Indonesian Journal of Biotechnology

VOLUME 31(1), 2026, 18-25 | RESEARCH ARTICLE

Recombinant expression and preliminary characterization of a synthetic L-asparaginase from the marine bacterium *Pseudoalteromonas tetraodonis* GFC in *Escherichia coli* BL21 (DE3)

Wulan Pertiwi[1,*], Taufik Ramdani Tohari[2], Qori Atur Rodiah Suhada[1], Tiara Zahira[1], Nadila Suci Nugraha[1]

[1]Department of Biotechnology, Faculty of Science and Technology, Universitas Muhammadiyah Bandung, Jl. Soekarno Hatta No 752, Cipadung Kidul, Panyileukan, Bandung, Jawa Barat 40614, Indonesia
[2]Research Center for Molecular Biotechnology and Bioinformatics, Universitas Padjadjaran, Bandung 40133, Indonesia
*Corresponding author: wulanpertiwi@umbandung.ac.id

SUBMITTED 15 December 2025 **REVISED** 25 February 2026 **ACCEPTED** 6 March 2026

ABSTRACT The marine environment represents a promising source of diverse enzymes produced by marine microorganisms, including L-asparaginase. This has been widely studied due to its ability to hydrolyze extracellular L-asparagine, an amino acid required for the growth of certain cancer cells. In this study, a synthetic L-asparaginase gene derived from the marine bacterium *Pseudoalteromonas tetraodonis* GFC was recombinantly expressed in *Escherichia coli* BL21 (DE3). The gene was cloned into the pD861-SR expression vector and transformed into *E. coli* BL21 (DE3). Positive transformants were confirmed by restriction digestion using SapI and Sanger sequencing. Recombinant protein expression was induced with L-rhamnose under the control of the rhaBAD promoter. The expressed L-asparaginase was then purified using Ni-Sepharose affinity chromatography followed by membrane dialysis, yielding a protein purity of 73.6%. SDS-PAGE analysis revealed a prominent protein band at approximately 37 kDa, corresponding to the expected molecular weight of recombinant L-asparaginase. Enzymatic activity was evaluated using the Nessler method, and the purified enzyme exhibited a specific activity of 5.863 U/mg. These results demonstrate the successful recombinant expression and preliminary functional validation of L-asparaginase from *P. tetraodonis* GFC in *E. coli*, providing a basis for further optimization and characterization in future studies.

KEYWORDS L-asparaginase; Marine; Overexpression; Purification; Synthetic gene

1. Introduction

Blood cancer (leukemia) remains one of the leading causes of morbidity and mortality worldwide. According to GLOBOCAN 2022, leukemia accounted for 487,294 new cases and 305,405 deaths globally, ranking as the tenth deadliest cancer type (Ferlay et al. 2024). Leukemia is a malignant disorder of blood-forming tissues that originates in the bone marrow and disrupts normal hematopoiesis (GCO 2022). Among its subtypes, acute lymphoblastic leukemia (ALL) is the most prevalent in children, although it can also affect adults. In 2021, the global number of pediatric ALL cases reached 168,879 (Ding et al. 2025). The disease is characterized by uncontrolled proliferation of immature white blood cells, leading to the accumulation of blast cells in peripheral blood, displacement of normal blood cells, anemia, and impaired immune function (Kemenkes RI 2025).

A key metabolic vulnerability of ALL cells is their limited ability to synthesize L-asparagine due to low expression of asparagine synthetase (ASNS). Consequently, ALL cells depend on extracellular L-asparagine for survival and proliferation. This dependency underlies the clinical application of L-asparaginase, an enzyme that catalyzes the hydrolysis of L-asparagine into L-aspartate and ammonia, thereby depleting circulating L-asparagine levels (Chiu et al. 2020; Trimpont et al. 2022). L-asparaginase is widely distributed in nature and has been identified in organisms ranging from microorganisms to higher eukaryotes (Zuo et al. 2014). By reducing the concentration of extracellular asparagine in plasma, L-asparaginase induces cancer cell death, as these cells are unable to synthesize proteins without sufficient asparagine.

Currently, clinically used L-asparaginase preparations are primarily derived from *Escherichia coli* and *Erwinia chrysanthemi*. Despite their effectiveness, these enzymes exhibit L-glutaminase co-activity, which has been associated with adverse effects, including hepatotoxicity, pancreatic dysfunction, neurological complications, hypersensitivity reactions, and other systemic toxicities (Ardalan et al. 2018; Abdullah 2024). These limitations have motivated ongoing research to identify alternative L-

asparaginase sources with improved biochemical properties.

Marine microorganisms represent a largely unexplored reservoir of enzymatic diversity and have attracted increasing interest as potential sources of novel biocatalysts. Pertiwi et al. (2025) reported the isolation of a marine bacterium from the coastal area of Rancabuaya, Garut, Indonesia, producing L-asparaginase with relatively low glutaminase co-activity. Based on 16S rRNA gene sequencing, the isolate showed 99% sequence similarity to *P. tetraodonis* GFC. The crude extracellular and intracellular L-asparaginase activities of this isolate were reported as 72.30 U mg^{-1} and 67.18 U mg^{-1}, respectively.

Members of the genus *Pseudoalteromonas* are widely distributed in marine environments, including biofilms, sediments, and extreme habitats such as deep-sea and polar regions (Lee et al. 2015; Jo et al. 2017; Rajeev et al. 2021). These bacteria are frequently associated with eukaryotic hosts, such as algae and crustaceans, and are known to produce a variety of bioactive compounds (Eze et al. 2023). Notably, *P. tetraodonis* has been reported to produce tetrodotoxin, a potent neurotoxin (Simidu et al. 1990; Holmström and Kjelleberg 1999), which raises safety considerations for direct enzyme production from the native organism.

Recombinant DNA technology provides an effective strategy to produce enzymes of interest while avoiding the need to culture potentially hazardous native strains. By expressing target genes in well-characterized heterologous hosts such as *E. coli*, recombinant approaches enable controlled production, simplified purification, and improved biosafety (Jayakrishnan et al. 2024). In this context, the present study focuses on the recombinant expression of a synthetic L-asparaginase gene derived from *P. tetraodonis* GFC in *E. coli* BL21 (DE3), as an initial step toward the biochemical characterization and further optimization of this marine-derived enzyme.

2. Materials and Methods

2.1. Bioinformatics and structural analysis

The amino acid sequence of *P. tetraodonis* GFC L-asparaginase 1 (UniProt ID: A0AA37S3L8) was retrieved from UniProt (https://www.uniprot.org) and analyzed for structural and functional features. Homology modeling was performed using SWISS-MODEL (https://swissmodel.expasy.org) to generate a high-confidence model. Physicochemical properties, including molecular weight and isoelectric point, were calculated using ExPASy ProtParam (https://web.expasy.org/protparam). Predictions of secondary structure, solvent accessibility, and intrinsic disorder were performed through the DTU HealthTech server using NetSurfP-2.0 and IUPred2A, providing an integrated analysis of structural stability and His-tag accessibility (https://services.healthtech.dtu.dk).

2.2. Cloning of the recombinant of L-asparaginase

The L-asparaginase sequence (UniProt ID: A0AA37S3L8) was synthesized and cloned into the expression vector pD861-SR (3266 bp) using the ATUM gene synthesis service (ATUM, USA). The recombinant plasmid pD861-SR[L-asparaginase] was transformed into *E. coli* BL21(DE3) competent cells prepared by the $CaCl_2$ method, followed by heat shock treatment. Transformation was carried out with 50 ng of plasmid DNA, and transformants were selected on LB agar plates containing kanamycin at a final concentration of 75 µg/mL (Sambrook and Russell 2001). Positive colonies were verified by antibiotic resistance screening, followed by plasmid DNA isolation and Sanger sequencing to confirm the presence of the L-asparaginase insert.

2.3. Expression of the recombinant L-asparaginase

This procedure followed standard protocols for bacterial protein expression as described in Molecular Cloning: A Laboratory Manual (Sambrook and Russell 2001). Recombinant *E. coli* BL21(DE3)-pD861-SR[L-asparaginase] construct was cultured in 50 mL Terrific Broth (TB; HiMedia, India) supplemented with kanamycin (Sigma-Aldrich, USA) at 37 °C until the culture reached an OD_{600} of 0.8–1.0. Protein expression was induced with 4 mM L-rhamnose (Sigma-Aldrich, USA), and incubation was continued for 8 h at 37 °C. Cells were harvested by centrifugation at 5,000 × g for 10 min at 4 °C (TOMY, Japan), resuspended in sodium phosphate buffer pH 7.5 as a lysis buffer (Promega, USA), and disrupted by sonication at 60% amplitude for 15 min (3s on and 2s off, on ice bath) (Bio-Base, China). The lysate was clarified by centrifugation at 10,000 × g for 10 min at 4 °C. The supernatant was collected as the soluble protein, whereas the pellet was solubilized in 8 M urea (Sigma-Aldrich, USA), incubated at 2–10 °C for 1 h, and centrifuged at 10,000 × g for 30 min at 4 °C to recover inclusion bodies as the insoluble fraction.

2.4. Purification of the recombinant L-asparaginase

Purification of recombinant L-asparaginase followed the manufacturer's instructions for Ni-Sepharose His-tag purification (Cytiva 2022). The protein was isolated from the insoluble fraction under denaturing conditions using immobilized metal affinity chromatography (IMAC) on a 1 mL Ni-Sepharose column (Cytiva, Sweden). A 2.5 mL inclusion body sample was loaded onto the column equilibrated with binding buffer containing 0.05 M sodium phosphate (Merck, Germany), 0.5 M sodium chloride (Merck, Germany), 8 M urea (Sigma-Aldrich, USA), and 5 mM imidazole (Biosharp, China), pH 7.5. The column was washed with 15 column volumes (CVs) of the same buffer, and bound proteins were eluted stepwise with 5 CVs of imidazole at 10, 20, 40, 60, 80, 100, and 250 mM. Fractions containing recombinant L-asparaginase were pooled and dialyzed using a cellulose membrane (Sigma-Aldrich, USA) against phosphate buffer (pH 7.5) at 4 °C

with multiple buffer changes to gradually remove urea and facilitate protein refolding. The effectiveness of buffer exchange was monitored by ensuring stable pH values of the dialysis buffer, assuming effective urea removal under these conditions.

2.5. L-asparaginase activity assay

Enzyme activity was tested using the Nessler method. Nessler reagent (K_2HgI_4) reacts with ammonia (NH_3) produced from the hydrolysis of L-asparagine by L-asparaginase, producing a brownish-yellow complex compound. The intensity of the color produced was measured using a UV-vis spectrophotometer at a predetermined wavelength (Pertiwi et al. 2025). The test was conducted on samples of pure L-asparaginase enzyme, negative control (distilled water), and positive control (protein of *E. coli* ATCC 8739). The reaction mixture, consisting of 900 µl of substrate (10 mM L-asparagine in 50 mM Tris-HCl, pH 8.6) and 100 µl of purified enzyme, was incubated in a water bath at 37 °C for 30 min. Afterwards, 100 µl of 1.5 M TCA was added to stop the reaction, then centrifuged at 10,000 rpm at 4°C for 5 minutes. A total of 100 µl of supernatant (containing ammonia from the reaction) was taken, then 200 µl of Nessler's reagent and 3.7 mL of distilled water were added (Muzuni et al. 2024). The mixture was homogenized using a vortex, then incubated at room temperature for 20 min. The amount of ammonia released was measured using a UV-Vis spectrophotometer at a wavelength of 420 nm. One enzyme unit is defined as the amount of enzyme required to release one µmol of ammonia per minute under treatment conditions. The specific activity of L-asparaginase enzyme is determined by dividing the L-asparaginase activity value by the total protein content of the enzyme (Dastmalchi et al. 2024). Enzyme activity is calculated using the following formula:

$$\textit{Enzyme Activity } (IU\ mL^{-1}) = \frac{y-b}{a} \times \frac{V_{total}}{V_{analysis}} \times \frac{1}{V_{enzyme}} \times \frac{1}{T_{incubation}} \quad (1)$$

y : Absorbance
a : Slope
b : Intercept
V_{total} : Volume of enzyme + substrate + buffer + TCA
$V_{analysis}$: Analyzed volume
V_{enzyme} : Volume of enzyme
$T_{incubation}$: Incubation time

3. Results and Discussion

3.1. Bioinformatics and structural analysis

In silico analysis of L-asparaginase I from *P. tetraodonis* GFC (UniProt ID: A0AA37S3L8) was performed to characterize the structural, physicochemical, and functional features of the target protein presented in Figure 1. Homology modeling successfully produced a high-confidence three-dimensional (3D) model using SWISS-MODEL, which became the structural basis for further studies. Methionine at position 1 (Met1) defines the N-terminus, whereas arginine at position 342 (Arg342) corresponds to the C-terminus.

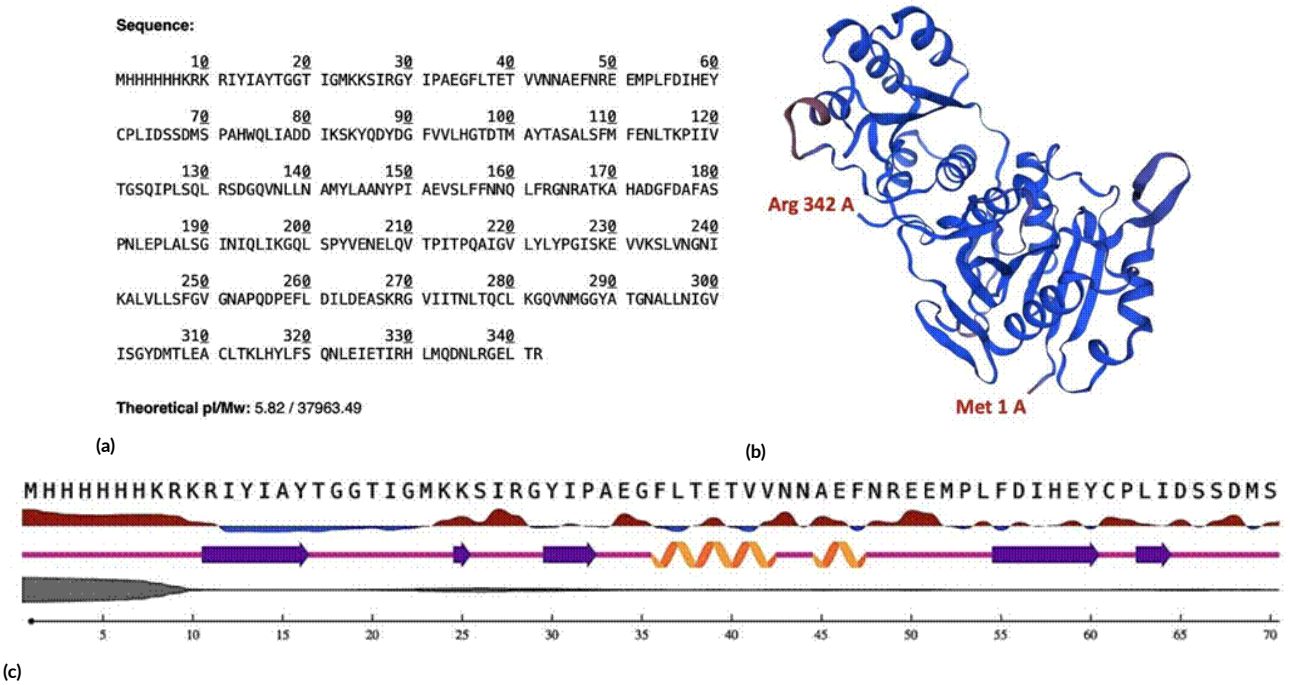

FIGURE 1 Structural characterization of recombinant L-asparaginase. (a) Theoretical pI/Mw. (b) Predicted 3D structural model. (c) Secondary structure prediction.

Modeling was carried out to determine the best location for the 6×His-tag, which was added to simplify purification using IMAC. The His-tag was chosen because its small size reduces the chance of interfering with protein structure and function (Cao and Lin 2009). Structural modeling revealed that both the N- and C-termini are in open conformations, indicating that the tag can be placed at either end and remain accessible for purification (Zhao et al. 2013). However, the N-terminus was selected. Additional analyses confirmed that the N-terminal His-tag region is flexible, highly disordered, and strongly exposed to solvent, conditions that ensure the tag remains accessible and supports efficient purification using Ni-Sepharose His-tag chromatography (Bornhorst and Falke 2000).

The basic physicochemical properties of L-asparaginase (UniProt ID: A0AA37S3L8) were predicted using the ExPASy ProtParam server (Figure 1a). The protein contains 340 amino acids (including the 6×His-tag) and has an estimated molecular weight of around 37 kDa. Its predicted isoelectric point (pI) is 5.82, which is the pH at which the protein carries no net charge. Below this pH the protein becomes positively charged, and above it, negatively charged. Because physiological pH ($\approx$ 7.4) is higher than the pI, L-asparaginase will carry a negative charge in the body, helping maintain its solubility in blood and cytoplasm. This solubility supports the protein's stability and effectiveness as a therapeutic agent (Tokmakov et al. 2021).

3.2. Cloning of the recombinant of L-asparaginase

The successful transformation of the recombinant pD861-SR [L-asparaginase] plasmid into competent *E. coli* BL21 (DE3) cells was confirmed through a series of selection steps. Initial selection was performed on LB agar plates containing 75 µg/mL kanamycin. The growth of colonies on this selective medium, as shown in Figure 2a, confirmed that *E. coli* BL21 (DE3) cells had taken up the recombinant plasmid, because the plasmid carried a kanamycin resistance gene that allowed the bacteria to grow on media containing kanamycin (Liu et al. 2020). The viable cell concentration was also determined to be 2.628×10^6 CFU/mL.

To confirm the presence of the gene insert, plasmid DNA was isolated from five positive single colonies. From the plasmid isolation results, the DNA bands produced were still varied due to different plasmid conformations (Sambrook and Russell 2001). Thus, the size of the isolated plasmid could not be determined accurately. Therefore, a restriction process was necessary to cut the plasmid into a linear form, ensuring a single DNA band could be obtained (Figure 3b), confirming the plasmid size to be approximately 3266 bp. This size was consistent with the size of the constructed plasmid shown in Figure 3a.

Plasmid isolate purity was checked using a NanoDrop spectrophotometer (ALLSHENG, China). Based on the highest concentration and purity, colonies two and three were selected to definitively confirm the presence of the L-asparaginase insert using Next Generation Sequencing. The sequencing results were translated into proteins, then analyzed using BLAST (Basic Local Alignment Search Tools) online at the NCBI website (https://www.ncbi.nlm.nih.gov/). The results showed that the sequences from colonies two and three had identical amino acid sequences (100% identity) with the amino acid sequence of the ordered L-asparaginase. This confirmed the successful construction and transformation of the recombinant pD861-SR [L-asparaginase] plasmid into competent *E. coli* BL21 (DE3) cells.

3.3. Expression of the recombinant L-asparaginase

The expression of the L-asparaginase gene was confirmed by SDS-PAGE analysis, which demonstrated clear differences in protein band intensity between L-rhamnose induced and non-induced samples. The induced cultures carrying the pD861-SR[L-Asp] plasmid exhibited a prominent band at approximately 37 kDa, indicating successful expression of recombinant L-asparaginase. In contrast, the non-induced pD861-SR[L-Asp] cultures did not produce detectable levels of the target protein. As expected, *E. coli* cells lacking the plasmid were also unable to express L-asparaginase, even when exposed to L-rhamnose, since the inducer can only activate transcription through the rhaBAD promoter present on the recombinant vector.

The rhaBAD promoter system is specifically designed to minimize basal (leaky) expression, ensuring that transcription of the target gene occurs only in the presence of L-rhamnose (Stargardt et al. 2021). This regulatory system involves two operons, rhaR and rhaS. RhaR is ac-

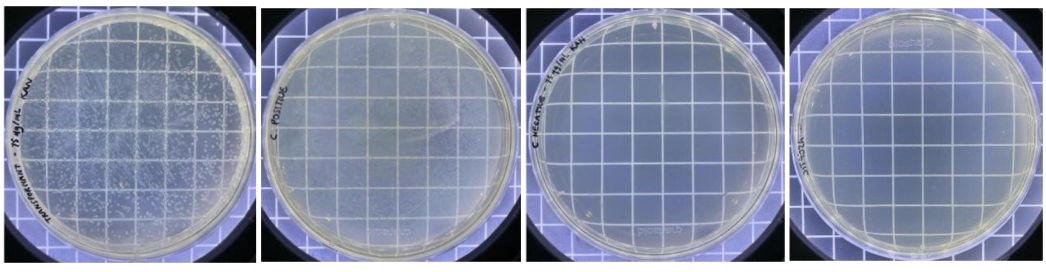

FIGURE 2 Transformation of recombinant *E. coli* BL21(DE3). (a) Transformants on selective LB-kanamycin agar (75 µg/mL). (b) competent *E. coli* BL21 (DE3) cells were spreading on LB agar (without kanamycin), (c) competent *E. coli* BL21 (DE3) cells were spreading on LB agar with 75 µg/mL kanamycin, and (d) aseptic controls.

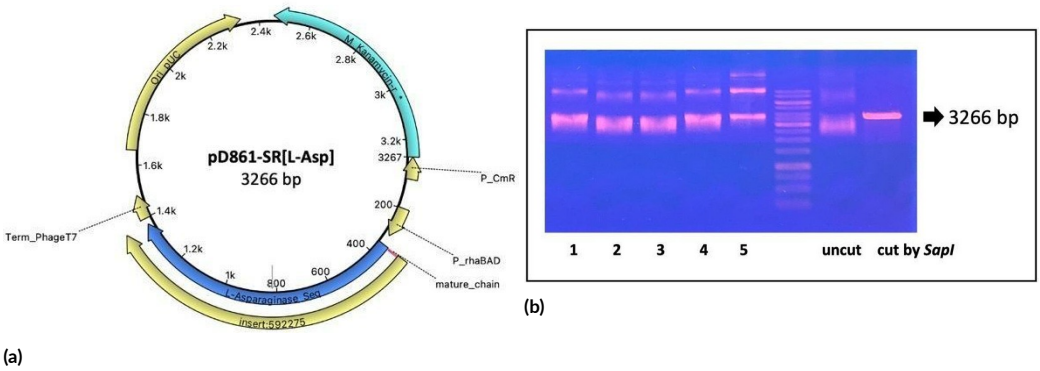

FIGURE 3 Plasmid construction and characterization of pD861-SR[L-Asp]. (a) Map of recombinant plasmid pD861-SR[L-Asp] (3266 bp). (b) Agarose electropherogram of plasmid isolation. Lanes 1–5: colonies 1–5; Lane uncut: intact plasmid; Lane cut by SapI: linearized plasmid at 3266 bp.

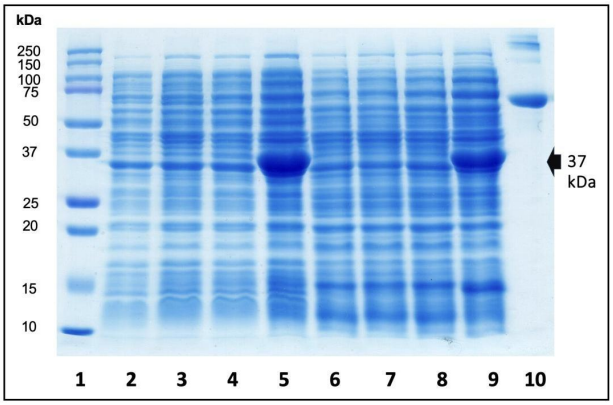

FIGURE 4 SDS-PAGE Electropherogram of recombinant L-asparaginase expression. Lane 1: protein molecular; Lane 2: soluble fraction (SF) of uninduced *E. coli* BL21(DE3); Lane 3: SF of induced *E. coli* BL21(DE3); Lane 4: SF of uninduced *E. coli* BL21(DE3)-L-Asp; Lane 5: SF of induced *E. coli* BL21(DE3)-L-Asp; Lane 6: inclusion body (IB) of uninduced *E. coli* BL21(DE3); Lane 7: IB of induced *E. coli* BL21(DE3); Lane 8: IB of uninduced *E. coli* BL21(DE3)-L-Asp; Lane 9: IB of induced *E. coli* BL21(DE3)-L-Asp; Lane 10: BSA standard.

tivated upon exposure to L-rhamnose and subsequently binds to the rhaSR promoter, inducing expression of RhaS. Activated RhaS then binds to the rhaBAD promoter, initiating transcription of the downstream gene. These two regulatory steps collectively reduce unintended background expression (Schweikert et al. 2021). The use of the rhaBAD promoter allows fine regulation of protein expression levels within individual cells. Protein expression can be increased by supplying higher concentrations of rhamnose. This differs from IPTG-induced systems, where increasing IPTG concentration primarily raises the proportion of cells expressing the protein, rather than the amount of protein produced per cell (ATUM 2025).

As shown in Figure 4, the target protein band appears more prominent in the soluble fraction than in the insoluble fraction, suggesting that recombinant L-asparaginase folds efficiently in the cytoplasm of *E. coli* BL21 (DE3).

This observation is consistent with the characteristics of BL21 (DE3), a protease-deficient strain widely used for high-yield recombinant protein production (Hausjell et al. 2018). Nevertheless, some portion of the protein was also detected in the insoluble fraction as inclusion bodies, which can form when expression levels exceed the host cell's folding capacity. Under such conditions, misfolded polypeptides aggregate into dense inclusion bodies, a well-documented phenomenon in bacterial expression systems (Bhatwa et al. 2021).

3.4. Purification of the recombinant L-asparaginase

Immobilized metal affinity chromatography (IMAC) is used for recombinant proteins that have a His-tag expressed in *E. coli* by allowing selective binding to nickel-charged agarose beads, followed by washing and elution with imidazole (Jiang et al. 2025; Cytiva 2022). In this study, purification was carried out under denaturing conditions using 8 M urea to fully unfold the recombinant protein, expose the His-tag, and solubilize aggregated proteins from inclusion bodies. SDS-PAGE analysis (Figure 5) showed a clear band around 37 kDa, consistent with the expected size of the target protein. Protein quantifica-

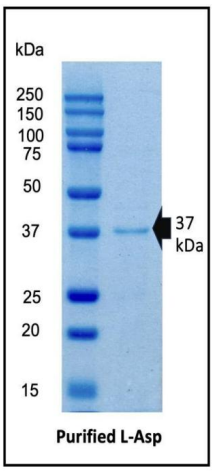

FIGURE 5 SDS-PAGE Electropherogram of recombinant L-asparaginase purified under denaturing conditions.

TABLE 1 Hydrogen bonds and hydrophobic interactions in docked protein-protein complexes.

No	Sample	Target Protein (mg)*	Protein Total (mg) **	Purity (%)
1	Purified L-Asparaginase	0.3843	0.514	74.8
2	Purified L-Asparaginase	0.3267	0.451	72.4
	Mean ± SD	-	-	73.6 ± 1.7

*Quantified using ImageJ densitometry
**Quantified using Bradford-Spectrophotometry
Values represent technical duplicates (n = 2).

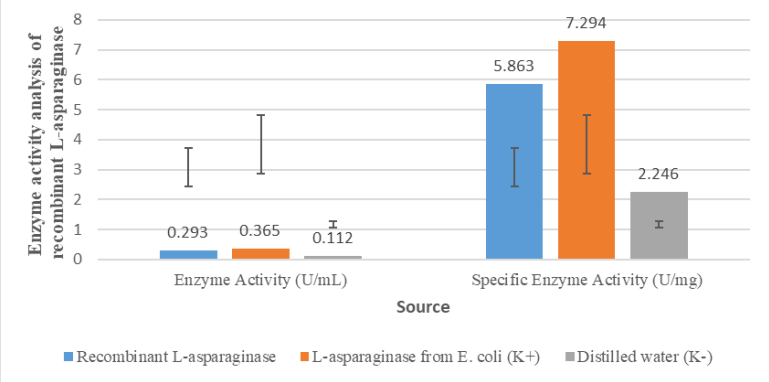

FIGURE 6 Enzyme activity analysis of recombinant L-asparaginase. Values are presented as mean ± SD (n=3).

tion using ImageJ and the Bradford assay indicated a final purity of 73.6% (Table 1). ImageJ is used to quantify the relative amount of target protein based on band intensity in gel electrophoresis, while Bradford method is used to determine total protein concentration. Protein purity is calculated by combining the relative band intensity from ImageJ with the total protein concentration obtained from the Bradford assay.

The success of purification is supported by structural analysis (Figure 1), which showed that the His-tag is located in an open and accessible region, allowing efficient binding to Ni-Sepharose resin (Riguero et al. 2020).

3.5. L-asparaginase activity assay

The ammonia produced by L-asparaginase is reacted with Nessler's reagent to determine enzyme activity. The standard curve was prepared using ammonium sulfate with concentrations 0, 2, 4, 6, 8, and 10 µmol/mL. The standard curve result showed a slope a is 0.1845 and an intercept b is 0.0165. In this preliminary assessment, the purified enzyme exhibited a specific activity of 5.863 U/mg, demonstrating that the recombinant protein remained catalytically active following expression in *E. coli* and purification under denaturing conditions (Figure 6). At this stage, the activity values reflect non-optimized experimental conditions. Factors such as incomplete protein refolding and residual impurities associated with the 73.6% purity level are expected to be improved in subsequent optimization steps (Shrief 2020; Booth et al. 2018).

The positive control, *E. coli* yielded a specific activity, consistent with previously reported values (Barati et al. 2016), thereby validating the assay conditions. However, the negative control (K-), which consisted of Nessler reagent, L-asparagine, and distilled water, exhibited a detectable increase in absorbance. This indicates the presence of residual ammonia, despite the absence of enzymatic hydrolysis. The most plausible explanation is that the distilled water used was not ammonia-free, allowing trace amounts of ammonium or ammonia to react with the Nessler reagent and produce a background signal. Another possible contributing factor is the spontaneous degradation or deamidation of L-asparagine, which can slowly release ammonia under certain conditions, particularly during prolonged storage or in suboptimal pH and temperature. In addition, contamination from glassware or pipette tips, especially if previously exposed to nitrogenous compounds, cannot be completely ruled out. Although this background activity did not exceed the absorbance values of the enzyme-containing samples, it highlights the importance of reagent purity and stricter quality control during colorimetric ammonia detection. In future experiments, the use of ammonia-free Milli-Q water, freshly prepared L-asparagine solutions, and acid-washed laboratory equipment is expected to minimize background signals and improve assay reliability.

4. Conclusions

This study successfully demonstrated the recombinant expression and purification of a synthetic type I L-asparaginase gene derived from the marine bacterium *Pseudoalteromonas tetraodonis* GFC in *Escherichia coli* BL21 (DE3). The recombinant enzyme was detected in both soluble and insoluble fractions and exhibited an apparent molecular weight of approximately 37 kDa. Purification using Ni-Sepharose affinity chromatography under

denaturing conditions yielded a protein purity of 73.6%.

The purified recombinant L-asparaginase displayed measurable catalytic activity, indicating that the expressed protein retained basic enzymatic functionality following purification and refolding. Although the current study represents an initial characterization, the results provide a basis for further optimization of expression conditions, refolding strategies, purification under native conditions, and more comprehensive enzymatic characterization in future studies.

Acknowledgments

The authors sincerely thank Universitas Muhammadiyah Bandung and the Research Center for Molecular Biotechnology and Bioinformatics at Padjadjaran University for providing laboratory facilities. The authors also thank the Ministry of Higher Education, Science, and Technology for providing funding for the research. Thanks also to the team that helped with data collection and data analysis.

Authors' contributions

WP and QARS designed the study. TRT, TZ, and NSN carried out the laboratory work. WP and TRT analyzed the data. WP, TRT, TZ, and NSN wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests regarding the research or the research funding.

References

Abdullah EM. 2024. Expression, characterization and cytotoxicity of recombinant L-asparaginase II from *Salmonella paratyphi* cloned in *Escherichia coli*. Int. J. Biol. Macromol. 279(4). doi:10.1016/j.ijbiomac.2024.135458.

Ardalan N, Mirzaie S, Sepahi AA, Khavari-Nejad RA. 2018. Novel mutant of *Escherichia coli* asparaginase II to reduction of the glutaminase activity in treatment of acute lymphocytic leukemia by molecular dynamics simulations and QM-MM studies. Med. Hypotheses 112:7–17. doi:10.1016/j.mehy.2018.01.004.

ATUM. 2025. pD861-SR. https://atum.bio/eCommerce/catalog/datasheet/397. Accessed 2025-11-30.

Barati M, Tabarzad M, Safarpour H, Asad AG, Ghaderi O. 2016. Validation of a simple and rapid method for assessment of intracellular bacterial asparaginase. Iran. J. Pharm. Sci. 12:33–42. doi:10.22034/IJPS.2016.22800.

Bhatwa A, Wang W, Hassan YI, Abraham N, Li XZ, Zhou T. 2021. Challenges associated with the formation of recombinant protein inclusion bodies in *Escherichia coli* and strategies to address them for industrial applications. Front. Bioeng. Biotechnol. 9:630551. doi:10.3389/fbioe.2021.630551.

Booth WT, Schlachter CR, Pote S, Ussin N, Mank NJ, Klapper V, Offermann LR, Tang C, Hurlburt BK, Chruszcz M. 2018. Impact of an N-terminal polyhistidine tag on protein thermal stability. ACS Omega 3(1):760–768. doi:10.1021/acsomega.7b01598.

Bornhorst JA, Falke JJ. 2000. Purification of proteins using polyhistidine affinity tags. Methods Enzymol. 326:245–254. doi:10.1016/s0076-6879(00)26058-8.

Cao H, Lin R. 2009. Quantitative evaluation of His-tag purification and immunoprecipitation of tristetraprolin and its mutant proteins from transfected human cells. Biotechnol. Prog. 25(2):461–467. doi:10.1002/btpr.121.

Chiu M, Taurino G, Bianchi MG, Kilberg MS, Bussolati O. 2020. Asparagine synthetase in cancer: beyond acute lymphoblastic leukemia. Front. Oncol. 9:1480. doi:10.3389/fonc.2019.01480.

Cytiva. 2022. Principles and methodology handbooks: affinity chromatography. Vol. 2: tagged protein. https://www.cytivalifesciences.com/en/us/support/handbooks. Accessed 2025-11-17.

Dastmalchi M, Hamzeh-Mivehroud M, Rezazadeh H, Farajollahi MM, Dastmalchi S. 2024. Investigating functional and folding stability of an engineered *E. coli* L-asparaginase harboring Y176F/S241C mutations. Adv. Pharm. Bull. 14(3):675–685. doi:10.34172/apb.2024.048.

Ding F, Deng L, Xiong J, Cheng Z, Xu J. 2025. Analysis of global trends in acute lymphoblastic leukemia in children aged 0–5 years from 1990 to 2021. Front. Pediatr. 13:1542649. doi:10.3389/fped.2025.1542649.

Eze OC, Berebon DP, Emencheta SC, Evurani SA, Okorie CN, Balcão VM, Vila MMDC. 2023. Therapeutic potential of marine probiotics: a survey on the anticancer and antibacterial effects of *Pseudoalteromonas* spp. Pharmaceuticals 16(8):1091. doi:10.3390/ph16081091.

Ferlay J, Laversanne M, Ervik M, Lam F, Colombet M, Mery L, Pineros M, Znaor A, Soerjomataram I, Bray F. 2024. Global cancer observatory: cancer tomorrow (version 1.1).

GCO. 2022. Cancer tomorrow. International Agency for Research on Cancer. Accessed 30 November 2025.

Hausjell J, Weissensteiner J, Molitor C, Halbwirth H, Spadiut O. 2018. *E. coli* HMS174 (DE3) is a sustainable alternative to BL21 (DE3). Microb. Cell Fact. 17(1):169. doi:10.1186/s12934-018-1016-6.

Holmström C, Kjelleberg S. 1999. Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. FEMS Microbiol. Ecol. 30:285–293.

Jayakrishnan A, Rosli WRW, Tahir ARM, Razak FSA, Kee PE, Ng HS, Chew YL, Lee SK, Ramasamy M, Tan CS, Liew KB. 2024. Evolving paradigms of recombinant protein production in pharmaceu-

tical industry: a rigorous review. Sci. 6(1):9. doi:10.3390/sci6010009.

Jiang X, Qin R, Ma T, Xu K, Lv F. 2025. Optimization of immobilized metal affinity chromatography for a recombinant protein expressed in CHO cells. J. Chromatogr. Open 8:100230. doi:10.1016/j.jcoa.2025.100230.

Jo J, Choi H, Lee SG, Oh J, Lee HG, Park C. 2017. Draft genome sequences of *Pseudoalteromonas tetraodonis* CSB01KR and *Pseudoalteromonas lipolytica* CSB02KR, isolated from the gut of the sea cucumber *Apostichopus japonicus*. Genome Announc. 5(28):49–50. doi:10.1128/genomeA.00627-17.

Kemenkes RI. 2025. Kenali kanker darah ancaman diam pada sistem kekebalan tubuh [Recognize blood cancer: a silent threat to the immune system]. URL https://keslan.kemkes.go.id/view_artikel/4171/kenali-kanker-darah-ancaman-diam-pada-sistem-kekebalan-tubuh. Accessed 5 December 2025.

Lee RD, Jospin G, Lang JM, Eisen JA, Coil DA. 2015. Draft genome sequence of *Pseudoalteromonas tetraodonis* strain UCD-SED8 (Phylum Gammaproteobacteria). Genome Announc. 3(6):2164. doi:10.1128/genomeA.01276-15.

Liu X, Li N, Jia M, Zhang S, Niu H, Li Q, Gu P. 2020. The effects of kanamycin concentration on gene transcription levels in *Escherichia coli*. 3 Biotech 10(3):93. doi:10.1007/s13205-020-2100-2.

Muzuni M, Jamaluddin J, Suriana S, Ardiansyah A, Yanti NA. 2024. Skrining bakteri termohalofilik penghasil L-asparaginase dari sumber air panas wawolesea Sulawesi Tenggara dan uji aktivitas enzimnya [Screening of thermohalophilic bacteria producing L-asparaginase from the Wawolesea hot springs, Southeast Sulawesi, and evaluation of its enzymatic activity]. Alchemy 20(1):12. doi:10.20961/alchemy.20.1.73523.12-21.

Pertiwi W, Nuraeni I, Namira AJ, Moeis MR, Fauzi M, Subroto T. 2025. Partially purified L-asparaginase with low glutaminase and urease co-activities of bacteria from the Rancabuaya Coast, Indonesia. J. Trop. Biodiv. Biotech. 10(2):jtbb16510. doi:10.22146/jtbb.16510.

Rajeev M, Sushmitha TJ, Toleti SR, Pandian SK. 2021. Draft genome sequencing of *Pseudoalteromonas tetraodonis* strain kknpp56, a potent biofilm-forming bacterium isolated from early-stage marine biofilm. Microbiol. Resour. Announc. 10(38):20–21. doi:10.1128/mra.00605-21.

Riguero V, Clifford R, Dawley M, Dickson M, Gastfriend B, Thompson C, Wang SC, O'Connor E. 2020. Immobilized metal affinity chromatography optimization for poly-histidine tagged proteins. J. Chromatogr. A 1629:461505. doi:10.1016/j.chroma.2020.461505.

Sambrook J, Russell D. 2001. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Schweikert S, Kranz A, Yakushi T, Filipchyk A, Polen T, Etterich H, Bringer S, Bott M. 2021. FNR-type regulator GoxR of the obligatorily aerobic acetic acid bacterium gluconobacter oxydans affects expression of genes involved in respiration and redox metabolism. Appl. Environ. Microbiol. 87(11):1–20. doi:10.1128/aem.00195-21.

Shrief EFH. 2020. Factors affecting enzyme activity. Department of Biochemistry and Nutrition, Ain Shams University.

Simidu U, Kita-Tsukamoto K, Yasumoto T, Yotsu M. 1990. Taxonomy of four marine bacterial strains that produce tetrodotoxin. Int. J. Syst. Bacteriol. 40(4):331–336. doi:10.1099/00207713-40-4-331.

Stargardt P, Striedner G, Mairhofer J. 2021. Tunable expression rate control of a growth-decoupled T7 expression system by L-arabinose only. Microb. Cell Fact. 20(1):27. doi:10.1186/s12934-021-01512-7.

Tokmakov AA, Kurotani A, Sato KI. 2021. Protein pI and intracellular localization. Front. Mol. Biosci. 8:775736. doi:10.3389/fmolb.2021.775736.

Trimpont MV, Peeters E, Visser YD, Schalk AM, Mondelaers V, Moerloose BD, Lavie A, Lammens T, Goossens S, Vlierberghe PV. 2022. Novel insights on the use of L-asparaginase as an efficient and safe anti-cancer therapy. Cancers (Basel) 14(4):902. doi:10.3390/cancers14040902.

Zhao X, Li G, Liang S. 2013. Several affinity tags commonly used in chromatographic purification. J. Anal. Methods Chem. 2013(1):581093. doi:10.1155/2013/581093.

Zuo S, Zhang T, Jiang B, Mu W. 2014. Recent research progress on microbial L-asparaginases. Appl. Microbiol. Biotechnol. 99:1069–1079. doi:10.1007/s00253-014-6271-9.