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# Cytotoxic activity of arginine deiminase purified from *Lactobacillus* sp

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## Abstract

The goal of this research was to characterize ADI isolated from Lactobacillus sp. and test its anticancer activity *in vitro*. Because the arginine-degrading enzyme is a strong anticancer agent, it was isolated in two phases from a Lactobacillus sp. clinical isolate: 70% saturated ammonium sulphate precipitation followed by gel filtration chromatography (2x35cm). The most active fractions in enzyme activity (7.3 U/ ml), Purified enzyme had a special action activity of (48.6 U/mg) with nine folds of purgation and 61.4% enzyme recovery, The cytotoxic activity of purified arginine deiminase on PC3,A375 and WRL68 cells for 24 was examined.

Purified arginine deiminase inhibited the proliferation of cancer cell lines PC3 and A375, with IC50 values of 68.64 g/ml and 136.3 g/ml, respectively.

At a concentration of 6.25 µg/ml, 94.83% and 95.68% cell viability were observed of PC3 and A375 after treatment with purified arginine deiminase, respectively. However, cell viability reached to 42.52% and 48.77% using 400 µg/ml concentration purified arginine deiminase, respectively while ADI didn't show a significant effect on the viability of normal cell line.

**Keywords:** Cytotoxic activity; Arginine deiminase; *Lactobacillus* sp; Anticancer activity

## 1. Introduction

Enzymes are nature's long-lasting catalysts since they are biocompatible, biodegradable, and made from renewable resources (Sheldon *et al*., 2013). Enzymes are big biological globular protein molecules that are responsible for thousands of metabolic processes that keep life going (Smithet al., 1997) and operate as catalysts for specific chemical reactions within the cell. These reactions are necessary for the organism's survival, and enzymes help life processes in virtually all living things, from viruses to humans.

The ADI pathway for arginine metabolism is extremely common in a variety of bacteria, allowing them to respond to sever environmental conditions and host immunity. (Xiong *et al*.,2014 and Choi *et al*., 2012 ).

Arc operons genes arcA,arcB, and arc C (Ryan et al., 2009) encode a mechanism (Ryan et al., 2009). Ammonia, CO2, and ATP are produced as byproducts of the digestion of arginine to ornithine. (Novák *et al*., 2016).

Cancer cells adopt a novel metabolic mechanism to maintain higher growth and tolerate some cell death signals, according to growing biotechnological developments(Tennant *et al*., 2010).. Protein & peptide treatments that target specific metabolic activities are now being studied in clinical trials all around the world. (Changou et al., 2014). The ADI

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enzyme has been extensively used in skin cancers and hepatocellular carcinoma target selective chemotherapy (HCC) during the recent decades (Yoon et al., 2012).

The lack of expression of argininosuccinate synthetase in tumor cells, particularly arginine auxotrophic cancer cells, prevents them from manufacturing their own arginine, giving ADI its anti-cancer properties. They do, however, need a lot of arginine to develop quickly. When ADI is given as a chemotherapy agents, the L-arginine in the systemic circulation is hydrolyzed into ammonia and L-citrulline (Wang and Li, 2014). DNA, RNA and protein synthesis are all hampered by arginine deficiency, Due to cell inhibition in the G0 phase and G1 phase of cell proliferation, this results in apoptosis(El-Sayed *et al.,* 2015).

## 2. Material and methods

Inoculation and the production of enzymes *Lactobacillus* sp was obtained from the medical laboratory of Al-Nahrain University and inoculated into an arginine deiminase medium pH 7.5 comprising 0.2 percent glucose, 0.5 percent arginine deaminase, Na2HPO4•2H2O 0.6 percent, KH2PO4 0.3 percent, NaCl (0.05 percent), MgSO4•7H2O (0.05 %), Peptone After 24 hrs. of incubation at 37 °C and 15 minutes of centrifugation at 6,000 rpm under refrigeration, crude enzyme was obtained (Takaku *et al*.,1992).

### 2.1. Arginine deiminase assay

The enzyme was fed L-arginine as a substrate, and Nesseler's reagent was employed to measure ammonia release. In a 0.5 ml sample, L-arginine solution (0.04M), distilled water, and phosphoric buffers were blended in an equal volume (0.1 M, pH 7).

After 30 minutes of incubation at 37 °C, using (0.5 ml) of 1.5 M TCA, the reaction was stopped. Then, to the 0.1 ml of combination, 3.7 ml DW and 0.2 ml Nessler's reagent were added. At 430 nm, the absorbance was measured, and the following formula was used to plot an ammonium chloride standard curve: (Holtsberg *et al*.,2002). The amount of enzyme that provided (1mol) under test circumstances was used to establish the standard unit of arginine deiminase.

### 2.2. Protein concentration measurement

Using a bovine serum albumin curve, protein content was evaluated accordance to the procedure (Bradford, M., 1979).

#### *2.2.1. A Precipitation of arginine deiminase*

Ammonium sulfate is used to precipitate enzymes. Solid ammonium sulfate was slowly added to 100 ml of crude extract at 4 °C in varied saturate ratios. For 45 minutes, the component was gently mixed. After centrifugation at 6000 rpm for (20 min), the residue was easy dissolved in an appropriate volume potassium phosphate buffer.

#### *2.2.2. Purification of arginine deiminase*

Pharmacia Fine Chemical Company approved Sephadex G-150 for manufacture. A volume of Sephadex G 150 was mixed in (0.05 M ) phosphate buffer (pH7.0 ) and heated at 90 °C for five hours to ensure that the beads swelled, degassed, and were packed in a glass column with same buffers. The column was loaded with the concentrated sample from the previous stage. Each portion's absorbance was assessed at a wavelength of 280 nm. In addition, the activity of enzymes in each fraction was evaluated, and protein content was determined using the Bradford method (1979).

#### *2.2.3. Evaluation of cytotoxic activity for Arginine deiminase*

MTT colorimetric used to determine the cytotoxic effect of Arginine deaminase on PC3 and the formation of formazan by mitochondrial enzymes was measured using an ELISA kit with a 570 nm absorbance values. (Ali et al., 2017).

A ten-milliliter MTT solution was placed into each well of a 96-well plate, which was then incubated at 37°C for four hrs. with the testing sample (The solution became yellow). After that, each well was filled with 200 µl of DMSO (dimethylsulfoxid) and shaken for 5 minutes (The DMSO solution became purple). With an ELISA reader The absorbance of the colored solution derived from living cells was red at 575 nm after full solvation of the pigment. For each group, mean absorbance was computed.

## 3. Results and discussion

Arginine deaminase purification from *Lactobacillus sp. results* in Table (1) represent the sequential purification steps for Arginine deaminase enzyme.

Ammonium sulfate precipitation with 70% saturation gave 9.2 U/mg protein specific activities, the elution profile for Arginine deaminase on SephadexG150 (Fig 1) showed the most active fractions in enzyme activity (7.3 U/ ml), purification fold (9), Enzyme specific activity in this step became 48.6U/ mg protein and with 61.4% enzyme recovery.

Kim et al., (2007) discovered that by combining sequential Q-Sepharose anion exchange with Sephacryl S-200 gel filtration columns chromatography, they were able to get the following results, The specific efficiency of the *Lactococcus lactis* spp. ADI enzyme was 140.3 U/mg.

**Table 1** Purity steps for Lactobacillus sp. arginine deiminase

| Purification steps | Volume (ml) | Enzymatic activity (U/ml) | Concentration of protein (mg/ml) | Specific activity (U/mg) | Total activity (U) | Purity | recovery (%) |
|---|---|---|---|---|---|---|---|
| Crude extract | 50 | 3.8 | 0.7 | 5.4 | 190 | 1 | 100 |
| Ammonium sulphate precipitation 70% | 30 | 6 | 0.65 | 9.2 | 180 | 1.7 | 94.7 |
| Dialysis | 29 | 6 | 0.3 | 20 | 174 | 3.7 | 91.5 |
| Sephadex G150 | 16 | 7.3 | 0.15 | 48.6 | 116.8 | 9 | 61.4 |

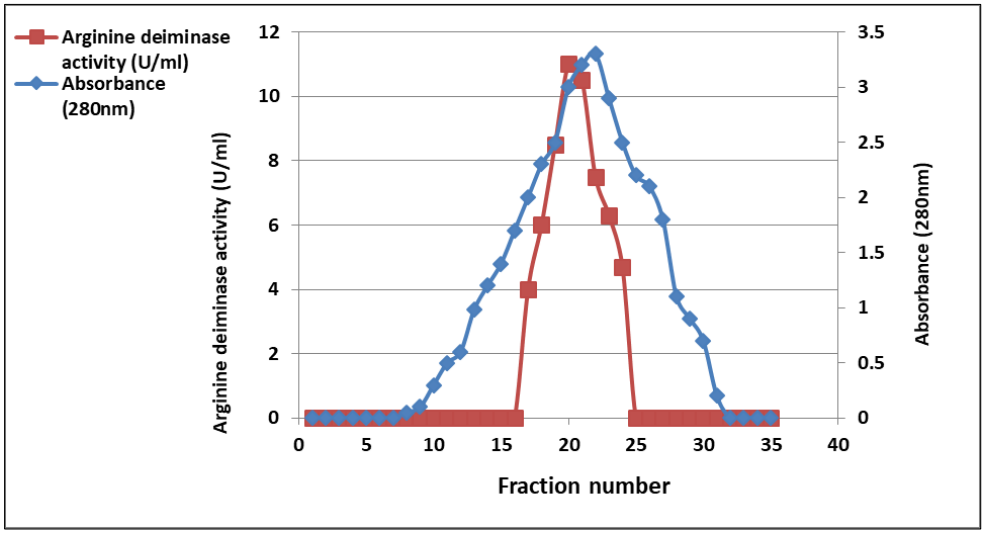


**Figure 1** Gel filtration chromatography of arginine deiminase produced by *Lactobacillus sp.* using SephadexG150

### 3.1. Cytotoxic activity of arginine deiminase

The method of MTT was used to evaluate the cytotoxic activity of purified arginine deiminase as a percentage of cell viability calculated for PC3. A375 and WRL68 cells treated with purified enzyme were shown in (Figure 2 and 3), purified enzyme have capability to reduce cell viability of PC3 and A375 with increasing the concentrations .

Purified arginine deiminase showed maximal inhibitory concentration( IC50) 68.64 µg/ml and an IC50 of 136.3 µg/ml against PC3 and A375 cell line, respectively.

At a concentration of 6.25 µg/ml, 94.83% and 95.68% cell viability were observed of PC3 and A375 after treatment with purified arginine deiminase, respectively. However, cell viability reached to 42.52% and 48.77% using 400 µg/ml concentration purified arginine deiminase, respectively ,while ADI didn't show a significant effect on life of ell line (Fig2 and 3).

The sensitivity of the human HCC cell line HEPG2 to ADI inhibition was examined by Yong-Mei Liu et al. in 2008. When the ADI activity was reduced to 0.05 U/ml, the inhibition rate for HEPG2 was 60. In a laboratory setting. ADI's efficacy in HCC and other malignancies, including as melanoma and renal carcinoma, has been verified in numerous investigations (El-Sayed et al.,2015). Furthermore, with maximal inhibitory concentration values ranging from 0.1–2 IU/ ml, effective in suppressing the growth of HCC cell lines, leukemia , pancreatic , prostate and Renal cancer cell lines (Zam *et al*., 2017). Arginine deprivation is a unique method for targeting malignancies lacking argininosuccinate synthetase (ASS) expression, according to Liu et al. (2014). Pancreatic tumors with lower ASS expression had increased survivin expression, as well as more lymph node metastases and local invasion. ASS-deficient PANC-1 cells were treated with arginine deiminase, which inhibited their growth in a dose and time-dependent manner.

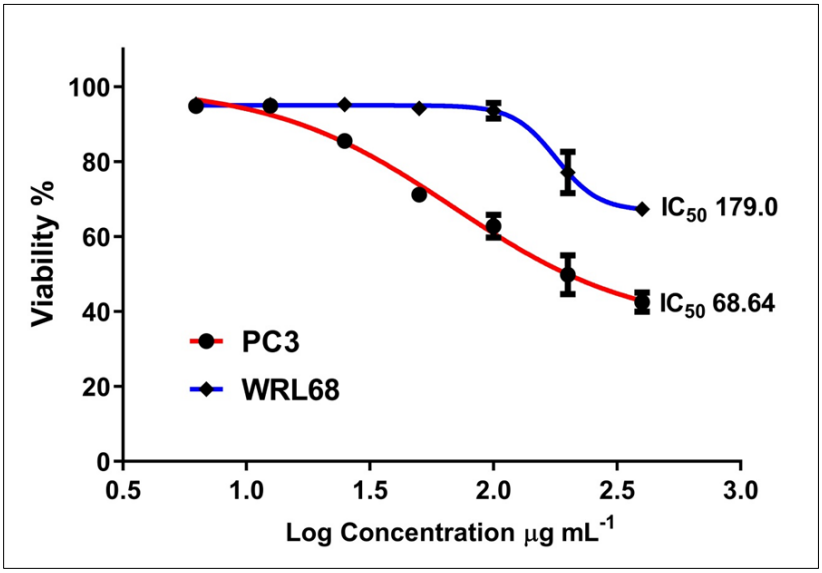


**Figure 2** Growth inhibition for purified Arginine deaminase on PC3 by using MTT assay

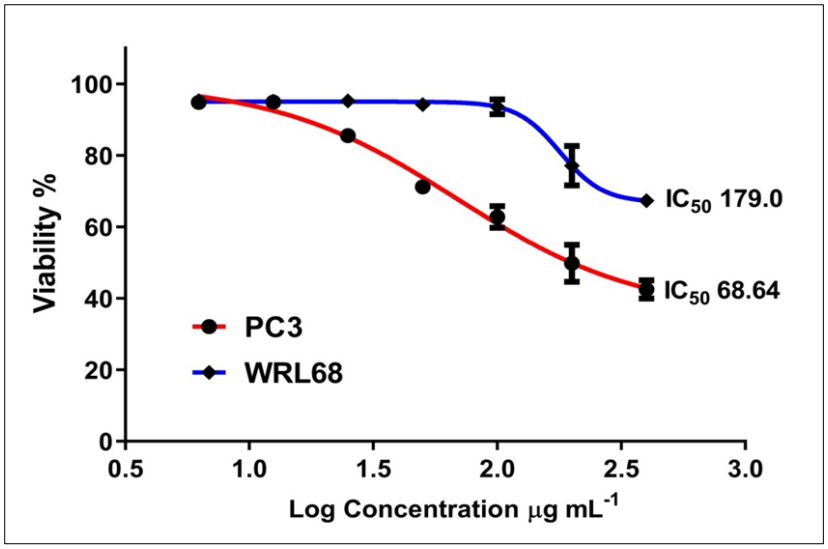


**Figure 3** Growth inhibition for purified Arginine deaminase on A375 by using MTT assay

## References


[1] Ali Al-Saffar, Fatimah A. Sabry, Firas Hassan and Noora A. Phytochemical Analysis, Antioxidant and Cytotoxic Potentials of Pelargonium graveolens Extract in Human Breast Adenocarcinoma (MCF-7) Cell Line. Asian J of Biochem 1917; 12(1):16-26.

[2] Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 1976; 72: 248-254.

[3] Changou, CA, Chen, YR, Xing, L, Yen, Y, Chuang, FYS, Cheng, RH, Boldg, RJ, Annc, DK, Kunga, HJ, 2014. Arginine starvation-associated atypical cellular death involves mitochondrial dysfunction, nuclear DNA leakage, andchromatin autophagy. Proc. Natl. Acad. Sci. 39 (14147-1452).

[4] Choi, Y, Choi, J, Groisman, EA, Kang, DH, Shin, D, and Ryu, S. Expression of stm4467-encoded arginine deiminase controlled by the stm4463 regulator contributes to salmonella enterica serovar typhimurium virulence. Infection and Immunity 2012; 80:4291–4297

[5] Delage B, Luong P, Maharaj L, O'Riain C, Syed N, Crook T, Hatzimichael E, Papoudou-Bai A, Mitchell TJ, Whittaker SJ, Cerio R, Gribben J, Lemoine N, Bomalaski J, Li CF, Joel S, Fitzgibbon J, Chen LT, Szlosarek PW: Promoter methylation of argininosuccinate synthetase-1 sensitises lymphomas to arginine deiminase treatment, autophagy and caspase-dependent apoptosis. Cell Death Dis 2012; 3: 1-9.

[6] El-Sayed, ASA, Hassan, MN, and Nada, HMS. Purification, immobilization, and biochemical characterization of l-arginine deiminase from thermophilic Aspergillus fumigatus KJ434941: Anticancer activity *in vitro*. Biotechnology Progress 2015; 31:396–405.

[7] Kim, JE, Jeong, DW, and Lee, HJ. Expression, purification, and characterization of arginine deiminase from Lactococcus lactis ssp. lactis ATCC 7962 in Escherichia coli BL21. Protein Expression and Purification 2007; 53: 9–15.

[8] Liu, J, Ma, J, Wu, Z, Li, W, Zhang, D, Han, L, Ma, Q. Arginine deiminase augments the chemosensitivity of argininosuccinate synthetase-deficient pancreatic cancer cells to gemcitabine via inhibition of NF-κB signaling. BMC Cancer, 2014; 14(1).

[9] Novák, L, Zubáčová, Z, Karnkowska, A, Kolisko, M, Hroudová, M, Stairs, CW, Simpson, AGB, Keeling, PJ, Roger, AJ, Čepička, I, et al. Arginine deiminase pathway enzymes: evolutionary history in of microgram quantities of protein using the principle of protein-dye. 2016.

[10] 1Ryan, S, Begley, M, Gahan, CGM, and Hill, C. Molecular characterization of the arginine deiminase system in Listeria monocytogenes: regulation and role in acid tolerance. Environmental Microbiology 2009; 11:432–445

[11] Sheldon, R. A and S. van Pelt, S.. "Enzyme immobilisation in biocatalysis: Why, what and how," Chemical Society Reviews, 42, 6223-6235Shibatani, T., Kakimoto, T., and Chibata, I. (1975). Crystallization and properties of L-arginine deiminase of Pseudomonas putida. The Journal of Biological Chemistry 2013; 250:4580–4583.

[12] Smith, AL Oxford dictionary of biochemistry and molecular biology: Oxford University Press. 1997.

[13] Tennant, DA, Duran, RV, Gottlieb, E, Targeting metabolic transformation for cancer therapy. Nat. Rev. Cancer 2010; 10: 267–277.

[14] Yoon, CY, Shim, YJ, Kim, EH, Lee, JH, Won, NH, Kim, JH, Park, IS, Yoon, DK, Min, BH, Renal cell carcinoma does not express argininosuccinate synthetase and is highly sensitive to arginine deprivation via arginine deiminase. Int. J. Cancer 2012; 120: 897–905.

[15] Wang, Y, & Li, Y-Z Cultivation to improve in vivo solubility of overexpressed arginine deiminases in Escherichia coli and the enzyme characteristics. BMC Biotechnology, 2014; 14: 53.

[16] Whitaker. JR. and Bernard, RA. Experiment for introduction to Ezymology. The Wiber Press Davis 1972.

[17] Xiong, L, Teng, JL, Watt, RM, Kan, B, Lau, SK, and Woo, PC. 2014. Arginine deiminase pathway is far more important than urease for acid resistance and intracellular survival in Laribacter hongkongensis: A possible result of arc gene cassette duplication. BMC Microbiology 14

[18] Zam, W. 2017. Arginine enzymatic deprivation and diet restriction for cancer treatment. Brazilian Journal of Pharmaceutical Sciences 53.