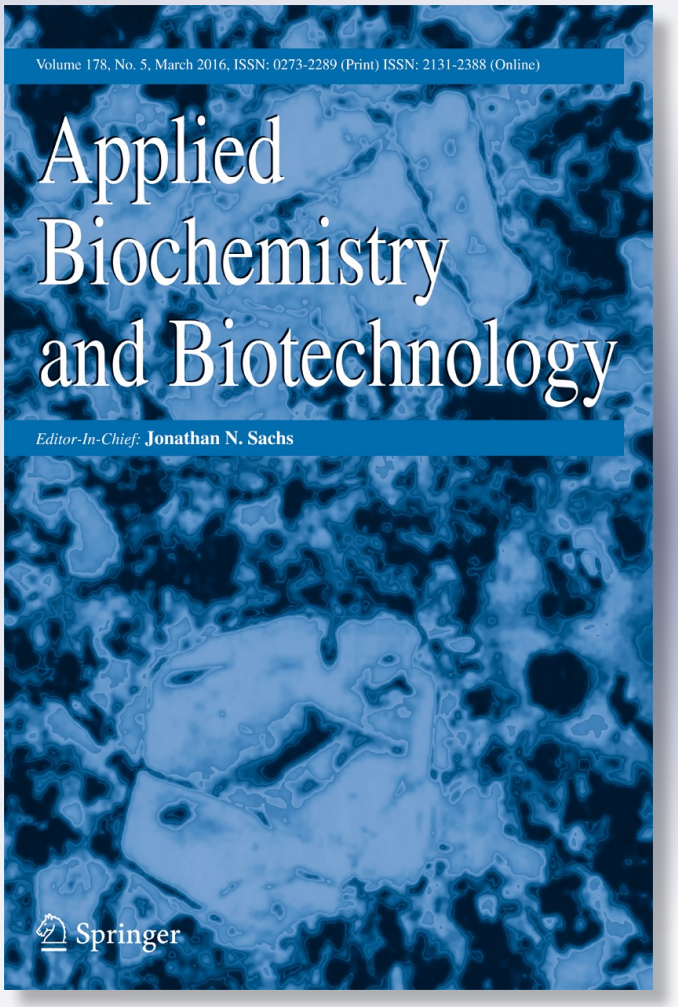
A Comprehensive Review on l-Asparaginase and Its Applications

Tahira Batool, Essam A. Makky, Muna Jalal & Mashitah M. Yusoff

Applied Biochemistry and Biotechnology
Part A: Enzyme Engineering and Biotechnology

ISSN 0273-2289
Volume 178
Number 5

Appl Biochem Biotechnol (2016)
178:900-923
DOI 10.1007/s12010-015-1917-3



Springer

Appl Biochem Biotechnol (2016) 178:900–923
DOI 10.1007/s12010-015-1917-3

A Comprehensive Review on L-Asparaginase and Its Applications

Tahira Batool[1] · Essam A. Makky[1] · Muna Jalal[1,2] · Mashitah M. Yusoff[1]

Received: 14 April 2015 / Accepted: 29 October 2015 /
Published online: 7 November 2015

Abstract L-asparaginase (LA) catalyzes the degradation of asparagine, an essential amino acid for leukemic cells, into ammonia and aspartate. Owing to its ability to inhibit protein biosynthesis in lymphoblasts, LA is used to treat acute lymphoblastic leukemia (ALL). Different isozymes of this enzyme have been isolated from a wide range of organisms, including plants and terrestrial and marine microorganisms. Pieces of information about the three-dimensional structure of L-asparaginase from *Escherichia coli* and *Erwinia* sp. have identified residues that are essential for catalytic activity. This review catalogues the major sources of L-asparaginase, the methods of its production through the solid state (SSF) and submerged (SmF) fermentation, purification, and characterization as well as its biological roles. In the same breath, this article explores both the past and present applications of this important enzyme and discusses its future prospects.

Keywords L-asparaginase · Lymphoblasts · Acute lymphoblastic leukemia · Solid state fermentation · Submerged fermentation

Introduction

All enzymes are proteins (and in some cases RNA molecules) with varied functions. They catalyze reactions used in normal development, help in the maintenance of the cell, and work as defense against diseases. Enzymes can operate intracellularly, extracellularly, or even on the surface of a cell membrane [1]. Several hundred different enzymes have been identified, and many of them have been characterized to a considerable degree. Quite a few of these enzymes

✉ Essam A. Makky
essam22001@gmail.com

[1] Faculty of Industrial Sciences & Technology, University Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia

[2] Department of Pathological analysis, Al-Haweeja Technical Institute, Foundation of Technical Education, Kirkuk, Iraq

have 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. According to Hammes, [2], such enzymes come up with special experimental advantages. One of such enzymes is L-asparaginase. L-Asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) has been the focus of several papers in the most recent three decades. Although it does have certain features of interest, it must be admitted that L-asparaginase is not especially remarkable as an enzyme. What is unusual about asparaginase and constitutes the feature remarkable for the considerable attention it has attracted is its antineoplastic activity. L-asparaginase also known as aminohydrolase pertains to the amidase group of enzymes and can break down amino acid L-asparagine into aspartate and ammonia [3] (Fig. 1).

L-asparaginase is the first therapeutic enzyme with antineoplastic properties that has been studied broadly by researchers and scientists far and wide. L-asparaginase was first observed by Lang in 1904 [4]. Research on physiological capabilities of L-asparaginase was under way for more than half century, and a real leap forward was accomplished in 1922 when Clementi [5] revealed the presence of L-asparaginase in blood serum of guinea pig. Kidd [6] in 1953 did a progression of experiments to prove the ability of guinea pig serum as tumor inhibitor. He carried out diverse sets of analysis where he concocted the findings that two sorts of subcutaneously executed lymphosarcoma in mice failed to proliferate once animals were given injections of guinea pig serum, while untreated controlled mice died because of carcinomas. In the second set of experiments, two different types of lymphomas namely mammary carcinoma and fibrosarcoma of mice failed to degenerate when treated with guinea pig serum. In 1961, yet another breakthrough was achieved when Broome [7] demonstrated L-asparaginase as an antitumor agent in guinea pig serum with substrate specificity. Later on, some other substrate-specific L-asparaginases were found to inhibit tumors [8–10]. McCoy through his in vitro experiments proved the centrality of amino acid asparagine for Walker carcinoma 256 [11]. Altenbern in 1954 and Broome in 1965 reported the antitumor activity of L-asparaginase in bacteria and yeast, respectively [12, 13]. L-asparaginase is found in the serum of guinea pig and rodents but is absent in humans [14]. Tumors in mice, dogs, and rats can be regretted by the asparaginase enzyme purified from guinea pig serum, *Escherichia coli*, and to a lesser degree by the chicken liver [15–17]. Two isozymes of L-asparaginase, namely type I and type II, have been identified by Ohnuma in 1967 [18]. Both type I and type II asparaginases are characterized by enzymatic activity for both L-asparagine and L-glutamine. However, type II asparaginase displays higher specific action against L-asparagine. Type II asparaginase precisely shows antitumor activity and is utilized as chemotherapeutics in ALL [19].

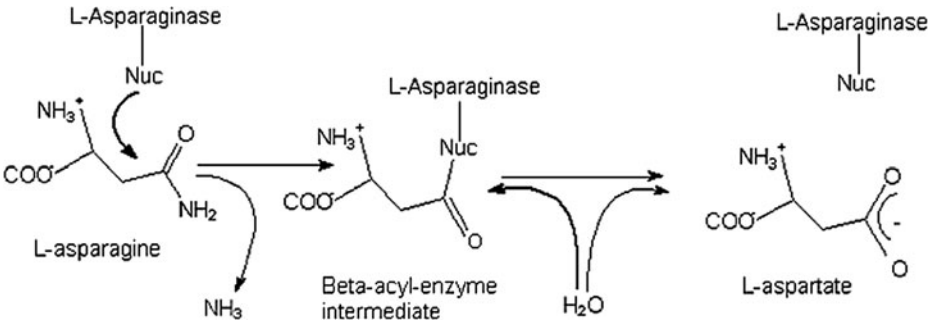


Fig. 1 Schematic illustration of the reaction mechanism of L-asparaginase

Commercially available asparaginase includes Colapase, Crasnitin, Crisantas, Pasum, Kidrolase, Elspar, Erwinaze PEG-asparaginase, and Pegasparagasum.

This paper reviews the different sources of L-asparaginase enzyme, methods that are being employed for its production, its assay methods, and its extensive applications in the health and food industry. Consequently, this review is organized in different sections. Section 1 sheds light on the structure, mode of action, and side effects of L-asparaginase. Section 2 gives insight into various sources of L-asparaginase enzyme including different microorganisms. Section 3 presents different production methods under submerged fermentation and solid state fermentation conditions and purification methods implemented for purification of L-asparaginase. Section 4 draws out the assay methods that are being used for detection of L-asparaginase. Section 5 discusses the applications in different areas including amino acid metabolism and clinical and food industry. Section 6 summarizes our conclusions and recommendations for future research on L-asparaginase.

L-Asparaginase Mechanism of Action

Because of its hydrolytic property, asparaginase is a cornerstone of treatment for ALL. Both normal and leukemic cells require the amino acid L-asparagine, for their metabolic needs. Normal cells can synthesize L-asparagine for their growth by utilizing transaminase enzyme that converts oxaloacetate into an intermediate aspartate, which later on transfers an amino group from glutamate to oxaloacetate producing α-ketoglutarate and aspartate. Finally, in healthy cells, aspartate is converted to asparagine by enzyme asparagine synthetase. Neoplastic cells lack the ability to synthesize the asparagine due to the absence of L-asparaginase synthetase enzyme, hence are dependent on the exogenous supply of asparagine for their existence and reproduction. Consequently, as a result, provision of L-asparaginase to tumor cells drains all circulating asparagine, which leads to starvation of cancer cells and ultimately they die off (Fig. 2). Clinical data published over the last two decades suggests asparaginase is a very important component of treatment regimens for ALL. L-asparaginase is prevalent among eukaryotes and microorganisms [20]. L-asparaginase also has a potential role in the food industry as a food processor [21].

L-Asparaginase Structure

Research has been carried out by many scientists to elucidate the structure of L-asparaginase enzyme at the molecular level. Usually, L-asparaginase exists as a tetramer but hexameric, dimeric, and monomeric forms are also found when isolated from different sources. Most bacterial L-asparaginases exhibit quaternary and tertiary structures [22]. Molecular structures of *E. coli* and *Erwinia* sp. are very well investigated, and their structural information is easily available [23, 24]. Both *E. coli* and *Erwinia* sp. have similar three-dimensional structures [25]. *Erwinia carotovora* enzyme consists of two tetramers (ABCD and EFGH) made up of four identical monomers (A to H) each. Three hundred twenty-seven amino acids assemble in each monomer with 14 α-strands, eight β-helices [26], and two domains, a big N-terminal domain and a small C-terminal domain [24]. The active site is positioned in between two adjacent monomers (A and C: B and D). The tetramer consists of four identical subunits. The whole molecule is considered as a dimer of dimers [27, 28]. Every active site is shaped by the conveyance of amino acids in two adjacent monomers. Following amino acids constitute

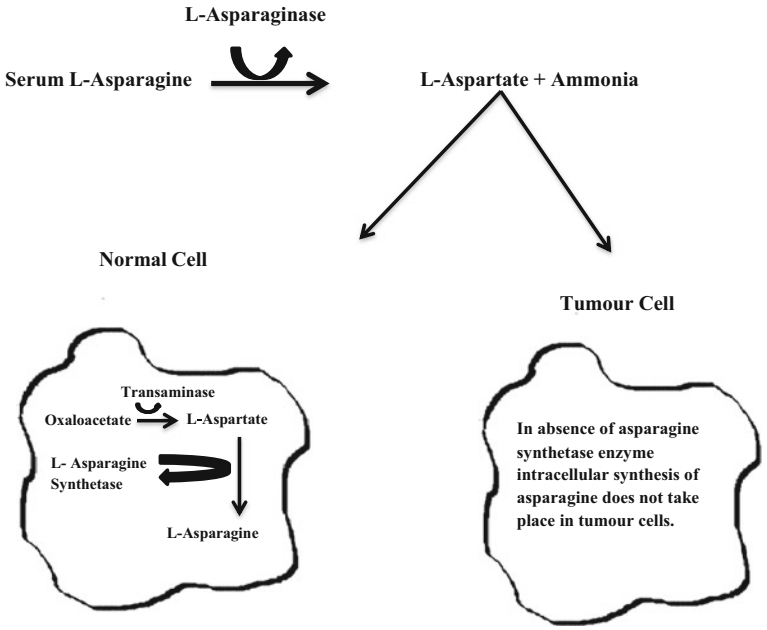


Fig. 2 Mechanism of Action of L-asparaginase in normal and tumor cells

active site: Thr15, Tyr29, Ser62, Glu63, Thr95, Asp 96, Ala120, and Lys168, while only one residue Ser254 is present in adjacent monomer [22, 27, 29–34]. Thr15 and Thr95 are the residues responsible for the catalytic activity of the enzyme.

L-Asparaginase Side Effects

L-asparaginase causes certain side effects, and despite its potential antileukemic activity, utilization of L-asparaginase by leukemic patients causes lethality to normal cells. L-asparaginase produces a broad range of symptoms such as edema, skin rashes, fever, hepatic dysfunction, diabetes, leucopenia, pancreatitis, neurological seizures, and hemorrhage [35, 36]. Moola reported that some hypersensitivity reactions, mild allergic reactions, and anaphylactic shock are also caused by the usage of asparaginase-based drugs [37]. Adolescents appear to be at higher risk of neurotoxicity caused by L-asparaginase, which results in depression, fatigue, lethargy, dizziness, and agitation [38]. The toxicity of L-asparaginases is believed to be brought about by its glutaminase action [39].

Sources of L-Asparaginase

The presence of L-asparaginase has been reported in various organisms, including animals, plants, and microorganisms (bacteria, fungi, algae, yeast, and actinomycetes) except humans. Even though L-asparaginase exists in several animal and plant groups, but owing to tiresome extraction procedures, other conceivable sources (bacteria, fungi, algae, yeasts, and actinomycetes) were explored by researchers. Large scale production of the enzyme from microbes is

much easier due to their facile production methods [40]. Here, we have outlined different microbial sources of L-asparaginase.

Bacterial Sources

L-asparaginase has been reported from both Gram-positive and Gram-negative bacterial species from the terrestrial and marine environment [41]. Gram-positive bacteria have gained less consideration as compared to Gram-negative [20]. More intensively studied species of both Gram-negative and Gram-positive classes are listed in Table 1.

L-asparaginase from most of the Gram-negative bacteria can be categorized into two main types: type I and type II L-asparaginase. Type I L-asparaginase is expressed quantitatively and possesses enzymatic activity on both L-glutamine and L-asparagine amino acids, while type II L-asparaginase possesses high specific activity on L-asparagine and is only induced in anaerobic condition [80]. Type II L-asparaginase produced from *E. coli* (Ec AII) and *Erwinia*

Table 1 Bacterial sources of L-asparaginase

Gram-negative bacteria	References	Gram-positive bacteria	References
Acinetobacter calcoaceticus	[42]	*Bacillus circulans*	[43, 44]
Azotobacter agilis	[45]	*B. coagulans*	[46]
Brevibacillus brevis	[47]	*Bacillus* sp.	[48]
Citrobacter sp.	[49]	*B. mesentericus*	[50]
Escherichia coli	[51]	*B. polymyxa*	[52]
Enterobacter aerogenes	[53]	*B. subtilus*	[54]
E. cloacae	[55]	*B. licheniformis*	[56, 57]
Erwinia aroideae	[58]	*B. circulans MTCC 8574*	[43]
E. cartovora	[59]	*Corynebacterium glutamicum*	[60]
E. chrysanthemi	[37]	*Mycobacterium bovis*	[45]
Helicobacter pylori	[61]	*M. phlei*	[62]
Klebsiella pneumoniae	[63]	*Staphylococcus* sp.	[64]
Pectobacterium carotovorum	[65]	*S. aureus*	[66]
Pseudomonas sp.	[67]	*Streptococcus albus*	[63]
P. fluorescens AG	[68]		
P. geniculate	[69]		
P. ovalis	[70]		
P. stutzeri	[71]		
Pyrococcus horikoshii	[72]		
Serratia marcescens	[73]		
Thermus thermophiles	[74]		
T. aquaticus	[75]		
Vibrio succinogenes	[76]		
Citrobacter freundi	[77]		
Proteus vulgaris	[78]		
Zymomonas mobilis	[79]		

chrysanthemi (Er A) has been used as an antitumor agent for the effective treatment of ALL for over 30 years [81]. L-asparaginase from two bacterial sources like *E. coli and E. carotovora* is currently in clinical use for the treatment of acute lymphoblastic leukemia [82].

Fungal Sources

Fungi are another potential source of L-asparaginase along with bacteria. Several adverse side effects linked with bacterial asparaginase often restrain their application. This impediment requests a quest for L-asparaginase from new sources. Human beings are more closely related to fungi as compared to bacteria. Hence, chances of immunological reaction against fungal asparaginase will be lesser [83]. Fungal asparaginase has acquired importance based on the fact that it is produced extracellularly, and it is a very easy to purify extracellular enzyme. Several L-asparaginase-producing fungi are being reported (Table 2).

Yeast Sources

L-asparaginase with less injurious effects is reported from the yeast. Asparaginases reported from the yeasts including *Saccharomyces* sp., *Candida* sp., Pichia sp., *Rhodotorula* sp., *Hansenula* sp., and *Spobolomyces* sp., exhibit antitumor activity [93]. L-asparaginase-producing yeasts are listed in Table 3.

Actinomycete Sources

L-asparaginase occurrence is also reported in actinomycetes [100]. Actinomycetes are pervasive worldwide in soil, water, and nature, but only those found in living animals especially in fishes are found to bear good enzymatic activity [101]. Moreover, actinomycetes are a better source of L-asparaginase when compared to bacteria and fungi [102]. Table 4 lists L-asparaginase-producing actinomycetes.

Algal Sources

Reports are available on the production of L-asparaginase from *Chlamydomonas* sp. [114] and a yellow green alga, *Vaucheria uncinata* [115].

Table 2 Fungal sources of L-asparaginase

Fungi	References
Alternaria sp.	[84]
Aspergillus nidulans	[85]
A. niger	[86]
A. oryzae	[87]
A. tamarii	[88]
A. terreus	[89]
Cylidrocapron obtusisporum	[90]
Mucor sp.	[91]
Fusarium roseum	[92]

Table 3 Yeast sources of L-asparaginase

Yeast	References
Candida utilis	[94]
C. guilliermondii	[95]
C. bombicola	[96]
Pichia polymorpha	[97]
Rhodosporidium toruloides	[98]
Rhodotorula sp.	[96]
Saccharomyces cerevisiae	[99]

Production and Purification of L-Asparaginase

Different methods reported for production and optimization of L-asparaginase from various microorganisms include solid state fermentation (SSF) and submerged fermentation (SmF) [93]. Reaction conditions vary from one organism to another for enzyme production, and it can be produced constitutively or after induction [116, 117]. SmF process has certain constraints like net yield is very low and costly. Whereas, SSF is cost-effective and yield of product is higher as compared to the SmF. Several methods have been reported for purification of L-asparaginase enzyme produced from various sources. The major suggested requisition of L-asparaginase is as an antileukemic drug that demands a very high level of purity for this enzyme. Most of the microbial asparaginases reported so far are intracellular in nature except for the few, which are extracellular [20]. Purification of intracellular asparaginases is tiresome as compared to extracellular enzymes. Detailed reaction conditions for production and purification of L-asparaginase using SSF and SmF from different microbes have been listed in Table 5 [94].

Table 4 L-asparaginase-producing actinomycetes

Actinomycetes	References
Actinomyces sp.	[82]
Streptomyces albidoflavus	[103]
S. aurantiacus	[103]
S. collinus	[104]
S. griseus	[105]
S. gulbargensis	[106]
S. karnatakensis	[107]
S. longsporusflavus	[108]
S. plicatus	[109]
S. tendae	[110]
S. venezuelae	[111]
Thermoactinomyces vulgaris	[112]
Noccardia sp.	[113]

Table 5 Different production, purification, and optimization methods of L-asparaginase from various sources (adapted from [94]

Organism	Production Conditions				Purification			Properties			Ref.
	Method	Medium	Temp (°C)	pH	Steps	Yield %	Purification fold	Optimum pH	Optimum temp (°c)	Mol. wt SDS-PAGE	
Aspergillus niger AK-10	SSF	Soybean meal	–	–	–	–	–	8.6	37	91.4 kDa	[118]
Bacillus sp.	SmF	Glucose	37	–	Ammonium sulfate	96.2	10.9	7.0	37	45 kDa	[119]
					IEC DEAE	43.1	11.2				
Bacillus sp.	SSF	Corn cob	37	5	–	–	–	5	37	–	[120]
Marine actinomycetes S3	SSF/SmF	Soybean meal/ tryptone glucose yeast extract broth	37	7	Ammonium sulfate	75	1.09	7.5	50	–	[121]
					Sephadex G 100	69.97	2				
Erwinia carotovora	SmF	Nutrient Broth	25	6.9	A. sulfate	85	6	8.6	35	33.5 kDa	[122]
					DEAE	76	88				
Marine actinomycetes PDK2	–	–	–	–	A. sulfate	65.83	1.09	8.0	60	140 kDa	[123]
					Sephadex G50	8.61	33.68				
					Sephadex G200	2.18	82.98				
Pseudomonas aeruginosa 50071	SSF	Soybean meal	37	7.4	A. sulfate	85	5.2	9.0	37	160 kDa	[124]
					Sephadex G100	60.8	27.7				
					CM Sephadex C50	43	106				
Corynebacterium glutamicum	SmF	Tryptone soya broth	30	7.3	Protamine sulfate	105	1.2	7.0	40	80±1 kDa	[60]
					DEAE Sephacel	35	6.6				
					A. sulfate	16	16.3				
					Sephacryl S 200	12.5	98				
Azotobacter vinelandii	SmF	Sucrose	30	7.4	Protamine sulfate	90	–	8.6	48	84 kDa	[125]
					A. sulfate	63	1.6				
					Sephadex G150	21	4.5				
					DEAE	5	12.5				

Table 5 (continued)

Organism	Production Conditions				Purification			Properties			Ref.
	Method	Medium	Temp (°C)	pH	Steps	Yield %	Purification fold	Optimum pH	Optimum temp (°c)	Mol. wt SDS-PAGE	
					Affinity chromatography	2.8	18.5				
Vibrio succinogenes	–	–	–	–	–	–	–	7.3	37	146 kDa	[126]
Streptomyces gulbargensis	SmF	Groundnut cake extract, 0.5 % maltose	40	8.5	A. sulfate	50.6	1.8	9.0	40	85 kDa	[106]
					Sephacryl S 200	37.8	26.88				
					CM Sephadex C50	32	82.12				
Pseudomonas stutzeri MB-405	–	–	–	–	–	–	–	9.0	37	34 kDa	[71]
Fusarium equiseti	SSF	Soybean meal	45	7	–	–	–	–	–	–	[127]
Flammulina velutipes	SmF	Minimal medium	–	–	CTAB	–	–	7.0	40	13 kDa,	[128]
					Superose 6	–	–			85 kDa	
Streptomyces sp. TA22	SmF	Sucrose	28	7	Ammonium sulfate	—	–	7.0	–	–	[129]
Staphylococcus s. 6A	SmF	Glucose	39	7.5	–	–	–	–	–	–	[130]
Thermus aquaticus strain T351	–	–	–	–	DEAE-Sepharose CL-6B column	86	18	9.5	–	80±2 kDa	[75]
					QAE-Sephadex A 50	83	29				
					Hydroxylapatite	38	75				
					Sephadex G-150	21	225				
Erwinia chrysanthemi 3937 (*Erl-L-Asparaginase* in *E. coli* BL21 (DE3) pLysS	SmF	–	–	–	S-Sepharose FF column	69.8	15.4	–	–	37.2 kDa	[19]
Vibrio succinogenes	SmF	Sodium fumarate, cysteine	37	7.3–7.4	–	–	–	–	–	–	[131]
Escherichia coli	SmF	Terrific broth	37	7.2	Ni-NTA affinity chromatography	–	3.3	–	–	37 kDa	[132]

Table 5 (continued)

Organism	Production Conditions				Purification			Properties			Ref.
	Method	Medium	Temp (°C)	pH	Steps	Yield %	Purification fold	Optimum pH	Optimum temp (°c)	Mol. wt SDS-PAGE	
Mycobacterium tuberculosis (tiasparagine amidohydrolases)	SmF	Youman's medium	–	–	Ammonium sulfate	–	7	–	–	–	[133]
Acinetobacter glutaminasificans (ATCC 27197)	SmF	L-glutamic acid	–	–	–	–	–	–	–	–	[134]
Staphylococcus aureus strain NCTC413	SmF	Cas amino acids	–	–	–	–	–	–	–	–	[135]
Fusarium tricinctum	SmF	Nutrient broth with asparagine	–	–	A. sulfate	67	16	8	–	161–170 kDa	[136]
					DEAE	35	33				
					Ampholine	22	110				
					Prep-disc electrophoresis	15	200				
Acinetobacter calcoaceticus	SmF	Xylose and L-asparagine	–	–	Precipitation with streptomycin	–	–	7–9	130 kDa		[42]
					DEAE	–	–	8.4–8.8	20–30	25 kDa, 105 kDa	[137]
					CMC chromatography	58	4				
					Agarose filtration	35	14				
					Phospho-cellulose	11.6	140				
					Streptomycin precipitation	–	–				
					DEAE-cellulose	50	3				
					Sephadex G-200	31	6				
					Affinity chromatography Sepharose 6B	15	80				
Serratia marcescens	–	Tryptic soy broth	–	–	A. sulfate	80	4.09	8.5	–		[138]

Table 5 (continued)

Organism	Production Conditions				Purification			Properties			Ref.
	Method	Medium	Temp (°C)	pH	Steps	Yield %	Purification fold	Optimum pH	Optimum temp (°c)	Mol. wt SDS-PAGE	
					P-150 BioGel filtration	47	95.15			37 kDa, 147 kDa	
					Isoelectric focusing	50	153.03				
					Prep-disc electrophoresis	27	684				
			37	–	DEAE	28.85	33.5	–	–	–	[73]
					BioGel P-300	13.45	130				
					Hydroxylapatite	9.5	240				
Bacillus coagulans	–	–	–	–	DEAE at pH 8.6 (11.8)	75.5	11.8	8.8–9.7	55	84 kDa	[139]
					DEAE at pH 6.6 (1.2)	55.2	1.2				
					Sephadex G-150 (1.2)	68.5	1.8				
					Hydroxyapatite-cellulose, pH 6.6	47.7	13				
					Overall	13.6	332				
Pseudomonas sp. strain GG13	–	Mono-Na glutamate and glucose	–	–	Butanol, A. sulfate, and zone electrophoresis	–	–	–	–	25 kDa	[140]
Bacillus brevis	SmF	Fructose, liquid paraffin	30	7	–	–	–	–	–	–	[47]
Erwinia carotovora (cloned in *E. coli*)	–	Yeast extract, soya bean meal, glucose, wood chips, asparagine, NaCl	–	–	Cation exchange and affinity chromatography	–	–	–	–	–	[141]
Pectobacterium carotovorum MTCC 1428	–	Glucose and L-asparagine	30	6.5	A. sulfate	70.15	6.86	8.5	40	36.5 kDa, 146 kDa	[142]
					DEAE	48.38	44.63				
					Sephadex G-100	42.05	72.12				
Pseudomonas aeruginosa	SSF	Corn steep liquor, asparagine, tryptone, and casein	37	8	–	–	–	–	–	–	[108]

Table 5 (continued)

Organism	Production Conditions				Purification			Properties			Ref.
	Method	Medium	Temp (°C)	pH	Steps	Yield %	Purification fold	Optimum pH	Optimum temp (°c)	Mol. wt SDS-PAGE	
Escherichia coli ATCC 11303	SmF	Lactose, tryptone, yeast extract, and L-asparagine	37	7.2	–	–	–	–	–	–	[143]
Helicobacter pylori CCUG 17874	–	–	–	–	Superdex 200 10/300 GL column	–	–	–	–	37 kDa, 140 kDa	[144]
Pseudomonas PO7111 (cloned in *E. coli*)	SmF	Glucose, peptone, and beef extract	–	–	–	–	–	–	–	–	[145]
Aspergillus niger	SSF	Bran of *Glycine max*	35	6.5	A. sulfate DEAE	92.05 72.04	1.14 1.44	6.5	40	–	[87]
Serratia marcescens	SmF	Glycerol peptone yeast extract	37	6.8–7.0	–	–	–	–	–		[146]
Erwinia aroideae NRRL B-138	Smf	Glucose yeast extract Tryptone K_2HPO_4	28	7	A. sulfate	–	–	7.5	–	–	[147]

Assay Methods

Different methods have been developed and reported to assay L-asparaginase activity. L-asparaginase activity can be evaluated by measuring the amount of ammonia or aspartic acid released during the reaction or by following the disappearance of asparagine [148, 149]. Both quantitative and qualitative techniques have been employed for estimation of L-asparaginase activity. Different methods are summarized in Table 6 [150].

Applications of L-Asparaginase

Anticancer Drug

L-Asparaginase is used to treat ALL in combination with vincristine and a glucocorticoid (e.g., dexamethasone) [159]. Due to its antileukemic properties, L-asparaginase has been considered as a therapeutically important antitumor drug. L-asparaginase is a well-known chemotherapeutic agent which in combination with other drugs is used in the treatment of certain malignancies such as ALL (mainly in children), Hodgkin's disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma, reticulosarcoma, and melanosarcoma [6, 7]. L-asparagine is an essential amino acid for many tumor cells for protein synthesis and cell growth, while L-asparaginase possesses the ability to convert L-asparagine to aspartate; so, in the presence of L-asparaginase, malignant cells are deprived of important growth factor that results in depletion of asparagine and ultimately tumor cells die off [160]. L-asparaginase is widely reported in animals, plants, and microorganisms, but only the asparaginase from *E. coli* and *E. chrysanthemi* was approved to be used as part of a multiagent chemotherapy to treat ALL [161]. The significant disclosure by Kidd in 1953 that guinea pig serum could stifle the development of Gardner 6C3HED lymphosarcoma cells established subcutaneously in mice opened new doors for the advancement of L-asparaginase as a treatment for ALL [160]. Table 7 [160] summarizes the breakthroughs achieved during different time periods for the development of L-asparaginase as an anticancer drug.

Role of L-Asparaginase in Biosensor

L-asparaginase is also used for the development of a biosensor to analyze asparagine levels either in leukemia or the food industry [161]. Several spectroscopy techniques such as XRD, XPS, SEM, and TEM are currently used for L-asparagine analysis, but high cost and tedious procedures make them less favorable [162]. Over such a situation, biosensor technology can be a reliable, cheap, and user-friendly approach. The mechanism of action of the biosensor is based on asparaginase activity, ammonium ions produced from the hydrolysis of asparagine cause a change in pH resulting in the change of color and absorption [163].

$$\text{L-asparagine} \quad \text{L- aspartic acid} + \text{ammonia}$$

Table 6 Summary of assays for L-asparaginase activity determination [150]

No.	Assay	Substrate	Product analyzed	Principle	Remarks	References
1.	Spectrophotometry	L-Asparagine	Ammonia	The OD at 500 nm after the colored reaction is measured and compared to a standard curve prepared from solutions of ammonium sulfate as nitrogen source	The method involves the use of highly toxic reagents. Simple and quantitative but low activities of L-asparaginase cannot be measured	[147]
		L-Asparagine	Ammonia	Ammonia produced is degraded by glutamate dehydrogenase with concomitant oxidation of beta-NADH; disappearance of β-NADH is then monitored spectrophotometry at 340 nm	Simple and continuous assay	[151]
		L-Asparagine	Ammonia	The aspartate concentration measured spectrophotometry through coupling with L-glutamic oxaloacetic transaminase and L-malic dehydrogenase	Linear results for ammonia concentrations between 0 and 200 μM	[152]
		L-Aspartic acid beta-(7-amido-4--methylcoumarin)	7-Amino-4-methylcoumarin	The excitation and emission wavelengths of the released 7-amino- 4-methylcoumarin are measured at 37 °C using a fluorometer	Rapid assay but such procedures require diligent attention	[153]
2.	Titrimetry (direct Nesslerization)	Asparagine	Ammonia	The ammonia released is quantitatively measured by titration	Good reproducibility is achieved but the method requires meticulous care and disparate results are obtained from different labs analyzing the same sample	[154]
3.	Kinetic enzymatic method (gas sensing electrode)	Asparagine	Ammonia	The ammonia released is measured using an ammonia gas sensing electrode	Expensive but very accurate and instantaneous quantification of ammonia	[155]

Table 6 (continued)

No.	Assay	Substrate	Product analyzed	Principle	Remarks	References
		Asparagine	Ammonia	The ammonia released is measured using an ammonia gas electrode (ISE)	Direct quantification of ammonia	
4.	Paper electrophoresis	Asparagine	Aspartic acid	L-Aspartate was well separated from L-asparagine by electrophoresis. The section of the strip-bearing aspartate was cut out and counted in a scintillation counter	Good sensitivity but time consuming	[8, 156]
5.	Chromatography	Asparagine	Aspartic acid	The conversion of L-asparagine to L-aspartic acid is followed by rapid chromatography on ion exchange paper	Detection of as small as a few pmoles of [14C] L-aspartic Acid. Therefore, a very less costly substrate required. But the method is time consuming and not continuous	[157]
6.	Conductimetry	Asparagine or aspartate hydroxamate	Ammonia and/or aspartate	Increased conductivity owing to high yield of ammonia or aspartic acid in reaction mixture	Measurement is simple and instantaneous	[158]

Table 7 Development of L-asparaginase (LA) as antileukemic medication [Adapted from 161]

Year	Advancement
1953	Kidd: discovery of antileukemic effect of guinea pig serum
1963	Broome: identification of L-asparaginase as antileukemic agent in guinea pig serum
1964–1967	Suppression of tumor cell growth by *Escherichia coli*-derived L-asparaginase; isolation and purification of active *E. coli* isoform
1966	Dolowy: first clinical use of L-asparaginase
1968	Wade: isolation of L-asparaginase from *Erwinia carotovora (chrysanthemi*)
1978	Native *E. coli* asparaginase approved by FDA for use to treat ALL
1981	Kamisaki: initial development of pegylated *E. coli*-derived asparaginase
1985	Erwinia asparaginase authorized in the UK for use to treat ALL
1993	Asselin: identification of distinct pharmacokinetic properties
1994	Pegylated *E. coli* asparaginase (Oncaspar) approved by FDA for use to treat ALL
2006	Pegylated *E. coli* asparaginase approved by FDA for first-line use to treat ALL
2008	Start of COG ALL07P2 and compassionate use EMTP trials to evaluate asparaginase *Erwinia chrysanthemi*
2011	Asparaginase *Erwinia chrysanthemi* approved by FDA for use in patients with hypersensitivity to *E. coli*-derived asparaginase

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. Besides Kreb's cycle, aspartic acid that is a direct precursor of lysine, and threonine, is also formed by the action of L-asparaginase enzyme [164].

Role of L-Asparaginase in Food industry

Besides, L-asparaginase is also used widely as a food processing aid. Recent developments in food technology exhibited that a colorless and odorless crystalline solid, acrylamide (2-propenamide), is produced as a result of Millard reaction, when starchy foods are fried or baked at 120 °C [165, 166]. Acrylamide is a neurotoxin and has been categorized as a carcinogenic to human [167]. In the food industry, acrylamide is largely derived from heat-induced reactions (Fig. 3) [35] between the α-amino group of the free amino acid asparagine and carbonyl groups of reducing sugars such as glucose during baking and frying [168]. Due to its ability to convert L-asparagine to L-aspartate, L-asparaginase promises to be a possible way to reduce the number of precursors for Millard reaction by pre-treating the starchy foods (potato and bread dough) with L-asparaginase, hence reducing the risk of acrylamide formation [169]. However, complete removal of acrylamide is not possible due to other asparagine-independent formation [21]. Certain fungal asparaginase used in food industries nowadays includes L-asparaginase from *Aspergillus oryzae* and *Aspergillus niger* [170].

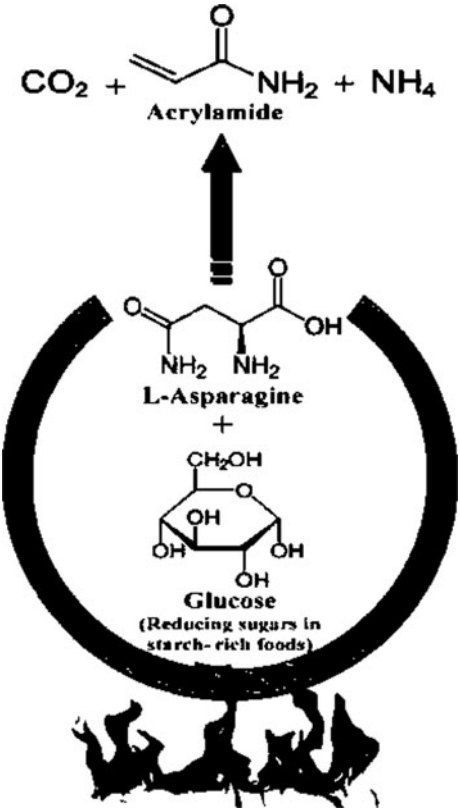


Fig. 3 Illustration of the formation of acrylamide

Conclusion

In this review article, we have presented both earlier and recent developments that continue to support the importance of L-asparaginase both in clinical and food industry. L-asparaginase, an amidohydrolase enzyme, is a significant enzyme that owes wide range of applications and is involved in deamination of asparagine and glutamine. From last 38 years, a lot of explorations have been carried out to establish the role of L-asparaginase in cancer therapy. Though distributed widely in bacteria, fungi, actinomycetes, plants, and animals, microbial L-asparaginase has drawn considerable attention due to cost-effective and easy production from microbes especially bacteria. However, certain limitations such as hypersensitive effects, resistance, immunogenic complications, and short life of enzyme are connected with its applications. Moreover, yields of L-asparaginase are low since gene expression rate of this enzyme is very low. Besides, L-asparaginase is utilized as food processing aid in the food industry for the elimination of carcinogenic compound acrylamide. Therefore, the desire of the time is to explore and optimize the production and purification parameters from new sources of the enzyme so that we can come up with more potential and specific L-asparaginase enzyme preparations. On the basis of better understanding of recombinant technologies like molecular cloning and genetic engineering, it may be possible to design enzyme with less immunogenic effects, novel functionalities, and better half-life. Besides, the use of latest recombinant

technologies could help to increase the net production yield of this therapeutically important enzyme.

Acknowledgments The authors are thankful to the Faculty of Industrial Sciences and Technology University Malaysia Pahang (UMP) for financial assistance under Doctoral Scholarship Scheme (DSS).

References

1. Maurer, H. R. (2001). Bromelain: biochemistry pharmacology and medical use. *Cellular and Molecular Life Sciences, 58*, 1234–1245.
2. Hammes, G. G. (2008). How do enzymes really work? *Journal of Biological Chemistry, 283*, 22337–22346.
3. Kumar, K., & Verma, N. (2012). The various sources and application of L-asparaginase. *Asian Journal of Biochemical and Pharmaceutical Research, 3*, 197–205.
4. Lang, S., & Uber. (1904). Desamidierung im Tierkorper. *Beitraege zur Chemischen Physiologie und Pathologie, 190*, 5321–345.
5. Clementi, A. (1922). La desemidation enzymatique de L-asparaginase chez les differentes especes animales et la signification physiologique de sa presence dans l organiasma. *Archives Internationales de Physiologie, 19*, 369–376.
6. Kidd, J. G. (1953). Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum. I. course of transplanted cancers of various kinds in mice and rats given guinea pig serum, horse serum or rabbit serum. *Journal of Experimental Medicine, 98*, 565–582.
7. Broome, J. D. (1961). Evidence that the L-asparaginase activity of guinea pig serum is responsible for its anti-lymphoma effects. *Nature, 191*, 1114–1115.
8. Broome, J. D. (1968). Factors which may influence the effectiveness of L-asparaginases as tumor inhibitors. *British Journal of Cancer , 22*, 595–602.
9. Capizzi, R. L, Peterson, R. Cooney, D. A. Creasey, W. A. Handschu, R. E. (1969) L-asparaginase therapy of acute leukemia-biochemical and clinical observations. Proc. Am. Asso.c Cancer. Res. 10,12.
10. Clubb, J. S., Neale, F. C., & Posen, S. (1965). The behavior of infused human placental alkaline phosphatase in human subjects. *Journal of Laboratory and Clinical Medicine, 66*, 493–507.
11. McCoy, T. A., Maxwell, M., & Neuman, R. E. (1956). The amino acid requirements of the Walker carcinosarcoma 256 in vitro. *Cancer Research, 16*, 979–984.
12. Altenbern, R. A., & Housewright, R. D. (1954). Stereospecific asparaginases in smooth *Brucella abortus* strain 19. *Archives of Biochemistry and Biophysics, 49*, 130–137.
13. Broome, J. D. (1965). Antilymphoma activity of L-asparaginase in vivo: clearance rates of enzyme preparations from guinea pig serum and yeast in relation to their effect on tumor growth. *Journal of the National Cancer Institute, 35*, 967–974.
14. Holmquist, N. D. (1963). Effect of normal sera of several related rodents on 6C3HED lymphoma in vivo. *Experimental Biology and Medicine, 113*, 444–445.
15. Boyse, E. A., Old, L. J., Campbell, H. A., & Mashburn, L. T. (1967). Suppression of murine leukemias L-asparaginase incidence of sensitivity among leukemias of various types: comparative inhibitory activities of guinea pig serum L-asparaginase and *Escherichia coli* L-asparaginase. *Journal of Experimental Medicine, 125*, 17–31.
16. Broome, J. D. (1963). Evidence that the L-asparaginase of guinea pig serum is responsible for its anti-lymphoma effects II Lymphoma 6C3HED cells cultured in a medium devoid of L-asparagine lose their susceptibility to the effects of guinea pig serum in vivo. *Journal of Experimental Medicine, 118*, 121–148.
17. Ohnuma, T., Bergel, F., & Bray, R. C. (1967). Enzymes in cancer asparaginase from chicken liver. *Biochemical Journal, 103*, 238–245.
18. Campbell, H. A., Mashburn, L. T., Boyse, E. A., & Old, L. J. (1967). Two L-asparaginases from *Escherichia coli* B. Their separation purification and antitumor activity. *Biochemistry, 6*, 721–730.
19. Kotzia, G. A., & Labrou, N. E. (2007). L-Asparaginase from *Erwinia chrysanthemi* 3937: cloning expression and characterization. *Journal Biotechnology, 127*, 657–669.
20. Savitri, N. A., & Azmi, W. (2003). Microbial L-asparaginase: a potent antitumor enzyme. *Indian Journal of Biotechnology, 2*, 184–194.

21. Dhanam, J. G., & Kannan, S. (2013). L-asparaginase: types perspectives and applications. *Advance Biology Technology, 13*, 1–5.
22. Ramya, L. N., Doble, M., Rekha, V. P. B., & Pulicherla, K. K. (2011). In silico engineering of L-asparaginase to have reduced glutaminase side activity for the effective treatment of acute lymphoblastic leukemia. *Journal of Pediatric Hematology/Oncology, 33*, 617–621.
23. Swain, A. L., Jaskólski, M., Housset, D., Rao, J. K., & Wlodawer, A. (1993). Crystal structure of Escherichia coli L-asparaginase, an enzyme used in cancer therapy. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 1474–1478.
24. Aghaiypour, K., Wlodawer, A., & Lubkowski, J. (2001). Structural basis for the activity and substrate specificity of *Erwinia chrysanthemi* L-asparaginase. *Biochemistry, 40*, 5655–5664.
25. Lubkowski, J., Dauter, M., Aghaiypour, K., Wlodawer, A., & Dauter, Z. (2002). Atomic resolution structure of *Erwinia chrysanthemi* L-asparaginase. *Acta Crystallographica D, 59*, 84–92.
26. Kozak, M., Jasloski, M., & Rohm, K. H. (2000). Preliminary crystallographic studies of 425F mutant of periplasmic *Escherichia coli* L-asparaginase. *Acta Biochimica Polonica, 47*, 807–814.
27. Jaskolski, M., Kozak, M., Lubkowski, J., Palm, G., & Wlodawer, A. (2001). Structures of two highly homologous bacterial L-asparaginases: a case of enantiomorphic space groups. *Acta Crystallographica D, 57*, 369–377.
28. Sanches, M., Barbosa, J. A., De, O., Abrahão, N., & Polikarpov, I. (2003). Structural comparison of Escherichia coli L-asparaginase in two monoclinic space groups. *Acta Crystallographica D, 59*, 416–422.
29. Asselin, B. L., Kreissman, S., Coppola, D. J., Bernal, S. D., Leavitt, P. R., Gelber, R. D., Sallan, S. E., & Cohen, H. J. (1999). Prognostic significance of early response to a single dose of asparaginase in childhood acute lymphoblastic leukaemia. *Journal of Pediatric Hematology/Oncology, 21*, 6–12.
30. Eden, O. B., Shaw, M. P., Lilleyman, J. S., & Richards, S. (1990). Nonrandomized study comparing the toxicity of *Escherichia coli* and *Erwinia asparaginase* in children with leukemia. *Medical and Pediatric Oncology, 18*, 497–502.
31. Vieira Pinheiro, J. P., Wenner, K., Escherich, G., Lanvers-Kaminsky, C., Wurthwein, G., Janka-Schaub, G., & Boos, J. (2006). Serum asparaginase activities and asparagine concentrations in the cerebrospinal fluid after a single infusion of 2500 IU/m2 PEG-asparaginase in children with ALL treated according to protocol COALL-06-97. *Pediatric Blood & Cancer, 46*, 18–25.
32. Narta, U. K., Kanwar, S. S., & Azmi, W. (2007). Pharmacological and clinical evaluation of L-asparaginase in the treatment of leukemia. *Critical Reviews in Oncology/Hematology, 61*, 208–221.
33. Janin, J., Rodier, F., Chakrabarti, P., & Bahadur, R. (2007). Macromolecular recognition in the Protein Data Bank. *Acta Crystallographica D, 63*, 1–8.
34. Park, Y. K., Abuchowski, A., Davis, S., & Davis, F. (1981). Pharmacology of *E. coli* L-asparaginase polyethylene glycols adducts. *Anticancer Research, 1*, 373–376.
35. Noura El-Ahmady, E. N., Sara, M. E. E., & Nancy, M. E. S. (2014). Microbial L-asparaginase as a potential therapeutic agent for the treatment of acute lymphoblastic leukemia: the pros and cons. *International Journal of Pharmaceutics, 10*, 182–199.
36. Duval, M. S., Suciu, A., Ferster, X., Rialland, B., Nelken, et al. (2002). Comparison of *Escherichia coli*-asparaginase with *Erwinia*-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organization for Research and Treatment of Cancer—Children's Leukemia Group phase 3 trials. *Blood, 99*, 2734–2739.
37. Moola, Z. B., Scawen, M. D., Atkinson, T., & Nicholls, D. J. (1994). *Erwinia chrysanthemi* L-asparaginase: epitope mapping and production of antigenically modified enzymes. *Biochemical Journal, 302*, 921–927.
38. Pochedly, C. (1977). Neurotoxicity due to CNS therapy for leukemia. *Medical and Pediatric Oncology, 3*, 101–115.
39. Howard, J. B., & Carpenter, F. H. (1972). L-asparaginase from *Erwinia carotovra* substrate specificity and enzymatic properties. *Journal of Biological Chemistry, 247*, 1020–1030.
40. Patro, K. K. R., Satpathy, S., & Gupta, N. (2011). Evaluation of some fungi for L-asparaginase production. *Indian Journal Of Fundamental And Applied Life Sciences, 1*, 219–221.
41. Izadpanah, Qeshmi, F., Javadpour, S., Malekzadeh, K., Tamadoni, Jahromi, S., & Rahimzadeh, M. (2014). Persian Gulf is a bioresource of potent L-asparaginase producing bacteria: identification & molecular differentiating. *International Journal of Environmental Research , 8*, 813–818.
42. Joner, P. E. (1976). Purification and properties of l-asparaginase B from *Acinetobacter calcoaceticus*. *Biochimica et Biophysica Acta, 438*, 287–295.
43. Hymavathi, M., Sathish, T., Rao, C. S., & Prakasham, R. S. (2009). Enhancement of L-asparaginase production by isolated *Bacillus circulans* MTCC 8574 using response surface methodology. *Applied Biochemistry and Biotechnology, 159*, 191–198.

44. Prakasham, R. S., Hymavathi, M., Rao, C. S., Arepalli, S. K., Rao, J. V., Kennady, P. K., & Sarma, P. N. (2010). Evaluation of antineoplastic activity of extracellular asparaginase produced by isolated *Bacillus circulans. Applied Biochemistry and Biotechnology, 160*(1), 72–80.
45. Wriston, J., & Yellin, T. O. (1973). L-asparaginase: a review. *Advances in Enzymology and Related Areas of Molecular Biology, 39*, 185–248.
46. Wriston, J. R. J. C. (1971). In P. D. Boyer (Ed.), *The enzymes, volume 4: L-asparaginase* (pp. 101–121). New York: Academic Press.
47. Narta, U., Roy, S., Kanwar, S. S., & Azmi, W. (2011). Improved production of l-asparaginase by *Bacillus brevis* cultivated in the presence of oxygen-vectors. *Bioresource Technology, 102*, 2083–2085.
48. Mohapatra, B. R., Sani, R. K., & Banerjee, U. C. (1995). Characterization of L-asparaginase from *Bacillus* sp. *isolated* from an intertidal marine alga *Sargassum sp. Letters in Applied Microbiology, 21*, 380–383.
49. Bascomb, S., Banks, G. T., Skarstedt, M. T., Fleming, A., Bettelheim, K. A., & Connors, T. A. (1975). The properties and large-scale production of L-asparaginase from *Citrobacter. Journal of General Microbiology, 91*, 1–16.
50. Tiul'panova, E. S., Eremenko, V. V., & Mardashev, S. R. (1971). Activity and properties of L-asparaginase from *Bacillus mesentericus* 43A. *Mikrobiologiia, 41*, 423–429.
51. Netrval, J. (1977). Stimulation of L-asparaginase production in *Escherichia coli* by organic and amino acids. *Folia Microbiologica Praha., 22*, 106–116.
52. Nefelova, M. V., Ignatov, S. G., Chigalenchik, A. G., Vinogradov, B. D., & Egorov, N. S. (1978). Biosynthesis of L-asparaginase-2 by cultures of *Bacillus polymyxa* var Ross. *Prikladnaia Biokhimiia i Mikrobiologiia, 14*, 510–514.
53. Mukherjee, J., Majumdar, S., & Scheper, T. (2000). Studies on nutritional and oxygen requirements for production of L-asparaginase by *Enterobacter aerogenes. Applied Microbiology and Biotechnology, 53*, 180–184.
54. Fisher, S. H., & Wray, L. V. (2002). *Bacillus subtilis* 168 contains two differentially regulated genes encoding L-asparaginase. *Journal of Bacteriology, 184*, 2148–2154.
55. Nawaz, M. S., Zhang, D., Khan, A. A., & Cerniglia, C. E. (1998). Isolation and characterization of Enterobacter cloacae capable of metabolizing asparagine. *Applied Microbiology and Biotechnology, 50*, 568–572.
56. Golden, K. J., & Bernlohr, R. W. (1985). Nitrogen catabolite repression of the L-asparaginase of *Bacillus licheniformis. Journal of Bacteriology, 164*, 938–940.
57. Sudhir, A. P., Dave, B. R., Prajapati, A. S., Panchal, K., Patel, D., & Subramanian, R. B. (2014). Characterization of a recombinant glutaminase-free L-asparaginase (ansA3) enzyme with high catalytic activity from *Bacillus licheniformis. Applied Biochemistry and Biotechnology, 174*(7), 2504–2515.
58. Tiwari, N., & Dua, R. D. (1996). Purification and preliminary characterization of L-asparaginase from *Erwinia aroideae. Indian Journal of Biochemistry and Biophysics, 33*, 371–376.
59. Maladkar, N. K. Singh, V. K. Naik, S. R. Fermentative production and isolation of L-asparaginase from Erwinia carotovora EC-113. Hindustan. Antibiot. Bull 35, 77–86.
60. Mesas, J. M., Gil, J. A., & Martin, J. F. (1990). Characterization and partial purification of l-asparaginase from *Corynebacterium glutamicum. Journal of General Microbiology, 136*, 515–519.
61. Stark, R. M., Suleiman, M. S., Hassan, I. J., Greenman, J., & Millar, M. R. (1997). Amino acid utilization and deamination of glutamine and asparagine by *Helicobacter pylori. Journal of Medical Microbiology, 46*, 793–800.
62. Pastuszak, I., & Szymona, M. (1976). Purification and properties of L-asparaginase EC-3511 from *Mycobacterium phlei. Acta. Biochemistry Pol., 23*, 37–44.
63. Reddy, V. K., & Reddy, S. M. (1990). Effect of carbon and nitrogen sources on L-asparaginase production by bacteria. *Indian Journal of Microbiology, 30*, 81–83.
64. Mikucki, J., Szarapińska-Kwaszewska, J., & Krzemiński, Z. (1977). Factors influencing L-asparaginase production by *Staphylococci. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, 132*, 135–142.
65. Kotzia, G. A., & Labrou, N. E. (2009). Engineering thermal stability of l-asparaginase by in vitro directed evolution. *FEBS Journal, 276*, 1750–1761.
66. Rozalska, M., & Mikucki, J. (1991). Staphylococcal L-asparaginase: catabolic repression of synthesis. *Acta Microbiologica Polonica, 41*, 145–150.
67. Zhang, N., Clarke, F. D., Trapani, G., Keough, D., & Beacham, I. (1995). Guinea pig serum L-asparaginase: purification and immunological relationship to liver L-asparaginase and serum L-asparaginases in other mammals. *Comparative Biochemistry and Physiology. B, 112*, 607–612.
68. Nilolaev, A., Sokolov, N. N., Kozlov, E. A., & Kutsman, M. E. (1974). Isolation and properties of a homogeneous L-asparaginase preparation from Pseudomonas fluorescens AG. *Biokhimiia, 40*, 984–989.
69. Wriston, J. C. Jr, (1985) *Methods Enzymol*, volume 113. Asparaginase, pp.608–618.

70. El-Din, S. M., & Foda, M. S. (1976). Kinetics and properties of L-glutaminase and L-asparaginase activities of *Pseudomonas ovalis*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, 131*, 489–496.
71. Manna, S., Sinha, A., Sadhukhan, R., & Chakrabarty, S. L. (1995). Purification characterization and antitumor activity of L-asparaginase isolated from *Pseudomonas stutzeri* MB-405. *Current Microbiology, 30*, 291–298.
72. Yao, M., Yasutake, Y., Morita, H., & Tanaka, I. (2005). Structure of the type I L-asparaginase from the hyperthermophilic archaeon *Pyrococcus horikoshii* at 216 angstroms resolution. *Acta Crystallographica D., 61*, 294–301.
73. Rowly, B., & Wriston, J. C. (1967). L-Asparaginase from *Serratia marcescens*. *Biochemical and Biophysical Research Communications, 28*, 160–171.
74. Pritsa, A. A., & Kyriakidis, D. A. (2001). L-Asparaginase of *Thermus thermophilus*: purification properties and identification of essential amino acids for its catalytic activity. *Molecular and Cellular Biochemistry, 216*, 93–101.
75. Curran, M. P., Daniel, R. M., Guy, G. R., & Morgan, H. W. (1985). A specific l-asparaginase from *Thermus aquaticus*. *Archives of Biochemistry and Biophysics, 241*, 571–576.
76. Disteasio, J. A., & Niedennan, A. (1976). Purification and characterization of L-asparaginase with anti-lymphoma activity from *Vibrio succinogenes*. *Journal of Biological Chemistry, 251*, 6929–6933.
77. Davidson, L., Burkom, M., Ahn, S., Chang, L. C., & Kitto, B. (1977). L-Asparaginases from *Citrobacter freundii*. *Biochimica et Biophysica Acta, 480*, 282–294.
78. Tosa, T., Sano, R., Yamamoto, K., Nakamura, M., & Chibata, I. (1972). L-Asparaginase from Proteus vulgaris; purification, crystallization and enzymatic properties. *Journal of Biochemistry, 11*, 217–222.
79. Pinheiro, I. O., Araujo, J. M., Ximenes, E. C. P. A., Pinto, J. C. S., & Alves, T. L. M. (2001). Production of L-asparaginase by Zymomonas mobilis strain CP4. *Biomater Diagnosi, 6*, 243–244.
80. Sanches, M., Krauchenco, K., & Polikarpov, I. (2001). Structure substrate complexation and reaction mechanism of bacterial asparaginases. *Current Chemical Biology, 1*, 175–186.
81. Borek, D., & Jaskólski, M. (2001). Sequence analysis of enzymes with asparaginase activity. *Acta Biochimica Polonica, 484*, 893–902.
82. Narayana, K. J. P., Kumar, K. G., & Vijayalakshmi, M. (2008). L-asparaginase production by *Streptomyces albidoflavus*. *Indian Journal of Microbiology, 48*, 331–336.
83. Shrivastava, A., Khan, A. A., Shrivastav, A., Jain, S. K., & Singhal, P. K. (2012). Kinetic studies of L-asparaginase from *Penicillium digitatum*. *Biochemistry and Biotechnology, 42*, 574–581.
84. Nagarajan, A., Thirunavukkarasu, N., Suryanarayanan, T. S., & Gummadi, S. N. (2014). Screening and isolation of novel glutaminase free L-asparaginase from fungal endophytes. *Research Journal of Microbiology, 9*, 163–176.
85. Saxena, R. K., & Sinha, U. (1981). L-asparaginase and glutaminase activities in culture filtrate of *Aspergillus nidulans*. *Current Science, 50*, 218–219.
86. Mishra, A. (2006). Production of L-asparaginase an anticancer agent from *Aspergillus niger* using agricultural waste in solid state fermentation. *Applied Biochemistry and Biotechnology, 135*, 33–42.
87. Hendriksen, H. V., Kornbrust, B. A., Ostergaard, P. R., & Stringer, M. A. (2009). Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from *Aspergillus oryzae*. *Journal of Agricultural and Food Chemistry, 57*, 4168–4176.
88. Sarquis, M. I. D. M., Oliveira, E. M. M., Santos, A. S., & Costa, G. L. D. (2004). Production of L-asparaginase by filamentous fungi. *Memórias do Instituto Oswaldo Cruz, 99*, 489–492.
89. Baskar, G., & Renganathan, S. (2009). Production of L-asparaginase from natural substrates by *Aspergillus terreus* MTCC 1782: effect of substrate supplementary nitrogen source and L-asparagine. *International Journal of Chemical Reactor Engineering, 7*, 1–16.
90. Raha, S. K., Roy, S. K., Dey, S. K., & Chakrabarty, S. L. (1990). Purification and properties of an L-asparaginase from *Cylindrocarpon obtusisporum* MB-10. *Biochemistry International, 21*, 987–1000.
91. Mohapatra, B. R., Bapuji, M., & Banerjee, U. C. (1997). Production and properties of L-asparaginase from *Mucor* species associated with a marine sponge *Spirastrella* sp. *Cytobios, 92*, 165–173.
92. Nakahama, K., Imada, A., Igarasi, S., & Tubaki, K. (1973). Formation of L-asparaginase by *Fusarium* species. *Journal of General Microbiology, 75*, 269–273.
93. Nagarethinam, S., Nagappa, A., Udupa, N., & Rao, V. (1995). Microbial L-asparaginase and its future prospects. *Asian Journal of Medical Research, 1*, 159–168.
94. Kil, J. O., Kim, G. N., & Park, I. (1995). Extraction of extracellular L-asparaginase from *Candida utilis*. *Bioscience, Biotechnology, and Biochemistry, 59*, 749–750.
95. Stepnyan, K. R., & Davtyan, M. A. (1998). Some questions of thermostability of L-asparaginase of yeast *Candida guilliermondii*. *Biology Zhurn. Armenii., 41*, 599–603.

96. Daverey, A., & Pakshirajan, K. (2010). Kinetics of growth and enhanced sophorolipids production by *Candida bombicola* using a low-cost fermentative medium. *Applied Biochemistry and Biotechnology, 160*(7), 2090–2101.
97. Foda, M. S., Zedan, H. H., & Hashem, S. A. (1980). Formation and properties of L-glutaminase and L-asparaginase activities in *Pichia polymorpha. Acta Microbiologica Polonica, 29*, 343–352.
98. Ramakrishnan, M. S., & Joseph, R. (1996). Characterization of an extracellular asparaginase of *Rhodosporidium toruloides* CBS14 exhibiting unique physicochemical properties. *Canadian Journal of Microbiology , 42*, 316–325.
99. Bon, E. P., Carvajal, E., Stanbrough, M., Rowen, D., & Magasanik, B. (1997). Asparaginase II of *Saccharomyces cerevisiae. Applied Biochemistry and Biotechnology, 63*, 203–212.
100. Balakrish-Nair, G., Selvakumar, N., Chandramohan, D., & Natarajan, R. (1977). Distribution & activity of L-asparaginase producing fungi in the marine environment of Porto Novo. *Indian Journal of Marine Sciences, 62*, 172–173.
101. Dhevendran, K., & Annie, K. (1999). Antibiotic and L-asparaginase activity of Streptomycetes isolated from fish shellfish and sediment of Veli estuarine lake along Kerala coast. *Indian Journal of Marine Sciences, 28*, 335–337.
102. Sahu, M. K., Sivakumar, K., Poorani, E., Thangaradjou, T., & Kannan, L. (2007). Studies on L-asparaginase enzyme of actinomycetes isolated from estuarine fishes. *Journal of Environmental Biology, 28*, 465–474.
103. Gupta, N., Mishra, S., & Basak, U. C. (2007). Occurrence of *Streptomyces aurantiacus* in mangroves of Bhitarkanika. *MJM, 3*, 7–14.
104. Mostafa, S. A., & Salama, M. S. (1979). L-Asparaginase-producing Streptomyces from the soil of Kuwait. *Zentralbl Bakteriol Naturwiss, 134*, 325–334.
105. DeJong, P. J. (1972). L-asparaginase production by *Streptomyces griseus. Applied Microbiology, 23*, 1163–1164.
106. Amena, S., Vishalakshi, N., Prabhakar, M., Dayanand, A., & Lingappa, K. (2010). Production purification and characterization of L-asparaginase from *Streptomyces gulbargensis. Brazilian Journal of Microbiology, 41*, 173–178.
107. Mostafa, S. A. (1979). Activity of L-asparaginase in cells of *Streptomyces karnatakensis. Zentralbl Bakteriol Naturwiss, 134*, 343–351.
108. Abdel-Fattah, Y. R., & Olama, Z. A. (2002). L-asparaginase production by *Pseudomonas aeruginosa* in solid-state culture: evaluation and optimization of culture conditions using factorial designs. *Process Biochemistry, 38*, 115–122.
109. Koshy, A. K., Dhevendaran, M. I., Georgekutty, P., & Natarajan. (1997). L-asparaginase in *Streptomyces plicatus* isolated from the alimentary canal of the fish *Gerres filamentosus* Cuvier. *Journal of Marine Biotechnology, 5*, 181–185.
110. Kavitha, A., & Vijayalakshmi, M. (2010). Optimization and purification of L-asparaginase produced by *Streptomyces tendae* TK-VL 333. *Zeitschrift für Naturforschung. Teil B, 65*, 528–531.
111. Mostafa, S. A. (1979). Production of L-asparaginase by *Streptomyces karnatkensis* and *Streptomyces venezuelae. Zentralblatt für Bakteriologie Orig A., 134*, 429–436.
112. Mostafa, S. A., & Ali, O. K. A. (1983). L-asparaginase activity in cell-free extracts of Thermoactinomyces vulgaris 13 MES. *Zentralblatt für Bakteriologie Mikrobiologie , 138*, 397–404.
113. Kavitha, A., & Vijayalakshmi, M. (2012). A study on L-asparaginase of Nocardia levis MK-VL_113. *Scientific World Journal, 96*(120), 160434–160434.
114. Paul, J. H. (1982). Isolation and characterization of a *Chlamydomonas* L-asparaginase. *Journal of Biochemistry, 203*, 109–115.
115. Usha, Pandey and J, Pandey. (2011) Food science and technology imprint, volume 2: Enhanced production of β-carotene and L-asparaginase from a tribal food alga Vaucheria uncinata (Anthony J Greco ed), pp. 195–208.
116. Barnes, W. R., Dorn, G. L., & Vela, G. R. (1977). Effect of culture conditions on the synthesis of L-asparaginase by *Escherichia coli* A-1. *Applied Environmental Microbiology, 332*, 257–261.
117. Ahmad, N., Pandit, N. P., & Maheshwari, S. K. (2012). L-asparaginase gene—a therapeutic approach towards drugs for a cancer cell. *International Journal of Biosciences, 2*, 1–11.
118. Dharmsthiti, S. C., & Luechai, S. (2010). Purification and characterization of asparaginase from solid state culture of *Aspergillus niger* AK10. *International Journal of Biochemistry And Biotechnology, 6*, 1083–1092.
119. Moorthy, V., Ramalingam, A., Sumantha, A., & Shankaranaya, R. T. (2010). Production purification and characterization of extracellular L-asparaginase from a soil isolate of *Bacillus* sp. *AGMR African journal of microbiology , 4*, 1862–1867.
120. Makky, S. E. A., Jee, J. O., Karim, M., & Lee, C. M. (2013). Production and optimization of L-asparaginase by Bacillus sp KK2S4 from the corn cob. *African Journal of Biotechnology, 12*, 2654–2658.

121. Basha, N. S., Rekha, R., Komala, M., & Ruby, S. (2009). Production of extracellular anti-leukaemic enzyme L-asparaginase from marine actinomycetes by solid state and submerged fermentation: purification and characterisation. *Tropical Journal of Pharmaceutical Research, 2009*, 8.
122. Kamble, V. P., Rao, R. S., Borkar, P. S., Khobragade, C. N., & Dawane, B. S. (2006). Purification of L-asparaginase from a bacteria *Erwinia carotovora* and effect of a dihydropyridine derivative on some of its kinetic parameters. *Indian Journal of Biochemistry and Biophysics, 43*, 391.
123. Dhevagi, P., & Poorani, E. (2006). Isolation and characterization of L-asparaginase from marine actinomycetes. *IJBT, 5*, 514.
124. El-Bessoumy, A. A., Sarhan, M., & Mansour, J. (2004). Production isolation and purification of L-asparaginase from *Pseudomonas aeruginosa* 50071 using solid-state fermentation. *Journal of Biochemistry and Molecular Biology, 37*, 387–393.
125. Gaffar, S. A., & Shethna, Y. I. (1977). Purification and some biological properties of asparaginase from *Azotobacter vinelandii. Applied and Environmental Microbiology, 33*, 508–514.
126. Kafkewitz, D., & Goodman, D. (1974). L-Asparaginase production by the rumen anaerobe *Vibrio succinogenes. Journal of Applied Microbiology, 27*, 206–209.
127. Hosamani, R., Hosamani, R., & Kaliwal, B. B. (2011). Isolation molecular identification and optimization of fermentation parameters for the production of L-asparaginase, an anticancer agent by *Fusarium equiseti. International Journal of Microbiology and Immunology Research, 3*, 108–119.
128. Eisele, N., Linke, D., Bitzer, K., Na'amnieh, S., Nimtz, M., & Berger, R. G. (2011). The first characterized asparaginase from a basidiomycete *Flammulina velutipes. Bioresource Technology, 102*, 3316–3321.
129. Priya, P. M., Radhakrishnan, M., & Balagurunathan, R. (2011). Production and optimization of L-asparaginase from Streptomyces sp TA22 isolated from Western Ghats, India. *Journal of Chemical and Pharmaceutical Research, 3*, 618–624.
130. Prakasham, R. S., Rao, C., Rao, R. S., Lakshmi, G. S., & Sarma, P. N. (2007). L-asparaginase production by isolated *Staphylococcus* sp.-6A: design of experiment considering interaction effect for process parameter optimization. *Journal of Applied Microbiology, 102*, 1382–1391.
131. Krautheim, P., Greenstein, T., & Kafkewitz, D. (1982). Asparaginase production by chemostat cultures of *Vibrio succinogenes. FEMS Microbiology Letters, 13*, 105–107.
132. Khushoo, A., Pal, Y., Singh, B. N., & Mukherjee, K. J. (2004). Extracellular expression and single step purification of recombinant *Escherichia coli* L-asparaginase II. *Protein Expression and Purification , 38*, 29–36.
133. Reddy, V. V., Jayaram, H. N., Sirsi, M., & Ramakrishnan, T. (1969). Inhibitory activity of l-asparaginase from *Mycobacterium tuberculosis* on Yoshida ascites sarcoma in rats. *Archives of Biochemistry and Biophysics, 132*, 262–267.
134. Steckel, J., Roberts, J., Philips, F. S., & Chou, T. C. (1983). Kinetic properties and inhibition of *Acinetobacter* glutaminase-asparaginase. *Biochemical Pharmacology, 32*, 971–977.
135. Mikucki, J., Sobiś, M., & Różalska, M. (1986). Localisation of staphylococcal L-asparaginase. *Microbiology Letters , 37*, 129–133.
136. Scheetz, R. W., Whelan, H. A., & Wriston, J. C. (1971). Purification and properties of an l-asparaginase from *Fusarium tricinctum. Biochemistry and Biophysics, 142*, 184–189.
137. Joner, P. E., Kristiansen, T., & Einarsson, M. (1973). Purification and properties of l-asparaginase a from *Acinetobacter calcoaceticus. Biophysics Acta, 327*, 146–156.
138. Boyd, J. W., & Phillips, A. W. (1971). Purification and properties of L-asparaginase from *Serratia marcescens. JB, 106*, 578–587.
139. Law, A. S., & Wriston, J. C. (1971). Purification and properties of Bacillus coagulans-asparaginase. *Archives of Biochemistry and Biophysics, 147*, 744–752.
140. Ramadan, M. E. D. A., Asmar, F. E., & Greenberg, D. M. (1964). Purification and properties of glutaminase and asparaginase from a pseudomonad: I. Purification and physical-chemical properties. *Archives of Biochemistry and Biophysics, 108*, 143–149.
141. Kotzia, G. A., & Labrou, N. E. (2005). Cloning expression and characterisation of *Erwinia carotovora* l-asparaginase. *Journal of Biotechnology, 119*, 309–323.
142. Kumar, S., Venkata Dasu, V., & Pakshirajan, K. (2011). Purification and characterization of glutaminase-free l-asparaginase from *Pectobacterium carotovorum* MTCC 1428. *Bioresource Technology, 102*, 2077–2082.
143. Kenari, S. L. D., Alemzadeh, I., & Maghsodi, V. (2011). Production of l-asparaginase from *Escherichia coli* ATCC 11303: optimization by response surface methodology. *Food and Bioproducts Processing, 89*, 315–321.
144. Cappelletti, D., Chiarelli, L. R., Pasquetto, M. V., Stivala, S., Valentini, G., & Scotti, C. (2008). *Helicobacter pylori* l-asparaginase: a promising chemotherapeutic agent. *Biochemical and Biophysical Research Communications, 377*, 1222–1226.

145. Ursi, D., Prozzi, D., Davison, J., & Brunel, F. (1987). Cloning and expression of aspartase and asparaginase from *Pseudomonas* PO7111. *Journal Biotechnology, 53*, 221–225.
146. Sukumaran, C. P., Singh, D. V., & Mahadevan, P. R. (1979). Synthesis of L-asparaginase by *Serratia marcescens* Nima. *Journal of Biosciences, 13*, 263–269.
147. Peterson, R. E., & Ciegler, A. (1969). L-asparaginase production by *Erwinia aroideae. Applied Microbiology, 18*, 64–67.
148. Meister A. (2009) Advances in enzymology and related areas of molecular biology, 11th ed.. John Wiley & Sons.
149. Makky, E. A., Loh, Y. C., & Karim, M. R. (2014). Purification and partial characterization of a low molecular weight l-asparaginase produced from corncob waste. *Biocatalysis and Agricultural Biotechnology, 34*, 265–270.
150. Kumar, D. S., & Sobha, K. (2012). L-Asparaginase from microbes: a comprehensive review. *Advances in Bioresearch, 3*, 137–157.
151. Balcao, V. M., Mateo, C., Fernández-Lafuente, R., Malcata, F. X., & Guisán, J. M. (2001). Structural and functional stabilization of L-asparaginase via multisubunit immobilization onto highly activated supports. *Biotechnology Progress, 17*, 537–542.
152. Dunlop, P. C., & Roon, R. J. (1975). L-Asparaginase of *Saccharomyces cerevisiae*: an extracellular enzyme. *Journal of Bacteriology, 122*, 1017–1024.
153. Ylikangas, P., & Mononen, I. (2000). A fluorometric assay for L-asparaginase activity and monitoring of L-asparaginase therapy. *Analytical Biochemistry, 280*, 42–45.
154. Meister, A. (1955). In N. O. Colowick SP Kaplan (Ed.), *Methods in enzymology II* (pp. 380–385). New York: Academic Press.
155. Tagami, S., & Matsuda, K. (1990). An enzymatic method for the kinetic measurement of L-asparaginase activity and L-asparagine with an ammonia gas sensing electrode. *Chemical & Pharmaceutical Bulletin (Tokyo), 38*, 153–155.
156. Peter, P. K. H., Elizabeth, B. M., Jesse, L. B., Edward, L. G., Philip, J. B., Bruce, H. F., Laverne, D. B., & Robert, W. S. (1970). Crystalline L-asparaginase from *Escherichia coli. B Journal Biology Chemistry, 245*, 3708–3715.
157. Cedar, H., & Schwartz, J. H. (1967). Localization of the two L-asparaginases in anaerobically grown *Escherichia coli. Biological Chemistry, 242*, 3753–3755.
158. Drainas, D., & Drainas, C. (1985). A conductimetric method for assaying asparaginase activity in *Aspergillus nidulans. European Journal of Biochemistry, 151*, 591–593.
159. Szymanska, B., Wilczynska-Kalak, U., Kang, M. H., Liem, N. L., Carol, H., & Lock, R. B. (2012). Pharmacokinetic modelling of an induction regimen for in vivo combined testing of novel drugs against pediatric acute lymphoblastic leukemia xenografts. *PloS One, 7*(3), e33894–e33994.
160. Salzer, W. L., Asselin, B. L., Plourde, P. V., Corn, T., & Hunger, S. P. (2014). Development of asparaginase *Erwinia chrysanthemi* for the treatment of acute lymphoblastic leukemia. *Annals of the New York Academy of Sciences, 1329*, 81–92.
161. Verma, N., Bansal, M., & Kumar, S. (2012). Whole cell based miniaturized fiber optic biosensor to monitor L-asparagine. *Journal of Applied Sciences Research, 3*, 809–814.
162. Zubavichus, Y., Fuchs, O., Weinhardt, L., Heske, C., Umbach, E., Denlinger, J. D., & Grunze, M. (2004). Soft X-ray-induced decomposition of amino acids: an XPS mass spectrometry and NEXAFS study. *Radiation Research, 161*, 346–358.
163. Kumar, K., Kataria, M., & Verma, N. (2013). Plant asparaginase-based asparagine biosensor for leukemia. *Artificial Cells, Nanomedicine, and Biotechnology, 41*, 184–188.
164. Sinha, R., Singh, H. R., & Jha, S. K. (2013). L-Asparaginase: present and future prospective. *IJIRSET, 2*, 7031–7051.
165. Lingnert, H., Grivas, S., Jagerstad, M., Skog, K., Tornqvist, M., & Aman, P. (2002). Acrylamide in food—mechanisms of formation and influencing factors during heating of foods. *Scandinavian Journal of Nutrition, 46*, 159–172.
166. Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Food chemistry: acrylamide is formed in the Maillard reaction. *Nature, 419*, 448–449.
167. Gokmen, V., & Palazoglu, T. K. (2008). Acrylamide formation in foods during thermal processing with a focus on frying. *Food and Bioprocess Technology, 1*, 35–42.
168. Friedman, M. (2003). Chemistry biochemistry and safety of acrylamide. *Journal of Agricultural and Food Chemistry, 51*, 4504–4526.
169. Pedreschi, F., Kaack, K., & Granby, K. (2008). The effect of asparaginase on acrylamide formation in French fries. *Food Chemistry, 109*, 386–392.
170. Morales, F., Capuano, E., & Fogliano, V. (2008). Mitigation strategies to reduce acrylamide formation in fried potato products. *Annals of the New York Academy of Sciences, 1126*, 89–100.