VOLUME 13 NOMOR 1 JUNE 2026 ISSN 2548 – 611X

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejournal.brin.go.id/JBBI/index

ANALYSIS OF *L-Asparaginase* II ENZYME ACTIVITY FROM NATIVE AND RECOMBINANT *Escherichia coli* DH10B FOR THE APPLICATION OF ACUTE LYMPHOBLASTIC LEUKAEMIA THERAPY

Analysis of *L-Asparaginase* II Enzyme Activity from Native and Recombinant *Escherichia coli* DH10B for The Application of Acute Lymphoblastic Leukaemia Therapy

Keysha Putri Febiona[1], Dian Pertiwi[1], Arni Amir[1], Lily Syukriani[2], Dessy Arisanty[1], Almurdi Almurdi[1], Lisana Shiddiqin Aliya[1], Jamsari Jamsari[3]

[1] Biomedical Sciences, Faculty of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia
[2] Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas, Padang West Sumatera, Indonesia
[3] Magister Program of Biotechnology, School of Postgraduate, Universitas Andalas, Padang, West Sumatera, Indonesia
*Email: jamsari@agr.unand.ac.id

ABSTRACT

Acute Lymphoblastic Leukemia (ALL) is a blood cancer that occurs due to abnormal proliferation of lymphoid progenitor cells. L-asparaginase II is an enzyme used to treat this disease by inducing apoptosis in cancer cells encoded by the *AnsB* gene. Previous studies have shown complications and toxicity in commercial L-asparaginase, necessitating exploration of L-asparaginase sources. *Serratia plymuthica* UBCF_13 is one of the microorganisms that encodes the *AnsB* gene. This study consisted of inoculation of native *E. coli* DH10B and *E. coli* DH10B *pET28a(+)_AnsB*, molecular validation, enzyme activity testing using direct nesslerisation method, and analysis of extracellular and intracellular protein profiles using SDS-PAGE. Enzyme activity and protein profiles were compared with *S. plymuthica* UBCF_13, and Isopropyl β-D-1-thiogalactopyranoside (IPTG) induction was performed on each bacteria as a comparison. The results showed the highest L-asparaginase II enzyme activity in *E. coli* DH10B *pET28a(+)_AnsB* induced with IPTG with an average enzyme activity of 0.392 ± 0.017 U/mL. Protein profile analysis using SDS-PAGE supported this finding, showing a thicker 36.5 kDa protein band compared to other bacteria. Statistical testing using One Way ANOVA showed a significant difference in L-asparaginase II enzyme activity in native *E. coli* DH10B and *E. coli* DH10B *pET28a(+)_AnsB*. It can be concluded that there was an increase in activity in recombinant *E. coli* DH10B compared to its native.

Keywords: *L-asparaginase II, Escherichia coli DH10B, Enzyme activity, SDS-PAGE*

ABSTRAK

Acute Lymphoblastic Leukemia (ALL) is a blood cancer that occurs due to abnormal proliferation of lymphoid progenitor cells. L-asparaginase II is an enzyme used to treat this disease by inducing apoptosis in cancer cells encoded by the *AnsB* gene. Previous studies have shown complications and toxicity in commercial L-asparaginase, necessitating exploration of L-asparaginase sources. *Serratia plymuthica* UBCF_13 is one of the microorganisms that encodes the *AnsB* gene. This study consisted of inoculation of native *E. coli* DH10B and *E. coli* DH10B *pET28a(+)_AnsB*, molecular validation, enzyme activity testing using direct nesslerisation method, and analysis of extracellular and intracellular protein profiles using SDS-PAGE. Enzyme activity and protein profiles were compared with *S. plymuthica* UBCF_13, and Isopropyl

***Received**: 27 October 2025* ***Accepted**: 05 May 2026* ***Published**: 09 June 2026*

β-D-1-thiogalactopyranoside (IPTG) induction was performed on each bacteria as a comparison. The results showed the highest L-asparaginase II enzyme activity in *E. coli* DH10B *pET28a(+)_AnsB* induced with IPTG with an average enzyme activity of 0.392 ± 0.017 U/mL. Protein profile analysis using SDS-PAGE supported this finding, showing a thicker 36.5 kDa protein band compared to other bacteria. Statistical testing using One Way ANOVA showed a significant difference in L-asparaginase II enzyme activity in native *E. coli* DH10B and *E. coli* DH10B *pET28a(+)_AnsB*. It can be concluded that there was an increase in activity in recombinant *E. coli* DH10B compared to its native.

Kata kunci: *L-asparaginase II, Escherichia coli DH10B, Enzyme activity, SDS-PAGE*

INTRODUCTION

Leukemia is a form of hematological malignancy characterized by uncontrolled proliferation of immature leukocytes. This disease derived from somatic mutations in hematopoietic stem cells (Romero-Morelos et al., 2024). This proliferation occurs in the bone marrow, leading to an increase in the number of cells from the affected hematopoietic pathway (Bispo et al., 2020). This can cause damage to red blood cells, bone marrow, and the body's immune system (Ghaderzadeh et al., 2021). There were 474,519 new cases of leukemia and 311,594 deaths from leukemia reported globally in 2020 (Huang et al., 2022). The prevalence of new leukemia cases in Indonesia in 2022 ranks 9th, with the mortality rate ranking 7th (Globocan, 2024). Acute lymphoblastic leukemia (ALL) is one of the most common types of acute leukemia, accounting for 75-80% of all childhood leukemia cases globally, and is also the leading cause of death in children (Mohammadian-Hafshejani et al., 2024).

L-asparaginase is an enzyme that hydrolyses asparagine into aspartic acid and ammonia (Shafqat et al., 2023). The L-asparaginase enzyme is used as enzyme-based therapy for acute lymphoblastic leukemia (ALL) and lymphosarcoma (Jia et al., 2021). Asparagine is a non-essential amino acid required by cells. ALL cells lack, or have very low, asparagine synthetase enzyme, so ALL cells require asparagine from external sources. The L-asparaginase enzyme can induce apoptosis in ALL cells by reducing asparagine levels (Nemkov et al., 2019). L-asparaginase approved by FDA is derived from *E. coli* and *Erwinia chrysanthemi* (Shishparenok et al., 2023), but there are complications and toxicities associated with L-asparaginase enzyme therapy, such as clinical hypersensitivity, severe hypoproteinaemia, allergic reactions, hepatotoxicity, hyperglycaemia, diabetes, pancreatitis, thrombosis, encephalopathy, and hypertriglyceridemia (Schmidt et al., 2021). This supports the need to explore L-asparaginase from other microbial sources to minimize risks and toxicity, such as *Serratia plymuthica* UBCF_13.

Serratia plymuthica UBCF_13 has the ability to produce the L-asparaginase II enzyme. Analysis of the whole genome sequencing of *S. plymuthica* UBCF_13 (Fatiah et al., 2021) shows the presence of the *AnsB* gene that codes L-asparaginase II enzyme. The idea of developing other therapeutic proteins that can be produced more quickly and affordably through *E. coli* has grown since the successful expression of recombinant insulin in *E. coli* K-12 bacteria in 1982 (İncir and Kaplan, 2024). In this regard, recombinant DNA technology with *E. coli* host cells was used to increase the production and expression of L-asparaginase II from *S. plymuthica* UBCF_13. One of the strains is DH10B. This strain is designed for large-scale propagation of target genes and is often used for DNA cloning because it has high DNA transformation efficiency and can stably maintain large plasmids (Durfee et al., 2008a). The DH10B strain was selected for this study because it is a substrain of *E. coli* K-12 that has previously been successful in expressing recombinant insulin (Durfee et al., 2008b).

This study focused on analyzing the activity of the L-asparaginase II enzyme from *S. plymuthic*a UBCF_13 produced by *E. coli* DH10B. To observe the increase in enzyme activity, the activity test was

compared with native *E. coli* DH10B and *S. plymuthica* UBCF_13 itself, as well as with the addition of IPTG to each bacteria. It is assumed that there will be an increase in L-asparaginase II activity in recombinant *E. coli* DH10B. Protein profile analysis with SDS-PAGE was performed to observe the increase in enzyme production between bacteria and between IPTG and non-IPTG.

MATERIALS AND METHODS

2.1 Rejuvenation of native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB*, and *S. plymuthica* UBCF_13

Bacterial rejuvenation was performed by growing each bacterial glycerol stock on Luria Bertani (LB) medium as a starter culture for 16-18 hours with 200 rpm agitation. *E. coli* was grown at 37°C and *S. plymuthica* UBCF_13 was grown at room temperature. Kanamycin was added to LB medium for the starter culture of *E. coli* DH10B *pET28a(+)_AnsB*. The starter cultures of each bacteria were inoculated onto LB agar using the 4-quadrant streak method. Kanamycin was added to LB agar for *E. coli* DH10B *pET28a(+)_AnsB*. Single colonies that grew were selected at random and subsequently used for molecular validation, activity testing, and protein extraction.

2.2 Single colony culture of native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB* and *S. plymuthica* UBCF_13

2.2.1 Without IPTG induction

Single colonies of native *E. coli* DH10B and *S. plymuthica* UBCF_13 were grown in LB agar without antibiotics, while single colonies of *E. coli* DH10B pET28a(+)_AnsB were grown in LB agar containing kanamycin. The cultures were grown with agitation at 200 rpm for 16-18 hours. *E. coli* DH10B was cultured at 37°C, while *S. plymuthica* UBCF_13 was cultured at room temperature.

2.2.2 With IPTG induction

The IPTG induction procedure in this study was based on the IPTG induction method used in the previous research by Zulfa (2024). Single colonies of each bacteria were first grown for 3-5 hours at 200 rpm agitation. The optimal density of the culture was measured with a spectrophotometer at a wavelength of 600 nm. IPTG induction was performed after the absorbance reached 0.8-1 by adding 0.2 mM IPTG to the culture, and incubation was continued with 180 rpm agitation for 24 hours. *E. coli* DH10B was cultured at 37°C, while *S. plymuthica* UBCF_13 was cultured at room temperature.

2.3 Molecular validation

2.3.1 *Native E. coli* DH10B

Native *E. coli* DH10B culture was isolated using a manual protocol from the GeneJET Genomic DNA Purification Kit (Thermo Scientific™). Molecular validation was performed by PCR using 16S rRNA-27F and 16S rRNA-1525R primers, electrophoresis on 1% agarose (0.5X TBE, 100V, 40 minutes), and followed by Sanger sequencing.

2.3.2 *E. coli* DH10B *pET28a(+)_AnsB*

E. coli DH10B *pET28a(+)_AnsB* plasmid was isolated using the protocol from the PrestoTM Mini Plasmid Kit (Geneaid). Molecular validation was performed by PCR using full-length *AnsB* primers and electrophoresis on 1% agarose (0.5X TBE, 100V, 40 minutes).

2.4 Harvest of cultures

The bacterial culture was harvested by centrifuging the culture at 8,000 rpm at 4°C for 10 minutes. The supernatant was then used for the L-asparaginase enzyme activity assay.

2.5 Ammonia standard curve

The standard ammonia curve was prepared by diluting ammonium sulfate solution with distilled water to a final concentration of 0.1 mM – 0.5 mM. Nessler's reagent was added to the diluted solution and incubated for 15 minutes. Absorbance was measured using a spectrophotometer at a wavelength of 425 nm.

2.6 L-asparaginase enzyme activity assay

L-asparaginase enzyme activity assay was performed using the direct Nesslerization method based on the procedure by Wriston (1985). A 100 µL supernatant was reacted with 50 µL of 50 mM Tris-HCl buffer pH 8.6 and 50 µL of 80 mM L-Asparagine substrate in a microtube. The sample was incubated at 37°C for 30 minutes in a water bath. The reaction was stopped by adding 30 µL of 20% TCA. The sample was diluted by adding 770 µL of distilled water. 100 µL of Nessler's reagent was added and the sample was homogenized by performing up-down movements using a micropipette. The sample was incubated for 15 minutes at room temperature. The absorbance of the sample was immediately measured using a UV-Vis spectrophotometer at a wavelength of 425 nm.

2.7 L-asparaginase enzyme activity calculation

Enzyme activity is calculated from the ammonia level produced by the sample. Based on calculations by Karim et al. (2020), the ammonia level of the sample can be determined by substituting the absorbance of the sample into the ammonia standard curve equation from the regression equation y= ax+b. Thus:

$x = \frac{(y-b)}{a}$

Where:
y = absorbance
x = ammonia concentration (µmol/L)
a = slope
b = intercept

The L-asparaginase enzyme activity value is obtained using the following equation:

$$\text{Enzyme activity (U/mL)} = \frac{y-b}{a} \times \frac{V.Total}{V.Analysis} \times \frac{1}{V.Enzyme} \times \frac{1}{t.Incubation} \times Diluting\ factor$$

Notes:
V. Total = Volume of enzyme + substrate + buffer + TCA + distilled water (1 mL)
V. Analysis = Total volume analyzed (1 mL)
V. Enzyme = Volume of enzyme reacted and incubated (0.1 mL)
Incubation time = Incubation time (30 minutes)
Dilution factor = 5x

2.8 Extracellular dan intracellular protein extraction

2.8.1 Extracellular

Extracellular and intracellular protein extraction was performed based on the procedure described in the study by Rohinda (2019). The supernatant from the culture harvest was used to obtain extracellular protein. The supernatant was filtered using a 0.2 µm syringe filter and transferred to a new falcon tube. The procedure continued with protein precipitation by adding a 20% TCA-acetone solution equal to 1 times of the supernatant volume. The sample was stored in a refrigerator at -15°C overnight for protein precipitation. The incubated extracellular protein was then centrifuged at 13,000 rpm at 4°C for 30 minutes. The supernatant is discarded, and the pellet, which is the protein, is washed with cold 80% acetone solution and then centrifuged at 13,000 rpm at 4°C for 10 minutes. This is done three times, replacing the cold 80% acetone solution after each wash. The liquid from the last wash is discarded, the tube cap is opened, and the tube is placed on ice to evaporate the acetone. The pellet is then resuspended with 500 µL of 50 mM Tris HCl pH 6.8 after the acetone has evaporated. The sample can be analyzed immediately by SDS-PAGE or stored at -20°C.

2.8.2 Intracellular

The pellets obtained from the culture were resuspended using 750 µL of lysis buffer (1 mM EDTA pH 8, 50 mM HEPES pH 7.4, and 150 mM KCl). The pellet was then lysed using the freeze-thaw method by incubating the sample at -80°C for 5 minutes, followed by incubation at 95°C for 5 minutes three times in a row. Next, the sample was centrifuged with agitation at 13,000 rpm at 4°C for 15 minutes. The supernatant was transferred to a 1.5 mL microtube (500 µL) and ammonium acetate-methanol solution (100 mM) was added at a 1:1 sample ratio. The mixture was incubated at -15°C overnight. The incubated intracellular proteins

were centrifuged at 13,000 rpm at 4°C for 20 minutes. The supernatant is discarded, and the pellet is washed using cold 80% acetone solution, then centrifuged at 13,000 rpm at 4°C for 20 minutes three times, replacing the cold 80% acetone solution after each wash. The final wash solution was discarded, the microtube cap was opened, and the microtube was placed on ice to evaporate the acetone. The pellet was then resuspended with 250 µL of 50 mM Tris HCl pH 6.8 after the acetone had evaporated.

2.9 SDS-PAGE

SDS-PAGE was used to analyze protein expression from the intracellular and extracellular fractions of each bacterium. SDS-PAGE was performed using a 12% polyacrylamide gel and stained with Commassie Brilliant Blue G-250. The molecular weight of L-asparaginase was calculated using Bioinformatics.org software. The protein code (protein ID) of the *AnsB* gene expressed by *S. plymuthica* UBCF_13 is QUY49937.1, while the protein code of the *AnsB* gene expressed by *E. coli* DH10B is WP_000394140.1. The protein codes of each bacterium were obtained from the NCBI database. Based on the results of the protein molecular weight calculation, the predicted molecular weight of the L-asparaginase II enzyme from *S. plymuthica* UBCF_13 is approximately 36.5 kDa, and the predicted molecular weight of the L-asparaginase II enzyme from native *E. coli* DH10B is approximately 36.86 kDa.

RESULTS

3.1 Rejuvenation of native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB*, and *S. plymuthica* UBCF_13

The growth results of each bacterial colony on agar media can be seen in Figure 1. The morphology of the *E. coli* DH10B colony observed is round, yellowish-white in color, opaque with a prominent surface, distinct edges, and a distinctive odor. The morphology of *S. plymuthica* UBCF_13 was round, whiter in color, opaque, and had a mucoid surface with a softer texture compared to *E. coli* colonies.

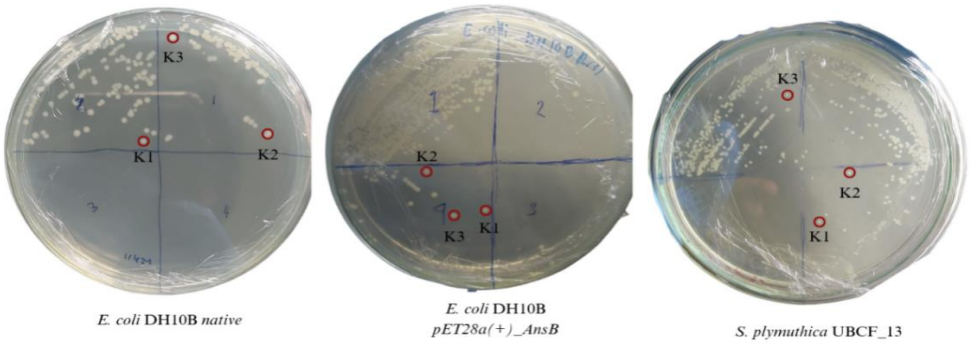


Figure 1 Single colonies of native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB*, and *S. plymuthica* UBCF_13 on agar medium (K1-K3 = single colony codes randomly selected for activity testing)

3.2 Molecular validation

3.2.1 Native E. coli DH10B

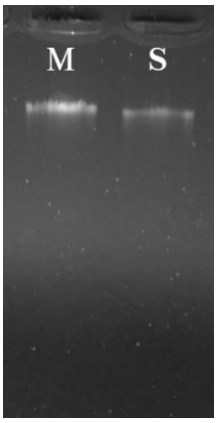
Native *E. coli* DH0B (Thermo Scientific) DNA successfully isolated using *GeneJET Genomic DNA Purification Kit* (Thermo Scientific) concentration and purity were measured using *Biodrop*. Measurement at absorbance ratio of 260/280 showed DNA concentration of 67.00 ng/µL with a purity value of 1.811. The results of native *E. coli* DH0B DNA isolation were electrophoresed on 1% agarose and visualized using GelDocThe isolated DNA was then analyzed by PCR using a pair of 16S rRNA 27F and 16S rRNA 1525R primers.

Gel electrophoresis showed a target band at a size of approximately 1498 bp, indicating that the isolated gene is a bacterial gene. The PCR results from the 16S rRNA primer could not confirm the species being analyzed, so molecular validation was continued with sequencing. The PCR product was sequenced using the services of 1st_Base in Singapore with the Sanger

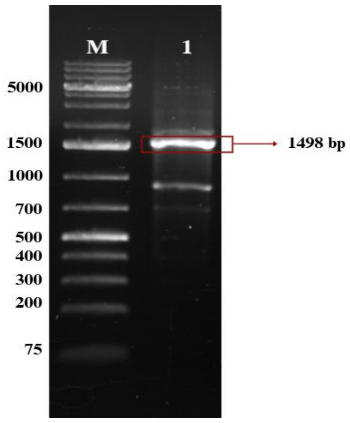
sequencing technique with bi-directional reading, namely sequencing with forward and reverse reading directions. Sequencing data was contigged using the SeqMan application. Contig was performed with the aim of combining the sequencing data and correcting overlapping fragments. When performing contig, the 16S rRNA gene data in NCBI with the code OLR78_RS21115 was added as a reference. The sequence was successfully contigged. The contigged sequences were then analyzed using BLAST to validate the DNA sequences from the isolation results by comparing the species similarity between the obtained DNA sequences and those in the NCBI database. Based on BLAST results, *native E. coli* DH0B DNA has similarities with *E. coli* DH10B species with 100% value of query cover and percent identity. BLAST aligned sequence result further analyze using MEGA 11 software to obtain pairwise distance and neighbor-joining phylogenetic tree.

A260/A230	1.811
A260/A280	1.811
Concentration	67.00 ng/µl

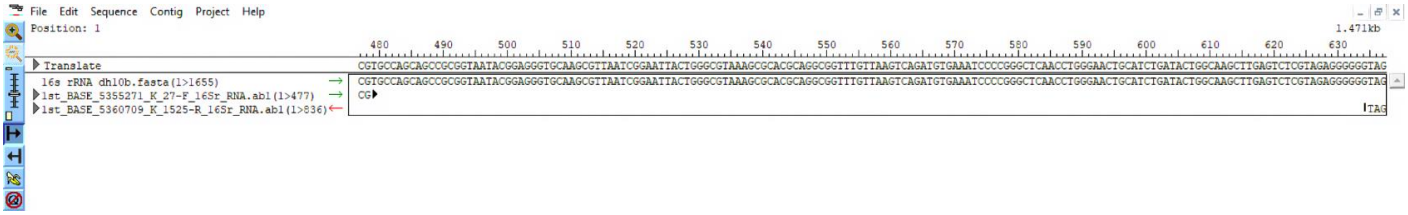
(A)



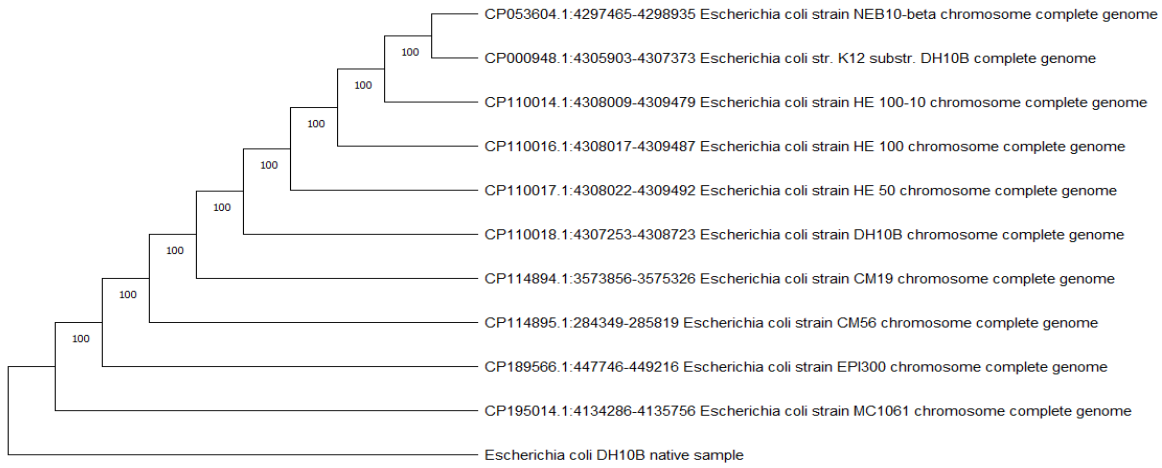
(B)



(C)



(D)



(E)

Figure 2 (A) Quantification result of isolated native *E. coli* DH10B DNA using BioDrop. (B) Electrophoresis results of native E. coli DH10B DNA (M = Lambda DNA, S = Native *E. coli* DH10B DNA.) (C) Visualization of PCR results from native *E. coli* DH10B DNA with 16S rRNA primers (M = 1kb marker, 1 = 16S rRNA sequence amplicon) (D) Contig sequences result (E) phylogenetic tree analysis displaying neighboring relations between native *E. coli* DH10B with the closest 16S rRNA aligned sequence from NCBI. Bootstrapping value was generated from 1000 replicates.

Table 1. BLAST result

Organism	Max score	Total Score	Query Cover	E value	Percent identity	Acc. Length	Accession
***E. coli* MC1061**	2717	18779	100%	0.0	100.00%	4567335	CP195014.1
***E. coli* EPI300**	2717	18779	100%	0.0	100.00%	4691561	CP189566.1
***E. coli* HE 100-10**	2717	18779	100%	0.0	100.00%	4688237	CP110014.1
***E. coli* HE 50**	2717	18779	100%	0.0	100.00%	4688252	CP110017.1
***E. coli* HE 100**	2717	18779	100%	0.0	100.00%	4688244	CP110016.1
***E. coli* NEB10-beta**	2717	18779	100%	0.0	100.00%	4667764	CP053604.1
***E. coli* CM56**	2717	18779	100%	0.0	100.00%	4578061	CP114895.1
***E. coli* CM19**	2717	18779	100%	0.0	100.00%	4591259	CP114894.1
***E. coli* K12 substr DH10B**	2717	18779	100%	0.0	100.00%	4686137	CP000948.1
***E. coli* DH10B**	2717	18779	100%	0.0	100.00%	4687487	CP110018.1

BLAST result shows that native *E. coli* DH10B sample has 100% query cover and percent identity. This means native *E. coli* DH10B 16S rRNA has exact similarities with *E. coli* MC1061, *E. coli* EPI300, *E. coli* CM56, *E. coli* CM19, *E. coli* DH10B, *E. coli* HE 50, *E. coli* HE 100, *E. coli* HE 100-10, *E. coli* NEB10-beta, and *E. coli* K12 substr DH10B.

Table 2 Pairwise comparison and genetic distance

	Sample	***E. coli* MC1061**	***E. coli* EPI300**	***E. coli* CM56**	***E. coli* CM19**	***E. coli* DH10B**	***E. coli* HE 50**	***E. coli* HE 100**	***E. coli* HE 100-10**	***E. coli* NEB10-beta**	***E. coli* K12 substr DH10B**
Sample											
***E. coli* MC1061**	0.0000										
***E. coli* EPI300**	0.0000	0.0000									
***E. coli* CM56**	0.0000	0.0000	0.0000								
***E. coli* CM19**	0.0000	0.0000	0.0000	0.0000							
***E. coli* DH10B**	0.0000	0.0000	0.0000	0.0000	0.0000						
***E. coli* HE 50**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000					
***E. coli* HE 100**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
***E. coli* HE 100-10**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
***E. coli* NEB10-beta**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
***E. coli* K12 substr DH10B**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

Pairwise comparison and genetic distance of native *E. coli* DH10B sample shows 0.000 genetic distance value compared with *E. coli* MC1061, *E. coli* EPI300, *E. coli* CM56, *E. coli* CM19, *E. coli* DH10B, *E. coli* HE 50, *E. coli* HE 100, *E. coli* HE 100-10, *E. coli* NEB10-beta, and *E. coli* K12 substr DH10B. This value shows exact similarity between bacteria compared.

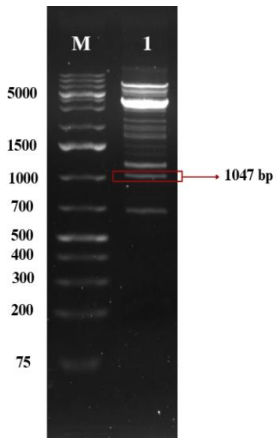
3.2.2 E. coli DH10B *pET28a(+)_AnsB*

E. coli DH10B *pET28a(+)_AnsB* plasmid DNA was measured for concentration and purity using Biodrop. Measurements with 260/280 absorbance ratio showed DNA concentration of 110 ng/µL with a purity of 2.000. *E. coli* DH10B *pET28a(+)_AnsB* DNA then electrophoresed on 1% agarose and visualized using GelDoc. DNA isolation then analyzed by PCR using full-length *AnsB* forward and reverse primers. Electrophoresis result show a target band in 1047 bp. This indicates that the plasmid DNA successfully isolated from the *E. coli* DH10B *pET28a(+)_AnsB* sample encodes the *AnsB* gene from *S. plymuthica* UBCF_13.

A260/A230	2.245
A260/A280	2.000
Concentration	110.0 ng/µl

(A)

(B)



(C)

Figure 3 (A) Quantification results of isolated *E. coli* DH10B *pET28a(+)_AnsB* plasmid DNA using BioDrop. (B) Electrophoresis results of isolated *E. coli* DH10B *pET28a(+)_AnsB* DNA (M = Lambda DNA, S = *E. coli* DH10B *pET28a(+)_AnsB* DNA) (C) Electrophoresis results of *E. coli* DH10B *pET28a(+)_AnsB* plasmid DNA isolate using full length *AnsB* primer. (M= 1kb Marker, 1= *E. coli* DH10B *pET28a(+)_AnsB* plasmid DNA)

3.3 Ammonia standard curve

Absorbance of the ammonia solution for ammonia standard curve was processed using Microsoft Excel. A standard curve equation was obtained with y = 2.6767x and R2 value of 0.9757, as shown in the figure 4.

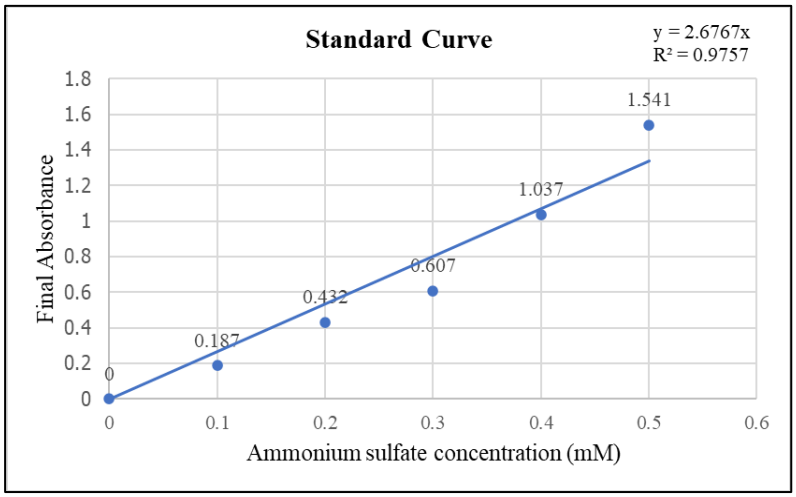


Figure 4. Ammonia standard curve.

3.4 L-asparaginase enzyme activity assay

L-asparaginase II activity assay with direct nesslerization method result in colour change from clear to yellow in incubated solution after Nessler reagent addition. The colour formed from the reaction can be seen in figure 5.

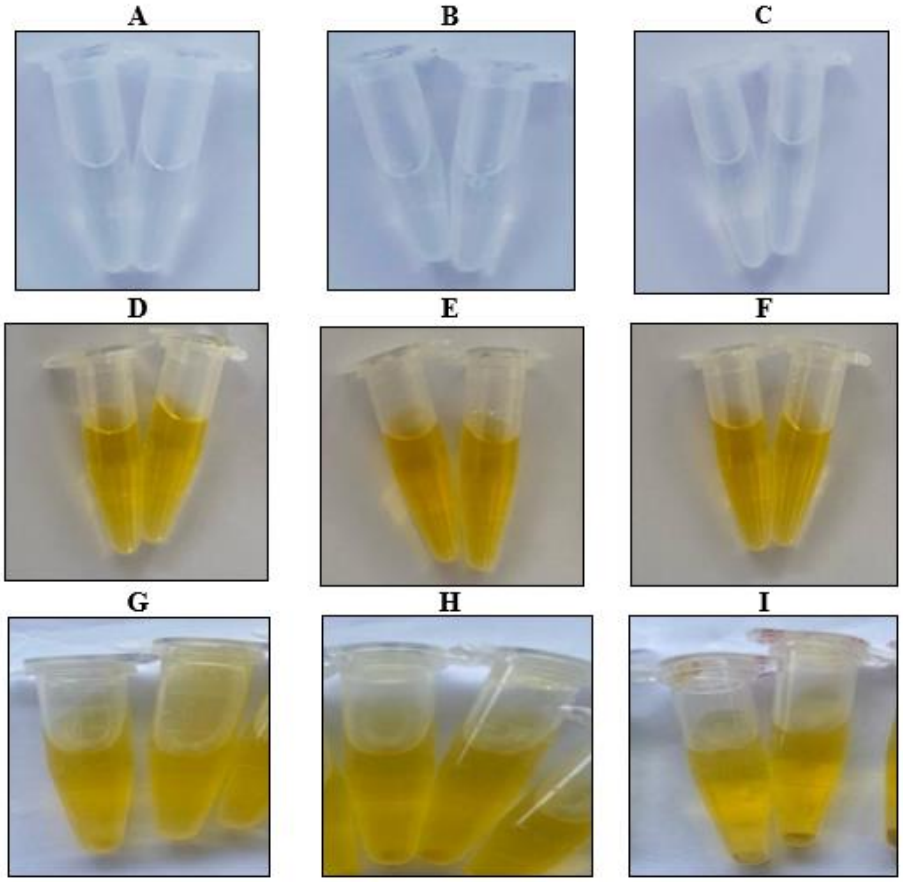


Figure 5 A-C: Sample before added Nessler reagent, D: *native E. coli* DH10B, E: *E. coli* DH10B *pET28a(+)_AnsB* F: *S. plymuthica* UBCF_13, G: *native E. coli* DH10B + IPTG, H: *E. coli* DH10B *pET28a(+)_AnsB* + IPTG, I: *S. plymuthica* UBCF_13 + IPTG.

Sample absorbance measured using a UV-Vis spectrophotometer at a wavelength of 425 nm. Based on L-asparaginase activity assay in sample without IPTG induction, *E. coli* DH10B *pET28a(+)_AnsB* has highest enzyme activity with mean value of 0.297 ± 0.011 U/mL, and followed with *S. plymuthica* UBCF_13 and native *E. coli* DH10B with L-asparaginase activity from each bacteria are 0.296 ± 0.001 U/mL and 0.193 ± 0.009 U/mL.

In sample with IPTG induction, *E. coli* DH10B *pET28a(+)_AnsB* also has the highest activity with mean value of 0.392 ± 0.017 U/mL and followed with native *E. coli* DH10B and *S. plymuthica* UBCF_13 with L-asparaginase activity from each bacteria are 0.356 ± 0.008 U/mL and 0.298 ± 0.012 U/mL. Mean value of L-asparaginase II activity from each bacteria in bar graph can be seen in figure 6.

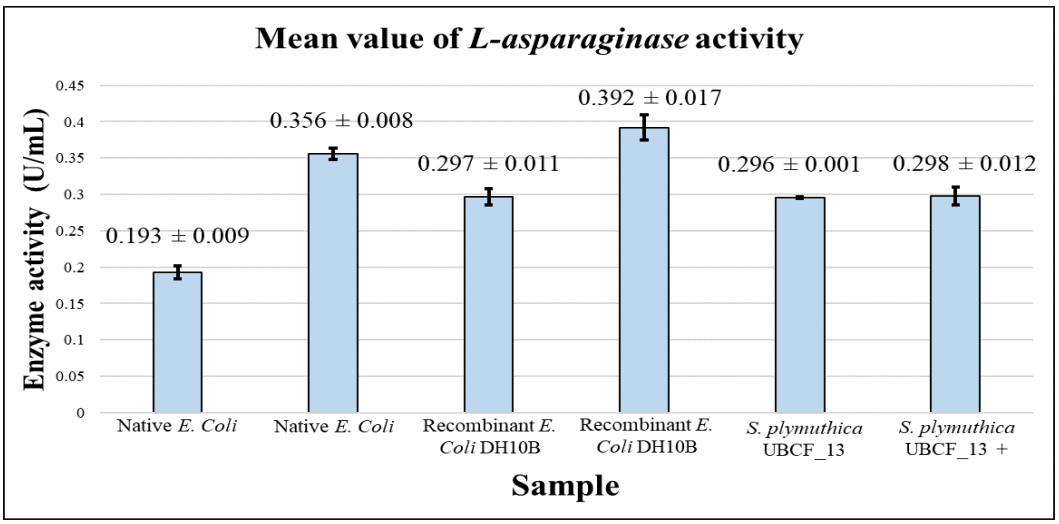


Figure 6 Bar graph of L-asparaginase II enzyme activity mean.

Statistical test was conducted to examine the differences between groups in L-asparaginase II enzyme activity data using the One-Way ANOVA statistical test. Result can be seen in table 3.

Table 3 One-Way ANOVA test of L-asparaginase II enzyme activity

Sample	Mean ± SD	Sig.	Note
E. coli DH10B *native*	0.193 ± 0.009	0.000	Significant difference
E. coli DH10B *native* + IPTG	0.356 ± 0.008		
E. coli DH10B *pET28a(+)_AnsB*	0.297 ± 0.011		
E. coli DH10B *pET28a(+)_AnsB* + IPTG	0.392 ± 0.017		
S. plymuthica UBCF_13	0.296 ± 0.001		
S. plymuthica UBCF_13 + IPTG	0.298 ± 0.012		

Based on the results of the One-Way ANOVA test in Table 3, there are at least two or more groups that have significant differences in L-asparaginase II enzyme activity between groups ($p < 0.05$). The statistical test was continued with the Post Hoc Games Howell test to determine the significant differences between each group. The results of the Post Hoc Games Howell test can be seen in Table 4.

Table 4 Post Hoc Games Howell test of L-asparaginase II enzyme activity

Sample	***Native E. coli* DH10B**	***Native E. coli* DH10B + IPTG**	***E. coli* DH10B *pET28a(+)_AnsB***	***E. coli* DH10B *pET28a(+)_AnsB* + IPTG**	***S. plymuthica* UBCF_13**	***S. plymuthica* UBCF_13 + IPTG**
***Native E. coli* DH10B**	-	0.000*	0.002*	0.002*	0.009*	0.002*
***Native E. coli* DH10B + IPTG**	0.000*	-	0.013*	0.206	0.017*	0.019*
E. coli* DH10B *pET28a(+)_AnsB	0.002*	0.013*	-	0.013*	1.000	1.000
***E. coli* DH10B *pET28a(+)_AnsB* + IPTG**	0.002*	0.206	0.013*	-	0.036*	0.013*
***S. plymuthica* UBCF_13**	0.009*	0.017*	1.000	0.036*	-	0.998

Sample	*Native E. coli* DH10B	*Native E. coli* DH10B + IPTG	*E. coli* DH10B *pET28a(+)_AnsB*	*E. coli* DH10B *pET28a(+)_AnsB* + IPTG	*S. plymuthica* UBCF_13	*S. plymuthica* UBCF_13 + IPTG
***S. plymuthica* UBCF_13 + IPTG**	0.002*	0.019*	1.000	0.013*	0.998	-

Description= *: significant difference ($p < 0.05$)

Based on table 4, there was a significant difference in some between groups. Data shows significant difference in L-asparaginase II enzyme activity of native *E. coli* DH10B with native *E. coli* DH10B + IPTG, *E. coli* DH10B *pET28a(+)_AnsB*, *E. coli* DH10B *pET28a(+)_AnsB* + IPTG, *S. plymuthica* UBCF_13, and *S. plymuthica* UBCF_13 + IPTG. There are also significant difference between native *E. coli* DH10B + IPTG with *E. coli* DH10B *pET28a(+)_AnsB*, *S. plymuthica* UBCF_13, and *S. plymuthica* UBCF_13 + IPTG, between *E. coli* DH10B *pET28a(+)_AnsB* with *E. coli* DH10B *pET28a(+)_AnsB* + IPTG, and lastly between *E. coli* DH10B *pET28a(+)_AnsB* +IPTG with *S. plymuthica* UBCF_13, and *S. plymuthica* UBCF_13 + IPTG.

3.5 SDS-PAGE

Visualization of extracellular and intracellular proteins using SDS-PAGE can be seen in the figure 7.

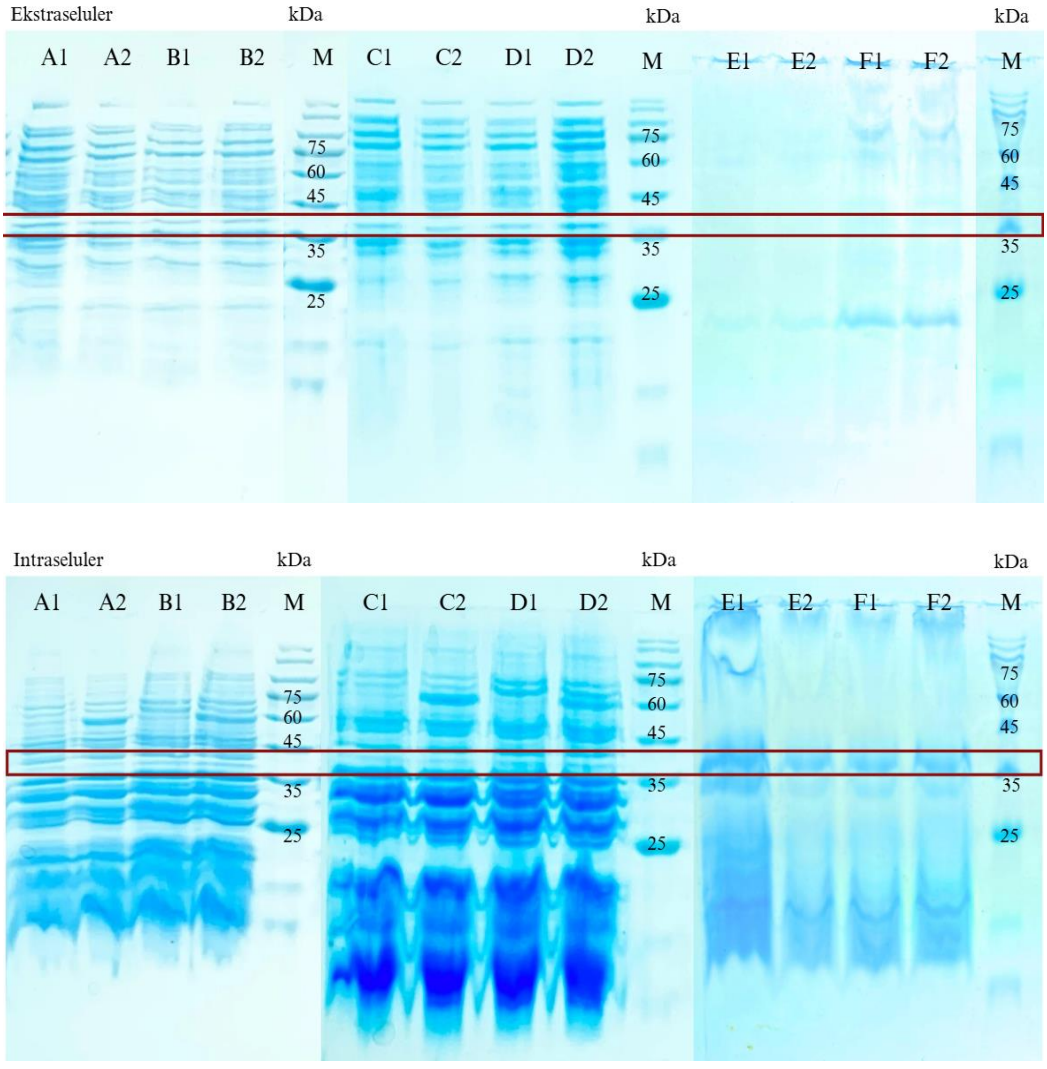


Figure 7 SDS-PAGE results (A= *Native E. coli* DH10B, B= *Native E. coli* DH10B + IPTG, C= *E. coli* DH10B *pET_AnsB*, D= *E. coli* DH10B *pET_AnsB* + IPTG, E= *S. plymuthica* UBCF_13, F=*S. plymuthica* UBCF_13 + IPTG)

SDS-PAGE result shows bands of target proteins corresponding to the estimated molecular weight of the L-asparaginase II enzyme, namely 36.5 kDa for *E. coli* DH10B *pET28a(+)_AnsB* and *S. plymuthica* UBCF_13, and 36.86 kDa in native *E. coli* DH10.

Discussion

This study successfully analyzed the activity of the L-asparaginase II enzyme from native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB*, and *S. plymuthica* UBCF_13. Molecular validation by sequencing the 16S rRNA gene showed that native *E. coli* DH10B was also identical to the *E. coli* DH10B species in the NCBI database, as indicated by 100% percent identity and query coverage. Further analysis using clusterW sequence alignment, pairwise comparison and genetic distance, and phylogenetic tree also support this finding. Validation by PCR using full-length *AnsB* primers showed that *E. coli* DH10B *pET28a(+)_AnsB* encoded L-asparaginase from *S. plymuthica* UBCF_13.

Enzyme activity testing using direct Nesslerization method showed that highest enzyme activity in bacteria induced with IPTG was found in *E. coli* DH10B *pET28a(+)_AnsB*, with an average enzyme activity of 0.392 ± 0.017 U/mL. There was a significant increase in enzyme activity observed in native *E. coli* DH10B and *E. coli* DH10B *pET28a(+)_AnsB*, but not in *S. plymuthica* UBCF after induction with IPTG. This is may occur due to the mechanism of IPTG that binding to the *lacI* repressor (Daber et al., 2007). Native *E. coli* DH10B has *lacI* repressor in the *lac operon*, the *pET28a(+)_AnsB* plasmid is designed with a *lacI* repressor, but *S. plymuthica* UBCF_13 does not have a *lacI* repressor, as supported by further analysis of the *S. plymuthica* UBCF_13 sequence in the NCBI database.

Highest L-asparaginase enzyme activity in bacteria that not induced by IPTG also found in *E. coli* DH10B *pET28a(+)_AnsB*, followed by *S. plymuthica* UBCF_13 and then native *E. coli* DH10B. *E. coli* DH10B *pET28a(+)_AnsB* had the highest activity because it expressed L-asparaginase from two sources, naturally and through the *pET28a(+)_AnsB* plasmid that transformed into this bacteria. The results of the Post Hoc statistical test with Games Howell in Table 4 show that there is a significant difference between the L-asparaginase II enzyme activity of *E. coli* DH10B *pET28a(+)_AnsB* and native *E. coli* DH10B, between native *E. coli* DH10B and *S. plymuthica* UBCF_13. There was no significant difference between *E. coli* DH10B *pET28a(+)_AnsB* and *S. plymuthica* UBCF_13. This may be because production conditions such as media selection, pH, temperature, purification stages, host bacterial strain selection, and optimization of the concentration of the inducer used in this study have not been optimized. These factors can be used as a reference to further increase the production of recombinant L-asparaginase II enzyme from *S. plymuthica* UBCF_13 expressed in *E. coli*.

Based on previous study by Wahab et al. (2023) that characterized the extracellular L-asparaginase enzyme from *Bacillus niacini spp*. found L-asparaginase activity of 3.78 U/mL. The enzyme was produced under optimal conditions using starch as a carbon source, potassium nitrate as a nitrogen source, and the bacteria were incubated at 35°C using M9 medium at pH 8. Enzyme activity was tested using the Nessler method with 1.5 mL of 0.04 M L-asparagine substrate and 0.05 M Tris-HCl buffer at pH 8.6, along with 0.5 mL of the sample. Research by Lashgarian et al. (2020) characterized the L-asparaginase enzyme from endophytic bacteria isolated from the roots of *Glycyrrhiza glabra*, showing enzyme activity of 0.051 IU/mL in the crude extract, which was the supernatant; furthermore, there was an increase in enzyme activity to 0.44 IU/mL after the crude extract was purified using the ammonium sulfate precipitation method. The bacteria were grown on M9 medium at 37 °C with agitation at 100 rpm for 48 hours. Enzyme activity was tested using the Nessler method with 1 mL of supernatant, 2 mL of 0.1 M sodium borate buffer (pH 8.5), and 1 mL of 0.05 M L-asparagine substrate.

A study by Hien-Trang et al. (2020) expressed the recombinant L-asparaginase enzyme from *Erwinia chrysantermi* in *E. coli* BL21 (DE3) using the pET26b and pET28a plasmids, demonstrating the highest

enzyme activity of 123.74 U/mL using an optimized IPTG concentration of 1.03 mM. In this study, bacterial inoculum was grown using Luria Bertani medium with 0.5% dipotassium phosphate at 37°C with agitation at 220 rpm. 1.03 mM IPTG was added after the optimal density reached 0.4–0.6. IPTG induction was performed for 6 hours at 28°C with agitation at 220 rpm. Enzyme activity was tested using the Nessler method. Research by Nadeem et al. (2022) expressed recombinant L-asparaginase from *Pyrococcus abyssi* in E. coli BL21 (DE3) using the pET28a(+) plasmid, demonstrating increased enzyme activity following purification via selective heat denaturation and ion exchange chromatography (IEC). Enzyme activity in the crude extract was 145 U/mL, and after the crude extract was purified, enzyme activity increased to 940 U/mL. In this study, bacterial inoculum was grown in LB medium at 37°C with agitation at 200 rpm. 0.5 mM IPTG was added after the optimal density reached 0.5–0.6, and the culture was continued for 3 hours. Enzyme activity was tested using the Nessler method. This previous research shows the importance of optimum condition of pH, temperature, IPTG induction and enzyme purification to produce highest enzyme activity.

pET28a is one of the plasmids for recombinant protein expression that is often used in *E. coli* host bacteria. This plasmid has a T7 promoter, lac operator, lac repressor, poly-histidine purification tag, and thrombin protease recognition site (Shilling et al., 2020). The T7 promoter can express target genes when recognized by T7 RNA polymerase, an enzyme that can transcribe five times faster than RNA polymerase from *E. coli* (Miao and Kompala, 1992). Research by Einsfeldt et al. (2016) states that there is an increase in the production of L-asparaginase enzyme expressed by *E. coli* BL21 (DE3) on the pET26b and pET28a plasmids when compared to its natural expression from the *Zymomonas mobilis* bacteria.

Based on SDS-PAGE visualization results, thickest extracellular protein band with a molecular weight of 36.5 kDa was found in the IPTG-induced *E. coli* DH10B *pET28a(+)_AnsB*. This indicates that the highest L-asparaginase production was found in these bacteria with IPTG induction. This is consistent with the activity test results, where IPTG-induced *E. coli* DH10B *pET28a(+)_AnsB* bacteria had the highest enzyme activity. The protein band appears thinner when compared to *E. coli* DH10B *pET28a(+)_AnsB* that was not induced with IPTG, but the protein band from these bacteria appears slightly thicker when compared to native *E. coli* DH10B at a molecular weight of 36.86 kDa and *S. plymuthica* UBCF_13 at a molecular weight of 36.5 kDa. But *S. plymuthica* UBCF_13 protein band in the extracellular fraction of is not visible at 36.5 kDa.

Intracellular protein fraction shows slightly thinner protein bands at the target molecular weight of native *E. coli* DH10B and *E. coli* DH10B *pET28a(+)_AnsB* when compared to its extracellular fraction. *S. plymuthica* UBCF_13 have the thickest protein band in the intracellular fraction with a size of 36.5 kDa.

Differences in secretion mechanisms may be one of the factors that make proteins in the intracellular fraction appear thicker when compared to the extracellular fraction in *S. plymuthica* UBCF_13. According to Kim et al. (2015), the *SecB*-dependent secretion pathway allows proteins to pass through the inner membrane and secretion is continued by the terminal branch of the general secretion pathway, which enables extracellular secretion in gram-negative bacteria. *AnsB* is secreted into the periplasm through the *SecB*-dependent pathway, which is one of the type II secretion systems in *E. coli*. Research by Suh et al. (1996) showed different secretion in *S. marcescens*, where proteins are first accumulated in the periplasm before being secreted into the medium. This is different from the mechanism found in other Gram-negative bacteria, where there is no prior accumulation in the periplasm.

Extracellular protein secretion is influenced by several factors, including the presence of a signal peptide in the target gene, bacterial growth conditions, and the secretion system of each bacteria (Jamali et al., 2025; Green and Mecsas, 2016). This limitation might be resolved by utilizing signal peptide engineering, enhancing secretion efficiency, and in silico screening using SignalP, TargetP and TMHMM. Research by

Kumar et al. (2025) utilizing secretory expression using pelB signal sequence of pET-26b, aspartate tags and Tween 80 in medium to enhances the extracellular expression of L-asparaginase II from *Pseudomonas sp*. PCH199 in *E. coli* BL21(DE3). Protein secretion increased from 0.33 to 0.77 mg/ml.

This study used IPTG with fixed concentration at 0.2 mM following method from Zulfa (2024). But optimal IPTG concentration for native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB* and *S. plymuthica* UBCF_13 is not yet been determined and its need to be optimized. Using optimum IPTG concentration for enzyme production is really important because while IPTG is widely used to induce protein expression since more than six decades (Simas et al., 2023). Increased IPTG concentration can also increase L-asparaginase expression, but further increased concentration of IPTG can lead to decrease in expression because of increased protein in insoluble fraction (inclusion bodies) (Abdelrazek et al., 2025)

This study still has its limitations, however additional characterization can be done in the next research by determine optimum pH and temperature, thermostability, pH stability and kinetic parameters measurement such as K_m and V_{max} to obtained the optimal alternative l-asparaginase source for treatment. Enzyme purification also needs to be performed before determine enzyme activity assay to obtain specific activity of this enzyme.

CONCLUSIONS

This study analyzed L-asparaginase enzyme activity of native *E. coli* DH10B, *E. coli* DH10B *pET28a(+)_AnsB*, and *S. plymuthica* UBCF_13, and performed extracellular and intracellular protein profile analyses on each bacteria with and without IPTG treatment. There was a significant increase in activity between native E. coli DH10B and E. coli DH10B pET28a(+)_AnsB, with the activity of each bacteria being 0.193 ± 0.009 U/mL and 0.297 ± 0.011 U/mL, respectively. The production and activity of the L-asparaginase enzyme can still be further improved by optimizing the medium, temperature, pH, and inducer concentration during culture.

ACKNOWLEDGEMENT

We would like to express our gratitude to Universitas Andalas, through the Research and Community Service Institute, for their assistance in providing financial support through the Final Project Recognition scheme, with the number 163/UN16.19/PT.01.01/2025 for the year 2025.

COMPETING INTEREST

All authors declare no conflict of interest.

REFERENCES

Abdelrazek, N. A., Saleh, S. E., Ali, A. E., Aboulwafa, M. M., & Raafat, M. M. (2025) 'Recombinant L-asparaginase from Stenotrophomonas maltophilia: a promising low-immunogenic anticancer agent', *Microbial cell factories*, 25(1), 16. https://doi.org/10.1186/s12934-025-02856-0

Bispo, J. A. B., Pinheiro, P. S., & Kobetz, E. K. (2020) 'Epidemiology and Etiology of Leukemia and Lymphoma', *Cold Spring Harbor perspectives in medicine*, *10*(6), a034819. https://doi.org/10.1101/cshperspect.a034819

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., *et al*. (2024) 'Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries', *CA: a cancer journal for clinicians*, *74*(3), 229–263. https://doi.org/10.3322/caac.21834

Daber, R., Stayrook, S., Rosenberg, A., & Lewis, M. (2007) 'Structural analysis of lac repressor bound to allosteric effectors', *Journal of molecular biology*, 370(4), 609–619. https://doi.org/10.1016/j.jmb.2007.04.028

Durfee, T., Nelson, R., Baldwin, S., Plunkett, G., Burland, V., Mau, B., *et al*. (2008) 'The complete genome sequence of *Escherichia coli* DH10B: insights into

the biology of a laboratory workhorse', *Journal of bacteriology*, *190*(7), 2597–2606. https://doi.org/10.1128/JB.01695-07

Einsfeldt, K., Baptista, I. C., Pereira, J. C., Costa-Amaral, I. C., Costa, E. S., Ribeiro, M. C., *et al*. (2016) 'Recombinant L-Asparaginase from Zymomonas mobilis: A Potential New Antileukemic Agent Produced in Escherichia coli', *PloS one*, 11(6), e0156692. https://doi.org/10.1371/journal.pone.0156692

Fatiah, R. Suliansyah, I. Tjong, D.H. Syukriani, L. Yunita, R. Trivano, R. Azizah, N. Jamsari J. (2021) 'Genome of *Serratia plymuthica* UBCF_13, Insight into diverse unique traits', *F1000Research*. https://doi.org/10.12688/f1000research.54402.1,

Ghaderzadeh, M., Asadi, F., Hosseini, A., Bashash, D., Abolghasemi, H., & Roshanpour, A. (2021) 'Machine learning in detection and classification of leukemia using Smear Blood Images: A systematic review', *Scientific Programming*, *2021*, 1–14. https://doi.org/10.1155/2021/9933481

Green, E. R., & Mecsas, J. (2016) 'Bacterial Secretion Systems: An Overview', *Microbiology spectrum*, *4*(1), 10.1128/microbiolspec.VMBF-0012-2015. https://doi.org/10.1128/microbiolspec.VMBF-0012-2015

Hien Trang, N. T., Thanh Hoang, L., & Tuyen, D. T. (2020) 'Optimization of L-asparaginase production from escherichia coli using response surface methodology', *Vietnam Journal of Biotechnology*, 16(4), 767–775. https://doi.org/10.15625/1811-4989/16/4/10861

Huang, J., Chan, S. C., Ngai, C. H., Lok, V., Zhang, L., Lucero-Prisno, D. E., 3rd, Xu, W., *et al*. (2022) 'Disease Burden, Risk Factors, and Trends of Leukaemia: A Global Analysis', *Frontiers in oncology*, *12*, 904292. https://doi.org/10.3389/fonc.2022.904292

İncir, İ., & Kaplan, Ö. (2024) 'Escherichia coli as a versatile cell factory: Advances and challenges in recombinant protein production', *Protein Expression and Purification*, *219*, 106463. https://doi.org/10.1016/j.pep.2024.106463

Jamali, H., Akrami, F., Layeghkhavidaki, H., & Bouakkaz, S. (2025) 'Bacterial protein secretion systems: Mechanisms, functions, and roles in virulence', *Microbial pathogenesis*, *206*, 107790. https://doi.org/10.1016/j.micpath.2025.107790

Jia, R., Wan, X., Geng, X., Xue, D., Xie, Z., & Chen, C. (2021) 'Microbial L-asparaginase for Application in Acrylamide Mitigation from Food: Current Research Status and Future Perspectives', *Microorganisms*, *9*(8), 1659. https://doi.org/10.3390/microorganisms9081659

Karim, H., Ahmad, A., Asmi, N., Natzir, R., Massi, M. N., Hatta, M., *et al*. (2020) 'Optimization of enzyme activity of L-asparaginase derived from Enterobacter agglomerans SB 221 bacterial symbiont of Brown Algae Sargassum sp.', *Rasayan Journal of Chemistry*, *13*(03), 1571–1579. https://doi.org/10.31788/rjc.2020.1335691

Kim, S. K., Min, W. K., Park, Y. C., & Seo, J. H. (2015) 'Application of repeated aspartate tags to improving extracellular production of Escherichia coli L-asparaginase isozyme II', *Enzyme and microbial technology*, *79-80*, 49–54. https://doi.org/10.1016/j.enzmictec.2015.06.017

Kumar, S., Kumar, V., & Singh, D. (2025) 'Secretory expression and optimization of type II L-asparaginase from Pseudomonas sp. PCH199' *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 1267, 124814. https://doi.org/10.1016/j.jchromb.2025.124814

Lashgarian, H. E., Marzban, A., Mirzaei, S. Z., & Karkhane, M. (2020) 'Isolation and anti-leukemic characterization of extracellular L-asparaginase from endophytic bacterium, brevibacterium sp.. M-R21 isolated Glycyrrhiza glabra root', *Biointerface Research in Applied Chemistry*, 11(2), 9113–9125.

https://doi.org/10.33263/briac112.91139125

Miao, F., & Kompala, D. S. (1992) 'Overexpression of cloned genes using recombinant Escherichia coli regulated by a T7 promoter: I. Batch cultures and kinetic modeling', *Biotechnology and bioengineering*, 40(7), 787–796. https://doi.org/10.1002/bit.260400706

Mohammadian-Hafshejani, A., Farber, I. M., & Kheiri, S. (2024) 'Global incidence and mortality of childhood leukemia and its relationship with the Human Development Index', *PloS one*, *19*(7), e0304354. https://doi.org/10.1371/journal.pone.0304354

Nadeem, M. S., Khan, J. A., Al-Ghamdi, M. A., Khan, M. I., & Zeyadi, M. A. (2022) 'Studies on the recombinant production and anticancer activity of thermostable L- asparaginase I from Pyrococcus abyssi', *Brazilian journal of biology = Revista brasleira de biologia*, 82, e244735. https://doi.org/10.1590/1519-6984.244735

Nemkov, T., D'Alessandro, A., & Reisz, J. A. (2019) 'Metabolic underpinnings of leukemia pathology and treatment', *Cancer reports (Hoboken, N.J.)*, *2*(2), e1139. https://doi.org/10.1002/cnr2.1139

Rohinda. (2019) 'Induksi Ekspresi dengan Ion Logam Mg, Cu, dan IPTG serta Aktivitas Kitinolitik Gen Kitinase Putatif (*Chi_Put*) Asal Bakteri *Serratia plymuthica* UBCF_13/-R_36. Skripsi Fakultas Pertanian Prodi Agroteknologi UNAND.

Romero-Morelos, P., González-Yebra, A. L., Bueno-Rosario, L. J., & González-Yebra, B. (2024) 'Leukemia Types and Subtypes Analysis: Epidemiological Age-Standardized Exploration in the Mexican Bajio Region', *Medicina (Kaunas, Lithuania)*, *60*(5), 731. https://doi.org/10.3390/medicina60050731

Schmidt, M. P., Ivanov, A. V., Coriu, D., & Miron, I. C. (2021) 'L-Asparaginase Toxicity in the Treatment of Children and Adolescents with Acute Lymphoblastic Leukemia', *Journal of clinical medicine*, *10*(19), 4419. https://doi.org/10.3390/jcm10194419

Shafqat, I., Shahzad, S., Yasmin, A., Chaudhry, M. T., Ahmed, S., Javed, A., *et al*. (2023) 'Characterization and applications of glutaminase free L-asparaginase from indigenous Bacillus halotolerans ASN9', *PloS one*, *18*(11), e0288620. https://doi.org/10.1371/journal.pone.0288620

Shilling, P. J., Mirzadeh, K., Cumming, A. J., Widesheim, M., Köck, Z., & Daley, D. O. (2020) 'Improved designs for pET expression plasmids increase protein production yield in Escherichia coli', *Communications biology*, 3(1), 214. https://doi.org/10.1038/s42003-020-0939-8

Shishparenok, A. N., Gladilina, Y. A., & Zhdanov, D. D. (2023) 'Engineering and Expression Strategies for Optimization of L-Asparaginase Development and Production', *International journal of molecular sciences*, *24*(20), 15220. https://doi.org/10.3390/ijms242015220

Simas, R. G., Pessoa Junior, A., & Long, P. F. (2023) 'Mechanistic aspects of IPTG (isopropylthio-β-galactoside) transport across the cytoplasmic membrane of Escherichia coli-a rate limiting step in the induction of recombinant protein expression', *Journal of industrial microbiology & biotechnology*, 50(1), kuad034. https://doi.org/10.1093/jimb/kuad034

Suh, Y., Jin, S., Ball, T. K., & Benedik, M. J. (1996) 'Two-step secretion of the Serratia marcescens extracellular nuclease', *Journal of bacteriology*, *178*(13), 3771–3778. https://doi.org/10.1128/jb.178.13.3771-3778.1996

Wahab, M. R. A., Palaniyandi, T., Wyson, J., Sivaji, A., & Thamada, S. (2024) 'Extracellular L-Asparaginase Synthesis Bacillus niacin Isolation, Optimization, and Characterization from Marine Saltern Sediment Sources', *Avicenna journal of medical biotechnology*, 16(1), 40–48.

https://doi.org/10.18502/ajmb.v16i1.14170

Wriston J. C., Jr (1985) 'Asparaginase', *Methods in enzymology*, *113*, 608–618. https://doi.org/10.1016/s0076-6879(85)13082-x

Zulfa, W. K. (2024) 'Kloning, Ekspresi, dan Purifikasi Protein Rekombinan *L-Asparaginase* dari Isolat Bakteri *Arthrobacter psychrolactophilus*', Skripsi Fakultas SAINS dan Matematika Prodi Biologi UNDIP.