagine and allowing these patients to continue benefiting from asparaginase treatment.

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