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Original Article

Purification of a chemotherapeutic agent, L-Arginase, from *Pseudomonas aeruginosa* isolated from soil

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Abstract

Introduction: *L. arginase* refers to the enzyme arginase found in the genus *Lactobacillus*, it plays a crucial role in the urea cycle, and has implications in various biological applications. This study aimed to purify arginase from *Pseudomonas aeruginosa*, isolated from soil, and apply it as an anticancer.

Methodology: 28 soil samples of *P. aeruginosa* were collected from different places of Baghdad, and rice lands in Najaf and Diwaniyah governorates. Different standard laboratory and biochemical assays, and Vitik system were used in diagnosis and growth of arginase enzyme under certain pH, temperature, incubation period.

Results: The purified enzyme was precipitated by ammonium sulfite (60-80%), dialyses bag 8000-1000KD, ion exchange by DEAE cellulose and sephadex G100 in gel filtration. Cytotoxicity of arginase against breast t cancer AJM-13 and rat embryo fibroblast REF normal cell line was evaluated for (48 and 72 hours). The inhibition rate increased in the low concentration of abnormal cell (AMJ-13) while decreased in the normal cell (REF), this study takes different concentration (0.392-12.5mg/mL), and low concentration (1562-0.048 mg/mL), the result in high concentration was IR 54.7% during 72 hours for AJM-13 and 14.3% for REF in the same time, while the low concentration was IR 91% in the 1562 mg/mL in the AMJ-13, and 51% in ERF, LD50 of arginase enzyme was 0.781 mg/mL that 41% during 72 hours for ERF, its save to normal cells.

Conclusions: Arginase enzyme, at low concentrations, may have an inhibitory effect on cancer cells, and simultaneously, protect normal cell lines.

Key words: Arginine depletion; L-arginase; AMJ-13; REF-Cell line.

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Introduction

Pseudomonas aeruginosa is a versatile bacterium found in various environments, including soil and sewage. *P. aeruginosa* that found in soil vary depending on factors such as location, soil type, and environmental conditions, it is known for its ability to survive and persist in diverse environments due to its metabolic versatility and resistance to stressors. In soil, *P. aeruginosa* can interact with other microorganisms, such as; plants, and organic compounds, and also play a role in nutrient cycling, decomposition of organic compounds, and bioremediation of pollutants. However, it can also be an opportunistic pathogen, particularly in individuals with weakened immune systems [1].

L-arginase is an enzyme that plays a crucial role in various physiological processes by catalyzing the hydrolysis of L-arginine into L-ornithine and urea. It is primarily involved in the urea cycle; a metabolic pathway and responsible for the elimination of toxic ammonia from the body [2]. L-arginase has been implicated in various physiological and pathological processes beyond the urea cycle, such as; it can influence nitric oxide (NO) produced by competing with nitric oxide syntheses (NOS) for their common substrate. The balance between L-arginase and NOS activity is critical for regulating NO levels and maintaining vascular health [3]. L-arginase, as an enzyme that catalyzes the hydrolysis of L-arginine, has been studied for its potential anti-cancer activity and its influence on tumor by depleting arginine, an amino acid required for T cell function and suppressing immune responses against cancer cells [4].

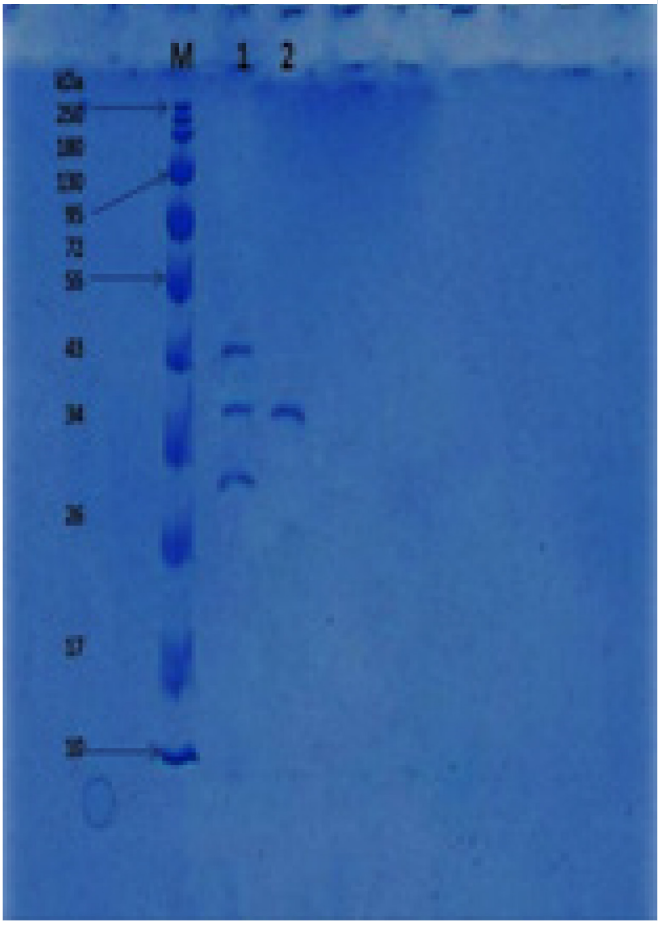
L-arginase can inhibit angiogenesis, the process of new blood vessel formation, which is essential for tumor growth and metastasis by depleting arginine, this enzyme disrupts the production of nitric oxide and other pro-angiogenic factors, leading to reduced blood vessel formation and tumor growth [5]. L-arginase has also been investigated as a potential adjunct therapy in combination with other anti-cancer treatments and has

shown promising results by enhancing treatment efficacy and overcoming resistance mechanisms [6].

Recent researches suggest the use of the effective therapeutic agent L-arginases as indicative *of P. aeruginosa* from soil through an extracellular arginase including characterization and purification *from P. aeruginosa* that has been assessed for anti-tumor action alongside section of human cell lines tumor [6,7]. There are some observations that purification of arginases induces selective apoptosis in cancer cells, which indicates aggregation [8]. Furthermore vastly articulated in several forms of tumor, including stomach, breast, colon, and liver [9].

The main objective of this study was to isolate arginase from *Pseudomonas aeruginosa*, extracted from soil, and investigate its potential as an anticancer agent.

Figure 1. Molecular weight of arginase by SDS gel electrophoresis in P. aeruginosa isolated from Soil (A)and Proteins marker 1 2 (M).



Methodology

Samples Collection

Between November 2022 and April 2023, 28 samples were collected from different locations in Iraq.

Culture and Isolation

For the purpose of isolating *P. aeruginosa*, the samples were grown on Brian heart infusion broth and nutrient medium, and then isolated by cultured on pseudomonas cetrimide agar medium at 37°C to obtain *P. aeruginosa* colonies.

Biochemical tests

Biochemical tests were carried out on *Pseudomonas aeruginosa* colonies. All isolates were determined as P. *aeruginosa* using VITEK 2 (Biomerieux).

Purification and Characterization

Purification and characterization of *P. aeruginosa* and L-arginase enzyme was carried out, as well as the precipitation in ammonium sulfite, ion exchange, and gel filtration. The molecular weight of arginase was determined using SDS gel electrophoresis.

Optimum condition of arginase production

Optimum condition of arginase production including PH, temperature, incubation time, media and substrate concentration

Cytotoxic effect of L–arginase on AMJ-13 & REF cell line

Cytotoxic effect of L–arginase (high and low concentration) on AMJ-13 and REF cell line was done according [10]. Cell adhesion or growth inhibition was calculated as a percentage of control cells, using the following equation:

$$\text{Inhibition Rate \%} = \frac{\text{Mean Abs. of Control} - \text{Mean Abs. of Treated Conc.}}{\text{Mean Abs. of Control}} \times 100$$

Results

The results are represented in four tables and one figure. Table 1 shows the optimum condition of arginase with their activity regarding pH, temperature, concentration, and incubation time. Table 2 shows the purification steps of L-arginase enzyme from the collected samples.

Figure 1 shows the molecular weight of arginase by SDS gel electrophoresis in *P. aeruginos*a isolated from soil, and proteins. Table 3 shows the cytotoxic effect of L-arginase enzyme on AMJ-13 and REF cell line using different concentrations and different incubation time,

while Table 4 shows the cytotoxicity using concentrations in nano-gram.

Discussion

The current research focuses on the extraction of ar ginase from Iraqi soil, figuring out the optimum extraction conditions, purifying it, characterizing and then testing its cytotoxic activity against cancer lines as well as against normal cell lines

The finding of the current study is represented in four tables and one figure. Table 1 shows the optimal conditions, and it was found the best yield can be reached at pH (8), temperature was 37°C, while the best substrate concentration (arginine) was from 0.01 to 2.5 g/L, and the incubation time was 96 hours, however, the media mixture gave 3.870μ/mL, these findings were reached via several trials which match the findings of Husain *et al.* and Al-Shammari *et al.* [7,10].

Table 2 shows the purification steps for L-Arginase that was collected from optimal conditions, and from the data that was listed in the table, it is clear that the activity of L arginase from crude enzyme is 3.022 μ/mL and rose to 4.231 μ/mL after precipitation with 80% saturation of $(NH_4)SO_4$, and the activity is decreased to 3.980 μ/mL after desalting and dialysis due to desalting and dialysis by which the unwanted proteins are removed as explained by Huang *et al.* [11]. Table 2 also shows that the activity reduced to 2.793 and 2.442 μ/mL after Ion exchange chromatography (DEAE-Cellulose)

Table 1. Optimum condition of arginase with their activity.

Condition	Result	Concentration enzyme of Soil
PH	8	2.99 U/mL
Temperature	37℃	3 U/mL
Substrate concentration (Arginine)	(0.1-2.5) g/L	2.5 U/mL
Incubation time	96 hours	4 U/mL
Media (M9B)	beef extraction yeast extraction medium	3.870 U/mL

Table 2. Purification steps of L-arginase enzyme from the soil sample

Purification step	Enzyme activity (U/mL) from soil
Crude enzyme	3.022
Precipitation with 80% saturation of $(NH_4)SO_4$	4.231
Desalting and concentration by dialysis	3.980
Ion exchange chromatography (DEAE-Cellulose)	2.793
Gel filtration G100	2.442

Table 3. Cytotoxic effect of L-arginase enzyme on AMJ-13 and REF cell line after different time incubation (mg/mL)

Concentrations (mg/mL)	Cytotoxic effect (IR%)			
	REF Incubation time		AMJ-13 Incubation time	
	48h	72h	48h	72h
0.392	17.2	14.3	47	54.7
0.784	15.6	13.3	41.6	47
1.575	14.8	12.6	34.5	41.4
3.125	13.2	10.2	27.7	38.2
6.250	12.9	0.9	22.2	27.9
12.5	11.4	6.2	20.3	29.4
Control rang	0.243-0.261	0.132-0.144	0.164-0.247	0.145-0.240

Table 4. Cytotoxic effect of L-arginase low concentration (mg/mL) on AMJ-13 and REF cell line after different time incubation.

(IR%) REF	REF		AJM-13	
Inc. time	48 h in (%)	72h in (%)	48h in (%)	72h in (%)
Con. (ng/mL)				
0.048	5.9	0	31	51
0.097	10.4	5.8	59	64
0.195	11.2	9.7	70	71.3
0.39 0	32.3	26.4	81	85.9
0.781	42.4	40	88.4	88.3
1.562	54.4	51	90.5	91
Control rang	0.01-0.495	0.01-0.520	0.029-0.623	0.072-0.465

and Gel filtration G100 respectively, this is due to ions removal [12,13] as well as undesired proteins as depicted by Ó'Fágáin *et al.* [14].

Figure 1 shows the molecular weight of arginase inspected by SDS gel electrophoresis. The M -Proteins marker 1 2 signify the presence of three enzymes at M 1, while at M 2, only one compound appears at 36KD, which belongs to the presence of arginase, this finding is in concordance with the the findings of Caldwell *et al.* [15].

Previous studies have investigated the impact of substrate concentration on arginase activity. For instance, research on arginase activity in microorganisms has demonstrated that the enzyme's activity is affected by the concentration of arginine substrate, these studies have shown that specific concentrations of the substrate result in higher arginase activity [16,17].

In the current study, the cytotoxicity of extracted and purified L arginine was tested against breast cancer cell line AMJ-13, and also its effect on normal cell line REF, Tables 3 and 4 represent the data of these assays that were carried out using different concentrations ranging from 0.392-12.50 mg/mL and from 0.048 to 1.562 ng/mL. All these concentrations were tested under incubation time 48 and 72 hours [18]. The best activity and best effect against cancer cell line AMJ-13 was found with a concentration of 0.392 mg/mL at incubation time 72 hours, however, the least effect on normal cell line REF was found with concentration 12.50 mg/mL at incubation time 72 hours. The best effect on cancer cell line and at the same time less effect on the normal cell line was found at 0.392 mg/mL for an incubation time of 72 hours (Table 3), while it is 0.195 ng/mL at incubation time 72 hours (Table 4) [19,20].

Conclusions

1. Soil is a good source of *Pseudomonas aeruginosa.*
2. *Pseudomonas aeruginosa* is a good source of effective arginase enzyme.
3. Optimal conditions of arginase extraction from *P. aeruginosa* were determined.
4. Arginase was purified and characterized successfully using SDS gel electrophoresis.
5. Arginase in very low concentrations can be used as a treatment against cancer cells.

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