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RESEARCH ARTICLE

# Partial Purification and Biochemical Characterization of L-lysine α-Oxidase from Clinical Isolates of *Pseudomonas aeruginosa*

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**Abstract**

Background: L-lysine α-oxidase (LO) is an oxidative enzyme with notable therapeutic and industrial potential due to its capacity to degrade lysine and generate cytotoxic by-products such as hydrogen peroxide. *Pseudomonas aeruginosa* is a known microbial source of LO, and its clinical prevalence in nosocomial infections supports its exploration for enzyme production.
Methodology: A total of 130 clinical samples were collected from various infection sites in Baghdad hospitals between October and December 2024. LO production was screened, and the most productive isolate (P6) was selected for purification and characterization. Purification involved ammonium sulfate precipitation, DEAE-cellulose ion-exchange chromatography, and Sephadex G-150 gel filtration. The enzyme was further characterized by SDS-PAGE, and its activity and stability were tested under varying pH, temperature, metal ions, and inhibitor conditions.
Results: A total of 130 clinical samples were collected from different infection sites, including wounds, burns, urine, and sputum. Out of these, 20 isolates (15%) were identified as *Pseudomonas aeruginosa*. The initial identification was based on colony morphology on cetrimide agar, characteristic pigmentation, and Gram staining. Further confirmation was achieved through standard biochemical tests (oxidase positive, growth at 42 °C, etc.) and automated identification using the VITEK 2 system. The specific activity of LO reached 80 U/mg protein after purification, with a purification fold of 10 and a yield of 49.5%. SDS-PAGE estimated the molecular weight of LO to be 54 kDa. Optimal enzymatic activity and stability were observed at pH 7 and 37 °C. Calcium and potassium ions enhanced enzyme activity, while mercury ions showed strong inhibition. The enzyme retained high activity in the presence of EDTA at low concentrations.
Conclusion: The successful isolation and purification of L-lysine α-oxidase from a local *P. aeruginosa* isolate demonstrate its potential for therapeutic and industrial applications. Its optimal activity at physiological conditions and tolerance to certain ions further support its applicability. Future studies should focus on scaling production and testing cytotoxicity against cancer cell lines

## Introduction

The bacterium *Pseudomonas aeruginosa* is a Gram-negative, opportunistic bacterium that causes a variety of nosocomial illnesses. Its high resistance to antimicrobial agents and ability to thrive in nutrient-poor environments contribute to its persistence in clinical settings [1]. This facultative aerobe can grow through aerobic respiration and anaerobically using nitrate or arginine as electron acceptors, though its fermentative capabilities are limited [2]. *P. aeruginosa* flourishes around 37°C and can endure a wide temperature spectrum (4–42°C). It causes infections in wounds, burns, the urinary tract,

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and the respiratory system, including pneumonia and otitis externa. Immunocompromised individuals, such as patients with hematological malignancies or cystic fibrosis, are particularly susceptible. In cystic fibrosis patients, mucoid morphotypes of *P. aeruginosa* are associated with chronic and progressive pulmonary infections [3]. Microorganisms such as bacteria, fungi, and yeast are possible sources of L-lysine α-oxidase, and the use of these microbes for L-lysine α-oxidase production is advantageous because they have simpler growth requirements, relatively low production costs, and easy processing. *Pseudomonas aeruginosa* is the most prevalent opportunistic pathogen in a spectrum of illnesses, ranging from superficial and moderate to severe. Moreover, immunosuppressed individuals, including those with acquired immune deficiency syndrome (AIDS), cancer, and severe burns, have a heightened susceptibility to infections by this bacterium [4]. *P. aeruginosa* infiltrates the host organism by breaching basic physical barriers, such as mucous membranes and skin, while also exploiting deficiencies in immunological defenses [5]. The antibacterial efficacy of L-lysine α-oxidase against the rec mutant of *Bacillus subtilis* was established. The antibacterial efficacy of LO diminished in vitro with the introduction of catalase, the enzyme responsible for the breakdown of hydrogen peroxide. The reduction in antiproliferative and antibacterial activity in the presence of catalase indicates that the degradation of the DNA molecule by hydrogen peroxide is likely the enzyme's principal impact on the cell [6].

L-lysine α-oxidase (LO) is an enzyme that shows promise for antitumor enzyme treatment due to the heightened sensitivity of tumor cells to the lack of growth stimulants, particularly amino acids [7]. L-lysine α-oxidase (LO EC 1.4.3.14), also known as L-lysyl α-oxidase, is a flavoenzyme belonging to the L-amino acid oxidase (LAAO) family. It catalyzes the oxidative deamination of L-amino acids, yielding α-keto acids, hydrogen peroxide, and ammonia [8]. LAAO enzymes are extensively found in nature and often play a role in defense systems against parasites and other dangers. The pharmacological potential of LAAOs to cause apoptosis and inhibit proliferation has been examined in several investigations. These effects are attributable not only to the elimination of amino acids but also to the secondary impact of hydrogen peroxide generated during the enzymatic process, which promotes oxidative stress in these cells [9]. Producing L-lysine α-oxidase from bacteria offers several advantages over fungal sources. Bacteria generally have faster growth rates, leading to quicker enzyme production and shorter cultivation times. They also require simpler and more controllable growth conditions compared to fungi, which often need specific environmental parameters. Additionally, bacteria can produce higher enzyme yields in a shorter period, making the process more cost-effective. Genetic manipulation of bacteria is easier, allowing for enhanced enzyme production and improved activity. Moreover, enzymes derived from bacteria tend to be purer with fewer contaminating substances, simplifying downstream purification processes. These factors collectively make bacterial production a more efficient and economical choice for industrial enzyme manufacturing [10].

## 2. Methodology

### 2.1. Samples Collection

Between October 2024 and December 2024, a total of 130 clinical isolates were obtained from burn, sputum, urinary tract infections (UTI), and wounds from Baghdad Teaching Hospital, Central Laboratories, Ghazi Al-Hariri, Specialized Burn Hospital in Medical City, and Al-Kaam Hospital. The collected specimens were carefully transferred to the laboratory using aseptic techniques for analytical procedures. The study was conducted in accordance with the ethical standards of the Iraqi Ministry of Health. Ethical approval for sample collection was obtained from the relevant institutional review board, and informed consent was obtained from all participants prior to sample collection.

### 2.2. Isolation of *Pseudomonas aeruginosa*

All isolates were cultivated in brain heart infusion (B.H.I.) broth and maintained at 37 °C for 24 hours to facilitate bacterial proliferation. The isolates were inoculated onto non-selective and differential culture media. Lactose non-fermenting colonies were isolated and streaked on MacConkey agar, followed by incubation at 37 °C for 24 hours. The isolates were then selected and re-cultured on new MacConkey agar plates to obtain pure, well-isolated colonies. The isolated colonies were streaked on cetrimide agar to examine the synthesis of fluorescein and pyocyanin pigments. Pure colonies from cetrimide agar were subcultured on blood agar plates and incubated at 37 °C for 24 hours to detect the type of hemolysis produced by the bacteria. Finally, bacterial species were identified and confirmed using the VITEK automated system, which provides rapid and accurate identification based on biochemical profiling.

#### *2.2.1. Aims of the study*

This study aims to isolate clinical strains of *Pseudomonas aeruginosa* capable of producing L-lysine α-oxidase, purify the enzyme using standard biochemical techniques, characterize its biochemical properties, and evaluate its potential for therapeutic and industrial applications.

### 2.3. Screening the Production of L-lysine α-oxidase

The ability of *P. aeruginosa* to produce LO was assessed according to the protocol described by [11] to determine the enzyme activity and specific activity using the following steps**:**

#### *2.3.1. Preparation of cell supernatant*

The bacterium *P. aeruginosa* was cultivated in 100 mL of pre-sterilized minimal salt broth medium and incubated at 37 °C overnight. After incubation, the culture was

centrifuged at 6000 rpm for 30 minutes using a refrigerated centrifuge, and the resulting cell-free supernatant was collected and used as the crude enzyme extract.

#### *2.3.2. Determination of protein concentration*

The protein concentration was determined using the Bradford method [12], following the procedure described by Bradford et al. [13].

#### *2.3.3. Determination of L-lysine α-oxidase activity*

*2.3.3.1 The Nesslerization reaction and the Wriston (1970)*

The enzyme activity was measured using a method that employs the Nessler reaction to quantify the ammonia produced from LO during the enzymatic process. The reaction was initiated by mixing 1.5 mL of 0.01 M L-lysine prepared in 0.05 M buffer, pH 7.6, with 0.5 mL of permeabilized cell suspension from the aqueous phase, followed by incubation for 1 hour at 37 °C. The reaction was stopped by adding 0.15 mL of 1.5 M TCA. The precipitate was removed by centrifuging the reaction mixture at room temperature for 5 minutes at 10,000 rpm. Ammonia in the supernatant was then quantified colorimetrically (A480) by adding 0.25 mL of Nessler reagent to tubes containing 0.5 mL of supernatant and 1.75 mL of $dH_2O$. After mixing, the tubes were allowed to sit at ambient temperature for 10 minutes, and the A480 values were compared to blanks that had been treated with TCA prior to the addition of the extract [14].

#### *2.3.4. Determination of specific activity*

The enzyme's specific activity was determined as follows [14].

$$\text{Specific activity}\left(\frac{\text{U}}{\text{mg}}\text{protein}\right)=\frac{\text{Enzyme activity}\left(\frac{\text{U}}{\text{ml}}\right)}{\text{Protein concentration}\left(\frac{\text{mg}}{\text{ml}}\right)}$$

### 2.4. Purification of L-Lysine α-Oxidase

#### *2.4.1. Ammonium sulphate precipitation*

The first stage in the purification of L-lysine α-oxidase included ammonium sulfate precipitation. The crude enzyme extract underwent progressive ammonium sulfate saturation levels from 20% to 80%. At each saturation stage, the solution was meticulously agitated with a magnetic stirrer for 3 hours at 4 °C to ensure homogeneous mixing. The solution was then centrifuged at 10,000 rpm for 15 minutes at 4 °C. The precipitated proteins were carefully resuspended in an appropriate volume of 0.05 M phosphate buffer (pH 7.0). Following each precipitation phase, protein content and specific enzyme activity were assessed to evaluate the efficacy of the precipitation process and the enrichment of L-lysine α-oxidase.

#### *2.4.2. DEAE-cellulose chromatography*

According to Whitaker and Bernhard (1972) [14], a DEAE-cellulose column was prepared, and 0.05 M potassium phosphate buffer (pH 7) was used to wash the concentrated enzyme, which was then applied to the resin. Fractions were eluted using increasing NaCl concentrations (0.1 to 1 M), and the flow rate was maintained at 30 mL/h. The absorbance of each fraction was measured at 280 nm using a UV-VIS spectrophotometer. The LO activity of each fraction was determined, and fractions exhibiting the desired LO activity were stored at 4 °C for further experiments.

#### *2.4.3. Gel filtration chromatography*

Based on the recommendations of Pharmacia Fine Chemicals, a Sephadex G-150 column was prepared. The column (1.5 × 40 cm) was equilibrated with 0.05 M potassium phosphate buffer (pH 7). Active fractions of L-lysine α-oxidase were applied to the column, and elution was carried out at a flow rate of 30 mL/h. The elution profile was monitored by measuring the absorbance of each fraction at 280 nm, and the target enzyme activity was assessed.

### 2.5. Characterization of L-Lysine α-Oxidase

#### *2.5.1. Evaluation of L-Lysine α-oxidase molecular weight*

SDS-PAGE was used to estimate the molecular weight and assess the purity of partially purified L-lysine α-oxidase based on the Laemmli method. A 7.5% resolving gel and a 5% stacking gel were prepared using standard acrylamide/bis-acrylamide solutions, SDS, buffers, APS, and TEMED. After polymerization, protein samples were loaded onto the gel, and electrophoresis was carried out at 40 V for stacking and 240 V for resolving. Gels were fixed, stained with Coomassie Brilliant Blue R-250, and destained to visualize the protein bands. The relative mobility (Rm) of the enzyme band was calculated by comparing the distance traveled with that of the tracking dye. A standard protein marker was used to create a calibration curve plotting log molecular weight versus Rm, and the molecular weight of L-lysine α-oxidase was determined from this curve.

#### *2.5.3. Effect of different pH values on L-Lysine α-Oxidase activity and stability*

Buffers with an ionic strength of 0.05 M were prepared to evaluate the effect of pH on the activity and stability of the partially purified L-lysine α-oxidase (LO). The buffers included acetate buffer (pH 5 and 6), phosphate buffer (pH 7 and 8), and Tris base buffer (pH 9). To assess the effect of pH on enzyme activity, the activity of the partially purified LO was measured at each pH value. For stability evaluation, the enzyme was incubated with each buffer at 37 °C for 30 minutes. After incubation, the tubes were immediately cooled in an ice bath, and the residual enzyme activity was subsequently determined. Enzyme activity was estimated by measuring the amount of ammonia released from L-lysine using Nessler's reagent. The absorbance was read at 480 nm, and the amount of ammonia produced was used to calculate the enzyme activity [15].

#### *2.5.4. Effect of different temperatures on L-Lysine α-Oxidase activity and stability*

A graph of enzyme activity versus temperature was constructed to determine the optimal temperature for

enzymatic activity. Various temperature ranges (27–47 °C) were used to analyze LO activity. Partially purified LO was pre-incubated for 30 minutes at different temperatures (32, 37, 42, 47, and 52 °C) in a water bath and then rapidly cooled in an ice bath. The remaining activity (%) was subsequently determined. Enzyme activity was measured by quantifying the amount of ammonia released from L-lysine using Nessler's reagent. The absorbance was read at 480 nm, and the amount of ammonia produced was used to calculate the enzyme activity [15].

#### *2.5.5. Determination the effect of various ions and inhibitors on L-Lysine α-Oxidase activity*

A variety of ions and inhibitors ($HgCl_2$, $CaCl_2$, $CuCl_2$, KCL and EDTA) were made at concentrations of 1M and 5M each. For 30 min, these agents were pre-incubated with LO at room temperature. Then, the enzyme's activity and remaining activity were measured [16, 17].

## 3. Results

The study results indicated that 20 out of 130 specimens (15%) tested positive for *P. aeruginosa* infections, while 110 specimens (85%) tested negative. The distribution of *Pseudomonas aeruginosa* isolates showed that the bacterium is associated with various patient infections: wound swab, 37; sputum, 33; urine, 41; and burn swab, 19. A total of 20 clinical isolates were obtained from different sources: 50% from urine samples (10/20), 25% from burn swabs (5/20), 15% from wound infections (3/20), and 10% from sputum samples (2/20), as summarized in Table 1. The morphological characteristics of *Pseudomonas aeruginosa* colonies grown on cetrimide agar included mucoid, smooth colonies with flat sides and a raised center. The colonies exhibited a characteristic fruity odor and displayed pigmentation ranging from yellow to green. Cetrimide agar served as a selective medium that inhibited the growth of other microorganisms, allowing specific isolation and identification of *P. aeruginosa* by promoting the production of distinctive pigments such as pyocyanin and fluorescein. In contrast, bacterial culture on MacConkey agar, which is used to distinguish between lactose-fermenting and non-lactose-fermenting bacteria, revealed the growth of single, small, pale colonies of *P. aeruginosa*, indicating that the bacterium does not ferment lactose. The isolates showed β-hemolysis on blood agar, as represented in Figure 1. The VITEK2 system was utilized to confirm the identification of the isolates according to bioMérieux's guidelines. The results presented in Table 2 indicate that these isolates exhibited 99% similarity to the characteristics of *P. aeruginosa* as identified by the standard GN (Gram-negative) card. In agreement with these findings, previous studies reported a correct identification rate of *P. aeruginosa* at 90.1% using the same VITEK2 system.

### 3.1. Screening the ability of *P. aeruginosa* for L-Lysine α-Oxidase production

L-lysine α-oxidase production from local *P. aeruginosa* isolates was screened quantitatively. Table 3 demonstrates that all isolates were capable of producing the enzyme, with specific activities ranging from 0.23 to 3.0 U/mg protein. *P. aeruginosa* (P6) exhibited the highest specific activity, yielding 3.0 U/mg protein, and was therefore selected for further studies.

### 3.2. Purification of LO from *P. aeruginosa*

The crude extract of LO underwent partial purification using ammonium sulfate precipitation at a saturation

**Table (1):** Number of isolates from each source.

| Sources of samples | Number of isolates |
|---|---|
| Burns | 5 |
| Sputum | 2 |
| Wounds | 3 |
| Urine | 10 |


**Figure (1):** Cultural characteristics of *Pseudomonas aeruginosa* colonies on (1) blood agar, (2) MacConkey agar, and (3) Cetrimide agar.

**Table (2):** Minimum inhibitory concentration for P. aeruginosa isolates using vitek2 identification system.

| Antimicrobial | MIC | Interpretation | Antimicrobial | MIC | Interpretation |
|---|---|---|---|---|---|
| + Temocillin | | R | +Cefotaxime | | R |
| + Amoxicillin | | R | Ceftazidime | >= | 64 R |
| + Ampicillin | | R | +Ceftizoxime | | R |
| + Amoxicillin/Clavulanic Acid | | R | +Ceftriaxone | | R |
| + Ampicillin/Sulbactam | | R | Cefepime | 32 | R |
| + Carbenicillin | | R | Imipenem | >= 16 | R |
| Ticarcillin | 32 | S | Meropenem | 1* | *I |
| Ticarcillin/Clavulanic Acid | 16 | S | Amikacin | 8 | S |
| + Azlocillin | | R | Gentamicin | >= 16 | R |
| Piperacillin | >= 128 | R | Tobramycin | 4 | S |
| + Ceftobiprole | | R | Ciprofloxacin | >= 4 | R |
| + Cefmetazole | | R | + Levofloxacin | R | |
| + Cefoxitin | | R | + Lo1nefloxacin | | R |
| + Cefixime | | R | +Norfloxacin | | R |
| + Cefpodoxime | | R | +Ofloxacin | | R |
| + Cefmenoxim | | R | Colistin | >= 16 | R |

**Table (3):** Specific activity of L-Lysine α-Oxidase produced by local isolates of *P. aeruginosa* incubation at 37°C after 24hrs.

| Isolate No. | LO activity (U/ml) | Protein conc. (mg/ml) | Specific activity (U/mg protein) | Isolate No. | LO activity (U/ml) | Protein conc. (mg/ml) | Specific activity (U/mg protein) |
|---|---|---|---|---|---|---|---|
| P1 | 1 | 0.4 | 2.5 | P11 | 0.3 | 0.39 | 0.76 |
| P2 | 0.1 | 0.6 | 0.16 | P12 | 0.7 | 0.4 | 1.75 |
| P3 | 0.85 | 0.5 | 1.7 | P13 | 0.6 | 0.47 | 1.7 |
| P4 | 0.7 | 0.3 | 2.3 | P14 | 0.9 | 0.45 | 2 |
| P5 | 1 | 0.4 | 2.5 | P15 | 0.1 | 0.42 | 0.23 |
| P6 | 1.2 | 0.4 | 3 | P16 | 0.3 | 0.4 | 0.75 |
| P7 | 0.92 | 0.35 | 2.6 | P17 | 0.4 | 0.4 | 1 |
| P8 | 1 | 0.4 | 2.5 | P18 | 0.95 | 0.46 | 2 |
| P9 | 1 | 0.34 | 2.9 | P19 | 0.6 | 0.44 | 1.3 |
| P10 | 1 | 0.4 | 2.5 | P20 | 0.5 | 0.4 | 1.2 |

level of 70%. According to the data in Table 4, the yield of LO was 65.9%, with a specific activity of 19 U/mg protein and a purification fold of 2.3. Subsequently, DEAE-cellulose ion-exchange chromatography was employed to further enhance the purity of LO. Figure 2A shows that one protein peak emerged during the washing stage, while another peak appeared after elution with gradient concentrations of sodium chloride. All peaks were analyzed to determine LO activity. The results indicated that the eluted fractions (42–78) contained the majority of LO activity, with a specific activity of 42.5 U/mg, a yield of 56.6%, and a purification fold of 5.3 (Table 4). Using Sephadex G-150 gel filtration chromatography, a single peak was observed (Figure 2B), indicating that the specific activity of LO increased to 80 U/mg protein with a 10-fold purification and a yield of 49.5%.

### 3.3. Characterization of LO

#### *3.3.1. Determination of molecular weight of LO*

SDS-PAGE was used to determine the molecular weight of LO. The results shown in Figure 3 demonstrated that the molecular weight of LO was 54 kDa. The migration distance of a protein complexed with the strong cationic detergent sodium dodecyl sulfate (SDS) in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) can be used to determine its apparent molecular weight (MW).

#### *3.3.2. Optimal pH for LO activity and stability*

To evaluate the optimal pH for L-lysine α-oxidase activity and stability, five different pH values (5, 6, 7, 8, and 9) were tested. The results, as presented in Figure 4A, revealed that the highest LO activity (8.5 U/mL) was

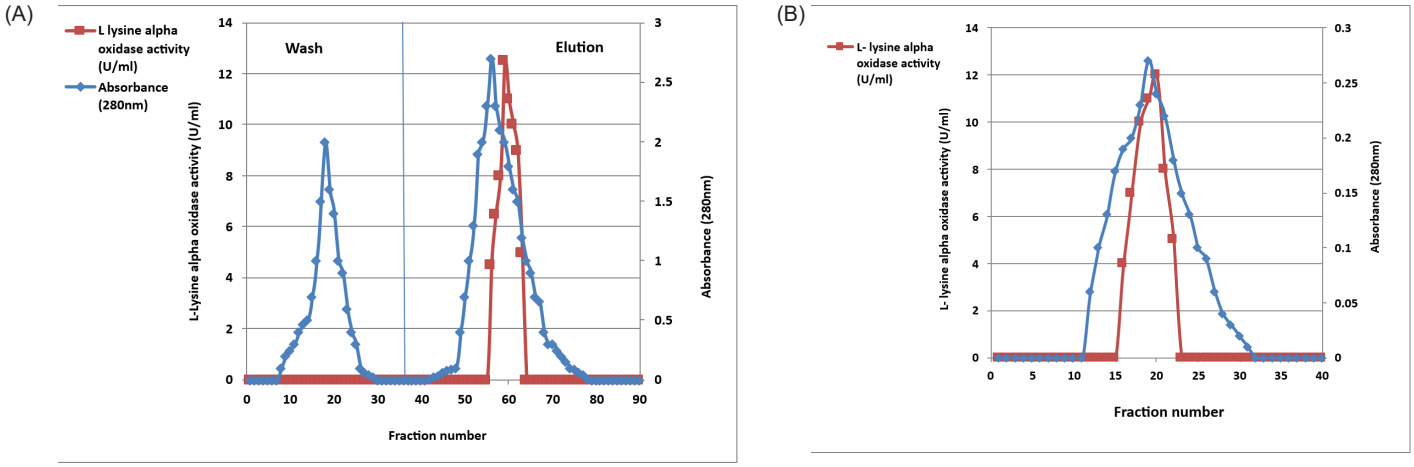


**Figure (2):** (A) Ion exchange chromatography for LO purification from *P. aeruginosa* using DEAE-Cellulose column (1.5 x 25 cm) equilibrated with potassium phosphate buffer (0.05 M; pH 7), eluted with NaCl gradient (0.1-1 M) in flow rate 30 ml/h for each fraction. (B) Gel filtration chromatography for LO purification from *P. aeruginosa* using Sephadex G-150 column (1.5 x 40 cm) equilibrated with potassium phosphate buffer (0.05 M; pH 7), eluted with the same buffer in flow rate 30 ml/h for each fraction.

**Table (4):** Purification steps for LO from P. aeruginosa.

| Purification step | Volume (ml) | Enzyme activity (U/ml) | Protein concentration (mg/ml) | Specific activity (U/mg) | Total activity (U) | Purification (folds) | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude enzyme | 100 | 3.6 | 0.45 | 8 | 360 | 1 | 100 |
| Ammonium sulphate precipitation 70% | 25 | 9.5 | 0.5 ` | 19 | 237.5 | 2.3 | 65.9 |
| DEAE-cellulose | 24 | 8.5 | 0.2 | 42.5 | 204 | 5.3 | 56.6 |
| Sephadex G150 | 21 | 8 | 0.1 | 80 | 178.5 | 10 | 49.5 |

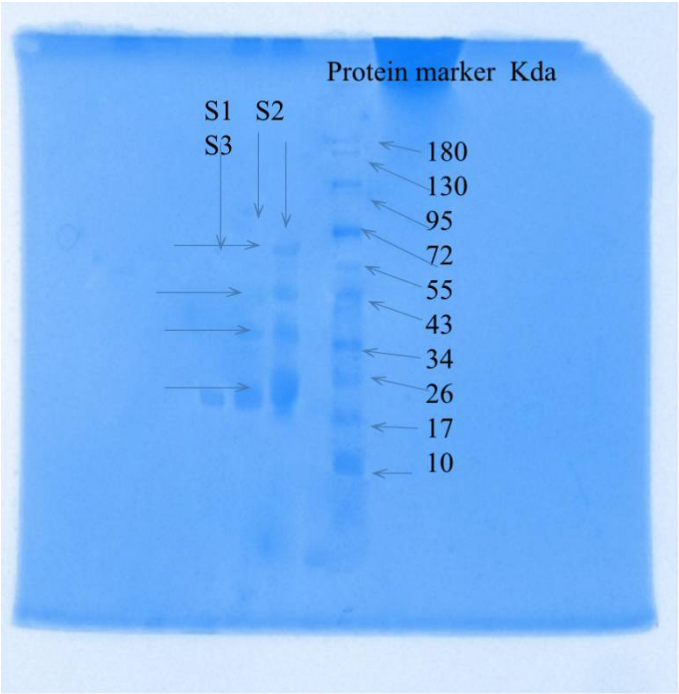


**Figure (3):** Molecular weight determination of *L-lysine α-oxidase* using SDS-PAGE. Lanes: (1) molecular weight ladder, (2) gel filtration protein band, (3) ion-exchange protein band. The enzyme was equilibrated and eluted with potassium phosphate buffer (0.05 M, pH 7) at a flow rate of 30 mL/h for each fraction.

observed at pH 7, indicating that this pH represents the optimal condition for enzyme function. A slightly lower activity (8 U/mL) was observed at pH 6, while enzyme activity dropped significantly at both acidic and alkaline pH values. Specifically, at pH 5 and pH 8, enzyme activity was reduced to 6 U/mL, and at pH 9, the lowest activity was recorded at 4 U/mL. Regarding enzyme stability, as illustrated in Figure 4b, L-lysine α-oxidase exhibited high stability within the pH range of 6 to 7, with residual activity reaching 95% and 100%, respectively. A moderate decline in stability was observed at pH 8 (70%), and further decreases occurred at more extreme pH values, with only 80% residual activity at pH 5 and 60% at pH 9. These results suggest that the enzyme maintains both high activity and stability under near-neutral conditions, with noticeable loss in performance under acidic or strongly alkaline conditions.

### *3.3.3. Optimum temperature for LO activity and stability*

To determine the optimal temperature for L-lysine α-oxidase (LO) activity and stability, the enzyme was evaluated at different incubation temperatures (27 °C, 32 °C, 37 °C, 42 °C, and 47 °C). As shown in Figure 5A, the enzyme exhibited increasing activity with rising temperatures up to 37 °C, where the highest activity of 8.5 U/mL was recorded, indicating this as the optimal temperature for LO function. Slightly lower activities were detected at

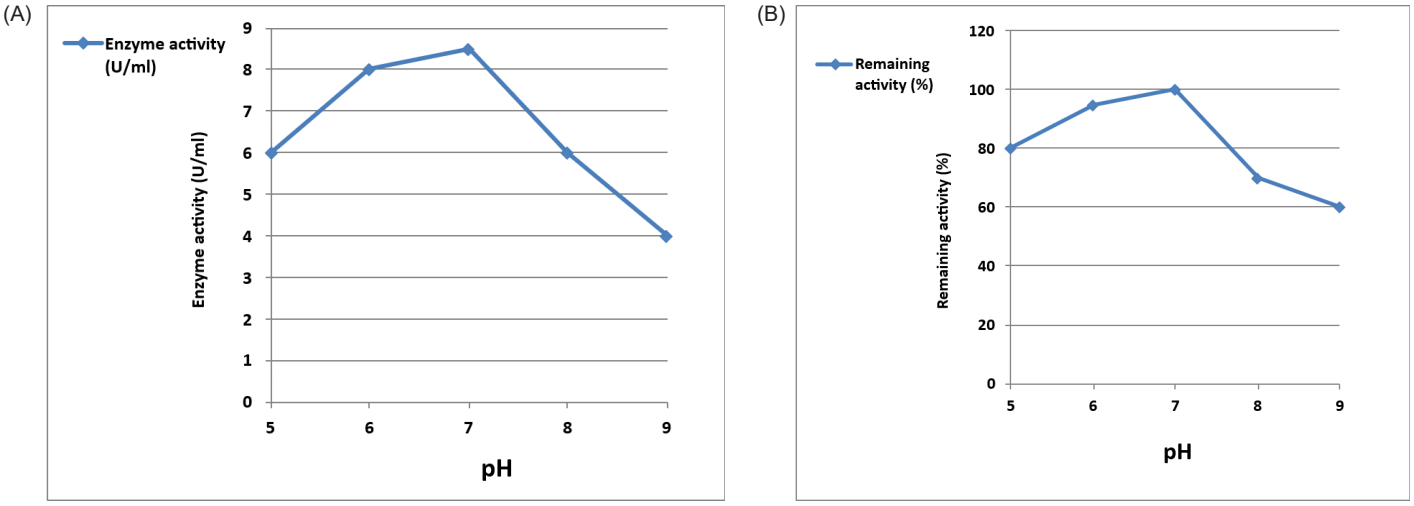


**Figure (4):** Effect of different pH values on (A) activity and (B) stability of partial purified LO produced by P. aeruginosa.

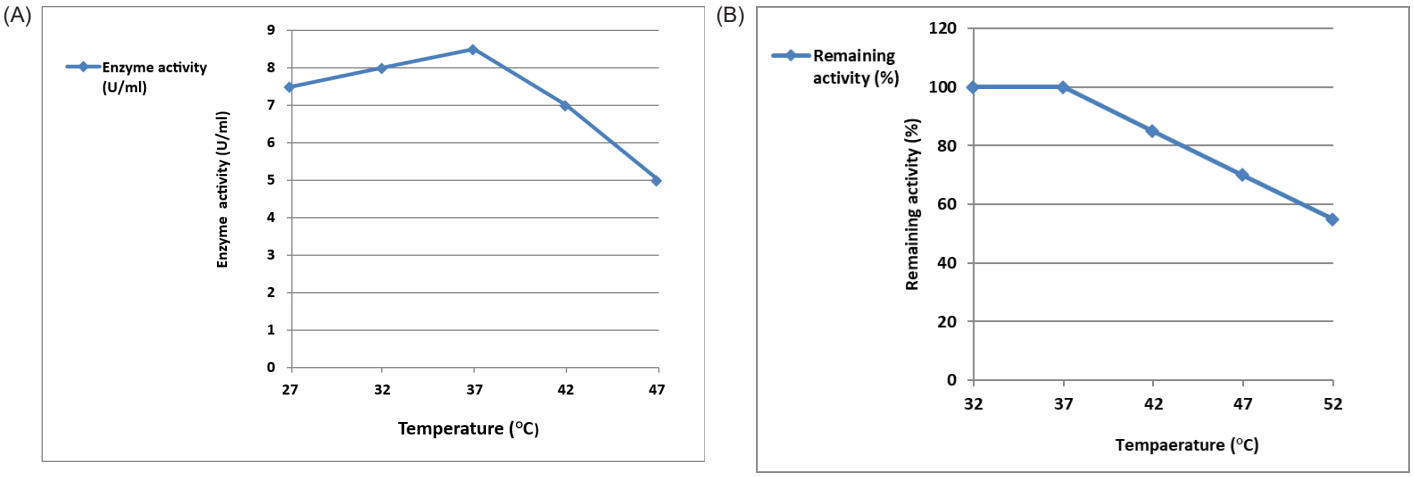


**Figure (5):** Effect of different temperature values on (A) activity and (B) stability of partial purified LO produced by *P. aeruginosa*.

32 °C (8 U/mL) and 27 °C (7.5 U/mL), suggesting that the enzyme retains good activity under moderately warm conditions. However, activity decreased notably at higher temperatures, dropping to 7 U/mL at 42 °C and reaching only 5 U/mL at 47 °C. Regarding enzyme stability, Figure 5B illustrates that LO retained full stability (100%) at both 32 °C and 37 °C, reinforcing that these are ideal temperatures for its use in biochemical processes. Stability began to decline beyond 37 °C, with 85% residual activity at 42 °C and a further drop to 70% at 47 °C. At 52 °C, the enzyme retained just 55% of its initial activity, indicating thermal denaturation at elevated temperatures.

**Table (5):** ions and inhibitors on LO stability.

| Reagent | Conc. (mM) | Remaining activity (%) | Conc. (mM) | Remaining activity (%) |
|---|---|---|---|---|
| Control (Enzyme) | - | 100 | | 100 |
| CaCl2 | 1 | 120 | 5 | 130 |
| KCl | 1 | 110 | 5 | 110 |
| CuCl2 | 1 | 100 | 5 | 100 |
| HgCl2 | 1 | 75 | 5 | 65 |
| EDTA | 1 | 100 | 5 | 90 |

### *3.3.4. Effects of ions and inhibitors on LO stability*

The impact of various metal ions and chemical reagents on the catalytic activity of L-lysine α-oxidase (LO) was assessed at two concentrations (1 mM and 5 mM), and the results are presented in Table 5. The findings revealed that $CaCl_2$ significantly enhanced the enzyme's activity, with residual activity increasing to 120% and 130% at 1 mM and 5 mM, respectively, suggesting that calcium ions may support the conformational stability of the enzyme's active site. Similarly, KCl exhibited a mild activation effect, with enzyme activity remaining at 110% across both concentrations, indicating a positive yet moderate ionic influence without disrupting the enzyme's structural integrity. In the case of $CuCl_2$, no significant

change in activity was observed, maintaining 100% activity at both concentrations, suggesting that copper ions at these levels neither activate nor inhibit the enzyme, possibly due to a lack of interaction with key functional groups. Conversely, $HgCl_2$ showed a strong inhibitory effect, reducing the enzyme's residual activity to 75% at 1 mM and 65% at 5 mM, likely due to mercury binding to thiol or other functional groups essential for enzymatic activity, resulting in conformational changes or direct interference with the catalytic site. Finally, EDTA, a metal-chelating agent, preserved full enzyme activity at 1 mM (100%) but caused a slight decrease to 90% at 5 mM, indicating that EDTA may chelate trace metal cofactors critical for LO activity at higher concentrations, mildly affecting enzymatic performance.

## 4. Discussion

In the current study, *P. aeruginosa* isolation and identification were performed based on morphological features, microscopic examination, biochemical tests, and the VITEK 2 system, which is specific for identifying *P. aeruginosa*. The results demonstrated that 20 isolates (15%) were obtained from patients presenting with various clinical manifestations, indicating that *P. aeruginosa* is associated with a wide range of infections. These findings are consistent with previous reports. For instance, Kadhim and Ali (2014) reported 60 *P. aeruginosa* isolates from hospitals in Baghdad, with sources including burns (36.66%), wounds (30%), sputum (15%), ear swabs (5%), urine (3.3%), and blood (10%). Similarly, a study conducted in Baquba, Diyala Province, identified 15 *P. aeruginosa* isolates (16.85%) out of 89 clinical samples collected from wounds, burns, ear swabs, and urine [18]. Furthermore, research from South Africa [19] also supports the prevalence of *P. aeruginosa* in diverse clinical samples.

The VITEK 2 system, when applied directly to positive aerobic blood culture broths containing a single Gram-negative bacillus, yielded accurate species-level identification in approximately 93% of cases, with no false identifications reported. A minority of isolates (around 7%) remained unidentified or were poorly discriminated. This direct approach showed slightly lower performance compared to conventional identification from pure cultures [20]. L-lysine α-oxidase is an oxidative enzyme that plays a key role in lysine catabolism and is found in a wide variety of organisms, ranging from microorganisms to higher eukaryotes. Due to its unique catalytic activity, it has attracted significant attention for potential applications in industrial and clinical settings, including cancer therapy and the production of bioactive compounds [7]. Fungi are among the most abundant sources of L-lysine α-oxidase. The enzyme has been characterized from several fungal species, which typically produce L-lysine α-oxidase as part of their metabolic processes. Fungal sources are particularly valuable due to their high yield and efficient enzyme production [21]. While fungi are considered primary sources of L-lysine α-oxidase, certain bacterial species, such as *Pseudomonas aeruginosa*, have also been reported to produce this enzyme. The use of bacterial isolates presents several advantages, including faster growth, ease of genetic manipulation, and potential production of unique enzyme variants. Therefore, the current study aimed to investigate local clinical *P. aeruginosa* isolates as a novel bacterial source of L-lysine α-oxidase**.**

Only a few of the many advantages provided by DEAE-cellulose resin include charge-difference-based separation, simplicity, high capacity, easy handling, strong resolving power, and excellent separation [22]. In agreement with these findings, various studies have used DEAE-cellulose ion-exchange chromatography and gel filtration chromatography to purify LO from different organisms. For instance, Japanese researchers extracted the homogeneous enzyme with an 8% yield and a specific activity of 66 U/mg from the wheat bran fungal culture of *Trichoderma viride* Y 2442 using an eight-step purification procedure. Another purification procedure facilitated enzyme separation with a yield of 56% and a specific activity of 31.5 U/mg by affinity chromatography [23]. German researchers developed an efficient method for purifying extracellular LO from the fungus *Trichoderma viride* i4, resulting in a 60% yield with a specific activity of 90 U/mg protein and a degree of purification of 300 [24]. The SDS-PAGE approach, developed in 1969, is still widely used due to its simplicity. The molecular mass of LO (115 kDa) was determined by gel filtration on the Toyo Pearl HW-55 column [25]. **Pseudomonas aeruginosa**-derived L-lysine α-oxidase (LO) exhibits optimal enzymatic activity at near-neutral pH, peaking at pH 7 (8.5 U/mL). This observation suggests that LO functions effectively under physiological conditions, making it a promising candidate for applications in biomedicine, including therapeutic enzyme development and antimicrobial approaches. The enzyme also maintained high stability at this pH, retaining up to 100% of its activity, which further supports its suitability for biological environments.

These findings are consistent with previous studies. For instance, Lukasheva et al. (2021) reported that fungal LO enzymes demonstrated maximum activity in the pH range of 6–7, confirming the preference of LO for mildly neutral environments [26]. Similarly, Kusakabe et al. (1980) showed that LO extracted from **Trichoderma viride** retained more than 90% of its activity between pH 6.0 and 7.5 [27]. The decline in activity observed at more acidic or alkaline pH levels in the current study may be attributed to changes in the ionization state of key amino acid residues at the enzyme's active site, as explained by Jang et al. (2020) [28]. Regarding temperature, LO showed its highest activity at 37 °C, close to human body temperature, and retained complete stability at both 32 °C and 37 °C. However, a gradual loss in stability was noted at higher temperatures, with only 55% activity retained at 52 °C, likely due to thermal denaturation. These results are supported by Costa and Silva (2022), who observed a similar thermal profile in LO derived from **Fusarium oxysporum** [29]. The impact of metal

ions varies depending on the type of metalloenzyme, as each enzyme exhibits specificity for the metal present in its native form. No explicit inhibitor for LO was identified. L-lysine (20 mM) does not impede the enzyme's activity. Thiol reagents (reduced glutathione, N-ethylmaleimide), ethylenediaminetetraacetic acid (EDTA), cyanide, and azide do not affect its action. Potassium chloride, barium chloride, calcium chloride, lithium sulfate, and magnesium sulfate marginally enhance its efficacy by up to 6%. In contrast, heavy metal ions ($ZnSO_4$, $CoSO_4$, $CuCl_2$, $HgCl_2$) and p-chloromercuribenzoate inhibit or completely inactivate the enzyme. All compounds were tested at a concentration of 10 mM, except for p-chloromercuribenzoate, which was added at 0.05 mL of a saturated solution per mL of reaction mixture. The effect of other substrates or substrate analogues on L-lysine oxidation was also examined. At concentrations below the saturation point for L-lysine, all substrate analogues exhibiting relative activity over 2%, including tryptophan, decreased the turnover rate of L-lysine by 5–54% when present at concentrations substantially higher than L-lysine (L-lysine: 0.2 mM; analogues: 20 mM) [30, 31].

## Conclusions

In this study, L-lysine α-oxidase was successfully isolated and partially purified from a clinical *Pseudomonas aeruginosa* isolate. The enzyme exhibited notable activity under physiological pH and temperature, indicating potential biological relevance. It also demonstrated moderate tolerance to certain metal ions, suggesting a degree of stability under variable conditions. SDS-PAGE analysis estimated its molecular weight to be approximately 54 kDa.

These findings highlight the potential of *Pseudomonas aeruginosa* as an alternative microbial source of L-lysine α-oxidase. However, the enzyme was only partially purified, and no functional assays were conducted in the current study.

## Author Contributions

MAA, AAH: Conceptualization; MAA: Data curation; MAA: Formal analysis; AAH Funding acquisition; MAA: Investigation, fMAA, AAH: Methodology; AAH: Project administration; AAH: Resources; AA: Software; MAA, AAH: Validation; MAA; Visualization; MAA: Writing-original draft; AAH: Writing-review & editing.

## Declarations

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### Conflicts of Interest

The authors declare no conflict of interest with respect to research, authorship and/or publication of this article. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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