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Microbial Extracellular L-asparaginase: An Enzyme of Therapeutic Interest

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Abstract: This review article encompases detailed information pertaining to microbial extracellular L-asparaginase, the introductory remarks on current status and future perspective of enzymes in general and overview of L-asparaginage in specific, underlying mechanism of action, therapeutic applications, microbial sources, effect of fermentation process parameters on its production, methods for purification, molecular weight and properties, biochemical aspects, assay methods side effects, therapeutic importance and scope of research. Effort has been made for inclusion of updated information and impact of newer parameters including fermentation processes, different methods for purification, properties and biochemical aspects of microbial extracellular L-asparaginase. L-asparaginase is still one of the most widely studied therapeutic enzymes by researchers and scientists worldwide.

Key Words: L-asparaginase, Acute Lymphoblastic Leukemia (ALL), Enzymatic Assay, Therapeutic Application.

Introduction

Biotechnology is gaining rapid importance as it offers several applications and advantages over conventional technologies. Enzymes are considered as the 'third wave' of biotechnology following the pharmaceutical and agricultural waves [56].

The global market for industrial enzymes is estimated at $3.3 billion in 2010 and expected to reach $4.4 billion by 2015. Technical enzymes are valued at just over $1 billion in 2010, will increase at a 6.6 % compound annual growth rate to reach $1.5 billion in 2015. The highest sales of technical enzymes occurred in the leather industries followed by the bioethanol, food and beverage industries [66].

The biotech industry in India registered only 2 % of global biotech markets. But due to newer applications in food and feed, detergent and textile processing, agriculture, dairy, leather, paper, healthcare, diagnostics and environment, utilization of renewable resources for synregising the efficacy and efficiency of industrial processes, investment oppurtunities and monitoring of

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environmental degradation delivers a more bio-friendly cum bio-based economy [55], the global viability, scope and existance is tremendously improved.

During the fiscal year 2010-11 the Indian biotech sector grew at 21.5 % to reach Rs 17,400 crores in revenues. The biopharma segment continues to maintain dominance followed by the bioindustrial, bioagricultural and bioinformatics [66].

Current status of L-asparaginase

L-asparaginase (L-asparagine aminohydrolase, E.C.3.5.1.1) exclusively present in guinea pig serum was proposed to be responsible for tumouricidal effects [3]. The enzyme L-asparaginase is enlisted under the World Health Organization's List of Essential Medicines, registered immense therapeutic importance as an anti-neoplastic drug used in acute lymphoblastic leukemia and lymphosarcoma chemotherapy [38]. The growth of a cell line derived from Walker carcinosarcoma was shown to be dependent on L-asparagine [65]. L-aspara-ginase purified from guinea pig serum was a tumouricidal agent [11] against implanted 6C3HED cells (serum from newborn guinea pigs lacking L-asparaginase was devoid of antitumour activity). It turned out that L-asparaginase is not an anticancer drug in general but is effective against certain types of tumours and it has become an essential component of chemotherapy strategies. Using salt precipitation, starch block electro-phoresis and DEAE-cellulose column chromato-graphy two isoforms of L-asparaginase were partially purified [105] and only one isoform had antilymphoma activity *in vivo*.

L-asparaginase is the first identified enzyme with antitumour activity in human beings. L-asparaginases are enlisted under cytotoxic and adjuvant medicines on WHO model list of essential medicine. It is an enzyme and drug of choice for acute lymphoblastic leukemia in children used in combination therapy [85]. L-asparaginase treatment for acute lymphoblastic leukemia is a major breakthrough in modern oncology as it induces complete remissions in over 90 % children within four weeks [28]. Since 1922, L-asparaginase has been considered as a therapeutic agent against malignant tumours [17]. The presence of L-asparaginase deprives tumor cells of an important growth factor and they fail to survive [43]. This fact suggested the development of this enzyme as a potent antitumour or antileukemic drug [80]. Elspar, Oncaspar, Erwinase Kidrolase, Colaspase and Crasnitin [24] are the brand names of L-asparaginase.

Mechanism of action and therapeutic applications of L-asparaginase

The FDA has approved L-asparaginase for the effective treatment of acute lymphoblastic leukemia and lymphosarcoma. L-asparaginase catalyzes the hydrolysis of L-asparagine into L-aspartic acid and ammonia. L-asparagine is a major requirement by the cells for the production of protein. It can be produced within the cell by an enzyme called asparagine synthetase or can be absorbed from the outside (consumed in the diet, absorbed into the body and made available to the body's cells). Tumor cells, more specifically lymphatic tumor cells, require huge amounts of asparagine to keep up with their rapid malignant growth [100]. Thus, the asparagine from the diet as well as what can be made by themselves (which is limited) is utilized by them to satisfy their large asparagine demand. Therefore L-asparagine is an essential amino acid for the growth of tumor cells, whereas the growth of normal cells is not dependent on its requirement as it can be synthesized in amounts sufficient for their metabolic needs with their own enzyme L-asparagine synthetase.

L-asparaginase also plays a very critical role in the biosynthesis of the aspartic family of amino acids. *Corynebacteria* producing amino acids are of great industrial interest as they excrete large amounts of various amino acids [57]. Lysine, threonine and methionine are commercially important amino acids produced by *C. glutamicum*, are derived from aspartic acid, which, under normal physiological conditions, might be limiting for lysine and/or threonine biosynthesis. Apart from Kreb's cycle (using glutamic acid as amino acid donor), aspartic acid is formed from asparagine by the action of asparaginase. L-asparagine was produced constitutively and its role may be that of an overflow enzyme, converting excess asparagine into aspartic acid, the direct precursor of lysine and threonine. A very active L-asparaginase was found in *C. glutamicum*

under lysine producing fermentation conditions [58]. L-asparaginase enzyme is usefull against Acute Lymphoblastic Leukemia (mainly in children), Reticlesarcoma, Hodgkin disease, Acute Myelocytic Leukemia, Acute myelomonocytic Leukemia, Chronic Lymphocytic Leukemia, Lymphosarcoma and Melanosarcoma [14,32]. The enzyme inhibited the growth of the two human cell lines including hepatocellular carcinoma (Hep-G2) and colon carcinoma (Hct-116) with IC50 value of 8.38µg/ml and 4.67µg/ml, respectively [23].

It is also used for the treatment of pancreatic carcinoma [107]. Treatment with L-asparaginase, polyphenoloxidase and low frequency laser decreased adhesion of uro-pathogenic Escherichia coli to human erythrocytes [49]. L-asparaginase like glutaminase and urease also plays an important role in the biogeocycling of carbon and nitrogen in natural waters and sediments [76]. L-asparaginase is a model enzyme for the development of new drug delivery system [93], L-asparagine biosensor for leukemia [100]. L-asparaginase production by using microbial systems has attracted considerable attention, owing to the cost-effective and eco-friendly nature [90]. For all clinical and non clinical uses, L-asparaginase has to be produced in larger quantities.

It is also used in food industry for the production of acrylamide (a potent carcinogen and a neurotoxic compound) free starchy fry food [69]. Preventase (DSM) and Acrylaway (Novozyme) produced from *Aspergillus niger* and *A. oryzae* respectively are commercially available L-asparaginase currently used in food industry [48].

L-asparaginase produced extracellularly is advantageous and preferred over intracellular type because of higher accumulation of protein, easy extraction and downstream processing [2,52]. The extracellular compartment in bacteria is protease deficient and the liberated protein exported to the medium is mostly soluble, biologically active and has an authentic N-terminus, relatively free from endotoxins that results in minimization of adverse effects [2]. Secretion also facilitates proper folding of proteins specially those requiring disulfide bridge formation, as it passes through a more favorable redox potential in the periplasmic space [52].

Micobial sources of L-asparaginase

L-asparaginase is broadly distributed among the plants, animals and microorganisms. The most economical and the most commonly used microorganisms to produce L-asparaginase are *Aerobacter*, *Pseudomonas*, *Xanthomonas*, *Photobacterium* [70], *Proteus* [95], *Serratia* [9], *Serratia marcescens* [19], *Vibrio* [42], *Aspergillus* [83], *Staphylococci*, *Aspergillus*, S*accharomyces* [100], *Erwinia* [50], *Bacillus cereus* MNTG-7 [91], P*ectobacterium carotovorum* MTCC-1428 [51], *Bacillus* [62] and *Bacillus subtilis strain hswx88* [71]. However the purified enzyme from *E. coli*, *Erwinia* sp. and *Serratia marcescens* has been used as anti-tumor and anti-leukemia agent.

Medium composition effecting L-asparaginase production

L-asparaginase producing microorganisms either produce this enzyme constitutively or after induction. The physicochemical conditions for L-asparaginase production vary with the microorganism.

Effect of Carbon source on L-asparaginase production

The synthesis of L-asparaginase in *E. coil* -W and *E. coli* K-12 was almost completely suppressed if glucose was added at a concentration of 0.5 % to the growth medium. This was because glucose caused catabolite repression and catabolite inhibition of the components involved in lactate transport [29] and lactate stimulated L-asparaginase synthesis.

Regulation mechanism of production of Staphylococcal L-asparaginase showed that carbon sources such as sucrose, maltose, galactose, lactose, mannitol and mannose inhibited L-asparaginase enzyme production [78].

Nocardia asterodies, an aerobic actinomycete, was grown in three different media, namely sabourand dextrose broth (SD), tryptic soy broth and synthetic medium, as a shake culture at 37°C for six days. The SD broth yielded maximum growth and showed maximum L-asparaginase production [33]. L-asparaginase is an inducible enzyme and is generally induced in the presence of glucose. 0.2 % glucose is used as the carbon source [32] which is proved to be a better isolation

method for the microbal cultures. The effect of glucose on optimum enzyme production has been reported by many workers [29,51]. Glucose was also regarded as a repressor for L-asparaginase production in bacteria [7,61].

According to literature maximum L-asparaginase production was observed with maltose (0.5 %), glucose (0.2 %), glucose (0.38 %) in *Streptomyces gulbargensis*, *Pectobacterium carotovorum* MTCC 1428 [51], *Serratia marcescens* SK-07 respectively [5].

Tapioca starch was the best carbon source with positive coefficient (6.390), where as fructose, mannitol, maltose and sucrose showed larger positive effects on the yields and the other carbon sources (Dextrose, Lactose, Dextrin white, Potato Starch, Starch soluble, Starch maize, Pharma starch, Xylose, Galactose, & Cellulose) were insignificant for L-asparaginase production in *Bacillus cereus* MNTG-7 [91].

Effect of Nitrogen source on L-asparaginase production

Organic acids and amino acids such as L-leucine and L-methionine were found to enhance production of L-asparaginase in *E. coli* [64]. High L-asparaginase activity has been observed in bacterial cultures growing in ample nitrogen [68]. In *Lupin arboreus*, plant parts such as leaves, root-tips, flower buds and developing seeds have been found to be sources of L-asparaginase [15]. L-asparaginase-I is constitutive and L-asparaginase-II is secreted in response to nitrogen starvation [21]. High L-asparaginase activity has been observed in bacterial cultures growing in ample nitrogen [67].

L-proline, Urea & L-asparagine [7], were found as significant and best nitrogen sources for the production of L-asparaginase by *Aspergillus terreus* MTCC 1782. Gelatin is the best nitrogen source having positive coefficient of 3.50 in comparison with Soyabean meal, Tryptone, gelatin, Yeast extract, Soya peptone, Yeast nitrogen base, Casein, Meat extract, Peptone, Casaminoacids, Malt extract, Beef extract, Urea & Albumin for L-asparaginase production in *Bacillus cereus* MNTG-7 [90]. Among the amino acids (D-Alanine, L-Cystine, L-Alanine, L-Lysine, D-Arginine, L-Asparagine, L-arginine, L-Histidine, L-Glutamic acid & L-Tryptophan), L-asparagine has the highest contribution (26.2 %) with positive coefficient of 4.75 for production of L-asparaginase in *Bacillus cereus* MNTG-7 [91].

C. glutamicum, produced L-asparaginase aerobically (pH 7.3) using casein and soya peptones as main nutrients [58]. The *Bacillus* sp, isolated from an intertidal marine alga, *Sargassum* sp, produces constitutive L-asparaginase in the cultivation media (pH 8.0) at 28°C using peptone as the main nutrient [59].

The maximum amount of L-asparaginase produced from the optimized carbon and nitrogen medium containing Asparagine (5.5g/l) has been reported [94]. The enzyme yielding capacity of *P. carotovorum* MTCC 1428 & *Bacillus sp.* BCCS 034 were found to be 14.2 IU/ml and 1.64 IU/ml in the supernatant [22,51].

In comparison to control with no carbon source at 37°C, lactose showed the maximum activity (16-fold) followed by monosodium glutamate (12-fold) by employing *E. carotovora* [53]. Out of different nitrogenous substances added to the fermentation medium, cornsteep liquor showed 14-fold higher activity followed by tryptone (13-fold) and yeast extract (11-fold).

Intracellularly expressed L-asparaginase was also detected from *Enterobacter cloacae* [62]. Gram negative bacteria like *E. coli* [102] and *E. cloacae* [63] utilized L-asparagine as the sole source of carbon and nitrogen. 0.1 % Maltose, Saccharose, Fructose and Galactose showed better enzyme production [63].

For the production of L-asparaginase by *Enterobacter aerogenes,* the most suitable C and N sources were sodium citrate and di-ammonium hydrogen phosphate, respectively [61]. Nitrogen catabolite repression on enzyme formation was absent in this bacteria but glucose was a repressor of this biosynthesis.

A pH of 7.9, casein hydrolysate (3.11 %) and corn-steep liquor (3.68 %) were the most significant factors for improving the enzyme production process by *Pseudomonas* [89].

Effect of fermentation process parameters

For Staphylococcus sp.-6A, incubation temperature, inoculum level and medium pH, among all fermentation factors, were the major

influential parameters at the individual level, and contributed to more than 60 % of total L-asparaginase production [72].

Optimization of temperature

The optimum temperatures for the production of enzyme by different Bacterial species have been reported as, 37°C for *Pseudomonas aeruginosa* 50071 6 and *Staphylococcus* sp.- 6A [71], 40°C for *Streptomyces Gulbargensis* [3] & 30°C for *Bacillus brevis* [61]. An organism identified as *Enterobacter cloacae* produced L-asparaginase (intracellularly which was resistant to a temperature range of 39-42°C and a good yield was obtained utilizing L-Fructose, D-Galactose, Saccharose or Maltose [63].

Optimization of pH

The initial pH of the medium plays a vital role in the production of L-asparaginase. The best enzyme production in so far reported strains was at neutral pH. While media with slightly acidic pH have been reported for L-asparaginase production by bacteria such as 7.5 for *Staphylococcus* sp.- 6A 71, pH 6.5 for *Pectobacterium cartovorum* 1428 [51] and *Serratia marcescens* [1]. Alkaline media have been reported for the production of L-asparaginase such as pH 8.5 for *Streptomyces gulbargensis* [3]. Production of L-asparaginase from a new *Erzoinia* sp. has been reported at optimum pH of 9.2 and the Km for L-asparagine was 2.8 mM [8]. This *Erzoinia* sp. L-asparaginase is thermostable and followed linear kinetics, even at 77°C.

Role of aeration, agitation and dissolve oxygen level

Aeration of the fermentation medium is necessary for the growth of *Serratia marcescens*, but not for the production of asparaginase [36]. Enhanced biosynthesis of asparaginase by *E. coli* K-12 with anaerobic growth conditions has been reported [13]. *Vitreoscilla* hemoglobin in *Erwinia aerogenes* and *Pseudomonas aeruginosa* respond differently to catabolic and oxygen repression for L-asparaginase production [30].

During the cultivation in a fermenter the dissolved oxygen level was the limiting factor for L-asparaginase production [61].

Maximum L-asparaginase production of 29.89 U/mg was achieved in a batch bioreactor with 40 % Dissolve Oxygen level by employing *Serratia marcescens* SK-07 [1].

Optimization of size of inoculum

The size of inoculum is also reported as one of the influencing factor for L-asparaginase production. 2 ml of inoculum having 0.8 absorbance of culture at 480 nm is the optimized inoculum for L-asparaginase production by *Staphylococcus* sp. - 6A [72].

Optimization of incubation period

The incubation period required for maximum production of the enzyme depends not only on the bacterium but also on the cultural conditions. The peak cell population and maximum L-asparaginase production by *Serratia marcescens* occurred simultaneously at 39 hr [36]. Maximum intracellular L-asparaginase production has been reported by *Pectobacterium carotovorum* [5] and *Bacillus brevis* [61] after 36 & 24 hours of incubation respectively.

Methods for purification of L-asparaginase

The major proposed application of L-asparaginase is as an injectible drug for the treatment of tumour or lymphoblastic leukemia in human beings. The sensitivity of application demands high degree of purity of this enzyme. L-asparaginase from *Mycobacterium phlei* was puriifed by fractionation with ammonium sulphate, absorption of contaminating proteins on calcium phosphate gel and chromatography on Sephadex G-150 and DEAE cellulose. The apparent Km for L-asparagine was 0.7 mM and the energy of activation was 9800 cal/mol [67]. Keiselguhr composite and CM sepharose has been used for large-scale production of asparaginase from *Erwinia chlysanthenzi* [31].

Extracellular L-asparaginase from *Candida utilis* was partially purified by acetone and by column chromatography on DEAE, Sephadex A-50 and Sephadex G-200. Optimum pH was 6 and the enzyme was stable for 10 minute at 50°C. Metal ions, SH inhibitor and chelating agents did not show any inhibition or activation of the enzyme [82].

Crude extracts of *Thermoactinomyces vulgaris* 13MES [60] were prepared after filtration of cultures and grinding the cells with sand, alumina or glass beads, by rapid freezing and thawing, by rapid mixing and also by exposure to ultrasonic waves. KSCN, NaC104 and Triton X-100 have been used for the solubilization of enzyme purified from *T. pyrilannis* [96].

Most of the microbial L-asparaginase is intracellular in nature except few, which are secreted outside the cells [46,59]. In case of extracellular L-asparaginase from *C. utilis*, the enzyme was extracted from the cell precipitate or cell culture broth by the treatment of 2-mercaptoethanol, dithiothreitol or cysteine as a reducing agent [46]. L-asparaginase from *Pseudomonas stutzeri*, after initial ammonium sulphate fractionation, was purified by consecutive column chromatography on sephadex G-200, calcium hydroxyapatite and DEAE sephadex A-50 [54].

Asparagine catabolism in Bryophytes has been studied and purification and characterization of two forms of L-asparaginase, L-asparaginase-I and L-asparaginase-II, obtained from *Sphagnum fallax* was carried out by anion-exchange chromatography [35]. They observed that the pH optimum of the enzyme was 8.2 and its molecular weight was 126,000. L-asparaginase from *Thermos thermophilus* has a dual L-asparaginase/ kinase activity. It was purified and its apparent molecular mass by SDS-PAGE was found to be 33 kDa [73].

L-asparaginase was typically extracted by incubating the cells at 50°C for 4 hrs in extraction solution containing 2-mercaptoehanol in potassium phosphate buffer. *E. coli* cell permeabilization was also carried out with the help of K_2HPO_4 and triton X-100 and the release of L-asparaginase was over 70 % [108].

Molecular weight and properties of microbial L-asparaginase

L-asparaginase produced by *Streptomyces Gulbargensis* [3], *Pseudomonas stutzeri* MB-405 [54], *Sphagnum fallax* [35], *Thermos thermophilus* [72] and *Erwinia carotovora* [103] have molecular weight of 82.12, 34, 126, 33 and 34.5 kDa respectively. SDS-PAGE analysis of purified recombinant L-asparaginase from *Escherichia coli* [44] and *Erwinia Chrysanthemi* 3937 [50] revealed single protein band migrating at 37 and 37.2 kDa protein respectively. This variation of molecular weight of L-asparaginase from different sources suggested that the molecular weight of the L-asparaginase is organism specific. The mol wt of *Corynebacterium glutamicurn* L-asparaginase determined by gel filtration was 81,000 Da [57] and the apparent Km of L-asparaginase for L-asparagine was 2.5 mM.

Comparative experimental evaluation of immuno-depressive and toxic effects of L-asparaginase from *E. coli* and from *Erwrinia carotovora* showed that L-asparaginase from *E. coli* is more immuno-depressive and immuno toxic than that from *E. carotovora* [12]. Sulphydryl groups (-SH gps) of L-asparaginase from *P flourescens* were reported to be essential for enzyme activity [88]. *E. coli* L-asparaginase has been shown to inhibit the growth of cultured pancreatic cells 103. Optimum pH of the *Rhodotorula rubra,* L-asparaginase enzyme was 7 and it was activated by Mg^{2+} and inhibited by Fe^{2+} and Pb^{2+} [25]. L-asparaginase of *Aspergillus nidulans* showed the clearest evidence of O_2 repression under N_2 metabolite derepressed conditions [85]. Four forms of L-asparaginase were isolated from *Tetrahymena pyriformis* [99].

Mostly L-asparaginases are intracellular and the pH and temperature optima for L-asparaginase production are the same as that for the growth of the enzyme-producing organism [58]. Presence of metal ions does not affect L-asparaginase production indicating that it is not a metalloprotein or does not require cofactors. Presence of chelating agents such as EDTA and compounds having thiol protecting groups such as glutathione, dithiothreitol, 2-mercaptoethanol etc. markedly enhance the enzyme activity [75].

L-asparaginase from *Tetrahymena pyriformis* was found in microsomal membranes. The enzyme exhibited an intrinsic phosphorylation activity with a Km value of 0.5 mM [99]. The pH optima of purified *Staphylococcal* L-asparaginase were found to be between 8.6 and 8.8 while the temperature optima were 30-32°C. [87].

L-asparaginase from *Erwinia carotovora* showed maximum activity at pH 8.0 and at 50°C temperature. The Km value of purified enzyme

was 1.8x10-5M. Purified enzyme showed significant antitumour activity on experimental animal models [53].

Two forms of L-asparaginase, L-asparaginase-I and L-asparaginase-II, were extracted and purified from *Thermos thermophilus* [98]. The two forms acted optimally at pH 8.6. The L-asparaginase activity from *Bacillus* sp. was found to be optimum at pH 8.0 [59] and temperature at 37°C. The enzyme activity decreased sharply above 40°C and the enzyme was inactivated at 50°C and exhibited a half-life period of about 1 hr. Km value of L-asparaginase from *Bacillus* sp was found to be $2.4x10^{-4}$ M. An extracellular asparaginase from *Rhodosporidium toruloides* has been reported to be a homodimer having pH optima of 6.35 and temperature optima of 37°C [76].

Biochemical aspects of L-asparaginase

The medical utilization of L-asparaginase from the reported sources suffer the limitations of eliciting immunological responses leading to hypersensitivity in the long-term usage [100], allergic reactions [77], anaphylaxis and instance of spontaneous resistance of the tumor cells [20,84]. Biochemical aspects play a vital role in enzyme production studies. Erwinia asparaginase is considered to be less toxic compared to *E. coli* asparaginase and hence is employed in the events of allergic reactions inspite of having a shorter half life than *E. coli* asparaginase. This enzyme was characterized by X-ray scattering (saxs) pattern of homo tetrameric asparaginase-II from *E.coli* was measured in solution in conditions resembling those in which its crystal form was obtained and compared.

L-asparaginase assay method

Many attempts have been made by researchers for the assay of L-asparaginase and for monitoring its activity. L-asparaginase was assayed based on the production of ammonia during hydrolysis of L-asparagine degraded by glutamate dehydroginase consequently with the oxidation of β-NADH [32]. Enzymatic assay of L-asparaginase is also done by Nesslerization method. The reaction is monitored by measuring the amount of ammonia released during reaction. The ammonia released is complexed with Nessler's reagent and the resultant reddish brown solution's optical absorbance is measured at 436 nm using UV-Visible spectrophotometer. Using ammonium standard curve, the enzyme activity will be calculated.

Automated enzymatic analysis with potentiometer ammonia detection by on-line gas analyzer for has been implemented 27 where an ammonia electrode was incorporated in conjunction with a pre-dialysis unit. A multi-analyte miniature conductance biosensor using enzymes such as urease and L-asparaginase and a three enzyme system consisting of urease, creatinase and creatininase for determining urea, L-asparagine and creatinine, respectively is also applicable [18]. An enzymatic method has been developed for the kinetic measurement of L-asparaginase activity and L-asparagine with an ammonia gas-sensing electrode. This method is based upon the deamination of L-asparagine by L-asparaginase from *E. coli* resulting in the formation of ammonia [92]. Garlic tissue electrode has been used for determination of L-asparagine [47]. Garlic tissue cells were employed for conversion of L-asparagine into ammonia. An ammonium gas electrode (ISE) was used as the detector. The combination of L-asparaginase in garlic tissue cells and gas electrode responds linearly to L-asparagine concentration. L-asparaginase from *Erwinia clnysanthemi* was assayed fluorometrically by incubating it with beta L-aspartic acid and measuring the release of 7-amino-4-methylcoumarin [106]. A thermostable recombinant asparaginase from *Archaeoglobus fidgidus* was cloned and expressed in *E. coli* as a fusion protein. It was later purified by an immobilized metal ion affinity chromatography method and its activity was determined by monitoring the change in the ammonia concentration in solution. The enzyme was immobilized and used with an ammonium selective electrode (ISE) to develop a biosensor for L-asparaginase [101].

Side effects

Besides minor side effects such as an allergic reaction and vomiting, L-asparaginase therapy of Acute Lymphoblastic Leukemia has some serious side effects. Onset of venous thrombosis in children undergoing histopathologic disease due

to Acute Lymphoblastic Leukemia therapy has been reported [81]. Neutropenic enterocolitis has been observed as an unusual acute complication of neutropenia, associated with leukaemia and lymphoma [74]. Hypersensitivity reactions to chemotherapeutic anti-neoplastic agents such as L-asparaginase [77] have been reported. Tubular and glomerular dysfunction due to Acute Lymphoblastic Leukemia chemotherapy have been observed [39]. Urethral obstruction was observed due to L-asparaginase induced pancreatitis during treatment of Acute Lymphoblastic Leukemia [16]. Myocardial ischemia has been observed in a patient with acute lymphoblastic leukaemia [84] due to L-asparaginase therapy.

An outburst of acute pancreatitis, called "drug induced pancreatitis" (DIP), has been reported by Trivedi, C.D., Pitchumoni, C., [97]. A cerebral thrombotic complication in adolescent leukaemia patients [40] has been attributed to L-asparaginase treatment. Acute hepatic dysfunction [4] and immunodeficiency in children with ALL [10] have been other major side effects. Ocular complications arise due to L-asparaginase treatment [26], but symptoms ease out by discontinuing treatment and carrying on treatment with heparin. Growth hormone deficiency in children receiving chemotherapy for acute lymphoblastic leukaemia has been reported [34]. Growth impairment after cranial radiation (CR) can result in diminished adult height. L-asparaginase treatment induced severe, acquired, and transient type I deficiency of antithrombin (and 1-antitrypsin) with intracellular accumulation of the nascent molecule, increasing the risk of thrombosis [37]. A study on the consequences of L-asparaginase on antithrombin levels in plasma from acute lymphoblastic leukemia patients, HepG2 cells, and plasma and livers from mice treated with this drug has been carried out by Hernandez-Espinosa, *et.al*., [37]. They reported that asparaginase treatment induced severe, acquired, and transient type I deficiency of antithrombin (and 1-antitrypsin) with intracellular accumulation of the nascent molecule, increasing the risk of thrombosis.

L-asparaginase an enzyme of medical interest and scope of research

L-asparaginase has been a major research subject for many researchers worldwide. The FDA has approved L-asparaginase for the effective treatment of acute lymphoblastic leukemia and lymphosarcoma. Its therapeutic potential is now well established, as it has remarkably induced remission in most of the patients suffering with ALL. The antileukemic action of the preparation made in the USSR was superior to the preparation (leumase) made in Japan. L-asparaginase made in USSR and Germany was recommended for clinical use.

Preventase (DSM) and Acrylaway (Novozyme) produced from *Aspergillus niger* and *A. oryzae* respectively are commercially available L-asparaginase currently used in food industry [48] for the production of acrylamide (a potent carcinogen and a neurotoxic compound) free starchy fry food [69].

The thermozymes from thermophilic organisms thrive under high temperature environments (45 to 100°C) are ideal microbiological niche to explore L-asparaginase production and preferred due to their guaranteed properties of stability in adverse conditions at which different industrial processes operate [21] and to combat adverse effects of the existing enzyme. Imanaka and his associates reported that the thermostability of protein will be made possible through a single amino acid substitution [41]. More research is required for the production of cost effective, eco-friendly, thermostable and a potent L-asparaginase with new antigenic properties. Thermozymes extracted from extreme environment might produce a serologically different L-asparaginase with novel immunological properties to that of proteins extracted at normal conditions.

Conclusion

The present review highlighted the key issues related to the use of L-asparaginase by researchers across the globe for patient benefits and effective control for the treatment of various types of malignancies, source, associated hypersensitivity and side effects to be addressed, properties, statistical optimization studies for bioprocess development of a novel, economically viable, safe, stable and potent extracellular enzyme with higher yield.

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