

Use of Microbial L-Asparaginase in Cancer Treatment: A Detail Review

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Abstract

The exceptional anticancer effects of L-asparaginases, which hydrolyze plasma L-asparagine and L-glutamine, have drew a lot of interest in recent years. This enzyme is effective against acute lymphoblastic leukemia (ALL) and lymphosarcoma, as well as ALL in children, neoplasia, and various other cancer. The L-asparaginase anticancer application technique has achieved amazing advances in modern oncology by depleting plasma L-asparagine to suppress cancer cell development, notably in youngsters. As bacterial alternative enzyme sources for anticancer therapy, high levels of L-asparaginase enzyme production by *Escherichia coli*, *Erwinia species*, *Streptomyces*, and *Bacillus subtilis* species are very desirable. Purification of L-asparaginase by Ammonium Sulphate precipitation and various chromatographic technique methods, and improvement of enzyme purity by dialysis confirmation of molecular weight with SDS page.

Keywords: L-asparaginases - Cancer treatment-Immune suppression - Food industry

1. Introduction

2. Enzymes are the biological substances or biological macro molecules that are produced by a living organisms. These are like the chemical catalysts in chemical reaction which helps to accelerate the biological or biochemical reactions inside as well as outside as well as the cell (Neelam Gaurang, Sumanta Ray *et al.*, 2013). Cancer may defined as unnecessary tissue growth that results from uncontrolled division of cells. The characterization of excessive multiplication of malignant and immature WBC (lymphoblast) is **Acute Lymphoblastic Leukemia (ALL)** also called as cancer of WBC.(Richa Jain *et al.*, 2011). This cells are also divided in to benign and . Leukemia's are tumors of hematopoietic cells that multiply rapidly initially in spleen, bone, lymph nodes and later in other tissue. Different types of leukemia like Acute Myeloid Leukemia (AML), Chronic Lymphoblastic Leukemia (CLL) and also hairy cell leukemia are observed to increase with age, especially in the sixties and seventies (Sanjotha .G ,2016). The first discovery of the tumor- inhibitory properties of L-asparaginase by Kidd who observed that guinea pig (spp of rodent) serum treated lymphoma-bearing mice (Kidd, J. G., 1953). **L-asparaginase** belongs to a group of homologous L-asparagine amino hydrolase (Vidhya Moorthy *et al.*, 2010). It is mostly used as effective **chemotherapeutic agent** also known as **antineoplastic agent** for the treatment of Acute Lymphoblastic Leukemia under the physiological condition. The enzyme converts L-asparagine to L-aspartate and ammonia (Richi. V. Mahajan *et al.*, 2012). The main principle of the use of asparaginase as an antitumor agent is that it takes advantage of the fact that all leukemic cells are unable to synthesize the non-essential amino acid asparaginase at their own, which is very essential for the growth of the tumor

cells. Whereas normal cells can synthesis their own asparagine; thus leukemic cells require high amount of asparagine. These leukemic cells depend on circulating asparagine for their ample nourishment and diet. Asparaginase however, catalyzes the conversion of l-asparagine to aspartic acid and ammonia. This deprives the leukemic cell of circulating asparagine and prevents them from the rapid malignant growth (Verma *et al.*, 2007). Though the enzyme is biodegradable, non toxic and also able to administered at the local site quite east, which make it more preferred for the purpose. Other agents are found quite painful when administered to the patient and also these are quite costly (K. D. Kamble *et al.*, 2012). Asselin *et al.*,(1989) have studied the cytotoxic action of L- Enzymes are the biological substances or biological macromolecules that are produced by a living organisms. These are like the chemical catalyts in chemical reaction which helps to accelerate the biological or biochemical reactions inside as well as outside as well as the cell (Neelam Gaurang, Sumanta Ray *et al.*, 2013). Cancer may defined as unnecessary tissue growth that results from uncontrolled division of cells. The characterization of excessive multiplication of malignant and immature WBC (lymphoblast) is **Acute Lymphoblastic Leukemia (ALL)** also called as cancer of WBC.(Richa Jain *et al.*, 2011). This cells are also divided in to benign and metastatic. Leukemia's are tumors of hematopoietic cells that multiply rapidly initially in spleen, bone, lymph nodes and later in other tissue. Different types of leukemia like Acute Myeloid Leukemia (AML), Chronic Lymphoblastic Leukemia (CLL) and also hairy cell leukemia are observed to increase with age, especially in the sixties and seventies (Sanjotha .G ,2016). The first discovery of the tumor- inhibitory properties of L-asparaginase by Kidd who observed that guinea pig (spp of rodent)serum treated lymphoma-bearing mice (Kidd, J. G., 1953). **L-asparaginase** belongs to a group of homologous L-asparagine amino hydrolase (Vidhya Moorthy *et al.*, 2010). It is mostly used as effective **chemotherapeutic agent** also known as **antineoplastic agent** for the treatment of Acute Lymphoblastic Leukemia under the physiological condition. The enzyme converts L-asparagine to L-aspartate and ammonia (Richi. V. Mahajan *et al.*, 2012). The main principle of the use of asparaginase as an antitumor agent is that it takes advantage of the fact that all leukemic cells are unable to synthesize the non-essential amino acid asparagine at their own, which is very essential for the growth of the tumor cells. Whereas normal cells can synthesis their own asparagine; thus leukemic cells require high amount of asparagine. These leukemic cells depend on circulating asparagine for their ample nourishment and diet. Asparaginase however, catalyzes the conversion of l-asparagine to aspartic acid and ammonia. This deprives the leukemic cell of circulating asparagine and prevents them from the rapid malignant growth (Verma *et al.*, 2007). Though the enzyme is biodegradable, non toxic and also able to administered at the local site quite east, which make it more preferred for the purpose. Other agents are found quite painful when administered to the patient and also these are quite costly (K. D. Kamble *et al.*, 2012). Asselin *et al.*,(1989) have studied the cytotoxic action of L- asparaginase producing micro oraganisms can be used which sensitive and rapid procedure that may directly give potential asparaginase activity. Production of L-asparaginase is accomplished by an increase in pH of the culture filtrates. The plate assay wad devised using this principle by incorporating the pH indicator phenol red in medium containing asparagine as sole source of nitrogen. At acidic pH, phenol red is yellow and at alkaline pH it turns in to pink. So the pink zone is formed around microbial colonies which are capable of prisuving6 l-asparaginase. Because when asparaginase acts upon asparagine, it is converted into aspartic acid and liberates ammonia, which is responsible for increase the pH of the medium (Pallem *et al.*, 2011)

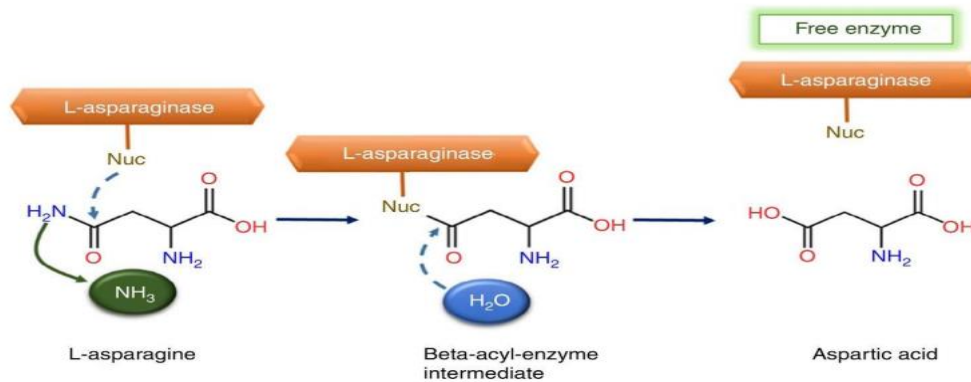


Figure:-1 .Hydrolysis of L-asparagine into aspartic acid and ammonia (El-Bessoumy et al., 2004)

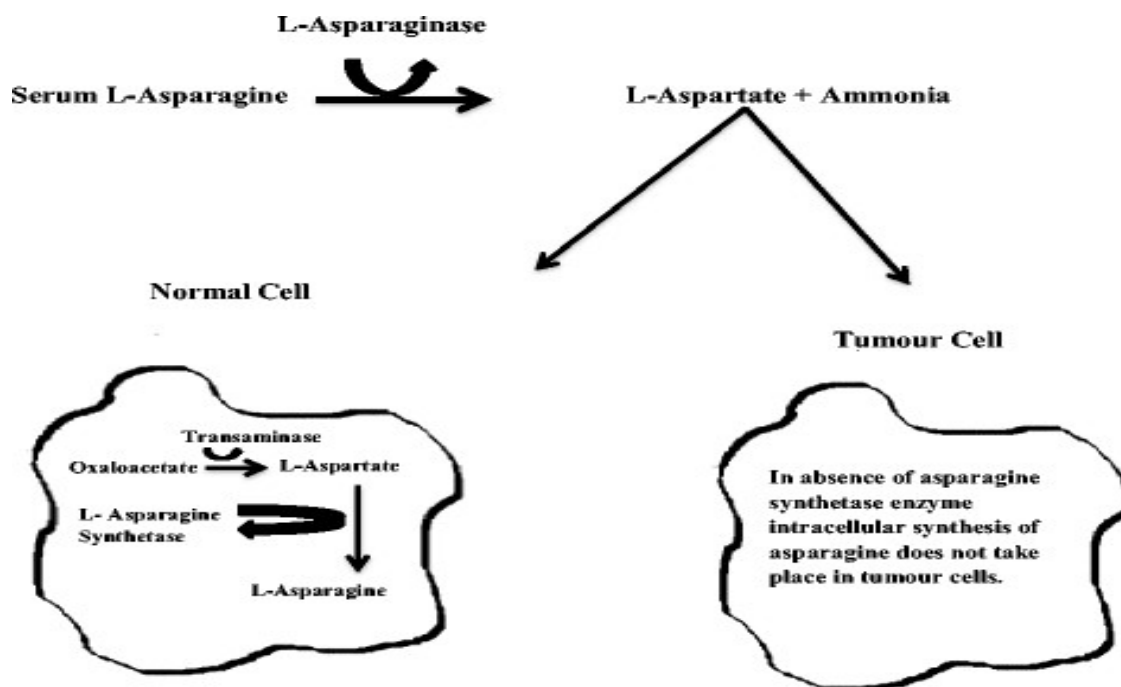


Figure:-2 Mechanism of action of L-asparaginase in normal cell and tumor cell

Biological Role of L-asparaginase in Normal cell and Tumor cell :-

L-asparagine is a very essential amino acid for the growth of tumor cells whereas the growth of normal cell doesn't of it's requirement and it is used by immature lymphocytes for their proliferation (Glibert *et al.*, 1970),(Hanna M Orabi.,2019). Human asparagine synthetase in healthy cell converts **aspartate to asparagine** by using ATP as energy source. It can be produced within the cell by an enzyme called **asparaginase synthetase**. Most of the normal tissue synthesizes l-asparaginase in amounts for their metabolic needs but the cancer or cells (especially Malignant and Carcinosarcoma cell) require external source of L-asparaginase for their growth and multiplication (Tahira Batool *et al.*, 2016). In the presence of L-asparaginase, the tumor cells deprived of an important growth factor and they may failure to survive. Thus, this enzyme can be used as a **chemotherapeutic agent** for the treatment of ALL (mainly in Children) as potent antitumor or anti leukemic drug (James B. Nachman *et al.*, 2021).

Mode of action of L- asparaginase :-

In both normal cells and tumor cells cannot synthesize L-asparaginase and L-glutamine. They need them in large amount for cell growth. Asparagine and glutamine both are non -essential amino acids used by immature lymphocytes for their proliferation and run as substrate for their proliferation and act as substrate for respiration . Nitrogen for the production of hexosamines, proteins , and macromolecules. In a healthy cell, l-asparaginase and glutamine synthetase catalyze the hydrolysis of L-asparagine to L-aspartate and ammonia and to lesser extent the hydrolysis of L-Glutamine to L-Glutamate respectively , using ATP as a energy source. While in cancer cells, they need remarkable high amount for the amino acid asparagine and glutamine otherwise it cannot synthesize. (Soni Yadav *et al.*, 2014).

Types of L-asparaginase :-

Two types of bacterial L-asparaginase have been identified :- In 1967, Ohnuma identified two isozymes of L-asparaginase , namely type I & type II. Both type I and type II asparaginase are characterized by enzymatic activity for both l-asparagine and L-Glutamine(Campbell H. A. *et al.*, 1967).

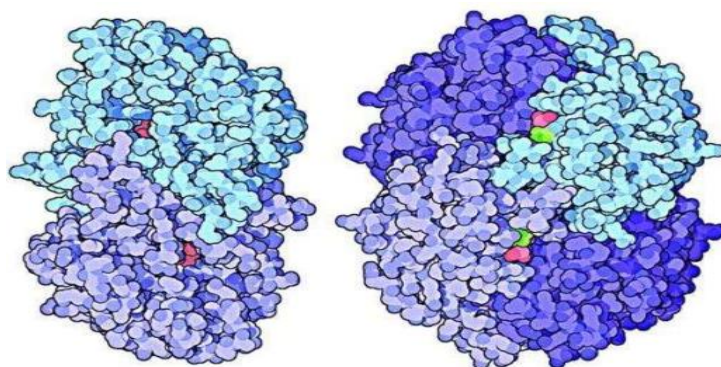
Type I :- Type I L-asparaginase are expressed constitutively in the cytoplasm and catalyze the hydrolysis of both L-Asn & L-Gln.

Type II :- Type II L-asparaginase are expressed under anaerobic condition in the periplasmic space of the bacterial membranes and display higher specificity for L-Asn hydrolysis (Howard Cedar and James H. Schwartz., 1968).

Structure of Asparaginase Synthetase and L-asparaginase :-

Asparagine Synthetase :-

Is a large enzyme composed of 2 identical subunits. The structure shown here has been referred to the enzyme from bacteria. It is responsible for the production of asparagine by combining an ammonia molecule directly to aspartate. In human, ,the enzyme uses glutamine to provide the amine instead of ammonia.



[A] Asparaginases synthetase. [B] L-asparaginase

L-asparaginase :-

Purified from bacterial cells is used for chemotherapy, composed of 4 identical subunits. The active sites grip asparagine (red) & use a well – placed threonine amino acid (green) to perform the cleavage reaction. The enzyme is also active with glutamine, cleaving its group off at a slower rate(Goodsell, D, S.2005). Usually, L-asparaginase remain as a tetramer but when isolated from different sources, hexameric, dimeric

and monomeric forms are also found. Most bacterial L-asparaginase exhibit quaternary and tertiary structure (Tahira et al., 2015), (Ramya L.N., 2011).

Bacterial sources of L-asparaginase :-

L-asparaginase has been reported from both Gram-positive and Gram-negative bacterial species from the terrestrial and marine environment. Gram-negative bacteria have gained more consideration as compared to the Gram-positive (Izadpanah Qeshmi et al., 2014). There are many reports regarding the presence of L-asparaginase in various distinct bacterial sources such as *Pseudomonas* GG13 (Ramadan et al., 1964), *Mycobacterium tuberculosis* (Jayaram et al., 1968), *Pseudomonas stutzeri* (Today T. et al., 1972), *Proteus vulgaris* (Manna, S. et al., 1995), *Enterobacter aerogenosa* (Mukherjee, J., et al., 2000), Marine actinomycetes (Basha, N.S. et al., 2009), *Pseudomonas acidovorans* (Davidson, et al., 1977), *Vibrio succinogenes* (Ammon et al., 1985), *Bacillus circulans* (Hymavathi, M. et al., 2010), *Pectobacterium cartovorum* (Arrivukkarasan, S. et al., 1975), *Citrobacter* sp. (Bascomb, S., et al., 1975), *Helicobacter pylori* (Saquis, M. et al., 2004), *Streptomyces albidoflavus* (El-Naggar N. E. et al., 2015), *Streptomyces griseus* (Dejong, P. J., 1972).

Fungal sources of L-asparaginase :-

Microorganisms like yeast and filamentous fungi are also used for the production of L-asparaginase (R. M. Stark et al., 1997). The fungal L-asparaginase from a strain *Aspergillus terreus*, isolated from decomposing vegetable substrate, has been adverse effect than the bacterial L-asparaginase that cause some allergic reaction, like difficulty in breathing, oblivion, skin rash, sweating or decreased blood pressure. There are some other fungal species which has capability to produce L-asparaginase in enough amount. Such as, *Aspergillus niger* (Abha Mishra., 2006), Endophytic fungi (Mohini P. Patil et al., 2012), *Fusarium equiseti* (Hosamani R. & Kaliwal B.B., 2011), *Bipolaris* sp. (Kodechakonn Lapmark et al., 2009).

Yeast source of L-asparaginase :-

L-asparaginase are currently in use are obtained from various member of yeast such as, *Candida utilis* (Ji Oeun Kil et al., 1995), *Pichia polymorph* (Foda M. S., 1980), *Saccharomyces cerevisiae* (Savitri et al., 2002).

Plant source of L-asparaginase :-

Like microorganisms, plants are also known to contain L-asparaginase. In plants, L-asparaginase is utilized by developing plant tissues for the nitrogen metabolism (Giannino, D., et al., 2008). L-asparaginase as an anti-tumor agent can be isolated from a number of sources. To fulfill the demand of medicine industry search of new and novel sources is needed. Green chillies (*Capsicum annum* L.) and tamarind (*Tamarindus indica*) has been reported to have adequate amount of L-asparaginase (Bano, M. & Sivaramakrishnan V M., 1980). Seeds of *Pisum sativum* was also used as a source of L-asparaginase. It was shown by Lea and Miffin that its activity is dependent on the presence of K⁺ ions. Activity was also affected by Na⁺ and Rb⁺ but it was not in sufficient amount. The concentration of K⁺ at which activity found it was at 20 mili mole. Presence of K⁺ preventes the enzyme from denaturation on heating (Sodek, L., et al., 1980). Other sources of plant for potent production of L-asparaginase such as, *Pinus pinaster*

(Bell, T. L. & Adams, M. A., 2004), *Sphagnum fallax* (Heeschen, V., *et al.*, 1996), *Arabidopsis thaliana* (Bruneau, L., *et al.*, 2006).

Sources of L-asparaginase :-

Organism name	Source	Reference
<i>Erwinia caratovora</i>	Bacterial strain	Deokar <i>et al.</i> (2010)
<i>Bacillus licheniformis</i> , <i>Bacillus circulans</i>	Bacterial strain	Gulati <i>et al.</i> (1997)
<i>Bacillus subtilis</i>	Bacterial strain	Pradhan <i>et al.</i> (2013)
<i>Pseudomonas aeruginosa</i>	Bacterial strain	Kamble <i>et al.</i> (2012)
<i>Aspergillus tamari</i> , <i>Aspergillus terreus</i>	Fungal strain	Sanjotha and Manawadi (2017; Sarquis <i>et al.</i> (2004)
<i>Penicillium notatum</i>	Fungal strain	Eisele <i>et al.</i> (2011)
<i>Aspergillus fumigatus</i>	Fungal strain	Benchamin <i>et al.</i> (2019)
<i>Spirulina maxima</i>	Algae strain	Baky (2016)
<i>Chlamydomonas spp.</i>	Algae strain	Paul (1982)

Screening of L-asparaginase :-

In 1997, R. Gulati and his colleagues reported a pH and dye-based fast procedure for screening L-asparaginase producing for screening of L-asparaginase producing microorganisms (R. Gulati *et al.*, 1997). The procedure is suitable for bacterial and fungal screening. The results are obtained within 24 and 48 hours for bacteria and fungi, respectively. The results correlate with quantitative estimation in culture broths. As reported by Dharmaraj – 2011, L-asparaginase is an anti-neoplastic agent used in chemotherapy of lymphoblastic leukemia. He worked with production of extra-cellular Lasparaginase from Marine

actinomycetes, using submerged fermentation. Marine *actinomycetes*, *Streptomyces* associated with marine sponge *Callyspongia diffusawas* isolated using specific ISP medium. Sponge-associated *Streptomyces* was characterized by conventional method and identified as *Streptomyces noursei*. Production of Lasparaginase by submerged fermentation was carried out using medium Tryptone Glucose Yeast extract (TGY) broth. The study suggest that marine *actinomycetes*, particularly *Streptomyces*, may be used as a potential source of L-asparaginase (Selvakumar Dharamra 2011). Kamble *et al.*, 2012, reported that the habitat chosen for screening the bacterial were farm soil, saline soil, agricultural soil and water. The activity was detected on a medium containing 1% peptone, 0.6% need extract, 0.33% KH₃PO₄, 0.1% L-asparagine and phenol red. L-asparaginase activity was detected on the basis of formation of pink color around the colony. Like wise efficient laspa5 producing bacteria were screened. These were then studied for routine Microbiological and biochemical characterization. The microorganisms characterized belongs to the general *E. coli.*, *Serratia spp.*, *Pseudomonas aeruginosa*, *Bacillus spp.*, *Aeromonas species* and *Proteus spp.* L-asparaginase from halophilic bacteria is expected to be non-allergenic and hence halophilic bacteria from saline soil can contribute to therapeutic value of this enzyme (Kamble *et al.*, 2012). In 1979, Sakumaran and colleagues reported the production of L-asparaginase by two mutants of *Serratia marcescens* grown on 14 different media was studied. The enzyme content increased from trace levels to 2.4 international units per ml when the organisms were grown in glycerol-peptone Yeast extract medium. Glucose was the best source under aerobic condition. The enzyme content increased when L-asparaginase was presenting the growth medium (C. P. Sukurmaran *et al.*, 1979).

Clinical availability of L-asparaginase :-

Current form of L-asparaginase therapy involves injecting the drug preparation either intravenously or intramuscularly. Preparation from *E. coli* and *Erwinia Chrysanthemiasparaginases* are clinically available in the market now along with pegylated form of *E. coli* asparaginase. *E. coli* and *Erwinia* asparaginases have identical mechanisms of action but their kinetics properties are different, and patients sensitive to one drug have often show tolerance to the other. There are several different types of Lasparaginase available commercially. Each derived from a different bacterium. Patients receiving treatment with l-asparaginase derived from *Escherichia coli* (*E. coli*), who develop hypersensitivity to that form of the enzyme, may be able to continue treatment with *Erwinase* as the enzymes are immunologically distinct. Immunologic cross-reaction between antibodies against various formulations of native L-asparaginase from *E. coli* and PEG L-asparaginase has been reported, but no such reaction has been found against *Erwinia* L-asparaginase (Avramis, V. I., & Panosyan ,E. H., 2005). Antibodies targeting *E. coli* derived Lasparaginase have been shown not to cross-react with *Erwinase*. *Erwinia* asparaginase is recommended to those patients who have high allergic reactions. The protein was conjugated with PEG group (PEG Asparaginases) ,to eliminate the high immunogenic reactions of *E. coli* Asparaginases , which reduce the side effects to a greater extend.(Muller, H. J., & Boos, J., 1998).

Applications of L-asparaginase :-

1. :- Anticancer Drug :-

L-asparaginase is used to treat ALL in combination with vincristine and a glucocorticoid (e.g., dexamethasone) (Szymanska, B., *et al.*, 2012). Due to its antileukemic properties, L-asparaginase has been considered as a therapeutically important antitumor drug. L-asparaginase is well-known chemotherapeutic agent which in combination with other drugs is used in the treatment of certain malignancies such as ALL

(mainly in Children), Hodgkin's disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma, reticulosarcoma, and (Kidd, J. G., 1953 & Broome, J. D., 1961). L-asparaginase is an essential amino acid for many tumor cells for protein synthesis and cell growth, while Lasparaginase possesses the ability to convert L-asparaginase to aspartate; so, in the presence of L-asparaginase, malignant cells are deprived of important growth factor that results in depletion of asparagine and ultimately tumor cells die off (Salzer, *et al.*, 2014). L-asparaginase is widely reported in animals, plants and microorganisms, but only the asparaginases from *E. coli* and *E. Chrysanthemi* was approved to be used as part of a multiagent chemotherapy to treat ALL (Verma, N., *et al.*, 2012). The significant disclosure by Kidd in 1953 that guinea pig serum could stifle the development of Gardner 6C3HED lymphosarcoma cells established subcutaneously in mice opened new doors for the advancement of L-asparaginase as a treatment of ALL.

2. :- Role of L-asparaginase in Biosensor :-

L-asparaginase is also used for the development of a Biosensor to analyze asparaginases levels either in leukemia or the food industry (Verma, N., *et al.*, 2012). Several spectroscopy techniques such as XRD, XPS, SEM and TEM are currently used for L-asparaginase analysis, but high cost and tedious procedures make them less favorable (Zubavichus, Y., *et al.*, 2004). Over such a situation, biosensor technology can be a reliable, cheap and user-friendly approach. The mechanism of action of the Biosensor is based on asparaginases activity, ammonium ions produced from the hydrolysis of asparagine cause a change in pH resulting in the change of color and absorption (Kumar, K., *et al.*, 2013).

3. Role of L-asparaginase in amino acid metabolism :-

L-asparaginase plays a vital role in the biosynthesis of an aspartic family of amino acids, namely lysine, threonine, and methionine. Besides Krebs's cycle, aspartic acid that is a direct precursor of lysine and threonine is also formed by action of Lasparaginase enzyme (Sinha, R., *et al.*, 2013).

4. Role of L-asparaginase in Food industry :

L-asparaginase is also used widely as a good processing aid. Recent developments in food technology exhibited that a colorless and odorless crystalline solid, acrylamide (C₃H₅NO, MW 71.08 g/mol), which also known as ethylene carboxamide, 2propenamide, propenamide, propanoic acid amide, or acrylic acid amide is produced as a result of Millard reaction, when starchy foods are fried or baked at 120° C (Lingnert, H., *et al.*, 2002 & Mottaram, D. S., *et al.*, 2002). Acrylamide is a neurotoxin and has been categorized as a carcinogenic to human. In the food industry, acrylamide is largely derived from heat-induced reactions (Noura ElAhmady *et al.*, 2014) between the α-amino group of the free amino acid asparagine and carbonyl groups of reducing sugars such as glucose during baking and frying (Friedman, M., 2003). Due to its ability to convert L-asparaginase to L-aspartate, Lasparaginase promises to be a possible way to reduce the number of precursor for Millard reaction by pre-treating the starchy foods (potato and bread dough) with L-asparaginase, hence reducing the risk of acrylamide formation (Pedreschi, F., *et al.*, 2008). However, complete removal of acrylamide is not possible due to other asparagine independent formation (Dhanam, J. G. & Kannan. S., 2013). Certain fungal asparaginases used in food industries nowadays includes L-asparaginase from *Aspergillus oryzae* and *Aspergillus niger* (Morales, F., *et Al.*, 2008).

FUTURE ASPECT

Purification of L-asparagine by Ammonium Sulphate precipitation and different chromatographic technique method and Improvement of purity of Enzyme by performing dialysis confirmation of molecular

weight with SDS page.

Potentially assess of *Bacillus spp.* for its clinical use after strain improvement.

Examination of the effect of enzyme L-asparaginase on cancer cell line.

Check the effect of activators and inhibitors on L-asparaginase activity.

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