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Microbial L-Asparaginases: Therapeutic and Industrial Applications

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ABSTRACT

L-asparaginase (EC 3.5.1.1) is an enzyme that mostly helps break down asparagine into L-aspartic acid and ammonium in water. This enzyme can be found in many living things, like bacteria, plants, and some animals, like the serum of some rodents. Especially for Acute Lymphoblastic Leukaemia (ALL) and Hodgkin's lymphoma, it is an important chemotherapeutic drug for treating lymphoproliferative diseases and lymphomas. When L-asparaginase comes in contact with water, it breaks down more easily. At the moment, biotechnological methods using certain microorganisms are mostly used to make L-asparaginase. Still, industrial manufacturing needs a study that focuses on both increasing production yields and coming up with new ways to do things, like using different microbes to make enzymes useful in more situations. This review gives an overview of L-asparaginase's uses and talks about how it is made by different microbes, as well as its limitations, current research, and issues that need to be fixed before it can be used in industry.

Keywords: L-asparagine; Leukaemia; Enzyme; Microorganisms; Physiochemical; Kinetic proerties

1. INTRODUCTION

L-asparaginase aminohydrolase (EC 3.5.1.1), which is used to treat cancers like acute lymphoblastic leukemia, malignant diseases of the lymphoid system, and Hodgkin's lymphomas, has gotten a lot of attention lately because it has so many important uses. As evidence, it is employed in the pharmaceutical sector as replacement therapy for several types of cancers.[1] Also, this enzyme is used in the food business to stop the formation of acrylamide when foods are heated up during processing.[2] Acrylamide is a poison that has been labeled as possibly cancerous to humans[3], so this use is very important.

All proteins that are enzymes catalyze several physiological as well as metabolic reactions that endow the growth and development of the cell. Enzymes can operate within a cell, outside of a cell, or on the outer layer of a cell membrane. Numerous enzymes with beneficial extracellular activity have been found in plants, animals, and microorganisms. Several of them have been explored for different industrial, agricultural, and health product designs and formulations. L-asparaginase, a crucial amino acid-degrading enzyme, is one of them and is constantly being researched as a potential medical treatment, particularly for the treatment of acute lymphoblastic leukemia, non-Hodgkin lymphoma, and Melano sarcoma as a potent anti-tumor agent[2]. L-asparaginase is a crucial chemotherapeutic drug and an enzyme that breaks down amino acids. Treatment for acute lymphoblastic leukemia is accomplished with it. L-asparaginase is an enzyme that changes L-asparagine from an unbound amino acid to L-aspartate and ammonia[3]. It is also known as L-asparagine amidohydrolase and has the Enzyme Commission number 3.5.1.1. Monomethoxy polyethylene glycol succinimidyl L-asparaginase is the formal name for the L-asparaginase enzyme. Due to their affordability, microorganisms such as *Erwinia carotovora* were traditionally used to produce L-asparaginase [4]. L-asparaginase is an essential component of several medicinal applications, including the therapy of melanosarcoma, Hodgkin's lymphoma, and acute lymphoblastic leukemia. L-asparaginase, on the other hand, offers enormous promise as a food processing aid.

Currently, fresh investigations are being conducted to improve the manufacturing process and develop new methods for enzyme synthesis. Consequently, this includes a discussion of some attributes and general information regarding the utilization of L-asparaginase in the pharmaceutical and food sectors.

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2. HISTORICAL BACKGROUND

As a potential treatment for cancer, L-asparaginase received a boost from the discovery made by the researcher. In this investigation, the activity of the guinea pig's serum L-asparaginase is being investigated[5]. It was found that enzymes from *Escherichia coli* had the same level of

activity as enzymes from guinea pigs when researchers were looking for sources of therapeutic L-asparaginase[2]. Other researchers' studies from 1966 and 1976 provided evidence in support of the conclusions[6]. As soon as it was clear that L-asparaginase could be made on a big scale, both preclinical and clinical studies were started. The first clinical test used L-asparaginase from guinea pig blood on people who had acute lymphoblastic leukemia. The serum of guinea pigs was used to partially purify two isoforms of L-asparaginase[7]. Many researchers have been working over the past few years to increase the production of L-asparaginase from various microorganisms. Many statistical screening designs for media, nutrients, and culture condition optimization have been reported during the last two decades. This was done in reaction to the need for more potential and exploitable L-asparaginase for drug discovery programs.

3. L-ASPARAGINASE PRODUCTION USING SUBMERGED AND SOLID-STATE FERMENTATION

3.1 Solid-State Fermentation (SSF)

L-asparaginase is derived from various bacteria and plants and is useful as an anticancer medication for treating lymphoid proliferative disorders. In recent times, the manufacturing of enzymes has been conducted through the utilisation of Solid-State Fermentation (SSF)[8]. Bacterial and filamentous fungal organisms establish symbiotic associations on solid substrates. Extracellular enzymes, which are crucial from an industrial standpoint, facilitate the extraction process. In terms of productivity, low capital expenditure, ease of use, energy requirements, water output, and product recovery, solid-state fermentation is preferable to submerged fermentation. SSF seeks to achieve the maximum substrate concentrations for fermentation by tightly contacting the cultured fungi or bacteria with the insoluble substrate. Even though it has only been used on a small scale thus far, this technique offers various processing benefits over SMF that have significant potential economic and ecological significance. The use of SSF for industrial production has been discouraged nonetheless, due to a number of its shortcomings[9].

3.2 Submerged Fermentation (SMF)

SMF (Submerged Fermentation) is the process of growing microorganisms in liquid broth media while maximizing the availability of key nutrients to promote the growth of bacteria. SMF has created equipment that makes use of the available microbes. Bacteria are often utilized as a source in conjunction with other microorganisms during this process due to their high moisture requirements. The generation of Asparaginase by SMF has been documented for fungi including *Aspergillus tamari*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium*, and *Penicillium*[10].

Table 1. Some numerous bacteria and fungi produce L-asparaginase, according to reports

Sources	Species bacteria	References
1.	Acinetobacter baumannii	[11]
2.	Bacillus aryabhattai	[12]
3.	Bacillus megaterium	[13]
4.	E. coli	[14]
5.	Pseudomonas fluorescens	[15]
6.	Rhizolibiumetli	[16]
7.	Serratia marcescens	[17]
8.	Thermococcus Kodakaraensis	[18]
9.	Vibrio cholera	[19]
10.	Zymomonasmobilis	[20]
	FUNGI	
11.	Aspergillus arenarioides EAN603	[21]
12.	Aspergillus fumigatus	[22]
13.	Aspergillus terreus BV-C strain	[23]
14.	Beauveria bassiana	[24]
15.	Cladosporium tenuissimum	[25]
16.	Colletotrichum gloeosporioides	[26]
17.	Emericellanidulans	[27]
18.	Fusarium culmorum	[28]
19.	Fusarium sp. LCJ324	[29]
20.	Lasiodiplodiatheobromae	[30]
21.	Meyerozymaguilliermondii	[31]
22.	Penicillium janthinellumBiourge	[32]
23.	Rhizomucormiehei	[33]
24.	Talaromycespinophilus	[34]

4. SOURCES OF L-ASPARAGINASE

Microorganisms have demonstrated notable efficacy and cost-effectiveness as providers of L-asparaginase. Table-1 lists the various microorganisms from which L-asparaginase can be derived, including bacteria, fungi, yeast, actinomycetes, and algae.

Table 2. Provides a summary of the needs and restrictions for the various bacterial and fungal strains

Micro organisms	Purification methods	Purification ratio-yield(%)/ fold(s)	Reference(s)
Aspergillus niger	Ammonium sulphate (80 %) DEAE cellulose	72.05/1.44	[35]
Cladosporium sp.	DEAE cellulose column, Methanol precipitation	190 fold	[36]
Penicillium digitatum	Ammonium sulfate precipitation, G-100 Column	60.94	[37]
Flammulina Velutipes	Ultrafiltration superpose 6	ND	[38]
Aspergillus terreus	DEAE sepharose and Sephacryl S-200 column	ND	[39]
Acinetobacter baumannii(R7)	Chromatography using isopropanol (1:2) and CM-Sephadex C-50	77/93	[11]
Cornyebacteriumglutamicum	Precipitation of protamine sulfate and filtration through Sephacryl S-200 gel	98 fold	[40]
Marine actinomycetes PDK2	Ammonium sulphate	65.83/1.09	[41]
	Sephadex G-50	8.61/33.68	
	Sephadex G-200	2.18/82.98	

4.1 Bacterial and Fungal L-asparaginase

Because bacteria can be controlled in a lot of different ways, the fabrication of L-asparaginase from a wide range of bacteria has been analysed in-depth for many years. ALL is clinically treated with L-asparaginase from *Erwinia chrysanthemi* and *E. coli*. There are some similarities in how toxic, anti-neoplastic, and immunogenic asparaginase is, but it is made by different bacteria and has different pH, molecular weight, stability, affinity, and

biochemical and serological properties. In the treatment of ALL, bacterial formulations are shown to exhibit significant immunogenicity. In addition to bacteria, fungi also provide a potential source of L-asparaginase. Bacterial asparaginase has several negative side effects that typically preclude its use. This obstacle necessitates a search for L-asparaginase from fresh sources. In contrast to bacteria, fungi exhibit a closer association with humans. Therefore, there will be less of a chance of an immune response against fungal asparaginase. Due to its extracellular production and ease of extracellular enzyme purification, fungus asparaginase has grown in prominence. Patients who are exposed to bacterial L-asparaginase frequently experience hypersensitivity reactions, some of which are deadly.

5. PURIFICATION STRATEGIES

A concentrated and purified enzyme must be used to assess an enzyme's physiological activity. The enzyme must be homogeneously purified to determine its structure and conformation. Once the enzymes are in their purest form, it is easy to look at their kinetic and thermodynamic characteristics, including how reactions are carried out. To study how L-asparaginase can help fight cancer and other health problems, it is important to use only pure forms of the enzyme. For use in major formulations for both industrial and medical applications, an enzyme must first be purified. A crude microbial extract can successfully provide purified L-asparaginase, contingent upon the specific microorganism employed, such as fungi or bacteria (Table 2).

6. L-ASPARAGINASE ALONG WITH ITS PHYSIOCHEMICALAND KINETIC PROPERTIES

Based on the many physiological and kinetic characteristics of the enzyme, microbial L-asparaginase is classified.

6.1 Physiochemical Properties of L-asparaginase

This is a list of the functions and properties of several microbial L-asparaginases that were studied from 2006 to 2018 (Table 3).

6.2 Kinetic Properties of Microbial L-asparaginase

Enzymatic kinetics are crucial to comprehending the speed and selectivity of most biological processes. To make efficient use of enzymes in industrial applications, it is necessary to have a firm grasp of the kinetic factors and enzyme properties present during a biochemical process (Table 4).

7. THE RAPEUTICINDUSTRIAL APPLICATIONS OF L-ASPARAGINASE

Both Non-Hodgkin Lymphoma (NHL) and Acute Lymphoblastic Leukemia (ALL) can be effectively treated with L-Asparaginase, which also makes the treatment more bearable. In addition to being beneficial against cancer, L-asparaginase is also useful against viral and autoimmune illnesses. L-asparaginase has a variety of applications, some of which include acting as an adjuvant in the treatment of cancer and lowering the levels of acrylamide in processed foods. However, each method of employing the enzyme is essential and directly contributes to human health and well-being.

7.1 Use of Polymers and Nanoparticles as Medication Delivery Vehicles for L-asparaginase

Many different types of nanoparticles and polymers have been created as drug-delivery agents in recent years. These include nanoparticles that are between 1

Table 3. L-asparaginases along with physiochemical properties

Micro organisms	Mass (kilo -Dalton)	pH optimum	Temp. optimum (°C)	References
Aspergillus flavus	100	7.0	37	[42]
Aspergillus fumigates WL002	35	9.0	50	[43]
Aspergillus sp.	56	6.0	47	[44]
Aspergillus terreus	136	9.0	40	[39]
Bacillus licheniformis	135	6.0-10	40	[45]
Bacillus sp.	45	7.0-8.0	37	[46]
Cladosporium sp.	121	6.3	30	[36]
Cornye bacterium glutamicum	80	7.0	40	[40]
Escherichia coli	153	6.0	55	[14]
Flammulina Velutipes	85	7.0	40	[38]
Penicillium cyclopean	55	8.0	37	[47]
Penicillium sp.	66	7.0	37	[48]
Pseudomonas fluorescens	140	7.5	37	[49]
Rhizo mucormiehei	133.7	7.0	45	[33]
Trichoderma viridae	99	7.0	37	[34]

Table 4. L-asparaginases along with kinetic properties

Micro organisms	Sub strate	*V* max	*K*m (mM)	Specific activity (U/mg)	Ref -erences
Flammul inavelu tipes	-	ND	ND	ND	[38]
Penicillium digitatum	-	ND	1 × 1 0 -5M	833.15	[37]
Aspergillus terreus	-	ND	ND	ND	[39]
Aspergillus flavus	-	ND	0.086	176.47	[42]
Penicillium cyclopean	-	3333 umol /ml /min	0.3	39480	[48]
Tricho derma viride	-	78.2	279.27 U/ml	2.56	[34]
Aspergillus oryzae CCT3940	-	282	0.66×10 -3 M	313 IU/ ml	[50]
Aspergillus niger AKV-MKBU	-	46.75	0.8141	6.228 µmol/ ml/min	[51]
Yersinia pseudo tuberculosis	L-asparagine	N.R	0.017 ±0.9	N.R	[53]
Therm ococcus gamma tolerans (EJ3)	L-asparagine	N.R	10	N.R	[52]
Yersinia pseudo tuberculosis	L-asparagine	N.R	0.017 ± 0.9	N.R	[53]

and 1000 nm in size and are used to treat cancer, HIV, infectious diseases, and other conditions. Pharmaceuticals' immunogenicity decreases, while their solubility, half-life, and therapeutic index all increase due to their nanoparticle form. Magnetic nanoparticles have recently been used to immobilize this enzyme. Additionally, poly (2-vinyl-4,4-dimethylazlactone), a biocompatible reactive polymer, functionalizes it. 95.7 % of the activity is still maintained even after ten uses[54]. Additionally, it keeps more than 72.6 % of the activity even after being stored for 10 weeks. An asparagine-carbon nanoparticle-methotrexate fluorescein isothiocyanate (FITC) nanobio composite was also developed by the researcher. Target medication distribution is aided by Mtx, a folic acid analog, and drug routes can be monitored with the aid of FITC's imaging capabilities. The reaction occurs within the pore of the hollow nanoparticles, while the antibody and protease stay outside. This prevents the immune system from destroying the enzyme that has been immobilized.

To treat Acute Lymphoblastic Leukemia (ALL), asparaginase (ASNase) is extensively utilised, however immune reactions and silent inactivation limit its bioavailability. Several solutions, including biobetters, have been proposed to address these issues, but only two are now available. Nano- and micro-encapsulation offer potential and innovative methods to enhance the in vivo effectiveness of ASNase by preventing direct exposure of the enzyme to the surroundings, shielding it from degradation by proteases, prolonging its catalytic activity, and decreasing its potential to provoke an immune response. Various literature review discusses ASNase nano- and micro-encapsulation techniques, their primary findings, limits, and knowledge gaps. Discussing the benefits and downsides of different nanocarriers aims to make ALL therapies safer and more successful[55-58].

7.2 Antimicrobial Activities

Industries are constantly looking for new L-asparaginase sources with improved yields and innovative properties. Its widespread use raises market demand, which is difficult to satisfy at the current rate of manufacturing. It has been claimed that the sources of this enzyme have antibacterial properties as well. For the synthesis of this enzyme, marine sources are more varied and have received less attention. It is anticipated that the microbes derived from this source will contain unique characteristics as a result of their ability to tolerate a wide variety of environmental variables, such as temperature, pH, and salinity[59].

Recent years have seen increased interest in this enzyme as an antibacterial. Previous studies described L-asparaginase's antibacterial activity and recommended its use in infection control. Meganathan (2016) found L-asparaginase antibacterial. Raj and Sathiyamurthy (2016) demonstrated l-asparaginase's antibacterial and antifungal activities and predicted its therapeutic use against pathogens[60-61]. higher L-asparaginase production strains and tested the antibacterial efficacy of the compound against a wide variety of gram-positive and gram-negative bacteria[62]. Effective formulation and eco-friendly methods using safe biomaterials can help make L-asparaginase an official antimicrobial medication. This viewpoint suggests that L-asparaginase could become an approved antimicrobial agent and encourages researchers to investigate its antimicrobial mode of action, directly acting phenomena on microbes or host-directed, biopolymer-mediated nano-delivery potential, etc.(Fig. 1).

7.3 Infectious Diseases

Patients suffering from necrotizing fasciitis, pharyngitis, glomerulonephritis, scarlet fever, toxic shock syndrome, meningitis, and rheumatic fever may experience varying degrees of severity, ranging from moderate to fatal. Endoplasmic reticulum stress is caused by the release of streptolysin toxins (streptolysin O and streptolysin S) by GAS when it clings to the host cell [63]. These steroids are responsible for the production of the toxins.

The literature suggests that the enzyme could treat more infectious diseases. Baruch et al. (2014) found metabolic alterations during pathogen infection. This could improve infectious illness therapy. Streptococcus pyogenes (GAS) causes necrotizing fasciitis, pharyngitis, glomerulonephritis, scarlet fever, toxic shock syndrome, meningitis, and rheumatic fever. L-asparagine also causes F. tularensis infection[64]. L-asparagine is used by gram-negative bacteria for cytosolic multiplication through the ΔansP transporter. L-asparaginase might also help your body fight this germ[65].

7.4 Autoimmune Diseases

A substance called L-asparaginase has been shown to help patients by weakening immune systems and reducing inflammation. It successfully stops B-cell responses that are triggered by T cells. Because it affects the lymphatic system, there is a greater chance that it will be used to treat inflammatory diseases where T cells react strangely. A study comparing it to cyclophosphamide, the most common drug used to treat CIA, found that it is more helpful and less dangerous. In the future, this enzyme may be used to treat inflammatory diseases, including rheumatoid arthritis, according to the study[66].

7.5 Food Industry

People need to eat, and thanks to industrialization, food is now available in a lot of different types and a lot of different packaging. Today, one of the biggest and most

significant industries in the global village is the food industry. In 2002, foods with a lot of carbohydrates that had been heated to a high temperature included acrylamide, according to the Swedish National Food Administration (SNF). Potatoes, coffee, and bakery goods (bread and biscuits) are foods that are associated with acrylamide exposure in humans (EFSA 2015). With a molecular weight of 71.08g/mol[67], acrylamide (C_3H_5NO) is alternatively referred to as acrylic amide, 2-propenamide, ethylene carboxyamide, propenamide, propanoic acid amide, acrylamide monomer, and acrylic acid amide. Food that has been boiled will not experience this phenomenon.

Recent research on the synthesis of acrylamide has revealed that L-asparagine provides the amide chain that is contained in the acrylamide structure. Reagents can be reduced or eliminated as one of the tested techniques for lowering the level of acrylamide in food. The authors of the study verified that cooking or baking food with the L-asparaginase enzyme could drastically reduce the level of acrylamide in the final product by more than 99 %. This is due to the enzyme's ability to reduce the initial feedstock's L-asparagine content by more than 88 %. Recent articles have discussed the use of L-asparaginase, which can lessen the deleterious effects of meals containing acrylamide without compromising their quality. Furthermore, it has been demonstrated that the enzyme's thermal stability, reusability, and catalytic efficiency are all improved through covalent immobilization on the surface of magnetic nanoparticles. The generation of acrylamide content in food was successfully decreased by L-asparaginase-linked magnetic nanoparticles[68].

7.6 Felines and Canines Undergoing Chemotherapy

One of the most common forms of cancer in canines and felines is lymphoma. In addition to humans, L-asparaginase is utilized as a chemotherapeutic agent to treat lymphomas in dogs and cats. Along with cyclophosphamide and prednisone, it is used in a multi-drug resistance strategy for canine cancer. Canine cancer relapses in the majority of treated cases, necessitating rescue therapy. Relapses may occur because specific anatomic locations, such as the central nervous system, do not reach lethal medication doses. L-asparaginase is given by a veterinary oncologist to temporarily reduce tumor size so that specialists can investigate alternate treatment options. However, due to its expensive price, it is not frequently used in veterinary applications[69-74].

7.7 Health and Pharmaceuticals

L-asparaginase derived from *Escherichia coli* is employed for the treatment of acute lymphoblastic leukemia., but the intended outcomes are not always achieved because of clinical hypersensitivity. To get around these problems, Researchers are currently utilizing clinically altered variants of the enzyme and exploring other sources of L-asparaginase to discover a less detrimental form of the enzyme. Both biotechnology and medicine are very interested in finding novel and scientifically improved medicinal enzymes. Although L-asparaginase has been used to treat a variety of cancers, it has several undesirable side effects, including toxicity and allergic reactions. As a result, the market needs L-asparaginase which is bio-better, or new medications created by strengthening and improving their qualities, including affinities, selectivity, and stability against degradation, peptides, or protein-based biopharmaceuticals[75-79].

8. CONCLUSION

The use of microbial L-asparaginase has garnered significant interest as a treatment for leukemia. and this review gives strategies about the manufacture, purification, characterization, and biological functions of L-asparaginase, as well as the main sources of this enzyme.

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