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Fungal L-asparaginases- A mini review

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ABSTRACT

L-asparaginase is an enzyme that catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonia. These enzymes are mainly produced by microbes. Microbial L-asparaginase has received much importance in the field of medicine for treating leukemia particularly acute lymphoblastic leukemia (ALL).This enzyme is also used extensively in food industries for reducing the formation of acrylamide in starch based foods. In this review we focus on the recent developments in the production (submerged and solid state fermentation), purification and applications of fungal L-asparaginases in medicine and acrylamide mitigation. Additional research is however required for exploring novel fungal L-asparaginase for confirming their potentiality in leukemia treatment and acrylamide mitigation.

Keywords: Fungi, Fermentation, L-asparaginase, Leukemia, Acrylamide

INTRODUCTION

Enzymes are proteinaceous molecules with biocatalytic potential and catalyze the biochemical reaction by speeding up the rate of reaction by reducing the energy for activation (Godfrey and West, 1996). Approximately 4000 enzymes are identified till today and many are in use commercially. Most of the enzymes used in industries are from microbial source. The enzymes from microbial source are more advantageous than other sources. Microbial enzymes are highly specific, stable and easy to produce in larger quantities (Wiseman, 1995). Enzymes from fungal origin are particularly well suited for industrial applications. Filamentous fungi are the preferred source of industrial and clinically important enzymes because fungi synthesize and secrete higher quantities of enzymes. More than 270 enzymes have been commercialized by Members of the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) of which 60% of the enzymes are of fungal origin (**Fig. 1**).

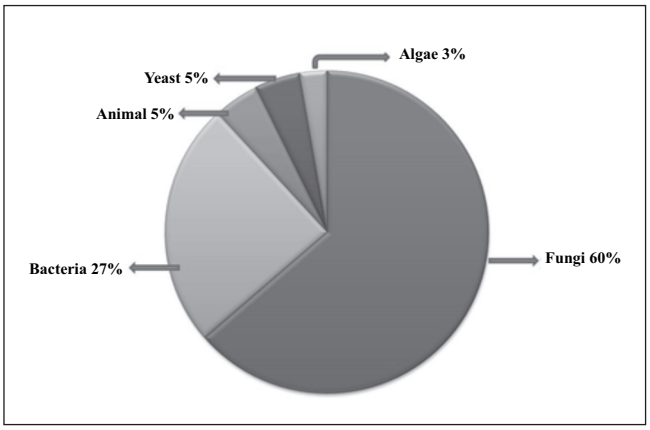

Fig. 1: Distribution of host organisms for production of industrial enzymes based on data from AMFEP (2009).

Fungal enzymes are extensively used in food, textile, detergent, leather, paper industries, and therapeutics. The enzymes which are medically used with other therapies for treating various metabolic disorders and disease like cancer are called therapeutic enzymes. The enzymes such as streptokinase, creatine kinase, L-asparaginase, chymotrypsin, urokinase, cytochrome oxidase and nattokinase are the commonly used therapeutic enzymes. Fungal enzymes with therapeutic potential are generally used as anticoagulants, anti-inflammatory, oncolytics and thrombolytics. The most promising use of therapeutic enzyme is in the treatment of cancer. The leading class of the therapeutic enzymes are L-asparaginases which accounts for 40% of the overall sales as anti leukemic agents (Warangkar *et al.,* 2009).

The enzyme L-asparaginase catalyze the breakdown of L-asparagine to L-aspartic acid and ammonia (**Fig. 2**). L-asparaginase (L-asparagine amido hydrolase E.C.3.5.1.1) belong to amidase group with anticancer properties and widely used for treating acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia, acute myelomonocytic leukemia, acute myelocytic leukemia, melanosarcoma, lympho and reticulosarcoma (Stecher *et al.,* 1999; Duval *et al.,* 2002). It is also widely used for lowering acrylamide levels from starchy foods in food and baking industries (Friedman, 2003; Pedreschi *et al.,* 2008).

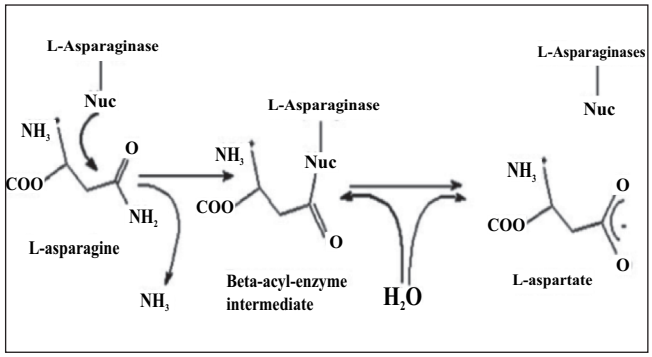

Fig. 2: Mode of action of L-asparaginase (Hill *et al.,* 1967).

Sources of L-asparaginase

L-asparaginase is broadly dispersed among microbes (bacteria, fungi, actinomycetes, yeast and algae), plants and animals. However, microbial L-asparaginase has received much interest as microbes can be cultured easily and production rates are higher (**Table 1**). The production of L-

Table 1: L-asparaginase producing microorganisms

Prokaryotes Bacteria	**Reference**	**Eukaryotes Fungi**	**Reference**
Bacillus brevis	Narta *et al*., 2011	*Alternaria sp.*	Nagarajan *et al*., 2014
Bacillus aryabhattai	Singh and Srivastava, 2013	*Aspergillus flavus*	Krishna *et al*., 2013
Bacillus cereus	Thenmozhi *et al*., 2011	*Aspergillus fumigatus*	Dutta *et al*., 2015
Bacillus subtilis	Jia *et al*., 2013	*Aspergillus nidulans*	Archana *et al*., 2014
Escherichia coli	Netrval, 1977	*Aspergillus niger*	Sharma and Mishra, 2021
Erwinia cartovora	Kamble *et al*., 2006	*Aspergillus oryzae*	Henrdiksen *et al*., 2009
Erwinia chrysanthemi	Moola *et al*., 1994	*Aspergillus terreus*	Baskar *et al*., 2010
Pseudomonas aeruginosa	Manikandan *et al*., 2010	*Bipolaris* sp.	Lampak *et al*., 2010
Pseudomonas fluorescens	Kishore *et al*., 2015	*Cladosporium* sp.	Kumar and Manonmani, 2013
Serratia marcescens	Agarwal *et al*., 2011	*Cladosporium tenuissimum*	Hamed *et al*., 2021
Staphylococcus sp.	Prakasham *et al*., 2007	*Mucor hiemalis*	Thakur *et al*., 2014
Actinomycetes		*Emericella nidulans*	Jayaramu *et al*., 2010
Streptomyces sp.	El-Sabbagh *et al*., 2013	*Fusarium culmorum* (ASP 87)	Meghavarnam *et al*., 2017
Streptomyces albidoflavus	Narayana *et al*., 2008	*Fusarium equiseti*	Hosamani and Kaliwal, 2011
Streptomyces brollosae	El-Naggar *et al*., 2017	*Fusarium solani*	Nakahama *et al*., 1973
Streptomyces griseoluteus	Kumari *et al*., 2013	*Fusarium oxysporum*	Niharika and Supriya, 2014
Streptomyces gulbargensis	Amena *et al*., 2010	*Penicillium* sp.	Soniyamby *et al*., 2011
Streptomyces noursei	Dharmaraj, 2011	*Penicillium brevicompactum*	Elshafei *et al*., 2012
Streptomyces rochei	El-Ahmady *et al*., 2020	*Penicillium digitatum*	Shrivastava *et al*., 2012
Streptomyces tendae	Kavitha and Vijaylakshmi, 2010	*Trichoderma viridae*	Linclon *et al*., 2015
Thermoactinomyces vulgaris	Mostafa and Ali, 1983	**Yeast**	
Nocardia sp.	Kavitha and Vijaylakshmi, 2012	*Candida bombicola*	Deverey an d Pakshirajan, 2010
		Leucosporidium muscorum	Freire *et al*., 2021
		Pichia polymorpha	Foda *et al*., 1980
		Rhodosporodium toruloides	Ramakrishnan and Joseph, 1996

asparaginase is reported in both prokaryotes and eukaryotes. L-asparaginase produced by the bacterial strains *Escherichia coli* and *Erwinia chrysanthemi* are commonly used for treating ALL (Yano *et al.,* 2008; Onishi *et al.,* 2010; Kenari *et al.,* 2011). L-asparaginase from the bacterial source are commercially available with the brand names Crasnitin, Crasnitine, Elspar, Erwinase, Kidrolase, Laspar, Leucogen, Leunase, Oncaspar, and Paronal which are used as medicines (Vimal and Kumar, 2022). Fungal L-asparaginase under the brand name Preventase and Acrylaway are used in food industries for reducing acrylamide formation (Halliday, 2007).

Long term use of bacterial L-asparaginase has few side effects such as acute pancreatitis (Weetman and Bachner, 1974), liver dysfunction, hyperglycemia (Oettgen *et al.,* 1970), immuno-suppression (Muller and Boss, 1998), leucopenia (Duval *et al.,* 2002), neurological seizures (Pochedly, 1977) and hyper-sensitive reactions resulting in allergy and anaphylaxis. (Rossi *et al.,* 2004; Pieters *et al.,* 2011; Ramya *et al.,* 2012; Noura *et al.,* 2014).

Hence, L-asparaginase from eukaryotic microbes has gained much attention. The fungal L-asparaginase was found to have lesser side effects than bacterial L-asparaginase (Raha *et al.,* 1990; Ali *et al.,* 1994). In fungal L-asparaginase production, the mechanism termed nitrogen catabolic repression exists and suppresses the synthesis of other catabolic enzymes (Sarquis *et al.,* 2004). Hence, the demand for fungal L-asparaginase has increased in the recent years due to its promising applications in food processing industries and in therapeutics.

APPLICATIONS OF L-ASPARAGINASE

In pharmaceutical industry

L-asparaginase is used to treat several types of lymphocytic diseases like non-Hodgkin lymphomas, acute and chronic lymphoblastic leukemia (Pieters *et al.,* 2011). The leukemic cells require an essential amino acid L-asparagine in higher amounts for their malignant growth and development. But these cells lack the enzyme asparagine synthetase which is necessary for the synthesis of L-asparagine. Hence, these cells depend on the exogenic supply of the asparagine present in the serum for their survival and proliferation (Oza *et al.,* 2011). However, the normal cells possess two key enzymes transaminase and L-asparagine synthetase. Initially trans-aminase catalyzes the conversion of oxaloacetate to aspartate later L-asparagine syntheatse converts aspartate to L-asparagine (Duval *et al.,* 2002). Hence, L-asparaginase treatment depletes the levels of serum L-asparagine by hydrolyzing it to aspartic acid and ammonia (**Fig. 3**). The depletion of L-asparagine does not affect the normal cells as they are able to synthesize. The depletion of L-asparagine in leukemic cell lines leads to mRNA and protein synthesis inhibition and also results in induction of cell cycle arrest and apoptosis (Broome, 1968; Patterson and Maxwell, 1970). The enzyme has also been used in developing a biosensor for analyzing the levels of L-asparagine in leukemic patients and in food industries (Verma *et al.,* 2007; Kataria *et al.,* 2015).

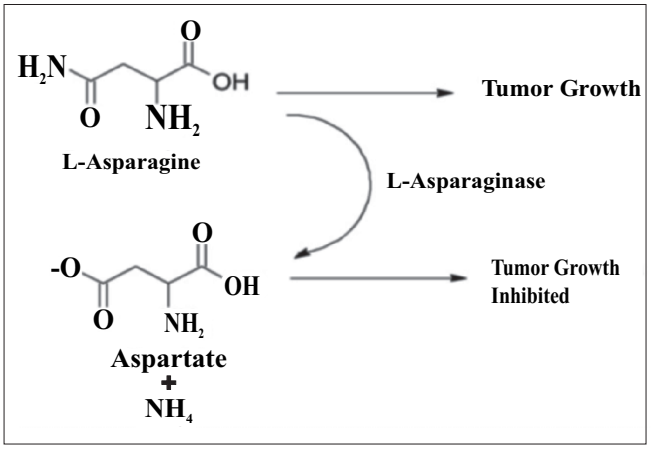

Fig. 3: Diagrammatic representation of mode of action of L-asparaginase inhibiting the growth of tumor cells (Narta *et al.,* 2007).

In food industry

L-asparaginase has received much interest in recent years as an aid in food processing industries for reducing acrylamide formation in starchy foods (Kukurova *et al.,* 2009; Pedreschi *et al.,* 2011). Acrylamide (2-propenamide) is a colourless, odourless, crystalline non volatile substance formed when starchy foods are baked or fried at 120°C or above and are grouped as "potentially carcinogenic to humans" (IARC 1994). Various foods like French fries, biscuits, fried potatoes, bread and coffee prepared by frying, baking or roasting contain high concentration of acrylamide, which affects human health and also proved to be carcinogenic, genotoxic and neurotoxic (Hogervorst *et al.,* 2007; Olesen *et al.,* 2008). Hence, reducing the concentration of acrylamide to moderate levels in these foods is gaining much importance (Tareke *et al.,* 2002). Recent research focuses on acrylamide formation mechanism in food during processing and alleviation of acrylamide by effective treatment. During food heating, acrylamide is formed by a process known as Maillard reaction where the carbonyl group of the reducing sugar reacts with the amino group of the L-asparagine to form acrylamide. Becalski *et al.* (2003) stated that the key precursor for the acrylamide formation is L-asparagine. The use of L-asparaginase in food processing selectively reduces the levels of free L-asparagine in food by hydrolyzing it to aspartic acid and ammonia, hence lowering the risk of acrylamide formation (**Fig. 4**) (Kornbrust *et al.,* 2009). Recently, the Joint WHO/FAO Expert Committee on Food Additives has universally accepted the L-asparaginase produced by *Aspergillus niger* and *Aspergillus oryzae* can be used for reducing the acrylamide formation in food processing (Kornbrust *et al.,* 2009; Hendriksen *et al.,* 2009; Anese *et al.,* 2011).

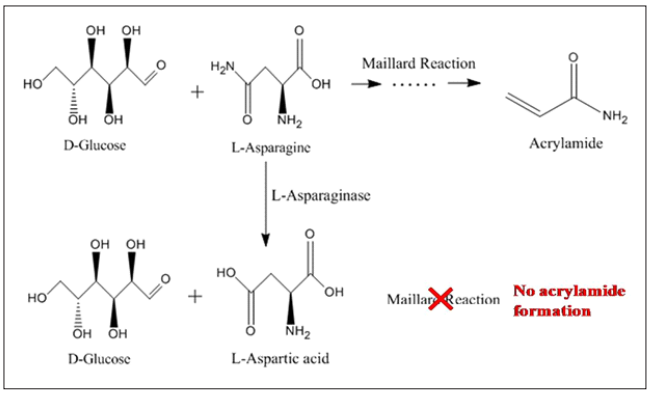

Fig. 4: Diagrammatic representation of L-asparaginase reducing acrylamide formation during food processing.

History of L-asparaginase

The first therapeutic enzyme identified with anticancer potential was L-asparaginase. The activity of L-asparaginase was first identified in beef tissues by Lang in 1904 (Lang and Uber, 1904). Furth and Friedman in 1910, studied the hydrolysis of L-asparagine in pig and horse organs and proved that its activity occurs in all animal tissues (Furth and Friedman, 1910). In 1922, Clementi reported L-asparaginase activity in omnivorous animals (serum of guinea pig) (Clementi, 1922). Forty years later, Kidd discovered that serum of guinea pig repressed a number of transplanted lymphomas in rats and mice (Kidd, 1953). Neuman and McCoy in 1956, reported that L-asparagine is required for the growth of Walker carcinosarcoma cell lines (Neuman and McCoy, 1956). In 1961, Broome demonstrated the anticancer potential of L-asparaginase in guinea pig serum (Broome, 1961). Broome (1965) recorded the anticancer activity of L-asparaginase from bacteria and fungi. First microbial L-asparaginase was extracted from *Escherichia coli* (Mashburn and Wriston, 1964). Oettgen *et al.* (1967) proved the effectiveness of L-asparaginase in treating human with leukemia. In recent years, use of L-asparaginase has expanded immensely in treating leukemia and other lymphoproliferative illness. L-asparaginase is approved by WHO and FDA as a promising agent for treating acute lymphoblstic leukemia (ALL).

Isolation and screening of L-asparaginase producing microorganisms

Microbes are considered as the best source of L-asparaginase from the time it was discovered in *E. coli* (Mashburn and Wriston, 1964). Since then, researchers all over the world started to screen microbes for L-asparaginase production. Any effective screening procedure should be fast, sensitive, rapid, stable, repeatable and cost effective. Gulati *et al.* (1997) designed a rapid pH and dye (phenol red) based plate assay for screening L-asparaginase producing microbes. This method was frequently used by researchers for L-asparaginase screening. Later, Mahajan *et al.* (2013) improved the screening method by using bromothymol blue (BTB) as pH indicator. Meghavarnam and Janakiraman (2015b) compared two pH indicator dyes phenol red and bromo cresol purple for screening L-asparaginase producers, and reported bromo cresol purple is an effective pH indicator than phenol red for screening L-asparaginase producing microbes. On the contrary, Doriya and Kumar, (2016) screened for L-asparaginase producers using two indicator dyes bromothymol blue and phenol red and reported bromothymol blue is more sensitive indicator dye than phenol red for L-asparaginase screening. Mihooliya *et al.* (2017) compared three pH indicator dyes (bromothymol blue, phenol red and cresol red) for screening L-asparaginase and reported cresol red can be used as an indicator which produced better results in contrast to other screening assays.

Production of L-asparaginase under submerged fermentation (SmF)

Metabolite production by microorganisms varies qualitatively and quantitatively depending on the strain used for production as well as on the cultural conditions and nutrition provided (Wang *et al.,* 2010). Any fermentation process is affected by many factors like pH of the medium, incubation temperature, composition and nature of the medium, dissolved oxygen and operational systems. Fluctuations in these factors will alter the rate of fermentation and formation of product. Therefore, formulation of the growth medium and optimization of fermentation parameters is necessary to enhance the enzyme yield.

L-asparaginase production by different microbes has been reported. The production of L-asparaginase is widely carried out by submerged fermentation. Studies were carried out for optimizing the nutritional and cultural parameters for L-asparaginase production in both batch and continuous fermentation. The production of L-asparaginase is affected by the types of carbon and nitrogen sources, temperature, pH, incubation time and oxygen transfer rate. Several techniques such as response surface methodology, genetic algorithm and Plackett Burman experimental design were used for optimizing the production conditions of L-asparaginase. The different methods and optimization techniques used for the production of L-asparaginase under submerged fermentation is given in **table 2**.

Production of L-asparaginase under solid state fermentation (SSF)

The fermentation process in which the organism is grown on insoluble substrate with sufficient moisture in the absence of water is known as solid state fermentation (SSF) (Lonsane *et al.,* 1992; Pandey, 1992). Solid state fermentation is gaining a lot of significance in the past decade and has come out as a cost effective, inexpensive method for the production of enzymes and other commercially valuable products by making use of industrial and agricultural wastes as substrates (Pandey, 1994). In industries, solid state fermentation has been broadly used for the manufacture of many products as this method has advantages like improved process control, lower contamination chances, maximal utilization of

Table 2: Summary of growth conditions for L-asparaginase production under submerged fermentation.

Microorganism	Medium	Statistical design	Carbon source (w/v)	Nitrogen source (w/v)	pH	Temperature (°C)	L-asparaginase activity U/ml	Reference
Aspergillus terreus	Modified Czapek-Dox	ND	ND	2% Proline	ND	ND	58	Sarquis *et al.*, 2004
Aspergillus terreus MTCC 1782	Modified Czapek-Dox	Latin square design	ND	Proline	6.2	30	26.47	Baskar and Renganathan, 2009
Bipolaris sp.	Modified Czapek-Dox	ND	0.4% glucose	1% asparagine	ND	30	6.3	Lapmak *et al.*, 2010
Aspergillus terreus MTCC 1782	Modified Czapek-Dox	Latin square design	0.2% glucose	1% urea	6.2	30	33.25	Baskar *et al.*, 2010
Aspergillus aculeatus	Czapek-Dox	ND	0.2% glucose	1% asparagine	6.2	30	380.14	Dange and Penshwe, 2011
Fusarium sp.	Asthana Hawkers medium	ND	Glucose	Proline	ND	ND	328 IU/ml	Tippani and Sivadevuni, 2012
Aspergillus niger	Czapek-Dox	ND	1% glucose	1% corn steep liquor	6.5	35	2.83	Zia *et al.*, 2013
Aspergillus terreus	Modified Czapek-Dox	ND	0.5% sucrose	1% asparagine	6.2	30	34.25	Anamika *et al.*, 2013
Trichoderma viride	Modified Czapek-Dox	ND	0.6% maltose	0.5% peptone	6.5	37	650	Lincoln and More, 2014
Mucor hiemalis	Modified Czapek-Dox	ND	0.4% glucose	1.25% asparagine	7	30	1203.55	Thakur *et al.*, 2014
Penicillium cyclopium	Modified Czapek-Dox	Plackett-Burman Design	ND	ND	6.2	30	160	El-Refai *et al.*, 2014
Aspergillus terreus	Modified Glucose Czapek-Dox	Plackett-Burman Design	0.2% glucose	0.3 % asparagine	6.0	35	10.97	Farag *et al.*, 2015
Fusarium culmorum (ASP-87)	Modified Czapek-Dox	One factor at a time	0.2% citric acid	0.5% ammonium chloride	7.2	30	3.12	Meghavarnam and Janakiraman, 2015c
Aspergillus oryzae CCT 3940	Modified Czapek-Dox	Plackett-Burman Design	0.5% glucose	2% Proline, 0.2% asparagine	8	30	67.49	Dias and Sato, 2016
Aspergillus terreus MTCC 1782	Modified Czapek-Dox	ND	0.2% glucose	1% asparagine	6.2	30	33.59	Doriya and Kumar, 2016
Aspergillus terreus CCT 7693	Modified Czapek-Dox	Central composite design	0.2% glucose	1% proline	9.49	34.6	13.81	Costa-Silva *et al.*, 2018
Fusarium oxysporum	Modified Czapek-Dox	Taguchi design	0.2% glucose	1% asparagine	5.0	40	143	Ali *et al.*, 2018
Aspergillus niger	Modified Czapek-Dox	Central composite design	Glucose	L-asparagine	ND	ND	13.1252	Vala *et al.*, 2018
Cladosporium tenuisssimum	Modified Czapek-Dox	Central composite design	ND	ND	6.2	37	2.6471	Hamed *et al.*, 2021

ND : No Data

substrate, better product stability and simple recovery and product purification steps. Though microbial L-asparaginases are generally produced by submerged fermentation, solid state fermentation too showed promising results. (Subramaniyam and Vimala, 2012; Meghavarnam and Janakiraman, 2017). Different solid substrates like sugarcane bagasse, rice bran, wheat bran, jowar bran, cotton seed oil cake, sesame oil cake, coconut oil cake, groundnut oil cake, bengal gram husk, green gram husk, red gram husk, black gram husk, corn pith, maize cob, paddy straw, maize straw, ragi straw, soya bean meal and carob pod are used as substrates for L-asparaginase production (El-Bessoumy *et al.,* 2003; Hymavathi *et al.,* 2009; Kumar *et al.,* 2013; Meghavarnam and Janakiraman, 2017). The solid substrates used for the production of L-asparaginase under solid state fermentation were summarized in **table 3**.

Purification and characterization of L-asparaginase

Purification of enzymes is an essential step for studying its properties and functions. The final quality of the biotechnological products is determined by their purification level. The purification step is regarded as the most important step in the entire production process. Since L-asparaginase is a therapeutic enzyme used as drug, its purity is of primary importance. For purification of L-asparaginase from microbes, various techniques have been developed (**Table 4**). Purification steps generally depended on techniques such as precipitation, chromatography and gel filtration (Muharram, 2014). Ammonium sulphate precipitation method has been employed initially for the purification of enzymes followed by different chromatographic techniques such as ion exchange chromatography, gel filtration chromatography, affinity chromatography and HPLC.

Recombinant L-asparaginase

Genetic engineering is a key approach for improving the yield of value added products. In recent years, researchers have used this approach for increasing the expression of L-asparaginase gene. Ferrara *et al.* (2006) reported the heterologous expression of *Saccharomyces cerevisiae* ASP3 gene in *Pichia pastoris* which resulted in increased L-asparaginase yield (1083 U/l). The *Rhizomucor miehei* L-asparaginase (RmAsnase) was cloned and expressed in *Escherichia coli* (Huang *et al.,* 2014).

Anticancer properties of fungal L-asparaginase

L-asparaginase is a key therapeutic agent used for treating a range of lymphoproliferative disorders and in particular acute lymphoblastic leukemia. L-asparaginase is used in combination with chemotherapy for treating pediatric acute lymphoblastic leukemia (ALL) for more than 30 years (Amylon *et al.,* 1999; Hann *et al.,* 2000). Anti-tumor activity of the enzyme was evaluated on various cell lines such as human myelogenous leukemia K-562 (Mahajan *et al.,* 2014), human myeloid leukemia HL-60 (Husain *et al.,* 2016), gastric cancer (AGS) (Selvam and Vishnupriya, 2013), human prostrate cancer PC-3 and human colon cancer CACO-2 (Mohamed, 2014), Hepato cellular carcinoma (Hep-G2) (Elshafei *et al.,* 2012), human breast cancer MCF-7 (Rani *et al.,* 2011) human T lymphoblastic leukemia cell line (MOLT-4) and human gastric cancer cell line (MKN-28) (Cappelletti *et al.,* 2008).

L-asparaginase from endophytic fungi *Colletotrichum* sp. E5T9 decreased the survival rate of CACO-2 cells (human colon adenocarcinoma) and HepG2 cells (human hepatocyte carcinoma) by 70% (Theantana *et al.,* 2009). The growth of hepatocellular carcinoma (Hep-G2) was inhibited by L-asparaginase purified from *Penicillium brevicompactum* with IC_{50} value of 43.3 µg/ml (Elshafei *et al.*, 2012). Loureiro *et al.* (2012) investigated the antiproliferative effects of L-asparaginase purified from *Aspergillus terreus* on two leukemia cell lines. Purified L-asparaginase exhibited cytotoxicity against HL60 cells and RS4;11 cells with an IC_{50} value of 200 µg/ml and 100 µg/ml respectively. L-asparaginase purified from *Mucor hiemalis* showed a positive scavenging activity against 2,2'-azinobis-(3-Ethyl-benzothiazoline-6-sulfonic acid (Thakur *et al.,* 2014). L-asparaginase purified from *Aspergillus niger* showed cytotoxicity against human leukemia cell line A431 after 96 h of incubation with IC_{50} value of 250 µg/ml (Dange and Peshwe, 2015). L-asparaginase purified from *Trichoderma viride* showed significant antioxidant activity (Linclon *et al.,* 2015). The L-asparaginase purified from *Fusarium culmorum* ASP-87 exhibited antiproliferative effects on Jurkat cell lines with IC_{50} value of 90 µg/ml (Meghavarnam *et al.,* 2017). The crude L-asparaginase from *Fusarium oxysporum* and *Aspergillus sydowii* exhibited cytotoxic effect against murine leukemic cell lines with IC_{50} value of 62.5 U/ml and 50.0 U/ml respectively (Ali *et al.,* 2018).

Acrylamide reduction potential of fungal L-asparaginase

Acrylamide is a possible source of a broad range of lethal effects and is grouped as possibly "carcinogenic in humans" (IARC 1994; Eriksson 2005; Manson *et al.,* 2005). In April 2002, the researchers from Swedish National Food Administration (SNFA) and University of Stockholm reported acrylamide formation in starchy or high temperature cooked foods such as baked and fried foods. Since then, related findings were recorded by researchers of many countries, including, USA, UK, Switzerland and Norway (FAO/WHO 2002). The acrylamide detection in food resulted in thorough exploring its mechanism of formation and its detection in foods. Acrylamide is formed in food by heat-induced reactions between the carbonyl group of reducing sugars and amino group of asparagines (Millard reaction). Various approaches for lowering the levels of acrylamide in food have been proposed, one approach is to remove the reactants i.e. L-asparagine. The asparagine content can be decreased by treating the foods with L-asparaginase (Taeyman *et al.,* 2004).

The impact of L-asparaginase on acrylamide formation in french fries was examined by Pedreschi *et al.* (2008). The

Table 3: Summary of growth conditions for L-asparaginase production under solid state fermentation.

Microorganism	Solid substrate	pH	Temperature (°C)	Moisture (%)	L-asparaginase activity U/gds	Reference
Aspergillus niger	Glycine max bran	6.5	30	70	40	Mishra, 2006
Aspergillus sp.	Groundnut oil cake	6.5	30	90	60	Sreenivasulu *et al*., 2009
Aspergilllus terreus (KLS2)	Carob pod	4.5	35	65	6.05 IU	Siddalingeshwara *et al*., 2010b
Fusarium equiseti	Soybean meal	7	45	70	8.51 IU	Hosamani and Kaliwal, 2011
Penicillium sp.	Sugarcane bagasse	6.2	30	60	8.2	Soniyamby *et al*., 2011
Fusarium oxysporum	Wheat bran	7.0	30	60	8.14 IU	Pallem *et al*., 2011
Aspergillus sp. KUFS20	Orange peel	6.2	30	50	70.67	Rani *et al*., 2012
Cladosporium sp.	Wheat bran	5.8	30	58	3.74	Kumar *et al*., 2013
Aspergillus niger	Gingelly oil cake	7.0	40	30	88	Vivekanandha *et al*., 2013
Aspergillus niger C4	Sesame oil cake	6.5	29.31	96.02	355.88	Uppuluri *et al*., 2013
Aspergillus niger	Sugarcane bagasse	9.0	37	ND	28.5	Rahiman *et al*., 2014
Trichoderma viridae F2	Rice husk and Wheat bran	5.0	28	75	113.43	Elshafei and El-Ghonmey, 2015
Aspergillus fumigates WL002	Wheat bran	5.0	37	ND	321.67	Dutta *et al*., 2015
Aspergillus niger LBA 02	Wheat bran, soybean meal, cotton seed meal	ND	30	50	89.22	Dias *et al*., 2015
Fusarium culmorum	Soybean meal	7.0	30	70	7.21	Meghavarnam and Janakiraman, 2017
Aspergillus sp.	Cotton seed cake, wheat bran and red gram husk	8.0	35	70	12.57 IU	Dorya and Kumar, 2018
Aspergillus niger LBA 02	Passion fruit peel	ND	30	60	3746.78	Cunha *et al*., 2018
Aspergillus niger	Niger de-oiled cake	6.2	30	62	34.65	Sharma and Mishra, 2021

ND: No Data

Table 4: Purification and characterization of fungal L-asparaginase

Microorganism	Purification method	Specific activity (U/mg of protein)	Purification fold	Molecular weight (kDa)	Optimum pH	Optimum temperature (°C)	K_m (mM)	V_{max}	Reference
Flammulina velutipes	Ultrafiltration Superose 6	ND	ND	85	7.0	40	ND	ND	Eisele *et al*., 2011
Aspergillus aculeatus	Ammonium sulphate precipitation, Dialysis, G-25 and G-150 column	207	267.75		9.0	30	12.5×10^{-3} M	104.16 IU/mL	Dange and Penshwe, 2011
Penicillium brevicompactum NRC 829	Heat treatment, gel filtration Sephadex G-100 and G-200 column	574.24	151.12	94	8.0	37	1.05	ND	Elshafei *et al*., 2012
Penicillium digitatum	Ammonium sulphate precipitation, gel filtration G-100 column	833.15	60.94	ND	7.0	30	1×10^{-5} M	ND	Shrivastava *et al*., 2012
Penicillium sp.	Ammonium sulphate precipitation, Sephadex G-100-120, DEAE column	13.97	1.9	66	7.0	37	4×10^{-3} M	ND	Patro and Gupta, 2012
Aspergillus terreus	DEAE-Sepharose and Sephacryl S-200 column	ND	ND	136	9.0	40	ND	ND	Loureiro *et al*., 2012
Cladosporium sp.	Methanol precipitation, DEAE cellulose column, Sepharose 6B	83.3	867.7	121	6.3	30	0.1	4.44 µmol/ml/min	Kumar and Manonmani, 2013
Mucor hiemalis	Acetone precipitation, Affinity chromatography with lectin-agarose	69.43	4.59	96.32	7.0	37	4.3	625 U/ml	Thakur *et al*., 2014
Rhizomucor miehei	Nickel-iminodiacetic acid column	1984.8	2.6	133.7	7.0	45	0.0253	3380.0 µmol/ml/min	Huang *et al*., 2014

Contd...

Contd...

Aspergillus flavus	Ammonium sulphate precipitation, Sephadex G-100-120	176.47	7.80	100	7.0	37	0.086	ND	Patro *et al*., 2014
Penicillium cyclopium	Acetone precipitation, Sephadex G-100	39480	52.3	55	8.0	37	0.3	3333 µmol/ml/min	Shafei *et al*., 2015
Aspergillus fumigates WL002	Ultrafiltration (30 kDa), Ammonium sulphate precipitation, DEAE-Sepharose column, Sephadex G-100	355.03	232.04	35	9.0	50	7.02	355.3 µmol/ml/min	Dutta *et al*., 2015
Trichoderma viride	Acetone precipitation, DEAE-cellulose column	78.2	13.0	99	7.0	37	2.56	279.27 U/ml	Lincoln *et al*., 2015
Fusarium culmorum (ASP-87)	Ammonium sulphate precipitation, Ion exchange, Gel filtration	16.66	14.03	90	8.0	40	3.57	0.5 µmol/ml/min	Meghavarnam and Janakiraman, 2015a
Aspergillus sp. ALAA-2000	Ammonium sulphate precipitation, Sephadex G-200	0.4	8.3	25	6.0	47	ND	ND	Ahmed *et al*., 2015
Aspergillus oryzae CCT 3940	Ammonium sulphate precipitation, dialysis, Q-Sepharose, SP Sepharose, CM Sepharose	282	28.6	115	8.0	50	0.66×10^{-3} M	313 IU/ml	Dias *et al*., 2016
Aspergillus niger AKV-MKBU	Ethanol precipitation, DEAE cellulose	46.75	10.36	90	7.0	30	0.8141	6.228 µmol/ml/min	Vala *et al*., 2018

ND: No data

efficient way to decrease the level of acrylamide in French fries is to soak the blanched potato strips 79 g/100 g of wet weight in asparaginase solution (10000 U/L) for 20 min at 37°C (Pedreschi *et al.,* 2008). Hendriksen *et al.* (2009) evaluated the acrylamide mitigation potential of L-asparaginase purified from *Aspergillus oryzae* in food products. Enzyme treated dough decreased the acrylamide level to 34-92% in ginger biscuits and crisp bread whereas, enzyme treated potato pieces reduced acrylamide content upto 60-85% in potato chips and french fries. Kumar *et al.* (2013) reported in potato chips acrylamide formation was reduced up to 96% by L-asparaginase purified from *Cladosporium* sp. He also reported the potential of L-asparaginase to decrease acrylamide levels in bakery products such as bread. The acrylamide concentration reduced with increasing concentration of L-asparaginase and at 300 U (enzyme treatment) there was 73% and 97% reduction of acrylamide levels in the crumb and crust region of the bread without any change in the rheological and physico-sensory characteristics of the bread (Kumar *et al.,* 2014). The acrylamide reduction potential of L-asparaginase purified from *Trichoderma viride* was evaluated by Lincoln *et al.* (2015). The purified L-asparaginase from *Fusarium culmorum* (ASP-87) inhibited the formation of acrylamide in bread and potato chips by 86% and 94% respectively (Meghavarnam and Janakiraman, 2018).

CONCLUSION

L-asparaginase finds major appliance in food as well as pharmaceutical industries. Since its discovery, it is broadly used in pharmaceutical industry for treating various types of leukemia and in the past two decade it is used in food industry for lowering the levels of acrylamide in starchy foods. L-asparaginase is efficiently produced from bacteria and widely used in industries. In spite of its ample applications, there are few disadvantages associated such as allergic reactions, short shelf-life, immune reactivity and low yield of enzyme. Hence, the quest for L-asparaginase from eukaryotic source with more potential features like specificity, increased shelf-life and reduced side effects are increasing. There is need for rapid screening methods for finding L-asparaginase with novel functions and for optimizing the fermentation conditions using statistical methods for enhancing yield of enzymes. Application of genetic engineering and cloning techniques may reduce the immune reactivity and increase the stability and shelf life of L-asparaginase. Therefore, in coming days L-asparaginase from fungi may be a potential candidate with all desirable features for application in pharmaceutical and food industries.

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