Current Trends in Biotechnology and Pharmacy
Vol. 20(2) 3112-3118 July 2026, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2026.3.32

Optimization, purification and characterization of L-asparaginase from clinical *Acinetobacter baumannii* isolated from hospitals in Ramadi city-Iraq

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Abstract

In the current study,32 isolates belonging to the bacteria *Acinetobacter baumannii* were isolated. It was diagnosed using several diagnostic methods, including microscopic, biochemical, culture and VITIK 2. Its ability to produce the L-asparaginase was also studied, and the optimal conditions for production were studied, where lactose was the best carbon source, casein the best nitrogen source, temperature 37, pH 8, and the incubation period was 24 hours, the best incubation period. The enzyme was also purified by ammonium sulfate precipitation, ion exchange chromatography, and gel filtration chromatography. High enzyme activity (0.85 Unit/ ml) and specific activity (4.25 U/mg. protein) were obtained in 70% ration saturation of ammonium sulphate After elution step in Gel filtration chromatography one peak was obtained and the activity of L-asparaginase was entirely associated with this peak. The L-asparaginase was purified from *A. boumamanni* using Sephadex G-150 as third step of purification with specific activity 13.7 U/ mg.

Key words: *A. boumamanni*, chromatography, L-asparaginase

Introduction

L-asparaginase is an amidohydrolase (EC 3.5.1.1), one of the amidase group enzymes catalyzing L-asparagine to aspartic acid with ammonia(1). this property confers several industrial and therapeutic use (2)**.**Bacteria are the most convenient source for the production of enzyme(3,4).The Major application of L-Asparaginase lies in chemotherapeutics for the treatment of lymphoproliferative and lymphoma diseases and more specifically, Acute Lymphoblastic Leukemia (ALL) which is the most causes of death among children worldwide (5).The anti-proliferative property of L-asparaginase makes it extremely desirable drug against cancer(6).Apart from ALL and lymphoma diseases, L-asparaginase has proven to be effective against various other forms of cancers –ovarian, lung, pancreatic, breast and anti-colon cancer being among them (7). Biological fluids or extracellular environments are sources of asparagine that is essential for cellular protein synthesis and thus for cell proliferation and survival. Moreover, normal cells express the activity of l-asparagine synthetase, which can be upregulated in response to asparagine depletion. In contrast to normal cells, malignant cells lack asparagine synthase activity that disables de novo synthesis of l-asparagine by different cancer cells and makes them susceptible to asparagine deficiency .Recently, there has been renewed interest in metabolic therapies for cancer including amino acid deprivation. l-Asparaginase (l-ASNase) is the basis of contemporary protocols used in the treatment of pediatric acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphomas. Whereas l-ASNase has been used very suc-

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cessfully for over 40 years, the therapy is not free of drawbacks. Recorded side effects include pancreatitis, liver dysfunction, allergic reactions(8). *Acinetobacter baumannii* has been described as an important pathogenic bacterial species responsible for hospital-acquired infections (9,10) that can cause health problems when it is highly resistant to antibiotics (11). Several recent studies have shown that asparaginase enzyme extracted from *Acinetobacter baumannii* may possess different immunomodulatory properties, reduce the likelihood of allergic reactions and be more stable under certain conditions. Therefore, this type of clinical isolation has been highlighted.

Materials and Methods

Methodology

In this study a total of 140 specimens were collected from the burns, wounds and UTI from patients who referred to AL-Ramadi Teaching hospital in Ramadi City during the period from 1/4/2022 to 1/7/2022. These included 50 swabs from burn patients, 50 swabs of wound infections 40 samples from urinary tract infections (UTI) patients for both sexes of different ages. Samples inoculated immediately on blood agar, MacConkey agar and , chocolate agar then incubated at 37°C for overnight aerobically (12) . The isolates were examined for their shape, size, color, pigments, and hemolytic activity. Isolates were diagnosed by morphological, cultural and biochemical characteristic and confirmed by VITIK-2 and molecular confirmation (16S rRNA sequencing). These isolates were cultured in different media with one variable factor to study the optimum requirement of bacteria to get the optimum production of L-Asparaginase enzyme. After enzyme optimization the enzyme was extracted and partial purified by precipitation by Ammonium sulphate and Ion exchange and gel filtration chromatography.

Results and Discussion

In this study, 32 strains was diagnosed as *Acinetobacter baumannii* by Cultural, Microscopic and Biochemical characteristic then confirmed diagnosis by VITIK II and16S rRNA .

Optimization of L-Asparaginase enzyme

Different sources of carbon (Starch, Maltose, Lactose, Sucrose and Glucose) were used to determine the optimum carbon sources for enzyme production by bacteria. Results showed that the optimum source of carbon was Lactose followed by Sucrose and Maltose. As shown in figure.1A. These results agree with (13,14) they found that Lactose is the best carbon source for L-Asparaginase production. It is known that bacteria use first carbon source that they have enzymes to degrade that source, or they produce new or induced hydrolysis enzymes for the purpose of using the carbon source present in the growth medium.

Different nitrogen sources (Casein,-Yeast extract, meet extract, Peptone and Tryptone) the Casein was the optimum nitrogen source for enzyme production as shown in figure .1B. These results agree with (14) and disagree with(15). All studies have shown that a Nitrogen source is the important limiting factor in the L-asparaginase enzyme production (16). The bacterial isolates were incubated in (25, 30, 37 and 42C^{o}) temperature. Results shown that 37C^{o} was the optimum temperature for enzyme production by *Acinetobacter baumannii* as shown in figure .1C. These results agree with (15,17). The increase of temperature led to activate growth of bacterial colonies which led to increase of enzyme production until arrive to optimum degree after that the increase in temperature led to effect on bacterial cells and kill it. Also the isolates were cultured in different PH media (6.0,6.5,7.0,7.5,8.0 and 8.5). The optimum PH was 8 as shown in figure .1D. The pH is a determining factor in the expression of enzymatic activity through change the ionization states of the substrate or amino acid chain (18) they reported that maximal L-Asparaginase activity purified was recorded at 37 °C. The effect of pH on enzyme activity due to the change of the shape and properties of an enzyme and/or the substrate is related to the change in pH and led to prevent the binding of enzyme with substrate. The results of our study show that the

optimum incubation time for L-Asparaginase synthesis by *Acinetobacter baumannii* was 24 hours with (1.0 U/mg protein) as shown in figure .1E. All enzymes synthesis are effected by the time of incubation which is range from 12 hours to a week depending on the microorganisms type, inoculum size, metabolic state of the cell, pH and temperature (18) .

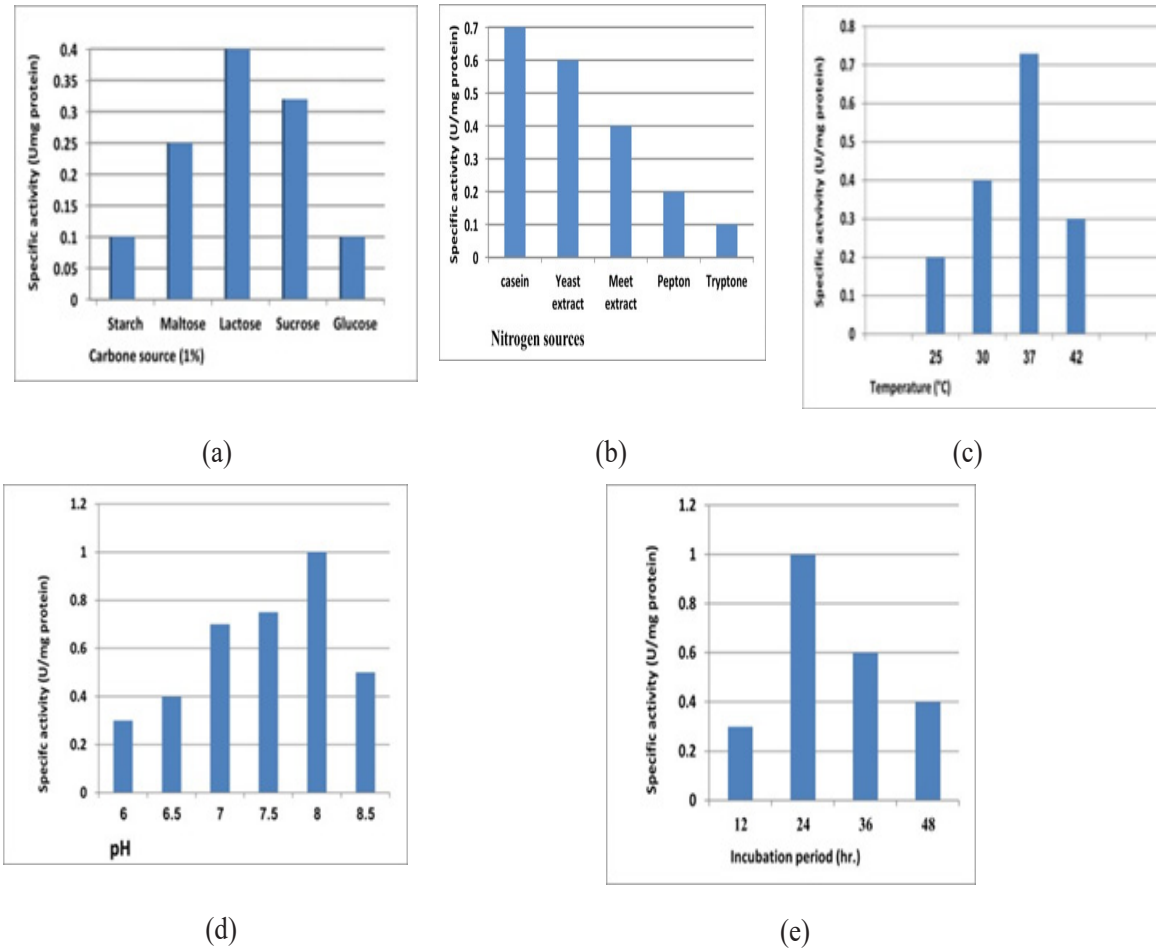

Figure.1 Effect of the **A-** carbon source **B-** nitrogen source **C-**temperature **D-**pH and **E-** incubation period on specific activity

L-Asparaginase purification

The enzyme purified by using three steps of purification (Precipitation with Ammonium sulphate, Ion exchange chromatography and Gel filtration Chromatography). High enzyme activity (0.85 Unit/ ml) and specific activity (4.25 U/mg. protein) were obtained in 70% ration saturation of ammonium sulphate with 4.25 purification fold and 52.8% of yield as shown in table .1. These results agree with (19) found L-Asparaginase enzyme was purified by 60-80% saturation of ammonium sulphate fractional precipitation.

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Table. 1. Partial purification steps for L-asparaginase produced by Acinetobacter baumannii

Purification step	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude enzyme	75	0.3	0.3	1	22.5	1	100
Ammonium sulphate precipitation 70%	14	0.85	0.2	4.25	11.9	4.25	52.8
DEAE-cellulose	21	0.5	0.09	5.5	10.5	5.5	46.6
Sephadex- G150	18	0.55	0.04	13.7	9.9	13.7	44

The results showed that two peaks were obtained from the washing steps and one peak obtained after elution by gradient concentrations of elution buffer (NaCl). The activity of L-asparaginase for all peaks were assayed. The eluted peak was contained most of enzyme activity (0.5 U/ml) at fractions numbered (60 to 70). Enzyme specific activity was measured to be 5.5 U/mg protein with purification fold 5.5 and 46.6% yield. As shown in figure .2.

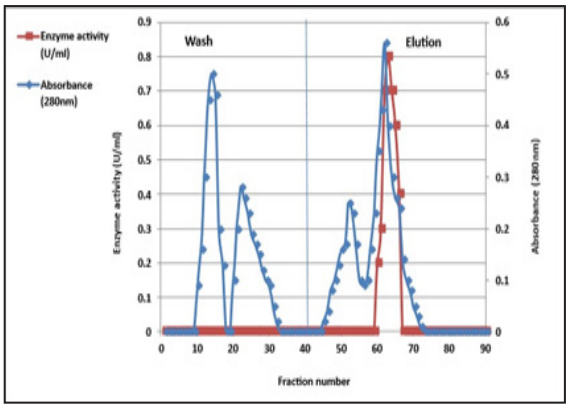


Figure .2. Ion exchange chromatography of L-asparaginase using DEAE-cellulose column (2 × 20 cm)

After elution step in Gel filtration chromatography one peak was obtained and the activity of L-asparaginase was entirely associated with this peak. The L-asparaginase was purified from *A. boumamanni* using Sephadex G-150 as third step of purification with specific activity 13.7 U/ml. protein with 13.7 purification fold. As shown in table .1 and figure .3.

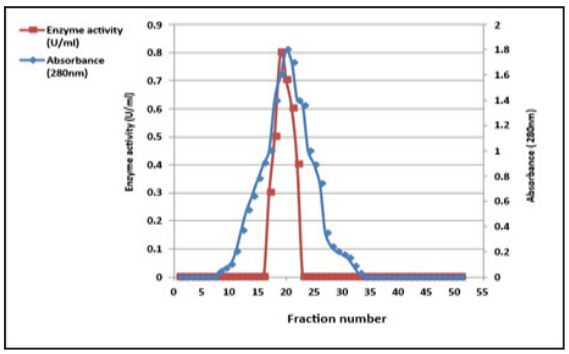


Figure . 3. Gel filtration chromatography of L-asparaginase produced by Acinetobacter baumannii using Sephadex G-150 column (1.5 ×35 cm)

Effect of pH on L-Asparaginase activity and stability

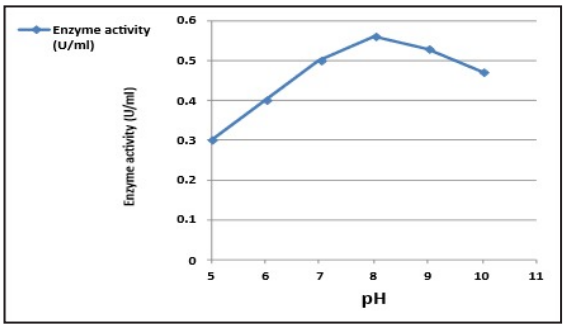
The result of L-Asparaginase characterization by pH showed that optimum activity of enzyme in pH 8 and the stability of enzyme after incubation for 30 min. at 37C around between 7-8. As show in figure. 4. All enzymes have optimum pH to perform catalytic activity. When the pH decreases or rises above the optimum level, it leads to changes in the structure and stereoscopic shape of the amino acids that make up the enzymes, which leads to an impact on the effectiveness and activity of the enzyme

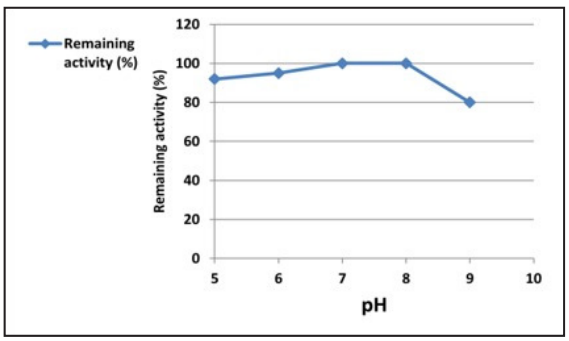
Effect of Temperature on L-Asparaginase activity and stability

As shown in figure.5 activity of L-asparaginase enzyme were at range temperature between (25-45 ˚C), highest activity was observed at 37 ˚C. And enzyme activity remaining 100% of its activity at temperature rage (32-42c) after

incubation at 37c for 30 min. This result agree with (20,21,22) which stated that bacterial L-asparaginase have maximum activity at 37 ° C. The temperature enhancing enzyme activity at the optimum degree, more or less temperature led to effects on enzyme structure and amino acid which is the building unit of all enzyme. low temperature inhibits the dynamic energy for enzyme molecule which means loss of activity, High temperature cause denature of enzyme and lose of its activity.

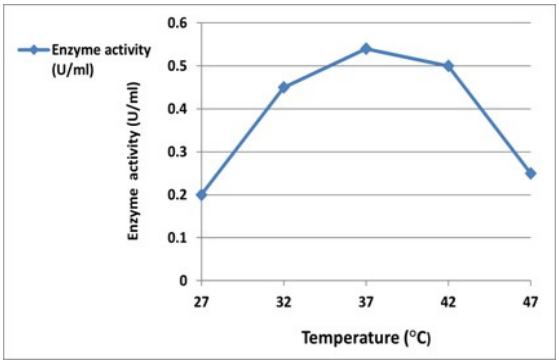
Figure 4. Effect of pH on L-asparaginase activity

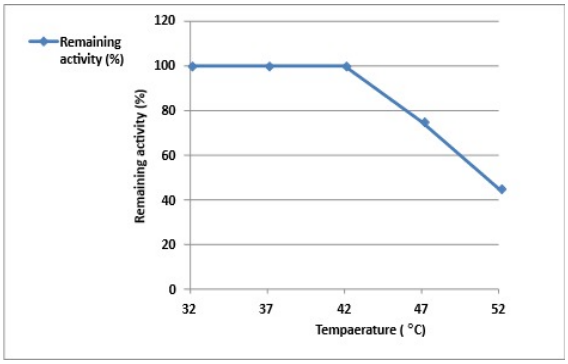




and L-asparaginase stability

Figure. 5 Effect of temperature on L-asparaginase activity and L-asparaginase stability





Conclusion

The optimum conditions for enzyme production were studied, with lactose as the best carbon source, casein as the best nitrogen source, temperature 37, pH 8, and 24 hours as the optimal incubation period. High enzyme activity (0.85 units/ml) and specific activity (4.25 units/mg protein) were obtained.

Conflict of interest

No conflict of interest,

Funding

Self

Author contribution

Ahmed Ghalib Jumaa: investigation, data curation, and methodology, and writing-original draft. Muthanna Hamid Hassan and Ahmed F. Alshanon: conceptualization, supervision, project administration, validation, as well as writing-review and editing.

Ethical Clearance

The Research Ethics Committee of the College of Science, University of Anbar (Ref. No. CSEC/1124/0084) gave an ethical approval. All patients freely consented to provide their specimens for research purposes. All isolates were anonymized before analysis.

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