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Review Article

Microbial L-Asparaginase Bio betters: Therapeutic Potential in Cancer Treatment and beyond

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ABSTRACT
Microbial enzymes are utilized in a variety of fields. They find applications in several industries, including baking, leather tanning and brewing, among others. Recently microbial enzyme bio betters have been used as therapeutic agents for numerous medical conditions. Notably, certain enzymes like L-asparaginase and L-glutaminase have shown effectiveness in combating particular types of cancers. Recent research and clinical investigations have identified L-asparaginase as a potent medication for managing lymphoblastic leukaemia. In future, improved L-asparaginase bio betters may be developed and become primary therapeutic drugs for lymphoblastic leukaemia. This review focuses on the mechanism of action, microbial sources, types, commercial production, therapeutic applications, and future potential of microbial L-asparaginases.

Keywords: Microbial Enzymes, Therapeutic Agent, L-Asparaginase, Lymphoblastic Leukaemia.

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INTRODUCTION

The enzymes are unique biomolecules that serve as catalysts and are produced in all forms of life. They catalyse metabolic reactions in humans, animals, plants and microbes. The application of enzymes derived from plants and animals for processing substrates (raw materials) has a long history. The initial discovery of enzymatic transformation dates back to 1783 when Spallanzani made his observations. Later, in 1814, Kirchhoff identified a compound in barley that transformed starch into sugars. The industrial use of enzymes commenced with Jokichi Takamine, who formulated enzyme preparation, which is a blend of proteases and carbohydrases.

The enzymes have been used for the processing of raw materials across numerous industries which include brewing, tanning, baking and dairy, for centuries. Currently, enzymes are regarded as key components of biotechnology, serving as essential instruments for implementing basic biotechnological approaches. They are not only the focal points for drug development but also crucial intermediates in various biotechnological processes. In addition, enzymes are unique in that they can also function as therapeutic agents themselves.[1] Microbial enzymes act as the therapeutic agents in the maintenance of human health and for combating human disorders. Based on their physiological, geographic and genetic diversity microorganisms produce a great variety of enzymes. In recent decades, medical and pharmaceutical research has focused on the development of bio betters (especially enzymes), with less purification criteria, reduced hypersensitivity reactions and increased enzyme stability. The bio betters are innovative therapeutic drugs derived from current biopharmaceutical drugs by enhancing their characteristics such as specificity, stability and affinity.[2,3] In recent years some of the enzymes of microbial source were proved to be extremely successful in curing various types of cancers.

The cancer is defined by the unregulated and invasive proliferation of cells, resulting from a breakdown in the regulatory mechanisms governing cell growth and division. While traditional cancer treatments, including surgical removal, radiation therapy, and chemotherapy, are effective in approximately 50% of cases, these non-specific approaches often yield numerous adverse side effects and may prove ineffective. Consequently, there is a growing interest in enhancing or substituting these invasive and non-specific conventional therapies with strategies that specifically target cancer cells. One promising approach is enzyme therapy, which offers a more precise and effective means of treating cancer. This method employs specific enzymes that can deplete cancer cells that of particular amino acids essential for their growth, while sparing healthy cells that do not rely on these amino acids for survival.[4] The microbial enzymes, such as L-asparaginase and L-glutaminase are emerging as potential cancer therapeutic agents. Notably, L-asparaginase has already

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received approval for the treatment of specific cancers, including leukaemia.

L-asparaginase is an innovative and developing therapeutic agent employed in the management of several blood cancers, particularly Acute Lymphoblastic Leukemia (ALL). This enzyme is classified within the Hydrolases category, which is characterized by hydrolytic cleavage. Functionally referred to as L-asparaginase amidohydrolase (E.C. 3.5.1.1), it basically acts as a hydrolytic enzyme that catalyzes the transformation of L-asparagine into L-asparatic acid and ammonia.[5] The microbial sources play a crucial role in the development of L-asparaginase bio betters, as they can produce the enzyme in significant quantities with high efficiency and cost-effectiveness. Bacterial species like *Escherichia coli* (*E. coli*) and *Erwinia chrysanthemi*, are capable of producing large quantities of L-Asparaginase in a short time and economically viable.[6] It is considered as a keystone in the treatment of leukaemia by inhibiting the cancer cell multiplication by reducing the availability of the amino acid Asparagine.[7] It is also utilized as a potent medication for the treatment of acute myelocytic leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, Hodgkin's disease, reticulosarcoma, lymphosarcoma and melanosarcoma. The prolonged administration of L-asparaginase can result in hypersensitivity reactions, which may manifest as mild allergic responses, pancreatitis, liver impairment, leukopenia, neurological seizures, and coagulation issues that could potentially result in intracranial thrombosis (haemorrhaging).[8,9] *E. chrysanthemi* L-asparaginase demonstrated low allergic reactions than the enzyme derived from *E.* coli; however, *Erwinia* L-asparaginase had a shorter half-life in comparison to E. *coli*.[10] L-asparaginase bio betters can be developed to improve their catalytic efficacy and stability *in vivo*, while also minimizing L-glutaminase activity and toxicities.

Furthermore, L-asparaginase yield can be enhanced by microorganisms, specifically bacteria and fungi, through genetic engineering. The simple cultivation methods and genetic modification processes allow enhanced production of L-asparaginase via microbial fermentation. The utilisation of genetically modified microorganisms, eliminates the ethical concern associated with animal-based production system and minimize the risk of contamination with animal derived pathogens providing a safe and controlled source of the enzyme. In the present review, the mode of action, bacterial and fungal sources, types, commercial production, therapeutic applications, clinical trials, challenges in development/production/use and future prospects of microbial L-asparaginases are discussed.

Mode of action of L-asparaginase

Clinical applications of microbial enzymes are categorised into anti-inflammatory agents, anticancer agents, fibrinolytic agents and enzybiotics.[11] Enzyme therapy is found to be more specific therapeutic method against cancer.[12] The basic principle behind the use of enzyme therapy is Amino Acid Depletion Therapy (AADT) by the use of specific enzyme it will deplete the amount of specific form of amino acid which is required by the malignant cells for multiplication.[13] Microbial enzymes used as anti-cancer agents are L-Asparaginase, Arginine deiminase, Methioninase, Lysine Oxidase and L-Glutaminase. L-asparaginase, which is the first bacterial enzyme utilized in anticancer therapy using AADT (amino acid deprivation therapy).[14] This is a hydrolytic enzyme that enhances the breakdown of L-asparagine into aspartic acid and ammonia (Figure 1).[15] All the cells require L-asparagine, which is a non-essential amino acid, for protein synthesis.

Normal cells synthesize L-asparagine from aspartic acid with the aid of L-asparagine synthase. In contrast, cancer cells do not produce L-asparagine synthase and must rely on external sources, such as diet, for their L-asparagine supply. Because of their rapid growth and proliferation, cancer cells require a greater amount of L-asparagine than normal cells.[16] Administering L-asparaginase decreases L-asparagine levels in blood, halting the cell cycle in G1 phase (in leukemic cells), which results in DNA (chromosome) breakage and triggers apoptosis in leukemic cells.[17]

Broome in 1961 first demonstrated the anti-cancer potential of L-Asparaginase by revealing its substrate specificity in guinea pig serum.[18] Before this, Altenbern in 1954, discovered the anti-tumor properties of L-asparaginase from bacterial sources, followed by Housewright's findings from yeast sources in 1965.[19] Ohnuma *et al*., in 1967, made a trailblazing discovery on L-Asparaginase isoenzymes: Type I L-asparaginase and type II L-asparaginase which exhibit distinct catalytic activity towards L-Asparagine.[20] Healthy cells remain unaffected by the treatment due to their capacity to synthesize asparagine endogenously facilitated by the catalytic Activity of Asparagine Synthetase (ASNS), which converts aspartate into L-asparagine.[21] L-Asparaginase is regarded as the key to managing Acute Lymphoblastic Leukemia (ALL), particularly in paediatric patients.

L-Asparaginase is considered as the cornerstone in the treatment of Acute Lymphoblastic Leukaemia (ALL) particularly in children. The survival rate in younger children is significantly high. According to the study of Koji *et al*., in 2024, complete remission was achieved in 72 patients (89%) out of 81 patients studied. The patients aged 16 to 35 years has shown remarkable improvement.[22] In children, long term improvement was reported to be 80% and overall survival rate is 90% while in adults it is 38% and 50% respectively.[23,24] To improve therapeutic effectiveness and clinical results in treating acute lymphoblastic leukemia, L-asparaginase is frequently used alongside other chemotherapeutic agents such as Vincristine, doxorubicin and Glucocorticoids.[25] The combination of L-asparaginase, vincristine and prednisone has been reported to achieve complete response rates of around 40% in patients with multiple relapses.[26]

Microbial sources of L-Asparaginase

L-asparaginase is commonly found in many organisms including bacteria, fungi, yeast, actinomycetes, plants, tissues of several animals, but it is absent in humans. Both Gram negative and Gram-positive bacteria were reported to produce bacterial L-Asparaginase,[27] which includes various species of *Acinetobacter, Escherichia, Bacillus, Klebsiella, Erwinia, Serratia* and *Pseudomonas.* Due to structural and functional differences, availability, efficiency, and specificity the L-Asparaginase synthesised by Gram negative bacteria gained more attention than Gram positive bacteria in therapeutic purpose.

Aspergillus nidulans,[28] *Alternaria* sp,[29] *Aspergillus niger*,[30] *Aspergillus terreus*,[31] *Mucor* sp,[32] *Fusarium roseum*[33] are several fungal species capable of producing L Asparaginase. Yeast such as *Candida, Saccharomyces., Hansenula, Rhodotorula, Pichia* and *Spobolomyces* species were reported to produce L-asparaginase.[34] The study of Sahu *et al.*, (2007) revealed that actinomycetes are recognized as superior sources of L-Asparaginase than bacteria and fungi.[35] L-asparaginase-producing actinomycetes include *Actinomyces* sp,[36] *Thermoactinomyces vulgaris*,[37] *S. griseus*,[38] *S. gulbargensis*,[39] *Streptomyces albidoflavus*,[40] *Noccardia* sp etc.[41] Table 1 below lists various bacterial and fungal sources of L-Asparaginase.

In plants, Asparagine plays a significant role in seed germination, nitrogen storage and act as an essential component for plant growth and development. Some of the important plant sources are *Tamarindus indica* (Tamarind), *Withania somnifera* (Ashwagandha or Indian ginseng), *Capsicum annum* (Paprika), *Vicia faba* (broad bean), *Phaseoulus vulgaris* (French bean), and *Pisum sativum* (Pea).[42-44] Compared to all the sources available, the bacterial L-Asparaginase is considered as superior due to the high production yield, ease of extraction, cost effective and well-studied characteristics.

The enzyme, L-Asparaginase plays a prominent role in both pharmaceutical and food sectors. This microbial enzyme is effective in treating Acute Lymphoblastic Leukaemia and reduces acrylamide formation, which is a carcinogen produced as a result of baking, frying and roasting starchy food. The biosensor technology utilizing Asparaginase is an innovative technology that measures the quantity of ammonium produced during the hydrolysis of L-asparagine.[45] The change in pH helps in the colorimetric detection and prove their potential with simple and fast response in L asparagine monitoring in ALL patients, L Asparagine concentration in food analysis.

Soil is the most common source for the isolation of microorganisms that produce L-asparaginase, in addition to marine and fresh water sediments, human flora especially from sputum.[46] *Erwinia chrysanthemi* and *Escherichia coli* are considered to be the most effective strains for producing L-asparaginase at large scale. For increasing the half-life, reduced toxicity and immunogenicity, enhanced pharmacokinetics the original product will be modified in chemical and molecular level. This new category of Bio betters gained the attention of pharma and therapeutic industries due to these clinical advantages.[47]

Type I L-asparaginase versus type II L-asparaginase

Type II exhibits a higher affinity for L-asparaginase than Type I. Hence Type II is considered to have more potent anticancer activity, and applied in the therapeutic management of Acute Lymphoblastic Leukemia.[48] The homodimeric cytoplasmic enzymes known as type-I L-asparaginases, which are encoded by *ansA*, have low affinity for L-asparagine. Type II L-asparaginases, characterized by their strong affinity for L-asparagine found in the periplasmic region and typically occur as homotetrameric form, are encoded by *asnB* gene. The Michaelis constant (Km) is a prerequisite for the therapeutic efficacy of an enzyme. The Km, or Michaelis constant, is defined as the substrate concentration at which the reaction rate reaches half of its maximum. This value indicates an enzyme's affinity for a substrate; a lower Km signifies a higher effectiveness of the enzyme in therapeutic applications and a stronger attraction to that substrate. For leukemic cell death to occur effectively, the normal concentration of L-asparagine in blood, which ranges from 40 to 80 µM to be lowered below 0.1 to 0.2 µM. An enzyme can only have such a powerful effect if its Km is approximately 10^{-5} M. Type I L-asparaginases usually have Km value in the millimolar range, and type II L-asparaginases have Km values that are two orders of magnitude less than that of type I L-asparaginases. Consequently, the only enzymes that have been employed as therapeutic agents are type II L-asparaginases.[49,50]

Commercial Production of L-Asparaginase by microbial fermentation

The fermentative production of L-Asparaginase is categorised into upstream and downstream processing. Upstream processing includes preparing and culturing the microbial cells used for enzyme synthesis. It includes the selection of media, fermentation parameters like pH, temperature, aeration, carbon and nitrogen

Table 1: Microbial sources of L-Asparaginase.

Source	Micro-organisms
Bacterial sources of L-Asparaginase	*Acinetobacter calcoaceticus, B. licheniformis, B. subtilus, Citrobacter freundi, E. chrysanthemi, Enterobacter aerogenes, Erwinia aroideae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas* sp., *Serratia marcescens, Staphylococcus* sp., *Thermus thermophiles.*
Fungal Sources of L-Asparaginase	*Aspergillus niger, Actinomyces* sp., *Fusarium equiseti, Fusarium tricinctum, Streptomyces albidoflavus, S. griseus, Noccardia* sp., *S. aurantiacus, S. gulbargensis, Saccharomyces cerevisiae, Thermoactinomyces vulgaris, Penicillium digitatum.*

Table 2: The activity of bacterial L-asparaginase on different cancer cell lines.

Cancer type	Cell lines	Effect of L-Asparaginase	Source of L-Asparaginase	References
Acute Lymphoblastic Leukemia (ALL)	CCRF-CEM, MOLT-4, Jurkat	Inhibits cell proliferation, induces apoptosis	*Escherichia coli, Erwinia chrysanthemi*	[74]
Pancreatic Cancer	PANC-1, MiaPaCa-2	Reduces tumor growth by blocking asparagine supply	*Escherichia coli, Pseudomonas* spp.	[75]
Breast Cancer	MCF-7, MDA-MB-231	Promotes cell cycle arrest and apoptosis	*Streptomyces albidoflavus, Bacillus* spp.	[76]
Lymphoma	Raji, Daudi	Induces apoptosis and inhibits tumor progression	*Bacillus licheniformis, Enterobacter* spp.	[77]
Lung Cancer	A549, H1299	Suppresses cell growth and enhances drug sensitivity	*Bacillus subtilis, Erwinia* spp.	[77]
Ovarian Cancer	SKOV-3, OVCAR-3	Depletes asparagine, leading to tumor suppression	*Pseudomonas aeruginosa, Serratia* spp.	[78]

Sources, Process Selection (SMF/SSF) and optimization.[51] The downstream processing involves all the necessary steps for enzyme purification and formulation of enzyme for therapeutic application. The type of medium, process parameters, purity methods, and other factors determine the characteristics of the enzyme produced.

The most common species used for Asparaginase production are *E. chrysanthemi* and *E. coli*. The production of Asparaginase depends on the cultural conditions also. Hence the media composition, temperature, pH, oxygen concentration, nitrogen sources and carbon sources are considered as the most influencing factors in enzyme production.[52] Submerged and solid-state fermentation are the primary approaches for producing L-asparaginase on a large scale commercially.[53] L-Asparaginase production can be improved by using synthetic culture media as an alternative method. The research conducted by Macauley-Patrick *et al.*, in 2005 indicates that utilizing specific media can effectively alter the physiochemical conditions and enhance the yield of heterologous proteins.[54] Fed batch culture is another process used to enhance the asparaginase production.[55] By employing fed batch cultivation, toxic byproduct formation can be reduced, downstream processing can be simplified, and the entire production cost can be decreased.[56] Optimization of the microbial growth process and adaptation of efficient purification approaches, increase the overall product yield and improve the enzyme quality.

The amount of enzyme released was first detected by qualitative analysis. The plate assay is considered as fast, efficient, sensitive and consistent method for analyzing and enumerating huge number of microorganisms.[57] Plate assay screenings rely on pH-sensitive indicators that undergo a colour change in response to the release of ammonium during the hydrolysis of asparagine.[58] The quantitative evaluation of enzyme activity is done using Nessler's reaction,[59] AHA (L-aspartyl-b-hydroxamic acid) method,[60] circular dichroism spectroscopy[61] and amplex Red method.[62]

Downstream processing accounts for approximately 60-80% of the overall production cost in bioprocessing systems. Hence the large-scale industries are focusing on the modified and advanced techniques for extraction and purification of the enzyme produced by reducing the number of downstream processing units.[63]

The L-asparaginase purification can be accomplished using low-resolution or high-resolution purification methods. Low resolution purification consists of centrifugation, aqueous biphasic systems, fractional precipitation and dialysis (membrane-based purification),[64-66] while high resolution purification is chromatography-based techniques. Because of robustness, scalability, specificity, effective elimination of impurities and simplified validation process, the chromatography is widely accepted method in comparison with other purification processes.[67] The advanced techniques like docking studies, protein engineering, site-directed mutagenesis is used for the development of bio betters improving the asparaginase properties.[68]

Bacterial L-Asparaginases vs Fungal L-Asparaginases

The bacterial and fungal L-Asparaginase have significant difference in structure, biochemical properties and applications. The bacterial L-Asparaginase is homogenic tetramers while fungal L-Asparaginase can exist as monomer, dimer or tetramer depending on species.[69,70] Compared to fungal asparaginase, bacterial L-Asparaginase have high glutaminase activity and

substrate specificity which can cause neurotoxicity in therapeutic use.[71] The stability in acidic pH makes the fungal L-Asparaginase more suitable for food industry, while bacterial L-Asparaginase shows optimal activity in alkaline pH. The fungal L-asparaginases are utilized in the food sector to reduce acrylamide production in baked products as they function well at lower pH. The Bacterial L-asparaginases are widely used in treating Acute Lymphoblastic Leukaemia (ALL) and some lymphomas.

The immunogenicity of bacterial L-Asparaginase is more compared to fungal asparaginase. It is evidenced by the studies of Tekeba Sisay *et al.*, (2023) who revealed that as fungi are eukaryotic organisms, their enzymes are structurally more similar to mammalian proteins, which is expected to result in reduced immunogenic responses.[72] To improve the quality of the asparaginase produced, different methods have been implemented which includes PEGylation, enzyme encapsulation, Recombinant Asparaginase synthesis, structural alteration etc. but the search for a new asparaginase producing microbe which is capable of producing enzyme with superior characteristics are still going on.[73]

Therapeutic applications of L-asparaginase

The antineoplastic property of L-asparaginase relies on its efficacy to induce cancer cell apoptosis by removing the essential amino acids and prevents cancer cells multiplication. The mechanism of action of L-Asparaginase on different cancer cell lines is depicted in Table 2.

Clinical trials on animal models and human subjects

The anti-cancer activity of L-asparaginase is extensively studied in both animal model and human clinical trial for testing its efficiency as an anti-cancer drug especially in treating acute lymphoblastic leukaemia. The animal models help in evaluating the therapeutic potential of L Asparaginase, by inhibiting tumor growth reducing the quantity of L Asparagine required for its proliferation. Clinical trials have investigated the effect of L Asparagine in human subjects also. The study made on the effect of PEGylated L-asparaginase in patients having multiple myeloma revealed a remarkable decrease in the concentration of asparagine in serum, indicating potential therapeutic benefits. However, significant toxicities, including allergic reactions and pancreatitis, were noted at higher doses.[79] According to a randomized study related to the clinical activity of ERY001(erythrocyte-encapsulated L-Asparaginase) compared to native L-Asparaginase in combination with the COOPRALL (Cooperative for Research on Acute Lymphoblastic Leukaemia) regimen in patients with relapsed ALL, ERY001 significantly decreased the incidence of hypersensitivity reactions and sustained therapeutic asparaginase activity for a longer duration than native L-Asparaginase.

The erythrocyte encapsulation of L-Asparaginase enhanced efficiency and the tolerability.[80] To reduce the silent inactivation and improve the enzymatic efficacy, the encapsulation of L-asparaginase in homologous red blood cells (GRASPA) has been put forward as a delivery system, wherein the red blood cells function as microbioreactors, shielding the enzyme from circulating antibodies. According to the study GRASPALL 2005-01 a single infusion of GRASPA achieved sustained

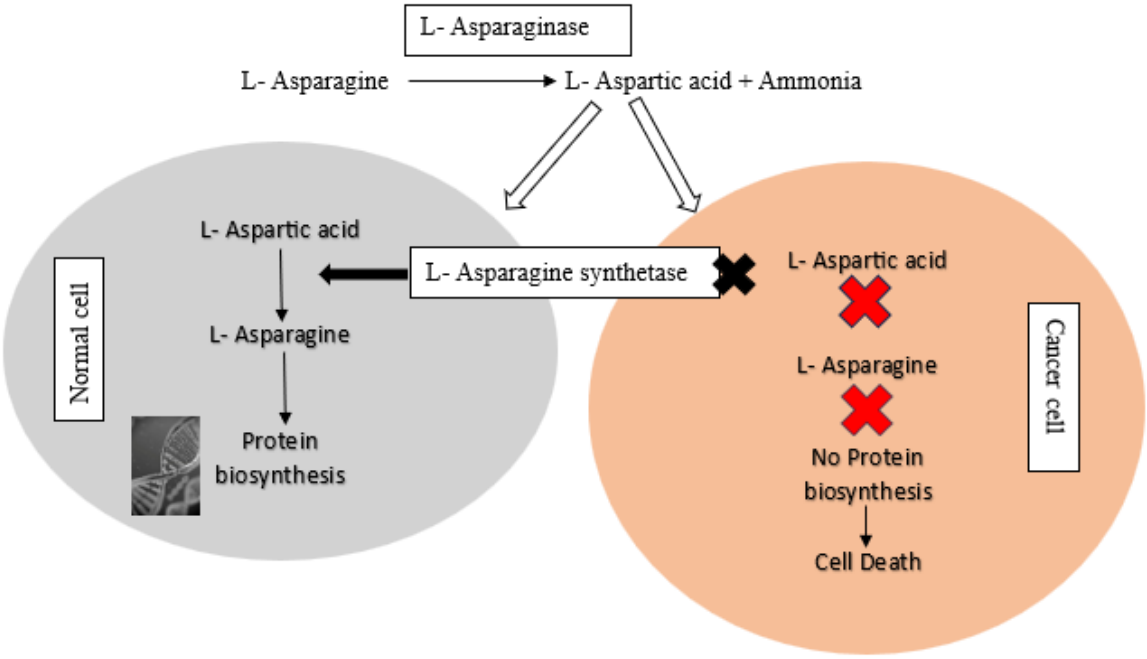

Figure 1: Mode of action of L-Asparaginase.

asparagine depletion comparable to multiple infusions of native L-Asparaginase.[81] In 2018, clinical trials were initiated for PEG-crisantaspase as a salvage treatment for patients exhibiting allergy to *E. coli*-derived asparaginase. The FDA has recently granted acceptance to the Biologics License Application (BLA) for Calaspargase pegol, a new and improved formulation of L-asparaginase.[82] However, all these alternative preparations still suffer from being immunogenic in one way or another. There is a need to create a novel and potent strategies for improving this biotherapeutic agent.

L-Asparaginase Bio betters for improved therapeutics

The L-Asparaginase produced by *Escherichia coli* and *Erwinia chrysanthemi*, is considered as a key stone for leukaemia therapy by inhibiting cancer cell proliferation by lowering the availability of the L-Asparagine.[83] It is employed as a prominent drug in the management of acute myelocytic leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, Hodgkin's disease, lymphosarcoma, melanosarcoma and reticulosarcoma. But long-term treatment leads to mild allergic reactions, pancreatitis, leukopenia, neurological seizures, liver dysfunction and coagulation abnormalities like haemorrhages or intracranial thrombosis.[84,85] To improve the therapeutic outcome and reduce the hypersensitivity reactions bio better forms of L-Asparaginase has to be developed. Bio betters are the engineered form of the enzyme which is designed to have superior properties compared to the original form.

One of the main challenges of using L-Asparaginase is short half-life in blood stream and increased hypersensitivity reactions. Although L-asparaginase produced by *Erwinia chrysanthemi* induces fewer hypersensitivity reactions than the *E. coli*-derived L-asparaginase, it is characterized by a shorter plasma half-life.[86] The bio better version of L-Asparaginase can be engineered to have improved specificity for cancer cells, enhancing the selectivity and minimising the damage to normal healthy cells. By modifying the enzymes catalytic properties bio betters can be designed to increase overall efficacy, making it more effective at lowering asparagine levels in cancer cells.

CHALLENGES IN DEVELOPING L-ASPARAGINASE BIO BETTERS

The development of L-Asparaginase bio better is not an easy task, it faces some challenges due to the complexity of biological products. Structural complexity is the main challenge in developing the bio similar products, especially glycosylation and protein conformation can alter the biological activity of the enzyme. As the enzymes are highly prone to degradation, the formulations, storage conditions, the administration to the patients and maintaining the activity will be the key challenge for the improved version of L Asparaginase.[87] Proteolytic degradation of the enzyme by serum proteases results in diminished enzymatic activity and heightened immunogenicity, primarily due to the cleavage and subsequent exposure of immunogenic epitopes.[88] Regulatory agencies like FDA and EMA have strict guidelines for approving bio betters/ bio similars in terms of pharmacodynamics, pharmacokinetics and clinical efficacy.

L-Asparaginase is an expensive drug, so developing a bio better using economically reasonable strategies, reducing production and purification costs is another important challenge for the pharma industries. To overcome these challenges, require close attention to the molecular structure, immunogenicity, clinical equivalence and cost effectiveness.

CONCLUSION AND FUTURE PROSPECTS

Microbial L-Asparaginase bio-betters represent a promising frontier for the management of cancer, more specifically in the treatment of Acute Lymphoblastic Leukaemia (ALL). Their therapeutic potential extends beyond cancer therapy with emerging applications in other diseases also, where asparagine depletion may play a crucial role. While the development of these bio-betters faces challenges such as ensuring structural and functional consistency, minimizing immunogenicity, and overcoming regulatory hurdles, and manufacturing processes. As these bio-betters undergo further clinical development, their broader clinical application could transform not only cancer therapies but also contribute to other areas of medicine. The continued refinement of L-Asparaginase-based therapies, along with a greater understanding of their molecular mechanisms, will likely unlock new therapeutic opportunities, marking a significant step forward in the evolution of microbial based cancer treatments.

Advanced technologies like gene therapy (CRISPR) and cellular engineering can develop better forms of L-Asparaginase from engineered mammalian cells or other sources with improved qualities. It can also produce better L-Asparaginase by direct modification of patient's cells within the body.[89] It reduces the need for repeated enzyme treatments, lowering the side effects and offers a personalized treatment program. With the help of genetic profiling, bio better L-Asparaginase can be used for personalized cancer therapy based on the individual's cancer type and genetic structure which can identify the patient's immune response to the enzyme.

Bio better L-Asparaginase can be combined with other cancer therapy to enhance the overall outcomes and reduce immunogenicity. Advanced Protein engineering can make modifications in the enzyme's structure to prevent triggering of immune response.[90] The combination of nano particles encapsulated with L-Asparaginase can deliver it directly to the tumor site significantly improve the targeting and efficacy of the enzyme.[91] The co-treatment with immunomodulators also

helps to reduce the immune related side effects and enhances the efficiency of the enzyme treatment. The study conducted by Tabandeh and Aminlari reported that conjugation of L-Asparaginase with oxidized inulin has been shown to enhance its pharmacokinetic and physicochemical properties, which include resisting the degradation associated with trypsin, improved heat resistance, extended half-life, superior reusability following repeated freeze-thaw cycles, and a wider optimal pH range than the original enzyme.[92]

The optimal protein engineering strategy prediction utilizing bioinformatics tools for modelling and L-asparaginase features include search heuristic, molecular docking studies, crystallographic structure analysis, three-dimensional structure modelling, structure-based multiple sequence alignment, binding free energy computation and prognosticate analysis like antigenic and allergenic peptide and hydrogen-bonded turn model forecasting, conformational stability assessment. The scope of bio better L-Asparaginase holds great potential in improving cancer therapy by reducing immunogenicity, resistance, targeting precision and pharmacokinetics making it as a powerful and patient friendly treatment regime.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Presently, microbial enzyme bio-betters have been utilized as therapeutic drugs for various medical conditions. Specifically, some enzymes like L-Glutaminase and L-Asparaginase have demonstrated efficacy in addressing certain cancer types. Recent clinical trials have recognized L-asparaginase as an efficient therapeutic agent for lymphoblastic leukaemia. In future, enhanced L-asparaginase bio-betters may be developed, potentially serving as an effective therapeutic agent for lymphoblastic leukaemia. This review covered the mode of action, various microbial sources, classification, commercial fermentative production, therapeutic applications and future prospects of microbial L-Asparaginases.

ABBREVIATIONS

ALL: Acute Lymphoblastic Leukemia; ***E. coli*:** *Escherichia coli*; **AADT:** Amino Acid Depletion Therapy; **ASNS:** Asparagine Synthetase; **AHA:** L-aspartyl-b-hydroxamic acid; **SMF:** Submerged Fermentation; **SSF:** Solid State Fermentation; **COOPRALL:** Cooperative for Research on Acute Lymphoblastic Leukaemia; **GRASPA:** Red Blood Cell Encapsulated L-Asparaginase; **FDA:** Food and Drug Administration; **EMA:** European Medicines Agency; **BLA:** Biologics License Application; **CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats; **CCRF-CEM:** Human T-lymphoblastic leukemia cell line; **MOLT-4:** Human T-lymphoblastic leukemia cell line; **PANC-1:** Human pancreatic carcinoma cell line; **MiaPaCa-2:** Human pancreatic carcinoma cell line; **MCF-7:** Human breast adenocarcinoma cell line; **MDA-MB-231:** Human breast adenocarcinoma cell line; **A549:** Human lung carcinoma cell line; **H1299:** Human non-small cell lung carcinoma cell line; **SKOV-3:** Human ovarian adenocarcinoma cell line; **OVCAR-3:** Human ovarian adenocarcinoma cell line; **µM:** Micromolar; **Km:** Michaelis constant; **ansA:** Gene encoding Type I L-asparaginase; **asnB:** Gene encoding Type II L-asparaginase.

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