Review Article

Marine L-Asparaginase: A Novel Microbial Therapeutic Approach for Cancer

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Accepted on: 29-06-2015; **Finalized on:** 31-07-2015.

ABSTRACT

The word cancer is a silent killer that creeps up on us. Cancer causes a total of 8.2 million deaths in worldwide. Secondary metabolites mainly enzymes are upgraded molecules for cancer therapy. A family of enzymes using for the treatment of human cancers is referred to as L-asparaginase. It hydrolyses the free aspergine to aspartic acid and ammonia. It is well accepted enzyme as a chemotherapeutic agent and antitumor activity against acute lymphoblastic leukemia and lymphosarcoma. There are several therapeutic asparaginase present in the market, but recent discoveries have indicated that the L-asparaginase from marine microbes might be more efficient and also to exhibit lesser side effects. So, the need for new therapeutic enzymes is of great interest in both biotechnology and medicine. This paper comprises a brief introduction about the enzyme, its sources, mechanism of action and as well as a detailed information about its applications.

Keywords: Cancer, L-asparaginase, Antitumor activity, Acute lymphoblastic leukemia.

INTRODUCTION

Cancer, the King of Maladies is the most serious challenge encountered by biomedical scientists. The therapies and treatments vary with types of cancer. However, antitumor compounds are always upgraded molecules by cancer biologists for therapy. Many cytotoxic compounds from natural resources were found to be having antitumor and anticancer activity. Many compounds with anticancer property were isolated and developed from various biological resources like plants and microbes. It can be assured that a focused approach and combined attempts would definitely accelerate the development of new marine antitumor drugs to be discovered with increased efficiency.[1]

Nature has been contributing extensively for drug discovery by providing remedial treatments to the mankind. Three quarters of the earth's surface is marine biotope which is one of the nature's treasures for medicines.[2,3] In biomedical research marine natural products play a growingly important role in drug development, either directly used as drugs or as lead structures for chemical drug synthesis.[4] The isolation and description of nearly 250 marine bacterial metabolites versus 150 isolated from terrestrial bacteria between 2000 and 2005, described by Laatsch. Research into marine microorganisms and their metabolites has therefore become a major task in the search for novel pharmaceuticals.[5] Although many compounds show promising biological activities. Currently, a large number of natural products are in preclinical investigations and 13 natural products isolated from marine microorganisms are being tested in different phases of clinical trials, thus highlighting the potential of marine natural compounds.[6]

Marine microbe's role in life sciences

A broad variety of diseases and medical problems perform a challenging risk to humans. 50% of the existing drugs that are acquired from terrestrial organisms, which are used to treat human diseases, are derived from natural products. However, due to continuous and exhaustive research, compared to land-based natural compounds, water-based natural compounds have become a more promising source, not only from industrial and commercial applications, but also a pharmacological view.[7]

Unique properties of marine microbes

Due to the impact of their particular environmental conditions marine and terrestrial micro floras differ from each other. Microorganisms living in the sea must be able to survive and grow in the water environment with low nutrition, high salinity, and high pressure. Marine microorganisms can be divided on the basis of habitat in to psychrophiles (living at low temperature), halophiles (Living at high salinity), and barophiles (living under high pressure). These characteristics highlight the differences between marine and terrestrial microorganisms. Marine microorganisms are attractive to researchers because they can probably produce compounds with unique biological properties.[2,3] Marine microorganisms have acquired special importance as the most potent source of antibiotics, enzymes and other bioactive secondary metabolites.[8] In clinical research enzymes have obtained great importance in recent years. L-asparaginase is one of them which are widely present in nature.[9]

L-asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) is an amidase group enzyme it catalyses the conversion of L-asparagine to L-aspartic acid and ammonia.[10] Asparaginase enzyme is mainly distributed in animals, plants and microorganisms. Mono methoxy polyethylene glycol succinimidyl L-asparaginase is the chemical name for L-asparaginase.[11] L-asparaginase is the

first enzyme with anti leukemic activity in human beings to be profoundly studied.[12] Cancer cells differentiate from normal cells in decreased expression of L-asparagine.[13] So, cancer cells are depends on external L-asparagine from the circulating blood plasma because, it's unable to produce L-asparagine.[14] As so far L-asparaginase, obtained from terrestrial bacterial sources, which is currently used for the treatment of leukemia, but it causes several side effects like anaphylaxis, pancreatitis, neurological disorders, bleeding or thrombotic events such as stroke, because of their continues administration.[15,16] Marine sample may be a good source for active asparaginase producing microorganisms, because marine environment, especially seawater, which is saline in nature and chemically closer to human blood plasma, it is anticipated that marine microorganisms could provide L-asparaginase with lesser side effects to human.[17] ELSPAR, CLOLAR, LEUKINE, ONCASPAR, ARRANON, ERWINASE and KIDROLASE are the brand names of L-asparaginase. FDA has approved L-asparaginase for the effective treatment of acute lymphoblastic leukemia and lymph sarcoma.[18]

The amido-hydrolytic activity of L-asparaginase was first observed by Lang S (1904) and further confirmed by Furth & Friedmann (1910) and Clementi (1922). In 1953, Kidd noted that guinea pig serum had antitumor activity against two strains of murine lymphoma and a strain of lymphosarcoma in rats. Neuman and McCoy (1956) demonstrated the metabolic difference between normal and malignant cells. Later, Broome in 1963 first identified that the tumor inhibitory activity was due to of L-asparaginase present in the serum. In 1966, De lowery purified guinea pig serum and treated a boy with acute lymphocytic leukemia and got positive result.[19-24]

Mechanism of Antitumor Action of L-Asparaginase

Normal cells have the asparagine synthetase for synthesis of asparagine in their diet and asparagine is an inessential amino acid for normal cells. In humans the gene asparagine synthetase was located on Chromosome number 7.[25] Tumor cells do not have the ability to produce asparagine due to the lack of asparagine synthetase hence it is a key amino acid for those tumor cells.[26] In the absence of asparagine RNA and protein synthesis is repressed and as a result cell cycle arrest and apoptosis is induced in leukemic cells.[27,28]

FDA has approved the drug can be used for the effective treatment of Acute Lymphoblastic Leukemia (ALL) and Lymphosarcoma. Therapeutic enzymes from other drugs are two main features; firstly that the enzymes act on their target with a great selectivity and with high affinity, secondly they are catalytic and able to convert a substrate into a desired product.[29] These features give the production of powerful drugs. Asparaginase are intended in many bacterial organisms, but only L asparaginase from *Escherichia coli* and *Erwinia chrysantemi* have been used as chemotherapeutics in Acute Lymphoblastic Leukemia (ALL).[30] (Figure 1)

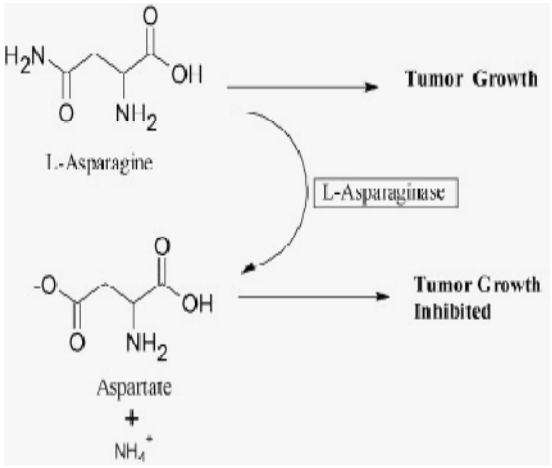

Figure 1: Reaction mechanism of L-asparaginase

Microbial Sources for L-Asparaginase

Over last 35 years the major sources of L-asparaginase are Bacteria, Actinomycetes, and fungi.

Microbes are a best source of L-asparaginase because of the following advantages

- Bulk production capacity
- Economical
- Microbes are safe to operate to acquire enzymes with desired characteristics
- Easy to withdraw the enzyme and purify

L-asparaginase: bacterial sources

Table 1: L-asparaginase from different bacterial sources

S. No	Name of the L-asparaginase producing Bacteria	Reference
1	*Coliform bacteria*	Shah (42)
2	*Erwinia cartovora*	Maladkar (43)
3	*Acinetobacter calcoaceticus*	Joner (44)
4	*Pseudomonas aeruginosa*	Ashraf (45)
5	*Erwinia aroideae*	Tiwari and Dua (32)
6	*Corynebacterium glutamicum*	Mesas (39)
7	*Thermus thermophilus*	Pritsa (34)
8	*Escherichia coli*	Netrval (31)
9	*P. stutzeri*	Manna (13)
10	*Bacillus circulans*	Hymavathi (36)
11	*B. mesentericus*	Tiul panova (46)
12	*Vibrio succinogenes*	Disteasio (47)
13	*Proteus vulgaris*	Rawaa (48)
14	*Pectobacterium caratovarum*	Sanjay (49)
15	*Tetrahymena pyriformis*	Tsirka (37)
16	*Cylindrocarpon obtusisporum MB-10*	Raha S.K. (40)

Bacterial sources verified to be an abundant source of L-asparaginase as they are easy to handle. There are many

reports concerning the presence of L-asparaginase in various definite bacterial sources such as *Escherichia coli, Erwinia aroideae* and most of the work has been carried out with gram negative bacteria such as *Thermus thermophilus, Vibrio succinogene*. Bokotky and Bezbaruah reported L-asparaginases produced from a new *Erwinia sp.* (2002). Production of L-Asparaginase from the isolated strain of *Bacillus circulans* MTCC 8574 by solid state fermentation reported by Hymavathi.[31-36]

Most of the Industrial researcher as well as Microbiological scientists are proposed to work with *Tetrahymena pyriformis,* because of its maximal activity of the enzyme has been found in stationary phase of growth.[37,38] Some current reports list *Pseudomonas stutzeri*, *Cylindrocarpon obtusisporum*, and *Rhodosporodium toruloides, Corynebacterium glutamicum* as sources.[39,40,13,41] (Table 1)

L-asparaginase: fungal sources

L-Asparaginase enzyme is also isolated from the various fungal sources other than the bacterial sources. Enzyme produced from the bacterial sources has been found to create the allergic reactions and anaphylaxis.[50] Enzyme isolated from fungal source does not have any allergic impacts.

A. terrus, isolated from decomposing of vegetable substrate can be used a better source of L-asparginase production form fungal source.[51] L-asparaginase from Mangrove ecosystem of Bhitarkanika by Gupta & Sarita 2009 has also been reported. Abha Mishra (2006) reported higher yield of the enzyme from a different isolate of *Aspergillus niger*, agrowaste from the leguminous crops as a source.[52] L-asperaginase has been studied in *Aspergillus nidulans*, *Mucor Sp.* and *Cylidrocapron obtusisporum.*[53,54,40] (Table 2)

Table 2: L-asparaginase from fungal sources

S. No	Name of the L-asparaginase producing Fungi	Reference
1	*Aspergillus nidulans*	Drainas and Drainas (53)
2	*Aspergillus tamari*	Verma (55)
3	*Mucor sp.*	Mohapatra (54)
4	*Aspergillus oryzae*	Hendriksen (56)
5	*Aspergillus niger*	Abha Mishra (52)
6	*Aspergillus terreus*	Balasuramanian (57)
7	*Cylidrocapron obtusisporum*	Raha (40)
8	*Penicillium sp.*	Soniamby (58)
9	*Fusarium equiseti*	Hosamani and Kaliwal (59)

L-asparaginase: Actinomycetes as source

Mostafa has reported that several Actinomycetes were present in different strains (*S. Karnatakensis and S. venezuelae*), were isolated from soil under different environmental and nutritional parameters.[60] Gunasekaran reported L-Asparaginase production by *Nocardia sp.*[61] Production of intracellular and extracellular Asparaginase from *Streptomyces longsporusflavus* has been described.[62] Actinomycete strain LA-29 was isolated from the gut contents of the fish, *Mugil cephalus* of the Vellar estuary, which was reported to have outstanding enzyme activity by Sahu.[63]

Dhevagi and Poorani reported the isolation of marine actinomycetes from the Parangipettai and Cochin coastal areas of South India, having enough L-Asparaginase activity.[64] *Streptomyces sp.* isolated from the gut of the fish *Therampon jarbua* and *Villorita cyprinoids* has L-Asparaginase activity.[65] (Table 3)

Table 3: L-asparaginase from different Actinomycetes source

S. No	Name of the L-asparaginase producing Actinomycetes	Reference
1	*Streptomyces karnatakensis*	Mostafa (60)
2	*Streptomyces venezuelae*	Mostafa (60)
3	*Streptomyces collinus*	Mostafa & Salama (66)
4	*Streptomyces griseus ATCC 10137*	Peter J. Dejong (67)
5	*Streptomyces tendae TK-VL_333*	Kavitha and Vijayalakshmi (68)
6	*Actinomycetes from estuarine fishes*	Maloy kumar sahu (69)
7	*Marine actinomycetes*	Dhevangi P. and Poorani E. (64)
8	*Streptomyces albidoflavus*	Narayana (70)
9	*Streptomyces sp. Strain EPD27*	Poorani (71)
10	*Actinomycete strain RAF 10*	Forer (72)
11	*Streptomyces longsporusflavus*	Abdel-Fatah (62)

Properties of L-Asparaginase

Several specifications such as pH, Oxygen, Temperature, several chelating agents such as EDTA play a very key role for the maximal growth of the enzyme producing organisms. Metal ions do not impact the production of L-asparginase. Using some of the agents like 2 mercaptoethanol and glutathione increase the activity of enzyme.[40] Physico-chemical parameters may vary due to their different sources of L-asparginases. L-asparginases from Guinea pig serum have molecular weight 1,38,000 Da and pH 7.5-8.5. It is stable for 6 months at heating for 10 min at 55^{o}C.[52]

Production of Microbial L-Asparaginase

Several research reports about the production of L-asparginase, can be produced from different sources of microorganisms either produce this enzyme constituently or after induction. Several parameters especially physical and chemical parameters for L-asparginase production

vary with the species of microbial source.[73] L-asparaginase is produced throughout the world by submerged fermentation (SmF). This methodology has many disadvantages, such as net yield is low and sequential handling, reduction and removal of large volumes of water during the downstream processing. Another alternative source to SmF is solid state fermentation (SSF) which is offering a wide range of advantages compared to SmF. Solid state fermentation is a very successful technique, as the yield of the product is many times higher and low capital investment, Better product recovery. Solid state fermentation holds incredible potential for the production of secondary metabolites has been increased in recent years.[74]

Activators and Inhibitors of L-Asparaginase

It has been reported that there are some elements which influence the activity of the enzyme, some activates the enzyme and increase the activity up to many folds and some inhibit the activity.

Some metal ions inhibit the enzyme activity, such as Zn2+, Hg2+, Fe2+, Cu2+, and Ni2+, while metal chelators enhanced the activity like EDTA, CN-, cysteine, etc., indicating that the enzyme was not a metalloprotein. Its activity was also increased in the presence of reduced glutathione but not with dithiothreitol and 2-mercaptoethanol.[41]

Other elements including Cu2+, Li+, diphosphate, EDTA, I-, Mg2+ has been reported to affect the activity of L-Asparaginase, isolated from different sources, directly or indirectly.

Applications of L-Asparaginase in Different Fields

Role in food processing

L-asparaginase has a remarkable role in food industry. Modern advances in food technology have demonstrated that fried and baked food (particularly fried potato) contains a significant amount of acrylamide.[75,76] Acrylamide is a significant toxic agent that causes neurotoxicity in humans and is present in adequate amounts in food items which are heat-derived, containing some reducing sugars.

A heat inducing reaction between the free amino acid asparagine and carbonyl group of reducing sugars like glucose, reaction is named as Maillard reaction.

The formation of acrylamide is significantly reduced by the hydrolyzing of asparagine catalyzed by the enzyme. Reduction in acrylamide content is reported as 90%.[77]

Kukurova K conducted an experiment on different parameters such as which influence the reaction, formation of acrylamide, temperature and time profile of frying process, moisture, sugars, amino acids and some indicators of Maillard reaction.

They got a 96-97 % of reduction in acrylamide content at different levels of asparagines.[78]

Making biosensor

The enzyme L- Asparaginase has also been used for modelling a diagnostic biosensor and as the amount of ammonia produced by the action of the enzyme that exactly correlates to the level of L- asparagine in a patient's blood.[55]

Role in aminoacid metabolism

L-Asparaginase also plays a very essential role in biosynthesis of the aspartic family of aminoacids. Lysine, threonine and methionine commercially important aminoacids produced by *C. glutamicum*, which are produced under normal physiological conditions, might be limiting for lysine and/or threonine biosynthesis.

Away from Kreb's cycle (using glutamic acid as aminoacid donor), aspartic acid is formed from asparagine by the action of asparaginase.[39]

As antitumor agent

L-asparaginase has been clinically acceptable enzyme for the treatment of different types of blood cancers such as acute lymphocytic leukemia (ALL, mainly in children), Hodgkin disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarcoma, and melanosarcoma.[79]

L-asparaginase is a medicinally suggestive enzyme used for treatment in all pediatric regimens and in the majority of adult treatment protocols.[80]

Among the number of treatments of acute leukemia such as steroids, radiation therapy, severe combined treatments including bone marrow or stem cell transplants etc, and chemotherapy is most preferable.

The drugs most usually employed for treatment includes, asparaginase, daunorubicin, cyclophosphamide, mercaptopurine, methotrexate etc.[81]

Many scientists have described the role of L-asparaginase in the treatment of cancer and cancer biology. A vast amount of investment has been made on the enzyme to discover the new successful ways.

Future Aspect

L-asparaginase enzyme has been a major research area for acute lymphoblastic leukemia and lymph sarcoma has been one of the major eminent diseases of recent times.

This paper shows that L-asparaginase has a great possible application in clinical research.

CONCLUSION

From the past 30 years, a lot of research had been done for the role of L-asparaginase as a powerful antitumor drug.

But, it is observed that the action of enzyme is combined with some side effects and the yield of enzyme was not enough to fulfill the demand of the drug.

So this review mainly focuses by using marine sources and to enhance the yield and decrease the side effects of the enzyme.

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Source of Support: Nil, **Conflict of Interest:** None.