ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Comprehensive Review On Microbial L-Asparaginase And Its Application.

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ABSTRACT

Over the past half century, cancer has posed a significant problem and a threat to human health. According to the WHO website, 8.2 million people died from cancer, and in 2012, 14.1 million cancer cases were recorded, with 60% of the incidents taking place in Africa, Asia, and Central and South America. Of the world's top 10 leading causes of death, lung cancer (2.7%, including trachea and bronchus cancer) was 7th and caused 1.5 million (2.7%) deaths in 2011, higher than 1.2 million (2.2%, 9th) deaths in 2000. In the United States, 1,660,290 new cancer cases, with 580,350 cancer deaths projected to occur in 2013, pedestalling cancer as the second leading cause of death, exceeded only by heart diseases.

Keywords: L-Asparaginase, human health, WHO, cancer

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ISSN: 0975-8585

OVERVIEW OF CANCER

A standard cell turns into a cancer cell when one or more mutations occur in its DNA, which can be acquired or inherited (Haber and Fearon, 1998). However, carcinogenesis is a complex multistage process, which involves more than one genetic change as well as other epigenetic factors (hormonal, carcinogenic and tumor-promoter effects) that do not themselves produce cancer but increase the chance of the genetic mutations resulting in this disease (Sundar, 2014).

The chemotherapy of cancer:

Since the 1950s, significant advances were apparent in the chemotherapeutic management of cancer. Unfortunately, the chemotherapy of solid tumors has had only limited effectiveness with a few exceptions. Thus, the majority of disseminated cancers do not respond to the current chemotherapeutic treatments (Sundar, 2014).

Cytotoxic drugs are not cancer-selective and are therefore active against both tumor and normal cells, which gives the drugs limited efficacy and significant toxicity (Minchinton and Tannock, 2006). Thus, identifying targets and agents, which are tumor-selective, is critical.

Role of Enzymes in cancer therapy:

Enzymes are biological catalysts (biocatalysts) that enhance the biochemical reactions in living organisms. They can also be extracted from cells and used to catalyze a range of commercially essential processes (Robinson, 2015).

Enzymes which function as drugs differ from all other types of drugs by two main features. First, proteins are known by their affinity and specificity when they act on their targets. Second, they catalyze and convert multiple target molecules to the desired products. These two features make enzymes specific and potent drugs that can accomplish therapeutic biochemistry in the body that small molecules cannot. These characteristics have resulted in the development of enzyme drugs for many diseases (Sundar, 2014).

Therapeutic enzymes (digestive and metabolic) are applied medically either isolated or adjunct with other therapies to cure diseases like cancer, cystic fibrosis, dermal ulcers, inflammation, gastrointestinal disorders, etc. Enzymes as direct pharmaceutical products have numerous applications, including their uses as antitumor (Kaur and Sekhon, 2012).

Research on cancer provides examples of the use of therapeutics enzyme (Sundar, 2014). Some tumors require the extracellular sources of some amino acids, which are considered as non-essential in normal cells, due to the metabolic lack (Stone et al., 2010; Kuoet al., 2010). Thus, when the enzymatic degradation happens on these amino acids, tumors can be suppressed (Shenet al., 2006). In the treatment of cancer, many enzymes were used, such as arginine-degrading enzymes consist of three main types of proteins: arginine deiminase, arginase and arginine decarboxylase which exist in archaea, bacteria, and eukarya (Zúñigaet al., 2002; Knodleret al., 1998). L-arginase (EC 3.5.3.1) is a therapeutic enzyme that catalyzes the irreversible hydrolysis of L-arginine to l-ornithine and urea. This enzyme found in bacteria, fungi, yeast, actinomycetes, algae and plants, and It has gained importance due to its potential effect as an anti-cancer agent against tumor cells (Unissaet al., 2015); L-methioninase (EC 4.4.1.11) as a pyridoxal 5'-phosphate-dependent multifunctional enzyme (Takakuraet al., 2004). L-methioninase catalyzes the breakdown of L-methionine to α-ketobutyric acid, methanethiol, and ammonia (Ronda et al., 2011). The activity of L-methioninase was extensively documented against several types of cancers including breast, kidney, colon, lung, and prostate cell lines (Tan et al., 1996). L-methioninase is ubiquitous in almost of organisms, including bacteria, fungi, protozoa, and plants, except mammals (El-Sayed, 2010),and L-glutaminase activity is widely distributed in an animal, plant tissues and microorganisms including bacteria, fungi, and actinomycetes. Microbial L-glutaminase (L-glutamine amido hydrolase EC 3.5.1.2) has received greater attention for its potential biotechnological applications and easiness in large-scale production (Binodet al., 2017).

The L-glutaminase has also shown good radical scavenging activity which could have used in the medical field, like anti-tumor agent (More et al., 2016).

L-asparaginaseis the example of amino acid depriving enzymes, which has been applied in the treatment of acute lymphoblastic leukemia for many years (Piatkowska-Jakubaset al., 2008).

L-asparaginase as an anticancer therapy

Hundred different enzymes have been identified, and many of them have been characterized to a considerable degree. Quite a few of these enzymes have a future of particular interest and command the attention of loyal bands of investigators. However, only a relatively small number have moved to the center stage and turned into the objects of extensive investigations. Such enzymes come up with unique experimental advantages, in particular, the protein ASNase (Batoolet al., 2016). ASNase (E.C. 3.5.1.1) is an amidohydrolase that widely distributed, which found in plants and microbial sources, barley rootlets, and animal tissues (livers of rat, pancreas,tissues of fish, liver, brain, ovary, spleen, lung, testes and kidney of mammals and birds) (Adamson andFabro, 1968; Cooney and Handschumacher, 1970).

L-asparaginase catalyzes the hydrolysis L-aspargine (amino acid) into aspartic acid and ammonia, and it is quite challenging to derive it from plants and animals. Therefore, microorganisms are assessed as potential sources for the production ASNase enzyme (Arimaet al., 1972). The history of the ASNase as therapeutic protein dates back to 1922 when (Clementi, 1922) reported that this enzyme is found in the blood of guinea pig serum. First said that guinea pig serum inhibits the growth of lymphosarcoma in mice and rats, providing conclusive evidence of its antitumor activity by (Kidd, 1953). Nevertheless, the search for the constituents present in guinea pig serum that is responsible for the inhibition of tumors in mice and rats was investigated, and it was found that ASNase is a substrate-enzyme, responsible for the hydrolysis of ASNase required for tumor cells (Broome, 1963). Later, it was shown that the amino acid L-asparagine is crucial for the growth of Walker carcinosarcoma 256 in vitro, and it was also demonstrated that cells of mouse leukemia had an absolute nutritional requirement of L-asparagine for growth in vitro (Neuman and McCoy, 1956; Neuman, 1956 ; Haley et al., 1961). As a result, this enzyme plays a significant role in the treatment of Acute Lymphoblastic Leukemia (ALL) (Boyseet al., 1967; Alpar and Lewis, 1985). Clinical trials undertook on ALL patients using ASNase from guinea pig serum, and Escherichia coli showed positive results (Dolowyet al., 1966; Hill et al., 1967). A breakthrough resulted when two researchers (Mashburn and WristonJr, 1964) stated that Escherichia coli ASNase could also inhibit the growth of tumors and this made it a primary interest as it was a well-characterized microorganism. Two ASNase have been found in Escherichia coli B. and are designated EC-1 (periplasmic) and EC-2 (cytoplasmic). One of these enzymes (EC-2) is a potent ant lymphoma agent (Campbell et al., 1967).

Acute lymphoblastic leukemia (ALL) is common among cancer in children. An estimated 6000 new cases of ALL are diagnosed annually in the United States; around 60% of the cases occur among people younger than 20 years (Inabaet al., 2013). L-asparaginase is a cornerstone of treatment protocols for (ALL) and is used for remission induction and intensification treatment in all pediatric regimens and the majority of adult treatment protocols (Pieterset al., 2011a). ASNase is an essential therapeutic enzyme, which combined with other drugs to treat acute lymphoblastic leukemia (mainly in children), acute myelocytic leukemia, acute myelomonocytic leukemia, Hodgkin disease, chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarbom and melanosarcoma (Stecheret al., 1999; Vermaet al., 2007b). ASNase is the first enzyme with anti-leukemic activity to be intensively studied in human beings (AsthanaandAzmi, 2003). Modern clinical treatments of childhood acute lymphoblastic leukemia (ALL) employ enzyme-based methods for depletion of blood asparagine in combination with standard chemotherapeutic agents (Richards andKilberg, 2006).

L-asparagine is a nonessential amino acid that is produced in human cells by an enzyme called L-asparagine syntheses. Tumor cells require a considerable amount of L-asparagine as an essential factor for their survival and growth. During the treatment of patients with ALL, ASNase is injected into the body and start hydrolysis of L-asparagine into aspartate and ammonia as a result of which L-asparagine starvation occur, and cancerous cells die (Broome, 1963).

OTHERS APPLICATIONS OF L-ASPARAGINASE

Food industry: acrylamide formation

Heating the amino acid L-asparagine, naturally found in starchy foods, leads to the Maillard browning reaction, which gives baked or fried food their brown color, crust and toasted flavor (Mottramet al., 2002).

Unfortunately, potential human carcinogens such as acrylamide (Gökmen and Palazoğlu, 2008) and some heterocyclic amines are formed in the Maillard reaction. Acrylamide is produced by L-asparagine and reducing sugars in starch-based foods that are heated above 120°C and prepared by frying or baking or broiling, including potato chips, French fries, cookies, reaction flavors (Tarekeet al., 2002), confirmed that using ASNase enzyme before frying or baking food could decrease more than 99% acrylamide level in the final product (Cachumbaet al., 2016).

Role of L-asparaginase in biosensor

L-asparaginase has been used as a diagnostic biosensor for L-asparagine, because of the amount of ammonia produced by the enzymatic reaction and its impact on the level of L-asparagine in a patient's blood (Vermaet al., 2007a)

Role of L-asparaginase in amino acid metabolism

L-asparaginase plays a vital role in the biosynthesis of an aspartic family of amino acids, namely lysine, threonine, and methionine.BesidesKreb's cycle, aspartic acid is a direct precursor of lysine, and threonine is also formed by the action of ASNase enzyme (Sinhaet al., 2013).

Role of L-asparaginase as an antioxidant:

Antioxidants are found in dietary supplements and have been under investigation to prevent diseases such as cancer, coronary heart disease and even altitude (Baillie et al., 2009). These oxidants can be harmful to the cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA could lead to mutations and potentially cancer, if not reversed by DNA repair mechanisms, and damage to proteins causes enzyme inhibition, denaturation and protein degradation (Stadtman, 1992). L-asparaginase has an anti-oxidant property (Maysaet al., 2010). Antioxidant properties, especially radical scavenging activities, are fundamental due to the harmful role of free radicals in food and biological systems. The reduction of DPPH absorption is indicative of the capacity of the sample to scavenge free radicals, independently of any enzymatic activity. (Rani et al., 2011). The production of L-asparaginase and antioxidant of the bacterial endophytes are associated with the ethnomedicinal plants of India. This takes place because of the isolation of microorganisms in culture is an essential procedure for the study and discovery of new biological activity from organisms. Hence, culture extracts for their bioproperties assessed the endophytic bacteria (Nongkhlawand Joshi, 2015).

Production of L-asparaginase by microorganisms

Microorganisms are the primary source of enzymes because they are stored in large quantities in a short period and genetic manipulations can be done on bacterial cells to enhance the enzyme production. Besides, the microbial enzymes have been paid more attention due to their active and stable nature than proteins from plant and animal (Anbuet al., 2013; Anbuet al., 2015; Gopinathet al., 2013). Microbial enzymes play a significant role in the diagnosis, treatment, biochemical investigation, and monitoring of various dreaded diseases (Gurunget al., 2013). Recent advances in biotechnology, particularly in protein engineering, have provided the basis for the efficient development of enzymes with improved properties. This yielded the establishment of the novel, tailor-made enzymes for entirely new applications, where catalysts were not previously used (Homaeiet al., 2013).

Microorganisms (bacteria, fungi, yeast, actinomycetes, and algae) proved to be proficient sources of this enzyme, although it was also isolated from plant and some animals sources. Microbes are a better source of ASNase because of being bulk in production capacity, Economic; Microbes are natural to form to obtain enzymes with desired characteristics, and easy to extract the protein and purify (Sinhaet al., 2013).

Despite the acknowledgment of the theory behind the utilization of the enzyme in malignancies, its use in therapeutic studies and implementation in treatment protocols met a few challenges in the beginning due to the low availability because at the time the primary source was guineas pigs' serum (Ali et al., 2016). Since the extraction of ASNase from the guinea pig serum in sufficient amounts was difficult, other sources were looked into (Nartaet al., 2007) With the development of microbial submerged cultures, which allowed for

the massive production of biomolecules, they became a luring alternative for being a very efficient and inexpensive source of biomolecules (Wegner, 1990). There is a wide range of microbes comprising bacteria, fungi, yeast, actinomycetes, and algae that produce this enzyme. However, the enzyme proprieties vary from which organism originates from and so it does the possibility of its usage in clinical treatment (Yadavet al., 2014).

L-asparaginase of Bacteria

L-asparaginase has been reported from both Gram-positive and Gram-negative bacterial species from the terrestrial and marine environment (IzadpanahQeshmiet al., 2014). These findings allowed the production of the enzyme in large quantities for the pre-clinical and clinical studies (Grigoropouloset al., 2013).

The dominant bacterial species that produce ASNase include E. coli (Kenariet al., 2011), Erwiniacartovora (Warangkar and Khobragade, 2010), Erwiniaaroideae (Peterson and Ciegler, 1969), Erwiniachrysanthemi (Plourdeet al., 2014), Thermus thermophiles (Pritsaet al., 2001),Staphylococcus aureus (Rozalska and Mikucki, 1992),Staphylococcus sp.–6A (Prakashamet al., 2007) Pseudomonas stuzeri (Manna et al., 1995) , Pseudomonas aeruginosa (Manikandanet al., 2010), Pseudomonas pseudoalcaligenes strain JHS-71 (Badoei-Dalfard, 2016), Vibrio succinogenes (Kafkewitz and Goodman, 1974),Citrobacterfreundi (Davidson et al., 1977) Proteus vulgaris (Tosaet al., 1972) Zymomonasmobilis (Menegatet al., 2016)Bacillus subtilis(Fengetal., 2017), Bacillus licheniformis (Mahajanet al., 2012), Bacillus circulans MTCC 8574 (Hymavathiet al., 2009), Bacillus brevis (Nartaet al., 2011), Enterobacter cloacae (Nawaz et al., 1998), Enterobacteraerogenes (Baskaret al., 2011; Mukherjee et al., 2000), Serratiamarcescens (Ghoshet al., 2013), Corynebacteriumglutamicum (Mesas et al., 1990), ASNase from E. coli and Erwiniacarotovora is currently in clinical use for the treatment of ALL (Asthanaand Azmi, 2003). Lactobacillus, species salivarius isolated from the marine showed potential ASNase activity (Bhargavi and Jayamadhuri, 2016).

Until today, only bacterial derived enzymes from E. coli and Erwiniachrysontheni (E.chrysontheni) have been approved for all treatment, so large ASNase production has just been done with these enzymes as they have been found to have the lowest toxicity. However, despite the lower toxic effects on the patient, the native or unmodified forms of ASNase from bacterial sources present immunogenic complications, resistance and a short half-life that make their clinical application challenging and limits its usage. The toxicity profile of ASNase falls under two main categories, the immunological sensitization to a foreign protein (hypersensitivity) and the adverse events related to inhibition of protein synthesis due to its activity that depletes the L-asparagine, such as neurotoxicity and the most common effect being pancreatitis (Masetti and Passion, 2009; Pieterset al., 2011b). The hypersensitivity reactions presented one of the main restrictions to the clinical use of ASNase, including several types of side reactions (Soareset al., 2002) that include anaphylaxis, pain, edema, Quincke's edema, urticaria, erythema, rash and pruritis (Pieterset al., 2011b).

It has been observed that about 60% of patients were suffering from hypersensitivity reactions during therapy with ASNase from E. Coli. However, the antibodies produced in response to ASNase do not always lead to clinical hypersensitivity, but may instead cause inactivation of the ASNase. The ASNase inactivation ("silent hypersensitivity" or "silent inactivation") leads to a lower concentration in the blood than that needed to achieve the optimal L-asparagine depletion (Pieterset al., 2011b; Shrivastavaet al., 2016) About 30% patients show silent hypersensitivity or silent inactivation. Both enzymes exhibited a high rate of immunogenicity. This immunogenic complication, resistance, and short half-life demanded alternative preparation of ASNase for removal or low frequency of allergic reactions. Researcher attempted to reduce the ASNase potential immunogenicity while preserving its enzymatic activity (Ali et al., 2016) and prolonging its half-life to reduce the frequency of administration, which also increases life quality. Therefore several strategies have been applied, such as protein engineering by site-directed mutagenesis (Ali et al., 2016) and chemical modifications. The chemical modifications that could be less immunogenic and achieve higher half-life were those that could hide the immunogenically active epitopes without compromising the antineoplastic property of the drug (Nartaet al., 2007). A new recombinant E. coli- ASNase preparation is currently undergoing clinical evaluation (Pieterset al., 2011b).

L-asparaginase of Fungai

It has been observed that eukaryote microorganisms similar to yeast and filamentous fungi are likely to produce ASNase (Pinheiroet al., 2001). An extensive profile of biologically active metabolites is described from filamentous fungi of terrestrial origin, mainly from three types: Penicillium, Aspergillus, and Fusarium (SithrangaBoopathy and Kathiresan, 2010). Aspergillus, Penicillium, Fusarium, Helminthosporium, Scophulariopsis, Paecilomyces, andPestalotiopsisscreened from Bhitarkanika mangrove forest soil of Orissa coast (India) were found to be a good source of ASNase (Guptaet al., 2009). Many fungal species producing ASNase were also isolated from soil, for example, Emericellanidulans from different grounds of Tumkur university campus (Jayaramuet al., 2010).The screening of Fusariumequiseti from rhizosphere soil of various plants around Dharwad campus (Hosamani and Kaliwal, 2011).

Certain ASNase producing fungal species also isolated and studied include Cylindrocarponobtusisporum (Rahaet al., 1990), separated the fungus Mucorsp. from the marine sponge Spirastrella sp. which produces the extracellular ASNase (Mohapatraet al.,1997). Aspergillustamari (Sarquiset al., 2004), Fusarium sp.(Tippani and Sivadevuni, 2012), Cladosporium sp. (Kumar et al., 2013), potent ASNase producing Mucorhiemalis was isolated from soil (Monica et al., 2013). The marine fungal Beauveriabassiana strain (MSS18/41) utilized wheat bran for the production of ASNase (Nageswaraet al., 2014) Aspergillusflavus (Patroet al., 2014), Aspergillusterreus (Farag et al., 2015).

L-asparaginase of Actinomycetes

L asparaginase, a bioactive compound obtained from marine Actinomycetes is gaining momentum as an anti-cancerous agent because of its stability in varied environments (Dhevagiand Poorani, 2016).

Streptomyces sp. is the most abundant actinomycetes producing ASNase from marine sources. Streptomyces species are vastly present in marine and terrestrial habitats and exhibit a unique ability for the production of novel metabolites, making them of commercial interest (Manivasaganet al., 2014). Micromonospora sp. is a potentially novel source for ASNase production (Raja et al., 2017).

Several researchers have reported ASNase production potential from various marine action mycetes. Among the actinomycetes, several Streptomyces species such as S. karnatakensis, S. venezualae, S. longisporusflavus, S. ginsengisoli, and a marine Streptomyces sp. PDK2 have been reported to produce ASNase (Deshpandeet al., 2014). Marine actinomycetes were enumerated in sediment samples from Parangipettai and Cochin coastal areas of South India (PDK7 and PDK2), the isolates showed potential ASNase activity (Dhevagiand Poorani, 2006). The actinomycete strain LA-29 separated from the gut contents of the fish, Mugilcephalus (Sahuet al., 2007). An actinomycete strain was isolated from the laterite soils of AcharyaNagarjuna University campus, and the culture was identified as Streptomyces albidofavus (Narayanaet al., 2008). Streptomyces sp. isolates obtained from mangrove area (Gupta et al., 2009). ASNase is an anti-neoplastic agent used in the lymphoblastic leukemia chemotherapy. A recent strain, Streptomyces gulbargensis was explored for the production of extra-cellular ASNase using groundnut cake extract (Amenaet al., 2010).

The isolated from sediment samples collected from the Pitchavaram mangrove ecosystem was identified as Streptomycetesparvulus KUAP106 can be used for the production of ASNase enzyme (Ushaet al., 2011). Streptomyces noursei MTCC 10469, isolated from marine sponge Callyspongiadiffusa (Dharmaraj, 2011). Streptomyces sp. (SS7) separated from the Bay of Bengal and evaluated for the ASNase production (Sivasankaret al., 2013). Rhodococcuserythropolis VLK-12 Isolated from South Coast of Andhra Pradesh, India (Naraganiet al., 2014).

L-asparaginase of Yeast

L asparaginase from yeast S. cerevisiae Administration of foreign proteins may cause immunological problems that limit their usage. Even with all the success given to the bacterial ASNase, there are still side effects, even with the chemical modifications already approved (Dunlop et al., 1978; Piatkowska-Jakubaset al., 2008; Shrivastavaet al., 2016) Therefore, there is a demand for new drug-protein with different immunological proprieties. There are many ASNase producing organisms, from microorganisms to mammals (Nartaet al., 2007; Ali et al., 2016; Lopes et al., 2017;) so it is possible that one of the enzymes might also be of clinical use. One of the many producing organisms is the yeast Saccharomyces cerevisiae. Yeast is the most studied

substance, and there is substantial information available concerning its genetics and cytology making it an excellent subject for the production of new ASNase. S. cerevisiae can do a post-translational modification to proteins such as glycosylation (Cregget al., 1993).

Similarly, to E. coli, S. cerevisiae can produce two distinctly different forms ofASNase. The first form is ASNase I, an internal constitutive enzyme located within the cell. The second one is ASNase II, an external glycoprotein enzyme in the cell wall which is secreted in response to nitrogen starvation (Jones, 1977; Dunlop et al., 1978). ASNase II of S. cerevisiae is a cell wall glycoprotein which has been shown to differ significantly in many aspects from the enzyme of bacterial sources, with lower allergenic potential that would reduce the secondary effects (Dunlop et al., 1980).

ASNase production is also reported by yeasts including Saccharomyces cerevisiae (Bon et al.,1997), Candida utilis (Kilet al.,1995), Candida guilliermondii (Stepanyan and Davtyan, 1998), Rhodosporodiumtoruloids (Ramakrishnan and Joseph, 1996), Pichiapastoris (de Castro Girãoet al., 2016).

L-asparaginase of Microalgae

More recently, production of ASNase from blue-green microalgae is receiving more attention due to its high nutrient contents, low cost of production, cost-effectiveness, no seasonal variation, top efficient producers, being easily cultured and harvested at large scales, and cheaper and easier extraction, and higher yields and purification of protein and enzymes by simple methods are available (Prihantoand Wakayama, 2014).

L-asparaginase is the first such enzyme to be purified from a marine microalgae Chlamydomonassp. (Paul, 1982) Chlamydomonas species and a yellow-green alga, Vaucheriauncinata are known to produce L-asparaginase under specific conditions of temperature, pH and other environmental factors (Ushaet al., 2011), Chlorella Vulgaris cells with the most ASNas activity (Ebrahiminezhadet al., 2014). Arthrospiraplatensis has been scarcely reported as a new candidate for ASNase production (Prihantoand Wakayama, 2014).Spirulinamaxima showed the high yields of ASNase production (Abd El Bakyand El Baroty, 2016).

Marine microorganisms as producers of bioactive compounds

Natural products are the source of the active ingredients of medicines, and this has been the case of drug discovery in ‘olden times' before the advent of high-throughput screening and the post-genomic era. More than 80% of drugs were made of natural products or inspired by a natural compound (Harvey, 2008).

Oceans occupy 75% of the earth's surface and harbor most ancient and diverse forms of organisms (algae, bacteria, fungi, sponges, etc.), serving as an excellent source of natural bioactive molecules, novel therapeutic compounds, and enzymes. The enzyme technology, its current state of the art, unique enzyme properties, and the biocatalytic potential of marine algal, bacterial, fungal, and sponge enzymes have boosted the Marine Biotechnology Industry. Researchers began exploring marine proteins, and today they are preferred over the chemical catalysts for biotechnological applications and functions, encompassing various sectors, namely, domestic, industrial, commercial, and healthcare (Parte et al., 2017).

The marine biosphere is one of the earth's richest many habitats. The oceans are highly complex environments and a diverse assemblage with extreme variations in pressure, salinity, and temperature. Marine microorganisms encompass a complex and diverse grouping of microscopic life forms of which it is estimated that only one percent has been cultured or identified considering the fact that the marine environment is saline and it could provide rare and unique microbial products, particularly enzymes which could be safely used for the human therapeutic purpose (Dhevagiand Poorani, 2006). The particular environmental conditions, involving low temperature, low light, high pressure, and high salinity, give marine residents multiple novel characteristic features, which have been attracting increasing attention from marine biologists (Wang et al., 2016). These features enable marine enzymes to catalyze chemical reactions under extreme conditions, that prove deleterious to most of their terrestrial counterparts and confer upon marine proteins a unique potential for relevant biotechnological applications, both in the manufacture of commercial products and in the health sector (Izadpanahet al., 2018).

This vast marine floral resource will offer great scope to discover novel drugs. It is known that ocean contains a considerable number of natural products and chemical entities with unique biological actions that may be useful in finding efficient and specific drugs for the treatment of human diseases (Haefner, 2003).

The marine environment is inhibited by more than 20,000 natural products discovered over the past 50 years. From these efforts, nine approved drugs and 12 current clinical trial agents have been found, either as natural products or as molecules inspired by the logical product structure. To a significant degree, these came from collections of marine invertebrates mostly obtained from shallow-water tropical ecosystems (Gerwickand Fenner, 2013). Marine organisms proved to be the potent sources of drugs. These are primarily invertebrates that include sponges, soft corals, sea fans, sea hares, nudibranchs, bryozoans, and tunicates. Researchers believed that microbial floras found in the insects are responsible for the production of medicinal elements. The search is mostly focused on marine faunal species, and herbaceous species are ignored. Compounds from marine organisms have antioxidant properties and anticancer activities, but they are primarily unexplored (Sithranga Boopathy and Kathiresan, 2011). It is undeniable that with 3.5 billion years of existence on earth and experience in biosynthesis, the marine microflora remains nature's best source of chemicals. As a result, ongoing research is undertaken to identify ASNase from marine microbial sources with such features, together with low toxicity (Izadpanahet al., 2018).

The biochemical diversity of marine microbial makes them reasonable sources of a wide variety of enzymes to be used in different industries including food, medicine and other biotechnological systems (Beygmoradiand Homaei, 2017).

Production of L-asparaginase under solid state fermentation and submerge fermentation

Solid State Fermentation

L-asparaginase is produced by various microorganism and plants and considered an essential antitumor agent to treat lymphoid proliferative disorders. In recent years, the production of the enzyme based on the solid-state fermentation (SSF) (Yadavet al., 2014). Bacteria and filamentous fungi grow on solid substrates, such as wood, seeds, stems, roots, and leaves of plants in symbiotic associations. Extracellular ease the extraction process, enzymes important from the industrial viewpoint (Hölkeret al., 2004).

The solidstate fermentation outweighs the submerged fermentation, including superior productivity, low capital investment, simple technique, low energy requirement, less water output and better product recovery (Datar, 1986). SSF aims to bring the cultivated fungi or bacteria into tight contact with the insoluble substrate and thus to achieve the highest substrate concentrations for fermentation. This technology results, although so far only on a small scale, in several processing advantages of vital potential economic and ecological importance as compared with SmF. However, several drawbacks of SSF have discouraged the use of this technique for industrial production. The central obstructions are due mainly to the build-up of gradients of temperature, pH, moisture, substrate concentration or pO2 during cultivation, which is difficult to control under limited water availability (Hölkeret al., 2004).

ASNase generated from P. aeruginosa in SSF has been found earlier on soybean meal, wastes from three leguminous crops-bran of Cajanuscajan, Phaseolusmungo and Glycine max (El-Bessoumyet al., 2004; Mishra, 2006). L-asparaginase produced by actinomycetes shows high activity by this technique (Bashaet al., 2009). Aspergillusniger used glycine as a solid substrate and provided a high amount of L-asparaginase (Ali et al., 2017). In cotton cake and wheat bran used for enzyme production by solid state fermentation using Bacillus sp. (Pawaret al., 2014).

Submerged Fermentation

Submerged fermentation (SmF), is where the growth of microbes takes place in liquid broth media, by optimizing essential nutrients to enhance cultivation of bacteria. During this procedure, selective microbes grow carefully in a closed reactor that has fermentation media and a high oxygen concentration. SmF has well-established equipment that makes use of the existing microorganisms. Among different microbes, bacteria are usually used as a source in this process because they need the high content of moisture. Fungal species such as Aspergillus tamari, Aspergillusniger, Aspergillusterreus, Fusarium, and Penicillium are reported for ASNase

production by SmF, and studies on the optimization of culture media and environmental conditions in both batch and ongoing SmF have been published (Patroand Gupta, 2012). Production of ASNase depends upon the type of the microorganism as well as on different factors: nutrient composition, temperature, pH of the medium, inoculation concentration, dissolved oxygen concentration and fermentation time (Kumar and Sobha, 2012). Submerged fermentation has disadvantages such as the low concentration production reduction and disposal of the large quantity of water during the downstream processing (El-Bessoumyet al., 2004).

L-asparaginase Side Effects

L-asparaginase results in specific side effects, and despite its potential antileukemic activity, using ASNase by leukemic patients causes lethality to normal cells. ASNase produces a range of symptoms such as edema, skin rashes, fever, hepatic dysfunction, diabetes, leucopenia, pancreatitis, neurological seizures, and hemorrhage (Duval et al., 2002). Moolaet al. (1994) stated that ASNase-based drugs lead to some hypersensitivity reactions, mild allergic reactions, and anaphylactic shock. Adolescents are prone to neurotoxicity caused by ASNase, which results in depression, fatigue, lethargy, dizziness, and agitation (Pochedly, 1977).

L-asparaginase in the commercial market

Enzyme drug "ASNase " is available in the market under the trade names of Elspar, Oncaspar (Pegaspargase, modified version), Crisantaspase, Kidrolase and, Erwinase. Elspar (ASNase) is of type EC-2, derived from E. coli which contains the enzyme L-Asparagine amidohydrolase. Crisantaspase is combined with other anti-cancer drugs and obtained from Erwiniachrysanthemi. Kidrolase indicated in the treatment of ALL, Leukemic meningitis and non-Hodgkin's lymphoma. Erwinase exists as a part of treatment protocols along with chemotherapy or radiotherapy. As the native preparations have various drawbacks, including hypersensitive reactions, modified forms of the drug achieve tremendous attention in clinical trials. Enzyme combined with polyethylene glycol (PEG) has been found to be safe to administer to patients suffered from an allergy to native preparations (Ramyaet al., 2012)

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