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REVIEW ARTICLE

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Bacterial L-asparaginases for cancer therapy: Current knowledge and future perspectives

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Abstract

L-Asparaginases hydrolyzing plasma L-asparagine and L-glutamine has attracted tremendous attention in recent years owing to remarkable anticancer properties. This enzyme is efficiently used for acute lymphoblastic leukemia (ALL) and lymphosarcoma and emerged against ALL in children, neoplasia, and some other malignancies. Cancer cells reduce the expression of L-asparaginase leading to their elimination. The L-asparaginase anticancerous application approach has made incredible breakthrough in the field of modern oncology through depletion of plasma L-asparagine to inhibit the cancer cells growth; particularly among children. High level of L-asparaginase enzyme production by *Escherichia coli*, *Erwinia* species, *Streptomyces*, and *Bacillus subtilis* species is highly desirable as bacterial alternative enzyme sources for anticancer therapy. Thermal or harsh conditions stability of those from the two latter bacterial species is considerable. Some enzymes from marine bacteria have conferred stability in adverse conditions being more advantageous in cancer therapy. Several side effects exerted by L-asparaginases such as hypersensitivity should be hindered or decreased through alternative therapies or use of immune-suppressor drugs. The L-asparaginase from *Erwinia* species has displayed remarkable traits in children with this regard. Noticeably, *Erwinia chrysanthemi* L-asparaginase exhibited negligible glutaminase activity representing a promising efficiency mitigating related side effects. Application of software such as RSM would optimize conditions for higher levels of enzyme production. Additionally, genetic recombination of the encoding gene would indisputably help improving enzyme traits. Furthermore, the possibility of anticancer combination therapy using two or more L-asparaginases from various sources is plausible in future studies to achieve better therapeutic outcomes with lower side effects.

KEYWORDS

bacterial L-asparaginase, cancer therapy, optimized production

1 | INTRODUCTION

Various therapeutic approaches have been verified and evaluated for the acute lymphoblastic leukemia (ALL; Gao, Dusenbery, Cao, Smith, & Yuan, 2018; Park et al., 2018), however, some poor clinical outcomes and side effects such as drug resistance, cytokine release syndrome and neurotoxic events have been observed. Many protein species exhibit tremendous cytotoxic activities which have been exploited to

develop new antitumor drugs (Serna et al., 2018). L-Asparaginases exert a powerful antilymphoma function. The maximum level function at physiological pH and temperature is one of the prerequisites of L-asparaginase for antitumor activity. For the first time, amidohydrolytic activity of L-asparaginase was observed by Lang and more often confirmed by Forsen and Friedman (Campbell, Mashburn, Boyse, & Old, 1967), while those from other mammals lacked this activity (Kidd & Sobin, 1966) and by observation of metabolites variety between normal and malignant cells in vitro (Neuman & McCoy, 1956; Oettgen et al., 1970). Although the theory of the enzyme application for cancer has been accepted, some difficulties were met with the clinical use of the enzyme, and until that time the guinea pig was the only source of the enzyme. L-Asparaginase was effective against only some cancer types but not all through cytokine production. In 1964, Wriston and Mashburn studied the L-asparaginase enzyme in *Escherichia coli* (Wriston & Yellin, 1973). Until today, several microbial sources for L-asparaginase have been revealed such as *E. coli*, *Erwinia cartovora*, *Erwinia chrysanthemi*, *Bacillus subtilis*, marine *Actinomycete* species, *Serratia marcescens*, *Vibrio cholerae*, *Corynebacterium glutamicum*, *Staphylococcus aureus*, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus nidulans*, and *Aspergillus terreus* (Avramis et al., 2002; Batool, Makky, Jalal, & Yusoff, 2016; Jaccard et al., 2011; Mitchell & Group, 2003; Pieters et al., 2011; Vrooman et al., 2010). However, not all have demonstrated cytotoxicity for leukemic cells and tumor inhibitory activity (Batool et al., 2016). Higher levels and sufficient quantity of L-asparaginase enzyme production by *E. coli* and *Erwinia cartovora* MTCC 1428 (0.176 U/8 hr) led to consideration of its large scale production for anticancer therapy. *B. subtilis* strain HSWX88 (23.8 IU/ml) and *E. coli* CTLS20 (30.22 IU/mg) were also exhibited high levels of L-asparaginase production (Kumar, Dasu, & Pakshirajan, 2011; Vrooman et al., 2013). It is notable that *Streptomyces acrimycini* with a total activity of 1510 U/ml with a specific activity of 10.79 U/mg of protein was reported from marine sources. Recently, two novel recombinant anticancer enzymes from *V. cholerae* and *Bacillus tequilensis* PV9WS (in BL21 or DE3) were expressed and their high efficiency with regard to long lasting effect, rapid time production, lower cost, higher activity, and lack of glutaminase activity were confirmed (Radha, Arumugam, & Gummadi, 2018; Shakambari et al., 2018). Several strains of other species such as *Pseudomonas stutzeri*, *E. aroideae*, and *Terreus*, *S. marcescens* have been isolated and screened to produce L-asparaginase. In general, the biochemical and functional properties of each enzyme vary according to related source of microorganism. Prokaryotic L-asparaginase is cost effective and eco-friendly nature compared with eukaryotic enzyme. It has been verified that marine bacteria produce L-asparaginases with higher efficiency and remarkably higher pH or thermal stability (Jiang et al., 2012; Shrivastava et al., 2016). Among these enzymes, only *E. coli* asparaginase, pegylated (PEG)-asparaginase (derived from *E. coli*; Mahajan et al., 2014) and *E. chrysanthemi* asparaginase have been used as chemotherapeutic drugs for the treatment of leukemia (Asselin & Rizzari, 2015; Shakambari et al., 2016). It is notable that commercially available L-asparaginases possess glutaminase activity, antigenicity and a very short half-life in blood conferring possible diverse side effects and severe allergic reactions in patients (Asselin & Rizzari, 2015).

Two types of asparaginase enzyme including the EC-1 (cytoplasmic type) and EC-2 (periplasmic type) have been produced by *E. coli*, of which the EC-2 demonstrates antilymphoma activity (Chang, 2013). The study on L-asparaginases from *E. coli* and *E. cartovora* has displayed a lack of cross-reactivity, but exhibited high levels of immunogenicity leading to hypersensitivity. Polyethylene glycol (PEG) is recognized as a material for prevention of the immune responses without changing the enzyme anticancer properties. This modified version of the enzyme in the animal model demonstrated a decreased induction of antibodies and displayed significantly more long-lasting effects. In 2006, asparaginase was introduced as an efficient drug for the treatment of leukemia by the European Medicines Agency. Asparaginase was eventually approved as a drug for the treatment of leukemia by the Food and Drug Administration (FDA; Domenech et al., 2011). The reason for the L-asparaginase application is its biodegradability and non-toxicity, though being costly (Kamble et al., 2012). L-asparagine, like other amino acids form *E. coli* (L-form), is naturally translated at the level of the ribosome in the structure of proteins, in contrast to the D-form amino acids, which occur during the posttranslational changes in the protein structure (Pisarewicz, Mora, Pflueger, Fields, & Marí, 2005).

Noticeably, thermo-tolerant L-asparaginase reduces acrylamide in food products conferring anticancer effect. Cloning and expression of L-asparaginase from *Thermococcus zilligii* AN1, TziAN1-1 could reduce the acrylamide in fries. The recombinant asparaginase was isolated by a reluctant nickel chromatography. The maximum activity of this enzyme is at pH 8.5 and $T = 90°C$, but the optimum enzymatic activity temperature is slightly >45°C. The enzyme has 73% of its core activity at 85°C range. When the fried potatoes were combined with 10 U/ml of L-asparaginase at 80°C for 4 min, the content of acrylamide in this sample was reduced in fries (Zuo, Zhang, Jiang, & Mu, 2015). During several research conducted to optimize the various products in the industry and products using the response surface methodology (RSM) software, many studies have been implemented. For example, on acetic acid as an important industrial food reservoir, the RSM method was used to analyze the required compounds, and some targeted studies and different variables were obtained. To increase the production of L-asparaginase enzymes and optimize culture conditions, first the L-asparagine enzyme expression is induced in *E. coli* and the expression conditions including isopropyl-β-D-thiogalactoside (IPTG), optical density (OD), and T_m is optimized to produce highest amount of the enzyme (Borah, Yadav, Sangra, Shahin, & Chaubey, 2012; Manikandan, Pratheeba, Pankaj, & Sah, 2010).

Considering the importance of L-asparaginase in the treatment of leukemia and the possibility of thrombotic complications during anticancer therapy, high level consumption but insufficient knowledge of its production, enzyme traits improvement alongside increasing the rate of production on the industrial scale using genetic recombination seems essential (Eden, Hipkins, & Bradbury, 2016). (Exerting the maximum asparaginase but minimum glutaminase activity with stable functionality over a long period or at different

times when administered at intervals) Given the fact that the enzyme is injected with different severity at different intervals, the enzyme's activity decreases when the intervals are prolonged, resulting in the presence of an enzyme with high activity. Therefore, production optimization and reducing adverse events by improving enzyme features with molecular techniques will provide promising outcomes in cancer therapy using efficient bacterial L-asparaginases. Additionally, *E. chrysanthemi* novel L-asparaginase exerted fewer hypersensitivity during anticancer therapy (Nguyen et al., 2018).

2 | L-ASPARAGINASE PROPERTIES AND ACTIVITY

All L-asparaginase produced by these bacteria are homo-tetramers (each subunit having C1377H2208N382O442S17) according to X-ray crystallographic data and their active sites include subunits, but the natural form of asparaginase produced by *Pyrococcus furiosus* is a dimmer (Bansal, Gnaneswari, Mishra, & Kundu, 2010). It hydrolyzes asparagine into aspartic acid and ammonia. ALL correspond to the most common childhood acute leukemia in 80% of children and 20% of adults' leukemia. Decrease in L-asparagine leads to inhibition of protein synthesis and cell cycle arrest in the G1-phase causing leukemia cell apoptosis. Because of lack of asparagine synthase in cancer cells, they need extracellular asparagine source. Asparaginase causes depletion of asparagine in bloodstream, causing death of cancer cells. To increase the production of the L-asparaginase enzyme by free cells in the culture medium, optimizing the conditions for the expression of the recombinant enzyme using the RSM software would be beneficial (Deokar, Vetal, & Rodrigues, 2010). The most anticancerous effect of L-asparaginase is in ALL cases particularly as a preservative in chronic forms. Owing to the rapid occurrence of resistance against its effect in the body, in the treatment of children with ALL, this drug is combined with vincristine and prednisolone as a combination of VPL-ASP. As a result, by reducing plasma asparagine, the apoptosis will occur in cancerous cells. L-Asparaginase has a specific inhibitory effect on the G1 phase of cell cycle (Agrawal et al., 2003; Avramis, 2014; Maggi, Chiarelli, Valentini, & Scotti, 2015). The *E. coli* CTLS20 and *Bacillus* sp. strains exhibited to produce high levels of enzyme production in solid state fermentation (SSF) method. *E. coli* L-asparaginase covalent binding with methoxy-PEG (PEG-ASP) provides the basis for the activity of this enzyme. Two productions are listed and the active sites of the enzyme for the asparagine are divided into two motifs (Place et al., 2015). The cytoplasmic type asparaginases mostly exert glutaminase activity, while periplasmic asparagine from anaerobic bacteria have a high affinity to asparagine, while exhibiting lower to negligible glutaminase activity (Bertrand et al., 2017). The half-life of the medication after intramuscular injection is 39–49 hr and after intravenous injection lasts 8–30 hr. The blood stream asparagine will recover again in plasma in 23–33 days after discontinuation of treatment (Arif & Hussain, 2014). The natural form of L-asparaginase is expressed in *E. coli* with a molecular weight of 138–141 kDa, with four similar subunits, each of which being a subunit of one active site in its center. The asparaginase purified from *E. coli* is a tetramer composed of 222 symmetric units. The enzyme consists of four subunits, each of which containing 326 amine acids and each active site interact with an enzyme substrate to produce aspartate molecule. Two amino acids (Thr12 and Thr89) are required for the activity of the enzyme (Palm et al., 1996). The Thr12 is involved in the formation of the acyl group, but Thr89 forms triad with three amino acids (T89, K162, and D90), which has a pattern similar to that of triad (S, H, and D) proteases. In addition to the chemical similarity of these three triad formed amino acids, the unusual form in the Lys twofold form has a more catalytic activity than Form 3. The factor that induces the asparaginase glutaminase activity is the transfer RNA and general control nonderepressible kinase 2 (GCN2; serine-threonine kinase). Asparaginase activity as glutaminase enzyme (asparagine elimination > glutamine elimination), leads to a response that results in the discharge of GCN2 (6002) (Kim et al., 2015). Both enzymes from *E. coli* and *Erwinia* species have activity and resistance to high concentrations of asparagine or ammonia. The enzymes obtained from the both bacterial species have different isoelectric points and the K_m for asparagine is higher than that for glutamine (Table 1). Although high concentrations of L-asparaginase can also decrease the serum glutamine, in a few minutes after prescribing, L-asparaginase completely catalyzes the serum asparagine, resulting in glutamine recovery. It was revealed that the blood cells have the ability to synthesize and metabolize asparagine to the required amount, but significant reduction in the asparagine in these cells is exerted by the L-asparaginase. In general, when the asparagine amino acid is lowered in the blood and injected from the outside asparagine reservoir, two pathways is stimulated in the body: 1) phosphorylation factor and 2) activation of the GCN2 pathway. Mammalian target of rapamycin induces the phosphorylation of the elongation factor translation into elf2a but inhibits elf2B, which hinders the recovery of elf2 during the protein synthesis process, thereby inhibiting the synthesis of protein (Covini et al., 2012). In glutamine high levels conditions, the mammalian target of rapamycin complex 1 (mTORC1) stimulation system causes the leucine degradation to synthesize important proteins (leucine is one of the important factors for protein synthesis). If blood glutamine level is low, the activity of mTORC1 would decreases, which reduces protein synthesis and activates the effects of asparagine deficiency in the process of apoptosis. Considering the importance of glutamine as the only source of amine in the blood, it is not necessary to synthesize many amino acids, such as asparagine. The factor causing asparagine synthesis of asparagine is synthase, which requires glutamine for activity. The expression of asparagine synthase is commonly found in the cell, but the GCN2-elfa system contributes to the transcription of activating transcription factor 4 inducing the expression of asparagine synthase. Therefore, glutamine, along with this enzyme, causes asparagine accumulation in cancer cells, which suppresses the GCN2 and inhibits the progression of apoptosis into cancer cells. So far, the efforts of some researchers have displayed that mutations in asparaginase though maintaining the activity of asparagine, would

TABLE 1 Specific properties of common bacterial L-asparaginases

Sources	MW (kDa)	OP Tem	OP pH	Specific activity (U/mg)	Km	Kcat (S^{-1})
Erwinia chrysanthemi	–	45	7.5	312.8	0.5	14,900
Erwinia carotovora	125–145	50	8.0	–	0.018	–
Escherichia coli	141	37	7.0–8.0	–	0.013	–
Cladosporium sp.	120	30	6.3	83.3	0.1	–
Enterobacteriaceae	150	37	6.0–7.0	–	0.89	–
Azotobacter vinelandii	84	48	8.6	2.47	0.11	–
Bacillus licheniformis	134.8	40	9.0	697.09	0.014	2,680
Bacillus tequilensis	–	35	8.5	10.19	0.045	–
Bacillus subtilis	–	40	7.5	–	0.43	–
Pectobacterium carotovorum	144.4	40	8.0–10.0	4,450	0.657	2,751
Vigna unguiculata	*70*	*40*	*8.0*	–	*1.25*	–
Pseudomonas fluorescens	141	34	6.3	0.94	109.9	
Pseudomonas aeruginosa	160	37	9.0	1,900	*0.147*	
Mycobacterium phlei	126	–	8.8–9.2	32.6	0.7	–
Helicobacter pylori	140	45	7.5	31.2	–	19.26
Vibrio succinogenes	146	–	7.3	202	0.048	–
Thermococcus kodakarensis	71	90	8.0	978.8	2.6	*694*
Corynebacterium glutamicum	80	40	7.0	–	2.5	–
Phaseolus vulgaris	79	37	8.0	846	6.72	–
Vibrio cholerae	132	37	7.0	2,120	1.1	4,424

Note. MW: molecular weight; OP: optimum; Tem: temperature of activity (°C); V_{max}: maximal velocity.

reduce the glutaminase activity of the enzyme, which is the goal known as targeted mutagenesis (Pokrovskaya et al., 2015). In addition, only enzymes derived from *Helicobacter pylori*, *Rhodospirillum rubrum*, *P. furiosus*, *Quinella*, and *Succinogenes* species have an activity of about 1% (Bansal et al., 2010; Gladilina, Sokolov, & Krasotkina, 2009). Further surveys unraveling the interactions of intracellular divalent cationic metal ions such as Ca^{2+} and Mg^{2+} with anticancer activity of L-asparaginases are essential as Ca^{2+} is also required.

3 | METHODS FOR THE PRODUCTION OF L-ASPARAGINASE ENZYME

Although various sources have been investigated for L-asparaginase production (Iraci et al., 2017), we focused on bacterial sources owing to some benefits. It has been observed that highest level of L-asparaginase is produced in anaerobic conditions. The production of L-asparaginase from microbial sources through fermentation is a promising procedure, given that it is cost-effective and environmentally friendly. L-Asparaginase is produced by submerged fermentation (SmF) worldwide. Although being expensive, SmF is still a common approach to produce L-asparaginase (Batool et al., 2016). The main drawbacks in the production of enzymes in the SmF include the low concentration of the product, thereby reducing the transportation and disposal of large amounts of water during the production process. Therefore, this method is very difficult and poorly feasible. In this regard SSF has been profoundly taken into consideration to overcome the problems of SmF fermentation. The SSF is more efficient as compared with SmF and has a multiplier function. SSF advantages over SmF fermentation, include less energy requirements, negligible risk of bacterial contamination, the lower water need, and less environmental concerns over the disposal of solid waste (Sunitha, Ellaiah, & Devi, 2010). Various microorganisms have been identified to be capable of producing asparaginase enzymes several of most common which have been depicted in Table 2.

Furthermore, marine Actinobacteria are a strong source of secondary metabolites, often derived from *Streptomyces* spp. (Dhevagi & Poorani, 2006). The production of various enzymes such as protease, lipase, ketamine, and alginate from marine *Streptomyces* spp. has been cornerstone for various applications. *Streptomyces* spp. also provide an appropriate source of L-asparaginase, applicable due to structural stability in harsh conditions, as an anticancer therapy regimen. The search for glutaminase free L-asparaginases is prominent as the depletion of plasma glutamine below critical levels may reduce the synthesis of important proteins such as fibrinogen, insulin and protein C leading to severe side effects in patients (Prabhu, Bhise, & Patravale, 2017).

4 | L-ASPARAGINASE FROM *E. coli*

Different isoenzymes of L-asparaginase have been isolated using different strains of *E. coli*. The purified *E. coli* L-asparaginase has a

TABLE 2 The most common microorganisms producing anticancer L-asparaginases

Producer microorganisms	Author
Streptomyces noursei MTCC 1046	Dharmaraj (2012)
Escherichia coli	Al-Jewari (2010)
Bacillus sp. *DKMBT10*	Moorthy, Ramalingam, Sumantha, and Shankaranaya (2010)
Bacillus cereus MNTG-7	Sunitha et al. (2010)
Streptomyces gulbargensis	Amena, Vishalakshi, Prabhakar, Dayanand, and Lingappa (2010)
Actinomycetes sp.	Basha, Rekha, Komala, and Ruby (2009)
Staphylococcus sp.	Prakasham, Rao, Rao, Lakshmi, and Sarma (2007)
Pseudomonas aeruginosa	El-Bessoumy, Sarhan, and Mansour (2004)
Aspergillus tamarii *Penicillium nigricans* *Bacillus subtilis* *Bacillus licheniformis* *Bacillus circulans*	Gulati, Saxena, and Gupta (1997)
Bacillus tequilensis	Shakambari et al. (2018)
Candida utilis	El-Bessoumy et al. (2004)
Corynebacterium glutamicum	Al-Jewari (2010)
Thermus thermophiles *Erwinia cartovora* *Enterobacter aerogenes* *Pisum sativum*	El-Bessoumy et al. (2004)
Erwinia chrysanthemi	Nguyen et al. (2018)
Vibrio cholerae	Radha et al. (2018)

molecular weight of 133–141 kDa. All L-asparaginase enzymes are composed of four subunits and an active site per unit, the molecular weight of which is reported for each of the sub units is 22 kDa. The molecular weight of each the *E. coli* L-asparaginase subunit is approximately 32 kDa and for *Erwinia* is 43 kDa (Duval et al., 2002). The molecular weight of L-asparaginase of *Erwinia* is 138 kDa and the specific activity of purified enzymes is between 300 and 400 mmol of substrate per min/mg of each protein. The isoelectric point of the *E. coli* L-asparaginase enzyme is between pH 4–5 (Vrooman et al., 2010). L-Asparaginase derived from *E. coli* has been being used in the treatment of ALL from 1960. More applications have been as an intravenous infusion or as an intramuscular injection. Preliminary studies of L-asparaginase were studied in ALL, the enzyme that was consecutive for 28 days (Narta, Kanwar, & Azmi, 2007). The half-life of enzyme activity in children is about 1–24 days. In adults with leukemia, the drug is prescribed intravenously or intramuscularly, usually given at a dose of 25,000 U/m^2 per day or in a diet with 6,000 U/m^2 of a day to 6 days. Reducing serum asparagine in most children is completed by taking 2,500–5,000 U/m^2 from Day 3 to 8. L-Asparaginase does not cross the blood–brain barrier, but it has efficiently reduced the L-asparagine in the spinal fluid.

5 | L-ASPARAGINASE FROM *ERWINIA* SPECIES

An efficient L-asparaginase enzyme should have a high affinity and binding capacity to asparagine substrate, low K_m, long half Life, high thermal stability, and low immunogenicity when injected into the body (Singh & Srivastava, 2012). L-Asparaginase is currently produced using *E. chrysanthemi*. *Erwinia* L-asparaginase can be administered intravenously or intramuscularly. The *Erwinia* bacterium is currently approved for the treatment of British cancer (Narta et al., 2007). The *Erwinia* L-asparaginase has a short half-life compared with common L-asparaginase and should be given at higher doses and in most cases L-asparaginase has been fully developed for asparagine elimination, and asparagine levels are rapidly improving in children taking *Erwinia* enzyme (Kotzia & Labrou, 2007). It is notable that L-asparaginase from the *Erwinia* species has proven effective in patients with severe allergic reactions to other L-asparaginase. Another benefit of that enzyme from *Erwinia* is probably in patients with the development of antibodies against *E. coli* L-asparaginase products. *Erwinia* L-asparaginase can quickly trap asparagine, thus being more efficient to reduce the plasma asparagine (Table 3).

6 | ENZYME PRODUCTION ENHANCEMENT APPROACHES

The L-asparaginase enzyme is an important source used for many years to treat children with ALL. The antineoplastic activity of this enzyme is due to its ability to reduce the storage of L-asparagine in the body. Cancerous cells that slowly produce asparaginase and are consumed as one of the essential amino acids themselves eliminate in oxygen deficient conditions (Goswami, Hegde, & Veeranki, 2015; Kambhampati, Ajewole, & Marsolais, 2017; Nguyen et al., 2018). However, demand for multiple use of this drug is the persistence and half-life of the drug, which leads to its rapid removal from the body and inevitably to reinject it to prevent its deterioration. The deficiency of this enzyme in terms of its sustainability has led to the creation of a framework for various studies to increase its

TABLE 3 Clinical Pharmacology of asparaginases with frequently administered doses

property	Natural	*Escherichia coli* PEG	*Erwinia* spp.
Activity (IU/mg protein)	280–400	280–400	650–700
K_m L-asparaginase	12	12	12
K_m L-glutaminase	3,000	3,000	145
L-Glu/L-Asp (maximal activity)	0/03	0/03	0/1
Molecular weight	141 kDa	–	138 kDa
PI	5	5	8.7

Note. PEG: polyethylene glycol; PI: isoelectric point.

sustainability (Spavor et al., 2016). Asparaginase used in the medical industry should be resistant to pH and thermal treatment processes with low cost of production and enough efficiency. The thermal resistance of L-asparaginases from *Bacillus* species and pH/salt stability of those from marine bacteria has been proved. Therefore, some studies have assessed the production optimization of L-asparaginases (Latha, Sivaranjani, & Dhanasekaran, 2017; Prabhu et al., 2017). Owing to the fact that L-asparaginase is produced at low levels at normal conditions by bacteria, optimization and set up of conditions for high-level enzyme production is critical.

The use of the expression system has numerous advantages for its high-level and inexpensive production. Functionalization of nanoscale drug vehicles has so far achieved a moderate targeting effect. The nanoscale size of drug preparations favors enhanced permeability and retention (EPR) and reduces renal filtration. Proteins are used as inert nanoscale carriers and as functional targeting agents in the form of antibodies or ligands that bind to tumor cell-surface markers. Protein engineering and recombinant DNA technologies allow cytotoxic proteins to be empowered with accessory domains for oligomerization, targeting, endosomal escape, and self-activation. Therefore, the production of self-assembling, self-delivered protein drugs for oncology is becoming feasible.

The application of pET expression system group members containing strong 7T promoter is beneficial. A study by Ghaderi and Ghezelbash (2017), *S. marcescens* produced higher levels of L-asparaginase in optimization conditions of 1.5% maltose (as a carbon source) and 1% ammonium sulfate (as a nitrogen source) and pH 6.8 in which no glutaminase activity was observed to be advantageous in industrial applications. According to our study (data not published), the optimal conditions included Time = 8 min, OD = 4.5, and IPTG = 0.277 mM. By increasing the IPTG concentration, cell efficiency would decrease due to the toxicity of high IPTG concentrations. However, gradual OD increase led to higher cell efficiency. The use of Mini Tab software was performed and provided 20 modes, in which time factors, IPTG, and OD rates were investigated and each of 20 modes was performed in laboratory conditions and at various conditions.

7 | ADVERSE EVENTS OF BACTERIAL L-ASPARAGINASES

Alongside with vast advantages, bacterial asparaginases are not free from limitations such as immune responses and allergic/hypersensitivity reactions to them and asparaginase depletion or inhibition of subsequent protein synthesis (Rau et al., 2018). Therefore, application of more efficient enzymes and alternative therapy such as immunosuppressing by steroids can help these conditions. The allergic reactions affect the central nervous system, the digestive system, and the urinary system and also adverse events occur on blood and plasma. This medication partially disrupts the synthesis of coagulation factors leading to severe deficiency in antithrombin and antityrosine, and ultimately the factors 9, 10, and 11 will reduce the coagulation factors (Patel et al., 2017; Avramis, 2014). Some side effects such as anaphylactic shock or neutralization of the drug effect following long-time administration have caused searching for novel more beneficial sources. The employment of recombinant technologies will also improve the shortages and drawbacks of L-asparaginase. Since several reports suggest that L-glutamine depletion correlates with many of the side effects of these drugs, enzyme variants with reduced L-glutaminase coactivity might be clinically beneficial if their antileukemic activity would be preserved (Nguyen et al., 2018).

8 | CONCLUSION

1. L-Asparaginase owes wide range of applications. A considerable attention has been drawn to L-asparaginase due to its cost-effective and easy production from microbes especially bacteria. High level of L-asparaginase enzyme production by *E. coli*, *E. cartovora*, *Streptomyces*, and *B. subtilis* species is highly desirable as bacterial enzyme alternative sources for anticancer therapy. High efficiency, higher asparagine affinity, and lower glutamine affinity are other key properties of efficient L-asparaginases.
2. Thermal or harsh conditions stability of those from the two latter bacterial species is considerable. Some enzymes from marine bacteria have conferred stability in adverse conditions being more advantageous in cancer therapy.
3. Several side effects of L-asparaginases such as hypersensitivity, resistance, immunogenic complications, and enzyme short life should be improved through alternative therapy or use of immune-suppressor drugs and recombinant technologies.
4. The L-asparaginase from *Erwinia* species has displayed desirable effects in children in this regard. Application of software such as RSM would optimize conditions for higher levels of enzyme production. Furthermore, the possibility of anticancer combination therapy using two or more L-asparaginases from various sources is plausible in future studies to achieve better therapeutic outcomes with lower side effects. Recent findings of low glutaminase L-asparaginases from *E. chrysanthemi* and *V. cholerae* represent attractive perspective and promising benefit and a clear advantage over previous enzymes toward decreasing side effects due to the enzyme properties and long-term and higher stability.
5. Targeting cytotoxic drugs in oncology is essential because side toxicities limit reaching effective local doses.
6. Functionalization of nanoscale drug vehicles has so far achieved a moderate targeting effect. The nanoscale size of drug preparations favors EPR and reduces renal filtration. Proteins are used as inert nanoscale carriers and as functional targeting agents in the form of antibodies or ligands that bind to tumor cell-surface markers.
7. Protein engineering and recombinant DNA technologies allow cytotoxic proteins to be empowered with accessory domains for oligomerization, targeting, endosomal escape, and self-activation. Therefore, the production of self-assembling, self-delivered protein drugs for oncology is becoming feasible.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

A. G., A.-H. A.-M., H. R. A.-A., and Y. A.: data collection and manuscript writing. S. A. K., S. K. S. M., Y. K. A., and A. F.: manuscript editing, support, and management.

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REFERENCES

Agrawal, N. R., Bukowski, R. M., Rybicki, L. A., Kurtzberg, J., Cohen, L. J., & Hussein, M. A. (2003). A Phase I–II trial of polyethylene glycol-conjugated L-asparaginase in patients with multiple myeloma. *Cancer*, *98*(1), 94–99.

Al-Jewari, H. (2010). *In vitro cytotoxic activity of the L-asparaginase extracted and purified from pathogenic Escherichia coli against four Leukemic cell lines*. (A thesis PhD in Genetic Engineering Biotechnology for Post Graduate Studies, University of Baghdad, Iraq).

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*(1), 173–178.

Arif, H. M., & Hussain, Z. (2014). Important sources and medicinal applications of L-asparaginase. *International Journal of Pharmaceutical Sciences Review*, *3*, 35–45.

Asselin, B., & Rizzari, C. (2015). Asparaginase pharmacokinetics and implications of therapeutic drug monitoring. *Leukemia and Lymphoma*, *56*(8), 2273–2280.

Avramis, V. I. (2014). Is glutamine depletion needed in ALL disease? *Blood*, *123*(23), 3532–3533.

Avramis, V. I., Sencer, S., Periclou, A. P., Sather, H., Bostrom, B. C., & Cohen, L. J., et al. (2002). A randomized comparison of native *Escherichia coli* asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: A Children's Cancer Group study. *Blood*, *99*(6), 1986–1994.

Bansal, S., Gnaneswari, D., Mishra, P., & Kundu, B. (2010). Structural stability and functional analysis of L-asparaginase from Pyrococcus furiosus. *Biochemistry*, *75*(3), 375–381.

Basha, N. S., Rekha, R., Komala, M., & Ruby, S. (2009). Production of extracellular anti-leukaemic enzyme lasparaginase from marine actinomycetes by solidstate and submerged fermentation: Purification and characterisation. *Tropical Journal of Pharmaceutical Research*, *8*(4), 353–360.

Batool, T., Makky, E. A., Jalal, M., & Yusoff, M. M. (2016). A comprehensive review on L-asparaginase and its applications. *Applied Biochemistry and Biotechnology*, *178*(5), 900–923.

Bertrand, Y., Baruchel, A., Thomas, X. G., Blin, N., Tavernier Tardy, E., & Perel, Y. (2017). Drug monitoring of ERY001 (erythrocyte encapsulated L-asparaginase) and native L-asparaginase (L-ASP) in combination with COOPRALL regimen in Phase 3 randomized trial in patients with relapsed acute lymphoblastic leukemia. *Journal of Clinical Oncology*, *4*, e18036–e18036.

Borah, D., Yadav, R., Sangra, A., Shahin, L., & Chaubey, A. K. (2012). Production, purification and process optimization of asparaginase. (an anticancer enzyme) from *E. coli*, isolated from sewage water. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(Suppl 3), 202–204.

Campbell, H., Mashburn, L., Boyse, E., & Old, L. (1967). Two L-asparaginases from *Escherichia coli* B. Their separation, purification, and antitumor activity. *Biochemistry*, *6*(3), 721–730.

Chang, T. M. S. (2013). *Biomedical applications of immobilized enzymes and proteins* 1). New York: Springer Science & Business Media.

Covini, D., Tardito, S., Bussolati, O., R. chiarelli, L., V. pasquetto, M., Digilio, R., … Scotti, C. (2012). Expanding targets for a metabolic therapy of cancer: L-asparaginase. *Recent Patents on Anti-cancer Drug Discovery*, *7*(1), 4–13.

Deokar, V. D., Vetal, M. D., & Rodrigues, L. (2010). Production of intracellular L-asparaginase from Erwinia carotovora and its statistical optimization using response surface methodology (RSM). *International Journal of Chemical Sciences*, *1*, 25–36.

Dharmaraj, S. (2012). Study of L-asparaginase production by Streptomyces noursei MTCC 10469, isolated from marine sponge Callyspongia diffusa. *Iranian Journal of Biotechnology*, *9*(2), 102–108.

Dhevagi, P., & Poorani, E. (2006). Isolation and characterization of L-asparaginase from marine actinomycetes. *Indian Journal of Biotechnology*, *5*, 514–520.

Domenech, C., Thomas, X., Chabaud, S., Baruchel, A., Gueyffier, F., Mazingue, F., … Bertrand, Y. (2011). L-asparaginase loaded red blood cells in refractory or relapsing acute lymphoblastic leukaemia in children and adults: Results of the GRASPALL 2005-01 randomized trial. *British Journal of Haematology*, *153*(1), 58–65.

Duval, M., Suciu, S., Ferster, A., Rialland, X., Nelken, B., & Lutz, P., et al. (2002). Comparison of *Escherichia coli*–asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: Results of a randomized European Organisation for Research and Treatment of Cancer—Children's Leukemia Group Phase 3 trial. *Blood*, *99*(8), 2734–2739.

Eden, D., Hipkins, R., & Bradbury, C. (2016). Cerebral thrombotic complications related to l-asparaginase treatment for acute lymphoblastic leukemia: Retrospective review of 10 cases. *Clinical and Applied Thrombosis/Hemostasis*, *22*(6), 589–593.

El-Bessoumy, A. A., Sarhan, M., & Mansour, J. (2004). Production, isolation, and purification of L-asparaginase from *Pseudomonas aeruginosa* 50071 using solid-state fermentation. *BMB Reports*, *37*(4), 387–393.

Gao, R. W., Dusenbery, K. E., Cao, Q., Smith, A. R., & Yuan, J. (2018). Augmenting total body irradiation with a cranial boost before stem cell transplantation protects against post-transplant central nervous system relapse in acute lymphoblastic leukemia. *Biology of Blood and Marrow Transplantation*, *24*(3), 501–506.

Ghaderi, F., & Ghezelbash, G. R. (2017). Isolation and optimization of glutaminase-free L-asparaginase enzyme producing *Serratia marcescens* isolated from natural sources. *Journal of Microbial World*, *10*, 49–58.

Gladilina, Y. A., Sokolov, N., & Krasotkina, J. (2009). Cloning, expression, and purification of Helicobacter pylori L-asparaginase. *Biochemistry*, *3*(1), 89–91.

Goswami, R., Hegde, K., & Veeranki, V. D. (2015). Production and characterization of novel glutaminase free recombinant L -asparaginase II of *Erwinia carotovora* subsp. atroseptica SCRI 1043 in *E. coli* BL21 (DE3). *British Microbiology Research Journal*, *6*(2), 95–112.

Gulati, R., Saxena, R., & Gupta, R. (1997). A rapid plate assay for screening l-asparaginase producing micro-organisms. *Letters in Applied Microbiology*, *24*(1), 23–26.

Iraci, N., Gaude, E., Leonardi, T., Costa, A. S. H., Cossetti, C., Peruzzotti-Jametti, L., … Pluchino, S. (2017). Extracellular vesicles are independent

metabolic units with asparaginase activity. *Nature Chemical Biology*, *13*(9), 951–955.

Jaccard, A., Gachard, N., Marin, B., Rogez, S., Audrain, M., Suarez, F., … Hermine, O. (2011). Efficacy of L-asparaginase with methotrexate and dexamethasone (AspaMetDex regimen) in patients with refractory or relapsing extranodal NK/T-cell lymphoma, a phase 2 study. *Blood*, *117*(6), 1834–1839.

Jiang, M., Zhang, H., Jiang, Y., Yang, Q., Xie, L., Liu, W., … Zou, L. (2012). Phase 2 trial of "sandwich" L-asparaginase, vincristine, and prednisone chemotherapy with radiotherapy in newly diagnosed, stage IE to IIE, nasal type, extranodal natural killer/T-cell lymphoma. *Cancer*, *118*(13), 3294–3301.

Kambhampati, S., Ajewole, E., & Marsolais, F. (2017). Advances in Asparagine, *Metabolism Progress in Botany* 79, 49–74. Darmstadt city, Germany: Springer.

Kamble, K., Bidwe, P., Muley, V., Kamble, L., Bhadange, D., & Musaddiq, M. (2012). Characterization of L-asparaginase producing bacteria from water, farm and saline soil. *Bioscience Discovery*, *3*(1), 116–119.

Kidd, J. G., & Sobin, L. H. (1966). The incorporation of l-asparagine-14C by lymphoma 6C3HED cells: Its inhibition by guinea pig serum. *Cancer Research*, *26*(2 Part 1), 208–211.

Kim, M., Kim, T. M., Kim, K. H., Keam, B., Lee, S. -H., Kim, D. -W., … Heo, D. S. (2015). Ifosfamide, methotrexate, etoposide, and prednisolone (IMEP) plus L-asparaginase as a first-line therapy improves outcomes in stage III/IV NK/T cell-lymphoma, nasal type (NTCL). *Annals of Hematology*, *94*(3), 437–444.

Kotzia, G. A., & Labrou, N. E. (2007). L-asparaginase from *Erwinia chrysanthemi* 3937: Cloning, expression and characterization. *Journal of Biotechnology*, *127*(4), 657–669.

Kumar, S., Dasu, V. V., & Pakshirajan, K. (2011). Purification and characterization of glutaminase-free L-asparaginase from Pectobacterium carotovorum MTCC 1428. *Bioresource Technology*, *102*(2), 2077–2082.

Latha, S., Sivaranjani, G., & Dhanasekaran, D. (2017). Response surface methodology: A non-conventional statistical tool to maximize the throughput of Streptomyces species biomass and their bioactive metabolites. *Critical Reviews in Microbiology*, *43*(5), 567–582.

Maggi, M., Chiarelli, L. R., Valentini, G., & Scotti, C. (2015). Engineering of Helicobacter pylori L-asparaginase: Characterization of two functionally distinct groups of mutants. *PLOS One*, *10*(2), e0117025.

Mahajan, R. V., Kumar, V., Rajendran, V., Saran, S., Ghosh, P. C., & Saxena, R. K. (2014). Purification and characterization of a novel and robust L-asparaginase having low-glutaminase activity from Bacillus licheniformis: In vitro evaluation of anti-cancerous properties. *PLOS One*, *9*(6), e99037.

Manikandan, R., Pratheeba, C., Pankaj, S., & Sah, S. (2010). Optimization of L-asparaginase production by Pseudomonas aeruginosa using experimental methods. *Nature and Science*, *8*(2), 1–6.

Mitchell, L. G., & Group, P. (2003). A prospective cohort study determining the prevalence of thrombotic events in children with acute lymphoblastic leukemia and a central venous line who are treated with L-asparaginase: Results of the Prophylactic Antithrombin Replacement in Kids with Acute Lymphoblastic Leukemia Treated with Asparaginase (PARKAA) Study. *Cancer*, *97*(2), 508–516.

Moorthy, V., Ramalingam, A., Sumantha, A., & Shankaranaya, R. T. (2010). Production, purification and characterisation of extracellular L-asparaginase from a soil isolate of Bacillus sp. *African Journal of Microbiology Research*, *4*(18), 1862–1867.

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*(3), 208–221.

Neuman, R. E., & McCoy, T. A. (1956). Dual requirement of Walker carcinosarcoma 256 in vitro for asparagine and glutamine. *Science*, *124*(3212), 124–125.

Nguyen, H. A., Su, Y., Zhang, J. Y., Antanasijevic, A., Caffrey, M., Schalk, A. M., … Lavie, A. (2018). A novel L-asparaginase with low L-glutaminase coactivity is highly efficacious against both T and B cell acute lymphoblastic leukemias in vivo. *Cancer Research*, *78*(6), 1549–1560.

Oettgen, H. F., Tallal, L., Tan, C. C., Murphy, M. L., Clarkson, B. D., Golbey, R. D., & Burchenal, J. H. (1970). Clinical experience with L-asparaginase, *Experimental and clinical effects of L-asparaginase* (219–235). New York: Springer.

Palm, G. J., Lubkowski, J., Derst, C., Schleper, S., Röhm, K. -H., & Wlodawer, A. (1996). A covalently bound catalytic intermediate in *Escherichia coli* asparaginase: Crystal structure of a Thr-89-Val mutant. *FEBS Letters*, *390*(2), 211–216.

Park, J. H., Rivière, I., Gonen, M., Wang, X., Sénéchal, B., Curran, K. J., … Sadelain, M. (2018). Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *New England Journal of Medicine*, *378*(5), 449–459.

Patel, B., Kirkwood, A. A., Dey, A., Marks, D. I., McMillan, A. K., Menne, T. F., … Fielding, A. K. (2017). Pegylated-asparaginase during induction therapy for adult acute lymphoblastic leukaemia: Toxicity data from the UKALL14 trial. *Leukemia*, *31*(1), 58–64.

Pieters, R., Hunger, S. P., Boos, J., Rizzari, C., Silverman, L., Baruchel, A., … Pui, C. H. (2011). L-asparaginase treatment in acute lymphoblastic leukemia: A focus on *Erwinia asparaginase*. *Cancer*, *117*(2), 238–249.

Pisarewicz, K., Mora, D., Pflueger, F. C., Fields, G. B., & Marí, F. (2005). Polypeptide chains containing d-hydroxyvaline. *Journal of the American Chemical Society*, *127*(17), 6207–6215.

Place, A. E., Stevenson, K. E., Vrooman, L. M., Harris, M. H., Hunt, S. K., O'Brien, J. E., … Silverman, L. B. (2015). Intravenous pegylated asparaginase versus intramuscular native *Escherichia coli* L-asparaginase in newly diagnosed childhood acute lymphoblastic leukaemia (DFCI 05-001): A randomised, open-label phase 3 trial. *The Lancet Oncology*, *16*(16), 1677–1690.

Pokrovskaya, M. V., Aleksandrova, S. S., Pokrovsky, V. S., Veselovsky, A. V., Grishin, D. V., Abakumova, O. Y., … Sokolov, N. N. (2015). Identification of functional regions in the *Rhodospirillum rubrum* L-asparaginase by site-directed mutagenesis. *Molecular Biotechnology*, *57*(3), 251–264.

Prabhu, R., Bhise, K., & Patravale, V. (2017). Marine Enzymes in Cancer: A New Paradigm, *Advances in food and nutrition research* 80, 1–14). Maharashtra, India: Elsevier.

Prakasham, R., Rao, C. S., Rao, R. S., Lakshmi, G. S., & Sarma, P. (2007). L-asparaginase production by isolated *Staphylococcus* sp.–6A: Design of experiment considering interaction effect for process parameter optimization. *Journal of Applied Microbiology*, *102*(5), 1382–1391.

Radha, R., Arumugam, N., & Gummadi, S. N. (2018). Glutaminase free L-asparaginase from Vibrio cholerae: Heterologous expression, purification and biochemical characterization. *International Journal of Biological Macromolecules*, *111*, 129–138.

Rau, R. E., Dreyer, Z., Choi, M. R., Liang, W., Skowronski, R., & Allamneni, K. P., et al. (2018). Outcome of pediatric patients with acute lymphoblastic leukemia/lymphoblastic lymphoma with hypersensitivity to pegaspargase treated with PEGylated *Erwinia* asparaginase, pegcrisantaspase: A report from the Children's Oncology Group. *Pediatric Blood and Cancer*, *65*(3), e26873.

Serna, N., Sánchez-García, L., Unzueta, U., Díaz, R., Vázquez, E., Mangues, R., & Villaverde, A. (2018). Protein-Based Therapeutic Killing for Cancer Therapies. *Trends in Biotechnology*, *36*(3), 318–335.

Shakambari, G., Birendranarayan, A. K., Angelaa lincy, M. J., Rai, S. K., Ahamed, Q. T., Ashokkumar, B., … Varalakshmi, P. (2016). Hemocompatible glutaminase free L-asparaginase from marine *Bacillus tequilensis* PV9W with anticancer potential modulating p53 expression. *RSC Advances*, *6*(31), 25943–25951.

Shakambari, G., Kumar, R. S., Ashokkumar, B., Ganesh, V., Vasantha, V. S., & Varalakshmi, P. (2018). Cloning and expression of L-asparaginase from Bacillus tequilensis PV9W and therapeutic efficacy of Solid Lipid Particle formulations against cancer. *Scientific Reports*, *8*(1), 18013.

Shrivastava, A., Khan, A. A., Khurshid, M., Kalam, M. A., Jain, S. K., & Singhal, P. K. (2016). Recent developments in L-asparaginase discovery and its potential as anticancer agent. *Critical Reviews in Oncology/Hematology*, *100*, 1–10.

Singh, Y., & Srivastava, S. K. (2012). Screening and characterization of microorganisms capable of producing antineoplastic drug, L-asparaginase. *International Journal of Biological and Medical Research*, *3*(4), 2548–2554.

Spavor, M., Halton, J., Dietrich, K., Israels, S., Shereck, E., Yong, J., … Mitchell, L. G. (2016). Age at cancer diagnosis, non-O blood group and asparaginase therapy are independently associated with deep venous thrombosis in pediatric oncology patients: A risk model. *Thrombosis Research*, *144*, 27–31.

Sunitha, M., Ellaiah, P., & Devi, R. B. (2010). Screening and optimization of nutrients for L-asparaginase production by *Bacillus cereus* MNTG-7 in SmF by plackett-burmann design. *African Journal of Microbiology Research*, *4*(4), 297–303.

Vrooman, L. M., Stevenson, K. E., Supko, J. G., O'Brien, J., Dahlberg, S. E., Asselin, B. L., … Silverman, L. B. (2013). Postinduction dexamethasone and individualized dosing of *Escherichia coli* L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: Results from a randomized study—Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. *Journal of Clinical Oncology*, *31*(9), 1202–1210.

Vrooman, L. M., Supko, J. G., Neuberg, D. S., Asselin, B. L., Athale, U. H., Clavell, L., … Silverman, L. B. (2010). *Erwinia* asparaginase after allergy to *E. coli* asparaginase in children with acute lymphoblastic leukemia. *Pediatric blood & cancer*, *54*(2), 199–205.

Wriston, J. C., & Yellin, T. O. (1973). L-asparaginase: A review. *Advances in Enzymology and Related Ares of Molecular Biology*, *39*, 185–248.

Zuo, S., Zhang, T., Jiang, B., & Mu, W. (2015). Reduction of acrylamide level through blanching with treatment by an extremely thermostable l-asparaginase during French fries processing. *Extremophiles*, *19*(4), 841–851.

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