AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 08, December 2022

# PURIFICATION AND CHARACTERIZATION OF ARGINASE PRODUCED BY PSEUDOMONAS AERUGINOSA

**Nour Abdulateef**
Ph.D Student, Al-Nahrain University, College of Biotechnology.

**Nedhaal S. Zbar**
Assist. Professor, Al-Nahrain University, College of Biotechnology.

**Dr. Ruqaya M. Ibrahim**
Assist. Professor, Al-Nahrain University, College of Biotechnology.

***Abstract:***

Arginase enzyme produced and purified from *Pseudomonas aeruginosa* by four steps, ammonium sulphate, dialysis, ion exchange and gel filtration and purified L-arginase showed the maximum activity at pH 8.0 in the presence of $Mn_2$+ ions and the enzyme activity was increased significantly by the addition of $ZnSO_4$, $MgCl_2$, and $MnCl_2$ ions separately in the enzymatic reaction mixture, and inhibited potently by chemicals such as ($CuCl_2$ and EDTA).

**Key words: arginase, Purification and protein.**

## *Introduction*

*Pseudomonas aeruginosa* freely living and distributed in nature in many different areas such as plants, soil, water and other moist locations. One of the most common causes of hospital-acquired urinary tract infections (UTIs), is *Pseudomonas aeruginosa* accounting for about 12 % of all hospital acquired infections (Kenneth, 2015).

Enzymes an important biocatalyst that play role in metabolic and biochemical reactions. Furthermore, the microbial enzymes have payment more attentiveness cause of activity and stability in nature than enzymes from plant and animal (Nigam, 2013; Anbu *et al.,* 2017).

L-Arginase (EC3.5.3.1) is thought to have appeared firstly in bacteria, but it has persisted through evolution and is found in yeasts, plants, invertebrates, and vertebrates. The L-arginine catalyzes a conversion of arginine to L-ornithine and urea, while consumption water molecule, as the last step in the urea cycle (Husain *et al.,* 2017). The main purpose of this study was to produce purified L-Arginase as one the medically important enzyme from locally isolated *pseudomonas aeruginosa*

## *Materials and methods*

### *Strain and growth conditions*

*Peudomonas aeruginosa* was used as the source of L-arginase. Cultures were maintained on nutrient medium. The production medium for the enzyme prepared according to (Zhang *et al*., 2013) contained 6 gm $Na_2HPO_4 \cdot 2H_2O$, 3gm $KH_2PO_4$ , 0.5gm NaCl , 2gm L-arginine , 2ml of 1 M

$MgSO_4 \cdot 7H_2O$ , 1ml of 0.1 M $CaCl_2 \cdot 2H_2O$, 10 gm Glucose, 0.009 % phenol red and 2% Agar (pH7). Cultivation was done at 37°C for 24 h in 100 ml of medium in 500-ml Erlenmeyer flasks on a rotary shaker. Cells were collected by centrifugation at 10,000X for 10 min at 4°C.

***Protein assay***

Protein was assayed by the method of (Bradford, 1979. Bovine serum albumin was used as a standard.

***Enzyme assay*** **(**Nadaf *et al*., 2019)

L-arginase activity was determined based on the amount of urea released in the reaction. Urea on heating reacts with α-isonitrosopropiophenone (Sigma-Aldrich, India) in the presence of ethanol produces a pink color which was estimated colorimetrically (Archibald *et al*., 2021). The reaction mixture consisted of 0.2 ml of glycine buffer (pH 9.0), 0.5 ml of an enzyme, and 0.1 ml of MnCl2. L-arginase was activated by incubating at 37 ºC for 10 min. L-arginine hydrolysis was achieved by incubating the activated arginase with 0.1 ml of L-arginine at 37ºC for 30 min 1 ml of perchloric acid was added to arrest the reaction. The urea liberated was estimated by addition of 0.1 ml of 4% α- isonitrosopriopophenone at 540 nm.

Enzyme activity (U/ml) = µmoles of urea released /Time of enzyme action × Volume of the enzyme (ml)

***Purification of arginase***

***Step 1. Precipitation of enzyme by ammonium sulfate:*** solid ammonium sulfate was gradually added in different saturation ratios (20 -85%) to 100 ml of crude enzyme at 4 Cº. The component was mixed gently on magnetic stirrer. Then it was centrifuged at 6,000 rpm for 20 min at cooled centrifuge, the supernatant was discarded and the precipitate was dissolved in a suitable volume potassium phosphate buffer. The activity of the enzyme and protein concentration were measured.

***Step2. Dialysis***: The enzyme solution was dialyzed after precipitation with ammonium sulfate against distilled water for 24 h under cooling conditions (4Cº) with stirring and changing the D.W for four times, then the enzyme activity, protein concentration, specific activity was measured

***Step3. DEAE-Cellulose column chromatography***: The dialyzed solution was put on a 2×20cm column of DEAE-cellulose equilibrated with buffer. The column was thoroughly washed with phosphate buffer while attached proteins were stepwise eluted with gradual concentrations of sodium chloride (0.1–1 M). Arginase activity was determined in each fraction. Fractions presenting arginase activity were pooled and kept for further steps of purification.

***Step4. Gel filtration****:* The enzyme preparation obtained in Step 3 was further purified by gel filtration using Sepharose equilibrated with 50 mM potassium phosphate buffer. Fractions containing arginase activity were combined, concentrated, and kept at 4°C.

***Characterization of enzyme***

***Effect of pH on arginase activity***

Purified enzyme was added to 20mM/ml of substrate solutions prepared at different pH values ranging from (5-10) including acetate buffer PH (4, 4.5, 5), potassium phosphate buffer PH (6, 6.5, 7, 7.5), and Tris-HCl buffer PH (8, 8.5, 9).The enzyme activity was assayed.

***Effect pH for purified arginase stability***

The purified enzyme was mixed with buffer solutions at different pH values ranging between 5 and 10 and incubated at 37Cº for 30 min. Then directly cooled in ice bath. The enzyme activity was measured and the remaining activity was calculated.

***Effect of temperature on arginase activity***

Arginase activity was determined after incubation of the purified enzyme with the substrate at different temperatures (30, 35, 40, 45, 50 ºC), and the reaction mixture then the enzyme activity was measured.

***Effect of temperature for arginase stability***

The Purified enzyme was incubated at different temperatures (30-80ºC) for 30 min, then immediately placed in an ice bath. Enzyme activity was assayed in each temperature .The remaining activity (%) for arginase was calculated and plotted against the temperature (ºC).

***Determination the effect of various ions and inhibitors on arginase activity***

The enzyme was incubated with an equal volume of each metal ions ($CuCl_2$, $ZnSO_4$, $MgCl_2$, $MnCl_2$, and EDTA) at a concentration of 10mM/mL at 37 ºC for 30 minutes. The enzyme activity was assayed for each treatment. The control was the enzyme solution without any of these compounds. The remaining activity was assayed for each treatment.

***Results***

***Enzyme purification***

The purification of arginase from *ps.aeruginosa* was done as described in Materials and Methods. The enzyme was extracted from the production medium by centrifugation and arginase was partially purified by ammonium sulfate precipitation and successive chromatographies using DEAE-cellulose and then purified by gel filtration. Rresults indicated in figure (1) showed that there is one peak of protein appeared in washing step, while there are two protein peaks appeared after elution by gradient concentration of sodium chloride. All those protein peaks were detected by measuring the absorbance at 280 nm of each elution fraction.

The three protein peaks were assayed to detected arginase activity. Result indicated in figure (1) showed that the first peak of eluted protein, eluted in fraction number 40 to 50, when arginase activity reached 9.5 U/ml. Those fractions were pooled and concentrated than protein concentration was measured.

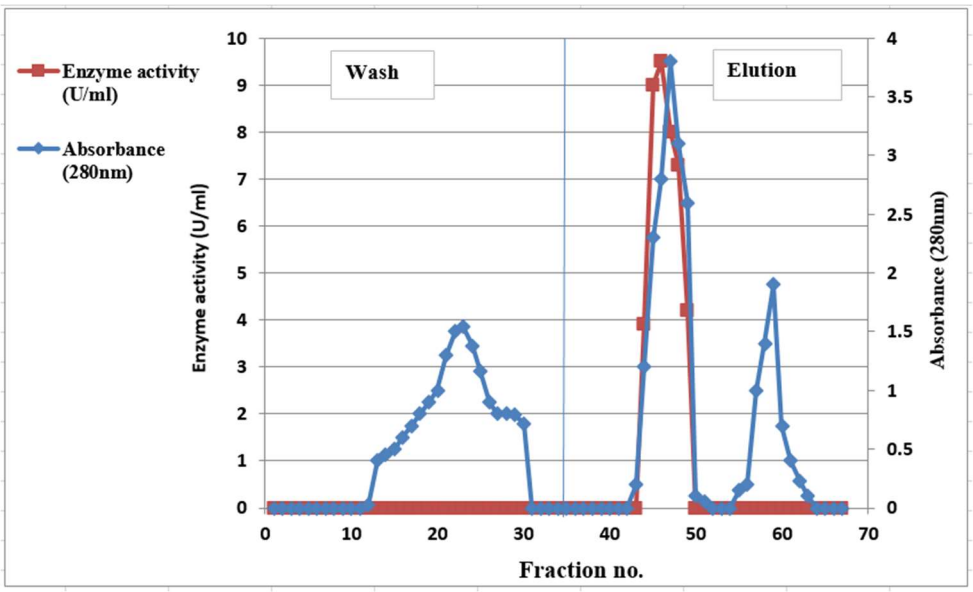


**Figure (1): Ion exchange chromatography of arginase produced by locally isolated *P.aeruginosa* ps2 using DEAE-cellulose column (2×20cm) chromatography equilibrated with Tris-Hcl buffer pH7 with 30ml/h flow rate.**

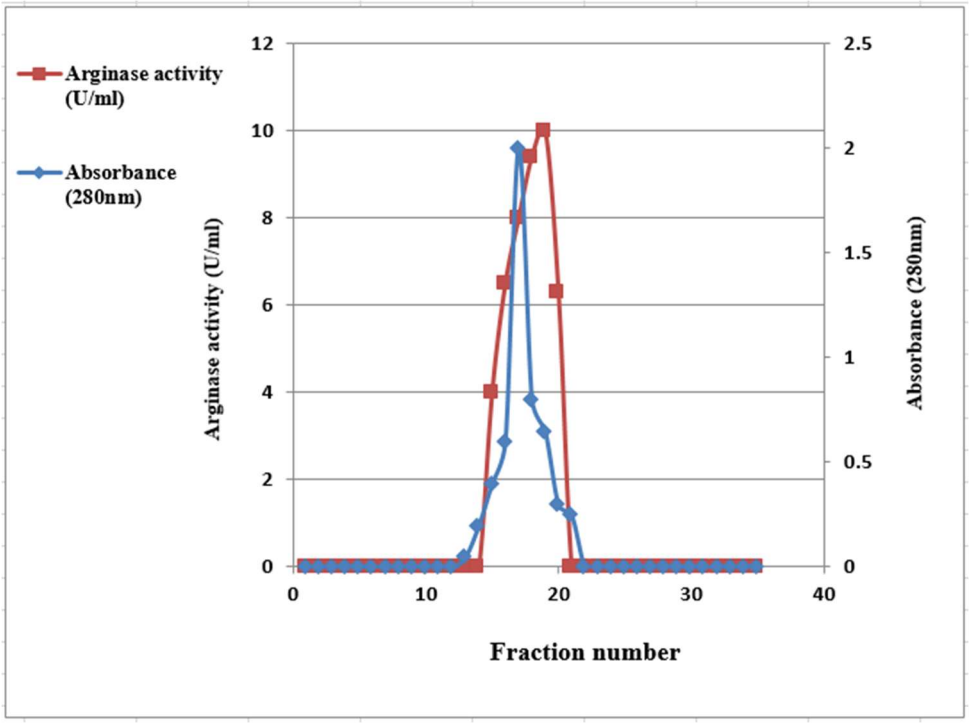


**Figure (2): Gel filtration chromatography of arginase produced by *P. aeruginosa* Ps2 using on Sephadex G100 column (1.5cmx40cm) equilibrated with 0.05M sodium phosphate buffer pH 7.0 at flow rate of 30ml/hr**

Sephadex G-150 represent the final step in the purification of arginase produced by the by the local isolate *P. aeruginosa* Ps2. Results indicated in (Fig.2) showed that only one peak represents arginase activity was appeared after elution with sodium phosphate buffer. Fractions represents

arginase activity were pooled. Protein concentration, activity and specific activity were measured. A summary of the purification procedure is given in (Table 1). The complete 4-steps procedure resulted in a11.5-fold purification with an activity yield of 50%.

**Table (1): Purification steps for arginase produced by *P. aeruginosa* Ps2**

| Purification step | Volume (ml) | Enzyme activity (U/ml) | Protein concentration (mg/ml) | Specific activity (U/mg) | Total activity (U) | Purification (folds) | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude enzyme | 50 | 6 | 0.9 | 6.66 | 300 | 1 | 100 |
| Ammonium sulphate precipitation 75% | 35 | 7.5 | 1 | 7.5 | 262.5 | 1.12 | 87.5 |
| Dialysis | 30 | 8.8 | 0.6 | 14.6 | 264 | 2.2 | 88 |
| DEAE-cellulose | 18 | 9.5 | 0.2 | 47.5 | 171 | 7.1 | 57 |
| Sephadex G150 | 15 | 10 | 0.13 | 76.9 | 150 | 11.5 | 50 |

***Effects of temperature on arginase stability and activity***

When incubated at various temperatures for 10 min in buffer (pH 7.0), the purified enzyme was quite stable up to 40°C (Fig 3). The activity of *P. aeruginosa* Ps2 arginase increased with the increasing temperature and reached its maximum at 40ºC (Fig 2)

***Effects of pH on the reaction rate***

The effects of pH on enzymatic activity are shown in Fig. 4 The enzyme showed the highest activity from pH 7 to 10, with a maximum at pH 8.

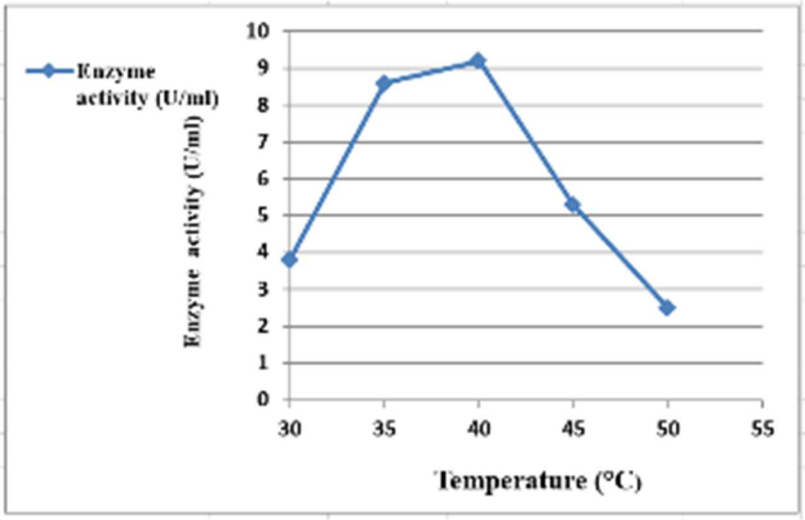

**Figure (4) Effect of temperature on the activity of purified arginase produced by *P. aeruginosa* Ps2**

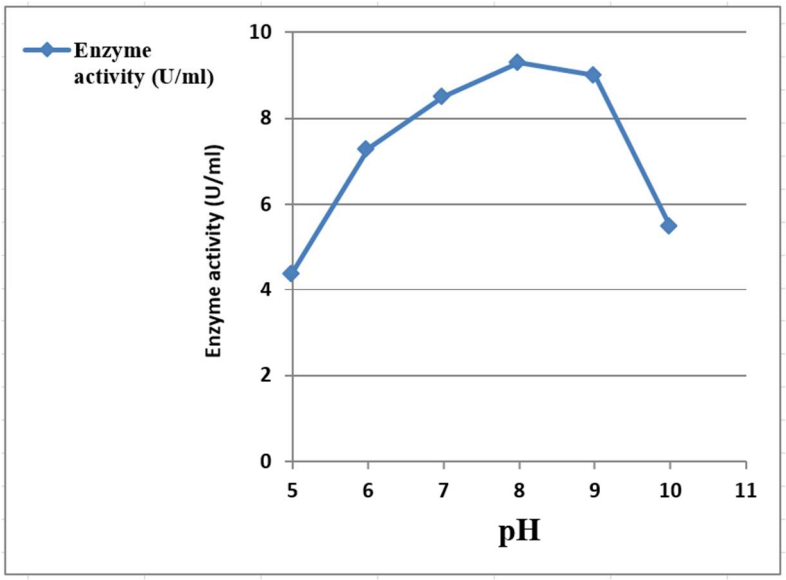


**Figure (4): Effect of pH on the activity of purified arginase produced by local isolate P. aeruginosa Ps2**

***PH stability for arginase***

The purified arginase was incubated at different pH values, Results indicated in figure (Fig5) showed that arginase was more stable at pH 6, 7 and 8. At those pH enzymes gain maximum remaining activity (100%) while the remaining activity was decreased when the enzyme was incubated with pH value less or more than optimum pH for stability (pH 6,7 and 8) as in glutaminase enzyme which keep all its remaining activity at pH 8 (Zbar *et al*.,2022).

These findings were similar to most previous findings which demonstrated the stability of arginase enzyme at different pH values (Kanda *et al.,* 1997). In contrast, Arginase of *Helicobacter pylori* was highly stable acidic and gained maximum remaining activity at pH 6.1 (McGee *et al.*, 2004).

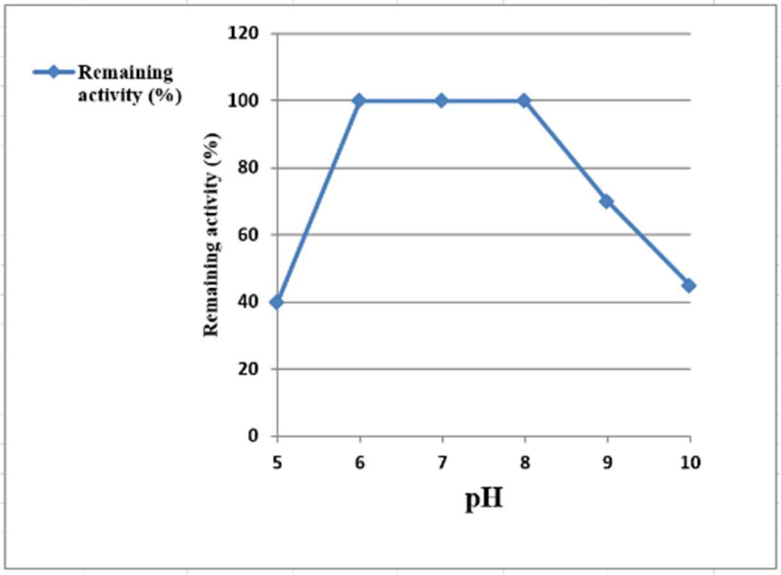


**Figure (5): Effect of pH on stability of purified arginase produced by local isolate *P. aeruginosa* Ps2**

***Effect of metal ions and inhibitors on arginase activity***

Results in table (2) show that arginase activity increased more than control when the enzyme was incubated with ZnSO4, MgCl2, and MnCl2gave a higher activity (123%, 107%, and 140%), respectively. However CuCl2, and EDTA were found that to the inhibit enzyme activity by 80 and 20 respectively. It is well known that a number of metal ion are essential to enzymes for their full catalytic activity and structural stabilization of the enzyme (Christianson, 2005).

On the other hand, yang, reported the incubation of arginase with Mn2+ caused a significant increase (33%) in arginase activity (Gursu, 2001).

**Table (2): Metal ions and inhibitors effect on arginase activity produce by *P.aeruginosa* Ps2**

| Reagent | Concentration (mM) | Remaining activity (%) |
|---|---|---|
| **Control (Enzyme)** | **-** | **100** |
| **CuCl2** | **2** | **80** |
| **ZnSO4** | **2** | **123** |
| **MgCl2** | **2** | **107** |
| **MnCl2** | **2** | **140** |
| **EDTA** | **2** | **20** |

References


- Archibald RM: Colorimetric determination of urea. Journal of Biochemistry1945; 157: 507-518
- Anbu, P., Subash C. B. Gopinath, Bidur Prasad Chaulagain, and Thangave Lakshmipriya (2017). Microbial Enzymes and Their Applications in Industries and Medicine. Hindawi, BioMed Research International Volume, Article ID 2195808, 3 pages

- Christianson, D.W., 2005. Arginase: structure, mechanism, and physiological role in male and female sexual arousal. Acc. Chem. Res. 38, 191 – 201.
- Crow, V. L., and Thomas, T. D. (1982). Arginine metabolism in lactic streptococci. J. Bacteriol. 150, 1024–1032.
- Gursu, M.F. (2001). Biochemical analysis of arginase and ornithine carbamoyltransferase in human vitreous humor. Arch. Med. Res. 32, 432 – 435.
- Husain, I., Bala, K., Wani, A., Makhdoomi, U, Malik, F., and, Sharma, A. (2017). Arginase Purified from Endophytic Pseudomonas Aeruginosa IH2: Induce Apoptosis through Both Cell Cycle Arrest and MMP Loss in Human Leukemic HL-60 Cells." Chemico-Biological Interactions 274: 35–49.
- Kanda, M., Ohgishi, K., Hanawa,T., and Saito, H. (1997). Arginase of Bacillus brevis Nagano: Purification, Properties, and Implication in Gramicidin S Biosynthesis. Vol. 344, No. 1, August 1, pp. 37–42, 1997 Article No. BB970174
- Kenneth, T. (2015). Online text book of bacteriology. http://textbookofbacteriology.net/ken_todar.html. Accessed 20 Jan.
- McGee DJ, Zabaleta J, Viator RJ, Testerman TL, Ochoa AC, Mendz GL. Purification and characterization of Helicobacter pylori arginase, RocF: unique features among the arginase superfamily. European Journal of Biochemistry. 2004; 271(10):1952-62.
- Nigam, S., P. (2013). Biomedical Microbial Enzymes with Special Characteristics for Biotechnological Applications. Biomolecules, 3, 597-611; doi:10.3390/biom3030597
- Zbar, N. S. (2022). Purification and characterization of glutaminase from Iraqi fruit of *Capsicum annuum*. Revis Bionatura 2022;7(3)
- Nadaf, P. D., Kulkarni, A. G. and Vedamurthy, A. B. (2020). INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY RESEARCH VOLUME 9, ISSUE 01, JANUARY 2020 ISSN 2277-8616
- Zhang T, Guo Y, Zhang H, Mu W, Miao M, Jiang B. (2013). Arginase from *Bacillus thuringiensis* SK 20.001: purification, characteristics, and implications for L-ornithine biosynthesis. Process Biochemistry; 48(4):663-8
- Archibald R., M.: Colorimetric determination of urea. Journal of Biochemistry1945; 157: 507-518.