Original Article

Purification and Characterization of Arginine Deiminase from *Klebsiella pneumoniae*

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Abstract

Background and Objectives: This study was aimed to characterize arginine deiminase (ADI) purified from *Klebsiella pneumoniae in vitro*. **Materials and Methods:** Precipitation with 70% saturated ammonium sulphate, ion exchange chromatography with a DEAE-cellulose column, and gel filtration chromatography throughout sepharose-6B were the three steps taken to isolate the arginine-degrading enzyme from a *K. pneumoniae* clinical isolate, which is a potent anticancer source. **Results:** After 5.9 folds of purification and 38.7% enzyme recovery, the specific activity of the purified enzyme reached 164.2 U/mg. When biochemical characteristics of the purified enzyme were studied, results showed that the activity was maximum at pH 6 and is most stable in pH ranging from (5–9), the optimum temperature for enzyme activity was observed at 37ºC and reach 11.5 U/mL. In contrast, ethylenediaminetetraacetic acid (EDTA), $NaNO_3$, and $ZnSO_4$ slightly inhibited ADI activity, whereas $MnCl_2$, increased the remaining activity of enzyme to 125%., as well as $NaNO_3$, EDTA, $ZnSO_4$, and $FeCl_3$ were found that they inhibit enzyme activity by 90, 70, 88, and 110, respectively. **Conclusion:** A locally isolated strain of *K. pneumoniae* N1 is a useful and potent arginine deiminase producer.

Keywords: Arginine deiminase, characterization, extraction, *K. pneumoniae*, purification

Introduction

Enzymes are nature's eco-friendly catalysts because they are non-toxic, biodegradable, and sourced from naturally occurring, renewable resources.[1] Enzymes are large, globular protein molecules in biology; they are involved in countless metabolic processes that are essential to life, and play catalytic roles in a variety of cellular processes. Enzymes play a crucial role in the metabolic processes of virtually every known form of life, from viruses to humans, and are adapted to work optimally under a wide range of physiological conditions; Build-up up new tissues,[2] renewing worn-out tissues,[3] process of transforming food into fuel,[4,5] toxic waste disposal.[6] Because tumor cells have an increased need for arginine, arginine deiminase is considered a promising anticancer agent due to its ability to inhibit tumor growth.[7] It is well established that a wide variety of microorganisms use arginine metabolism *via* the arginine deiminase (ADI) pathway to survive in extreme environments and evade host defenses.[8] There could be additional biological functions for ADI enzyme; numerous microorganisms ferment arginine (as an intracellular enzyme) to produce ATP, and the ADI enzyme promotes increased survival during the stationary phase.[9] Ammonium sulfate precipitation, Q-Sepharose fast flow anion exchange chromatography, and Sephadex-G75 gel filtration chromatography were used to successfully isolate and purify ADI from *Enterococcus faecalis*. Despite claims that the purified *E. faecalis* ADI is made up of four identical subunits, researchers instead found a single band at 46 KDa and an overall molecular weight of 190 KDa for the enzyme. Research has shown that at many different pH levels, the ADI enzyme remains active and stable.[10] *Mycoplasma arthritidis* ADI is particularly stable because of its unusual composition, which includes 36 half-cystine residues and a known oxidation state for those residues (16 disulfide and 4 sulfhydryl groups).[11]

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Materials and Methods

Sample collection

The samples collected from both the Al-Hilla Teaching Hospital and the Merjan Hospital in Babil, Iraq, provided a total of 150 different clinical samples for the study. From January 2, 2022, to February 8, 2022, we collected samples from a wide range of people of both sexes and ages.

Isolation and identification of *Klebsiella pneumoniae*

All of the isolates were found in clinical and hospital environmental samples and were cultured on MacConkey, eosin methylene blue, and blood agar plates. After an aerobic incubation period at 37ºC for a full day, the plates were examined for bacterial growth. Sub-culturing the pink colonies with a mucous texture onto MacConkey agar confirmed that they were lactose-fermenting bacteria.[12] Colony morphology, staining reaction, and polymerase chain reaction (PCR) were used to identify possible *Klebsiella pneumoniae* isolates.[13] Bacterial isolates were examined microscopically after staining by Gram stain to observe the color, size, shape, and arrangement of bacterial cells under a light microscope.[14] The isolate of *K. pneumoniae* was identified by using PCR with 16sRNA primer and gel electrophoresis.

Extraction of arginine deiminase enzyme from bacterial cells

After harvesting bacterial cells from the production medium, centrifuging them at (6000 r/min for 30 min in cooling centrifuge), and determining their wet weight, the bacteria were re-suspended in extraction buffer containing 0.2 mg/mL, 1 mM of mercaptoethanol to aid in the disintegration of the *K. pneumoniae* N1 cell wall. The bacterial cell wall was breached in multiple ways: First, a bacterial cell suspension containing 200 g/mL of lysozyme was incubated for 20 min at 37ºC, and then the suspension was centrifuged at 8000 r/min for 15 min in a cooling centrifuge. Two, a suspension of bacterial cells in extraction buffer was mixed with sterilized glass beads, vortexed for 20 min at cold temperatures, and then poured into a Pasteur pipette for removal and centrifugation (at 8000 r/min for 15 min in a cooling centrifuge). Suspension of bacterial cells was treated with 12.5% K_2HPO_4 and 2% Triton X-100, centrifuged at 8000 r/min in a cooling centrifuge after being incubated at 25°C for 30 min.[15] We threw away the pellet and saved the supernatant to determine the enzyme activity, protein concentration, and specific activity using the three different techniques we discussed.

Optimization of arginine deiminase synthesis

We estimated the arginine deiminase activity and protein concentration in the crude cell-free supernatant after inoculating 100 mL of the minimal salt medium pH 7 with 1 mL of fresh culture (optical density = 0.6) of the bacterial isolate and incubating the mixture at 37ºC for 24 h in a shaker incubator at 150 rpm. When an optimum value was found, it was used in subsequent optimization experiments.

Optimum carbon source

The minimal medium was supplemented with various carbon sources (glucose, galactose, starch, lactose, and maltose) one at a time. At a final concentration of 0.5%. Specific activity, protein concentration, and arginine deiminase activity were measured.

Optimum nitrogen source

After the optimum carbon source was added in the previous step, peptone, casein, tryptone, yeast extract, and gelatin were all added to the production medium with a 0.5% final concentration to serve as nitrogen sources. In addition, the total amount of protein, specific activity, and arginine deiminase activity were evaluated.

Optimum inoculum size

The best results were obtained by using inoculum sizes of (103, 104, 105, 106, 107, 108, and 109 colony forming unit) to inoculate the production medium on an individual basis.

Purification of arginine deiminase

A precipitation of enzyme by ammonium sulfate

At 4ºC, 100 mL of the crude enzyme was mixed with solid ammonium sulfate at varying saturation ratios (from 20% to 85%). For 45 min, we gave the ingredient a slow, steady stir. Next, the mixture was centrifuged at 6000 rpm for 20 min; the supernatant was thrown away, and the precipitate was dissolved in a potassium phosphate buffer.

Ion exchange chromatography

The enzyme was separated using a Diethylaminoethyl (DEAE)-Cellulose column, and then eluted with a 0.05 M Tris–HCl buffer pH 8 containing a stepwise addition of NaCl (0.1–1 M). Each fraction was pumped through the column at a rate of 3 mL/fraction, and its absorbance was measured at 280 nm with a ultraviolet–visible spectroscopy spectrophotometer. Each fraction's arginine deiminase activity was measured. For the sake of further purification, all of the fractions that showed arginine deiminase activity were combined.

Gel filtration chromatography

The column of Sepharose-6B (220 cm) was equilibrated with 50 mM potassium phosphate buffer before the concentrated enzyme was applied. The ion exchange step yielded partially purified enzyme, which was then applied to the matrix. Using the same equilibration buffer, we were able to achieve an elution flow rate of 3 mL/fraction. Protein volume and enzyme activity were determined after 280 nm measurements of optical density were taken on each fraction. Active fractions were then collected.

Arginine deiminase characterization

Effect of pH on purified arginine deiminase activity

20 mM/mL of substrate solutions were mixed with 10 mM/mL of purified enzyme in buffers of varying acidity (pH), from acetate (pH 4.5) to potassium phosphate (pH 6.5) to Tris-HCl (pH 9) to achieve the desired pH range (8, 8.5, 9). The enzyme activity was assayed.

Optimum pH for purified arginine deiminase stability

Once the enzyme was purified, it was incubated at 37ºC for 30 min with buffer solutions ranging in pH from 5 to 9. Then the enzyme was cooled quickly in an ice bath. Enzyme activity was measured, and the amount of active enzyme was computed.

Effect of temperature on arginine deiminase activity

After incubating the purified enzyme with the substrate at varying temperatures (from 20 to 80ºC), the enzyme's arginine deiminase activity was measured. The impact of heat on the stability of arginine deiminase after 30 min incubation at various temperatures (25–50ºC), the purified enzyme was chilled in an ice bath. At each temperature, the enzyme's activity was measured. We plotted the remaining ADI activity as a function of temperature in ºC.

Effect of temperature for arginine deiminase stability

After 30 min incubation at varying temperatures (between 30 and 80ºC), the purified enzyme was cooled in an ice bath. We measured the enzyme's activity at various temperatures. We plotted the remaining ADI activity as a function of temperature in ºC.

Determination of various ions and inhibitors effect on arginine deiminase activity

Enzyme activity was measured after incubating the protein with various metal ions [$FeCl_3$, $MgCl_2$, NaCl, $ZnSO_4$, $MnCl_2$, and ethylenediaminetetraacetic acid (EDTA)] at a concentration of 10 mM/mL at 37ºC for 30 min. The absence of these substances in the enzyme solution served as the control. Each treatment's residual activity was measured.

Ethical approval

The study followed the Declaration of Helsinki's guiding principles for conducting research with minimal risk to participants. Before any samples were taken, we made sure to get the patient's verbal and analytical consent. Document B220103, which includes the number and the date in January 17, 2022, indicates that the study protocol, subject information, and consent form were reviewed and approved by a local ethics committee.

Results

Isolation and identification of *Klebsiella pneumoniae*

After being cultured on MacConkey, eosin methylene blue agar, and blood agar plates, all isolates were recovered from clinical and hospital environmental samples. Bacterial growth was examined after an aerobic overnight incubation at 37ºC. After identifying pink colonies on eosin methylene blue agar, which indicated lactose-fermenting bacteria, they were subcultured onto MacConkey agar to distinguish them from colorless colonies, which indicated non-lactose-fermenting bacteria. Colonies of Klebsiella are bright pink and mucoid in texture, but they look pale and give a gamma-hemolysis result when grown on blood agar.[12]

The result showed that 95 isolates from bacteria had *K. pneumoniae* depending on the primer that was used for this bacteria where the result of the gel electrophoresis showed the presence of band that indicates the presence of *K. pneumoniae*.

Extraction of arginine deiminase from bacterial cells

Quantitative screening, which relies on the quantification of the arginine deiminase's specific activity, is used to screen *K. pneumoniae's* potential for arginine deiminase production. All *K. pneumoniae* isolates were tested positive for producing arginine deiminase, though to varying degrees. It was found that the specific activity of arginine deiminase in the culture filtrate of these isolates was anywhere from 0.8 to 12 U/mg. The specific activity of arginine deiminase in the crude filtrate of *K. pneumoniae* No. 1 was 12 U/mg, making this isolate the most effective producer of arginine deiminase among those tested. Consequently, the No. 1 isolate was chosen for further research into the production and application of arginine deiminase.

Optimal conditions for arginine deiminase production

One factor at a time was varied in order to maximize arginine deiminase production by *K. pneumoniae* N1, whereas the other was held constant.

Optimal carbon source

The metabolic activities of the isolate depend on carbon, which is why it is a major component of the medium.[16] The results of this study showed that lactose, when used

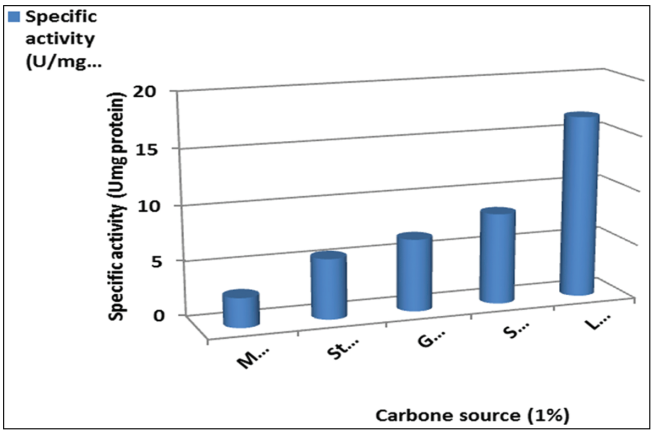

Figure 1: Optimal carbon source for arginine deiminase produced by *K. pneumoniae* N1 after incubation at 37°C for 24 h

as the sole source of carbon and energy in the production medium, yields the highest specific activity of ADI from *K. pneumoniae* N1 (16.6 U/mg protein), followed by sucrose, glucose, starch, and maltose [Figure 1].

Optimal nitrogen source

Next to carbon, nitrogen is an extremely important nutrient for microbial growth in the lab or in the wild. A key component of protein, nucleotides, enzymes, and a cofactor in metabolic processes, nitrogen is also an essential part of the human diet.[17] Yeast extract was the most effective nitrogen source for ADI production from *K. pneumoniae*, with specific activity reaching 22.2 U/mg protein. Other nitrogen sources included peptone, tryptone, yeast extract, casein, and meat extract. Peptone was the second nitrogen source suitable for the enzyme production, which gave a specific activity of 19.4 U/mg protein. However, meat extract represented the poor nitrogen source for ADI production and bacterial growth [Figure 2].

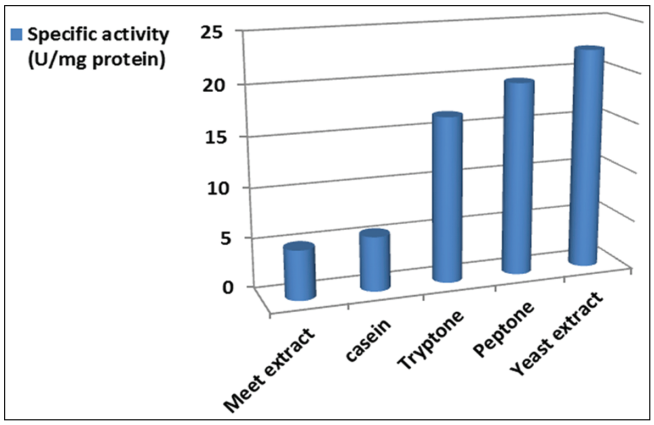

Figure 2: Optimal nitrogen source for arginine deiminase production by *K. pneumoniae* N1 incubated at 37°C for 24 h

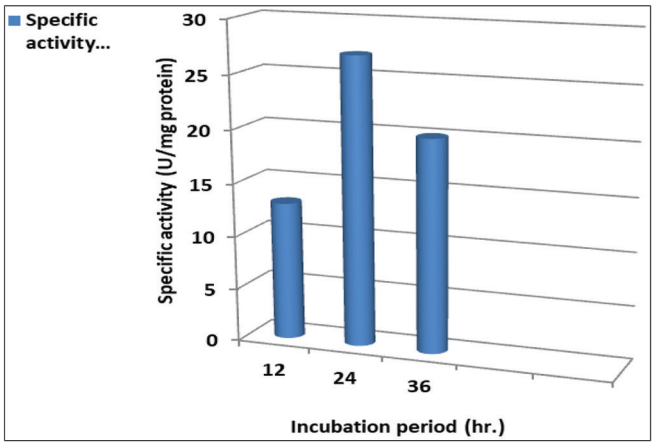

Figure 3: Effect of incubation period on arginine deiminase production by *K. pneumoniae* N1 after incubation at 37°C for 24 h

Optimum incubation period

Media with different Incubation period of arginine (12, 24, 36 h) were used for the production of ADI from *K. pneumoniae* N1. Figure 3 demonstrates the effect of these incubation period on the production of enzyme; 24 h arginine was the most suitable incubation period for enzyme production was (27 U/mg protein), whereas in other incubation period, the specific activity was less than this (13 U/mg protein) for 12 h and (20 U/mg protein) for 36 h.

Purification of arginine deiminase

To purify arginine deiminase produced by *K. pneumoniae* N1 under the optimum conditions, three purification steps were summarized in Table 1.

Ammonium sulfate precipitation

Precipitation of arginine deiminase activities at 70% ammonium sulfate yielded a high enzyme-specific activity (97 U/mg protein).

Ion exchange chromatography

An additional boost in enzyme activity was observed after purification via DEAE-cellulose chromatography. The activity and specificity of enzymes for *K. pneumoniae* are shown in Figure 4. The results showed that arginine deiminase activity (120 U/mL) was present in fractions 25–37 of the washing step. As there was no ADI activity in the second peak (which eluted at 0.4 of NaCl), it was disregarded. The arginine deiminase activity of the third peak protein in 0.5 M of NaCl ranged from 121 to 133 U/mL.

Gel filtration chromatography

Purification of arginine deiminase from a local isolate of *K. pneumoniae* N1 was completed using gel filtration chromatography. After going through an ion exchange purification step, the enzyme's final specific activity was 164.2 U/mg, with a purification fold of 5.9 and an overall yield of 38.7% [Figure 5]. It was determined by Lall *et al.*[18]

Table 1: Purification steps for arginine deiminase produced by *K. pneumoniae* N1

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	8.3	0.3	27.6	622.5	1	100
Ammonium sulphate precipitation 70%	15	19.4	0.2	97	291	3.5	46.7
DEAE-cellulose	21	12	0.1	120	252	4.3	40.4
Sephadex-G100	21	11.5	0.07	164.2	241.5	5.9	38.7

DEAE, diethylaminoethyl

Using sequential Q-Sepharose anion exchange and Sephacryl S-200 gel filtration column chromatography, we found that the specific activity of ADI enzyme purified from *Lactococcus lactis* spp. was 140.3 U/mg.

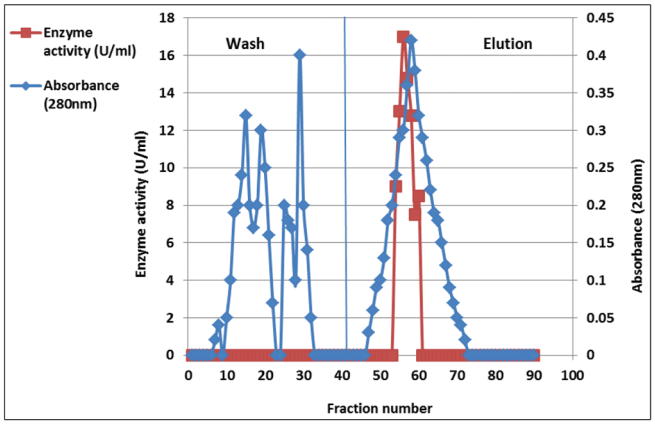

Figure 4: Ion exchange chromatography of arginine deiminase produced by *K. pneumoniae* N1 using diethylaminoethyl-cellulose column (2 × 20 cm) with a flow rate of 30 mL/h

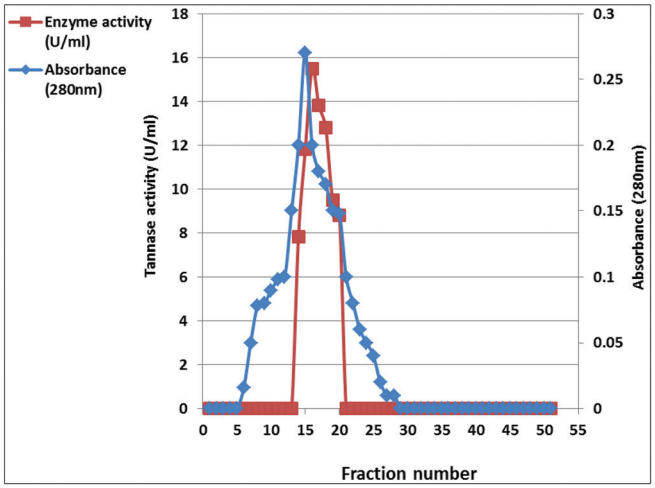

Figure 5: Gel filtration chromatography of arginine deiminase produced by *K. pneumoniae* N1 using sepharose-6B column (3 cm × 35 cm) equilibrated with 50 mM potassium phosphate buffer pH 7

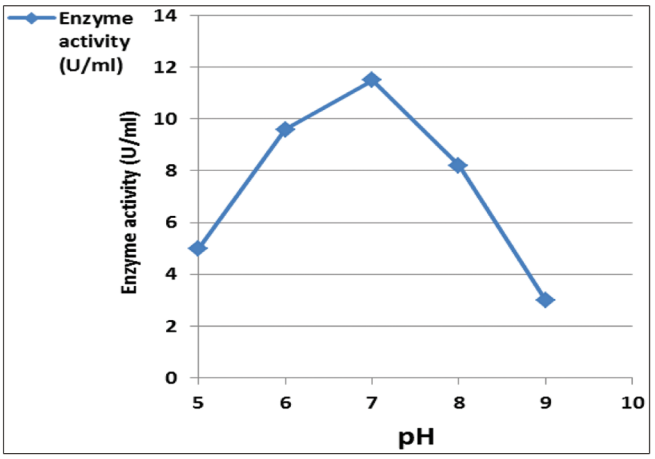

Figure 6: Effect of pH on the activity of purified arginine deiminase produced by *K. pneumoniae* N1

Characterization of arginine deiminase

Effect of pH on purified arginine deiminase activity

Researchers determined that pH 6 was the sweet spot for *K. pneumoniae* N1 ADI activity. Although inactive at lower pH levels, *K. pneumoniae* N1 arginine deiminase is still functional at higher pH levels (4–9) [Figure 6].

Optimum pH for arginine deiminase stability

Arginine deiminase is pH-stable. The enzyme was purified, and then incubated at different pH values for 30 min at 37ºC to find the optimal pH for ADI stability. Although the ADI of *K. pneumoniae* N1 was stable over a wide pH range (5.0–9.0), it was most stable at a neutral pH (6.0–7.0) [Figure 7].

Effect of temperature on arginine deiminase activity

Enzyme activity is significantly influenced by temperature. It's possible that various strains of bacteria have varying optimal temperatures for ADI activity. There was a linear increase in *K. pneumoniae* ADI activity up to 37ºC [Figure 8]. The enzyme activity was 2% U/mL at body temperature (37ºC).

Effect of temperature on arginine deiminase stability

Considering the data presented in Figure 9, it can be concluded that the enzyme was stable between 37 and

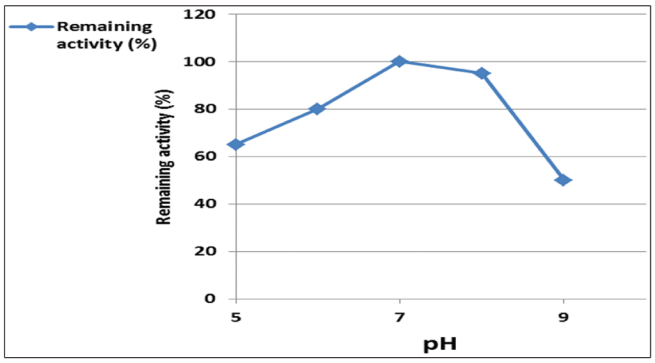

Figure 7: Effect of pH on stability of purified arginine deiminase produced by *K. pneumoniae*

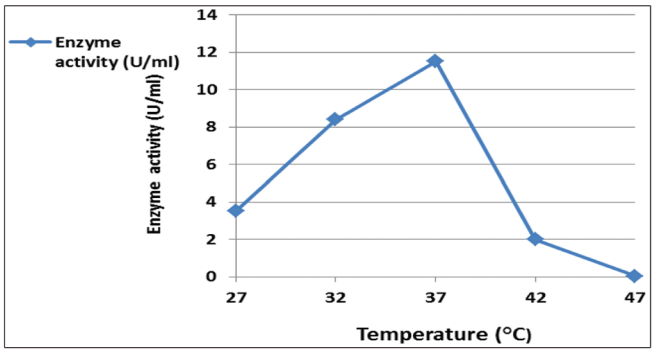

Figure 8: Effect of temperature on the activity of purified-arginine deiminase produced by *K. pneumoniae* N1

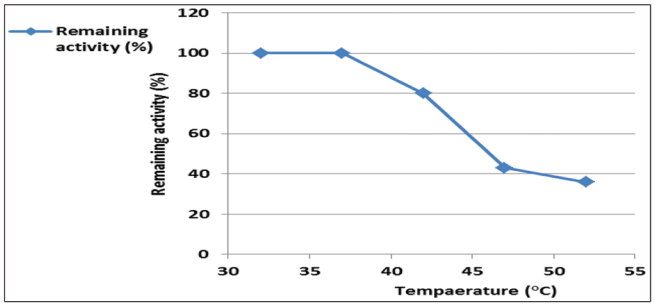

Figure 9: Temperature stability of arginine deiminase produced by *K. pneumoniae* N1

Table 2: Metal ions and inhibitors effect on arginine deiminase activity produce by *K. pneumoniae* N1

Reagent	Concentration (mM)	Remaining activity (%)
Control (enzyme)		100
$NaNO_3$	5	90
EDTA	5	70
$ZnSO_4$	5	88
$MnCl_2$	5	125
$FeCl_3$	5	110

EDTA, ethylenediaminetetraacetic acid

40ºC, and that its activity gradually decreased with increasing temperature beyond that point, although at 50ºC, about 93% of the activity was recalled;

Effect of metal ions and inhibitors on arginine deiminase activity

Results in Table 2 showed that ADI activity increased when the enzyme was incubated with $FeCl_3$, $MnCl_2$, $NaNO_3$, EDTA, and $ZnSO_4$ at 5 mM as ADI activators gave a higher activity. $MnCl_2$ was the best metal ion for increasing the activity of ADI enzyme which gave a maximum remaining activity reached 125%. However, $NaNO_3$, EDTA, $ZnSO_4$, and $FeCl_3$ were found that the inhibit enzyme activity by 90, 70, 88 and 110, respectively.

Discussion

The production of arginine deiminase by *K. pneumoniae* N1 was optimized using one factor at a time variable and keeping the other factor constant.

Optimal carbon source

The metabolic activities of the isolate depend on carbon, which is why it is a major component of the medium.[19]

Previous researchers found that ADI production was greatly influenced by the carbon source.[20] When the medium was supplemented with both arginine and lactose, ADI production skyrocketed. When maltose was added to the arginine medium, however, the effect was the opposite. In Kozai *et al.*[21] study, the enzyme production was improved when the production medium was supplemented with 1.5% maltose. Moreover, in our study pointed that maltose was found to induce the arginine catabolic pathway for an organism which was proceeded *via* citrulline.

Comparable to earlier results obtained for *Enterococcus faecium*, the addition of glucose-arginine reduced the activity of the arginine fermentation system.[22] Another study found that the enzyme yield was cut in half when glucose was present compared to galactose; this was likely due to the catabolic repressive nature of glucose.[23]

Optimal nitrogen source

Next to carbon, nitrogen is an extremely important nutrient for microbial growth in the lab or in the wild. A key component of protein, nucleotides, enzymes, and a cofactor in metabolic processes, nitrogen is also an essential part of the human diet.[17]

The synthesis and composition of microbes are profoundly impacted by dietary and environmental factors. Because of their close relationship with cell growth and metabolite synthesis, carbon and nitrogen sources play a pivotal role in most biological processes. Catabolic repression and other phenomena can also control the secondary metabolic pathways.[24] According to Lall *et al.*[25] tryptone stimulated the highest levels of arginine deiminase production in Enterococcus faecium.

Optimum incubation period

The incubation period, which can range from 24 h to a week depending on the microorganism and other culture conditions such as inoculum size, metabolic state of cell, pH, and temperature, has a major impact on enzyme production. These findings could be explained by the fact that this isolate thrived at this temperature. It has been observed experimentally that the incubation time and temperature have a significant impact on enzyme production.[26]

Purification of arginine deiminase

To purify arginine deiminase produced by *K. pneumoniae* N1 under the optimum conditions, three purification steps were used including:

Ammonium sulfate precipitation

The basic amino acids, the acidic amino acids, and the neutral hydrophilic amino acids all contribute to the solubility of proteins in water. By decreasing the amount of water available, salts like ammonium sulfate disrupt the interactions between the side-chains of amino acids and the water, thereby decreasing the protein's solubility. This allows the protein to interact with itself rather than with water, allowing it to precipitate out of solution. Different proteins would precipitate out at various saturation levels of ammonium sulfate, depending on their hydrophilicity. For its precipitation to be possible, the protein must

have a higher hydrophilicity, and the ammonium sulfate concentration must be higher to break the protein–water interactions. Duong-Ly and Gabelli[26] found that ADI was most active at saturations of 50%, 60%, 70%, and 80%, with specific activities ranging from 6.5 to 6.7 U/mg protein.

Ion exchange

Diethylaminoethylcellulose (DEAE-cellulose) is the resin used at this stage; it is a weak base. Anionic proteins can bind to the resin whereas cationic proteins can pass through it. The protein's pI value and the pH of the buffer solution in which the DEAE-cellulose is equilibrated determine how well the protein will bind to the resin. DEAE-cellulose resin has many advantages, including effective separation, high resolution power, simple separation based on charge difference, easy handling, high capacity, and the possibility of reactivation of using many times.[27] The enzyme activity was improved even further after purification via DEAE-cellulose chromatography. There may be more than one isoform of an ADI enzyme present in the bacterial extract, and this may account for the presence of enzyme activity during the washing step. The ability to bind with the ion exchangers may also be hindered by the presence of a hydrophobic moiety. Using DEAE-agarose chromatography, Unissa *et al.*[28] demonstrated the existence of two distinct enzyme forms, one of which (arginine deiminase I) was detected only in stationary-phase cultures. 30%–60% of the enzyme in late log cultures is the other form, deiminase II. Using DEAE-Sephadex column fast flow anion column chromatography, ADI was extracted from *E. faecalis* and eluted at NaCl concentrations (0.1–0.5). Recently, the arginine deiminase enzyme from *E. faecalis* SK32.001 was purified using Sepharose fast flow resin.[29]

Gel filtration chromatography

Column chromatography with anion exchange on Q-Sepharose and gel filtration on Sephacryl S-200 in sequential order, El-Sayed *et al.*[30] determined that the specific activity of ADI enzyme purified from *L. lactis* spp. was 140.3 U/mg.

Aspergillus fumigatus KJ434941 was shown to be capable of producing arginine deiminase in a separate investigation. To get the enzyme with specific activity of 26.74 U/mg and 11.2% yield, we used gel filtration with Sephadex G100 as the third purification step (following ammonium sulfate precipitation and DEAE-cellulose).[31]

Characterization of purified enzyme

Optimum pH for enzyme activity

According to Henningham *et al.*,[32] *Streptococcus pyogenes* ADI activity is most effective at a pH of 6, with a maximum enzymatic reaction rate, but the enzyme's activity drops off significantly between 5.0 and 9.

Optimum pH for arginine deiminase stability

This suggests that the ADI of *K. pneumoniae* prefers a neutral pH environment, as it retained the highest amount of activity (88%–100%). These results mirrored those of previous studies showing that ADI enzyme is stable across a wide range of pH.[33]

Optimum temperature for enzyme activity

In most cases, enzyme activity was lowest at low temperatures, then increased at moderate ones, and finally decreased at high ones due to protein denaturation, active site change, and slowed reaction rate.[34]

Temperature stability of arginine deiminase

The result agrees with previous research into the stability of ADI *K. pneumoniae* at various temperature.[35]

Effect of metal ions and inhibitors on arginine deiminase activity

It is common knowledge that enzymes cannot perform their catalytic function without a variety of metal ions. In contrast, their impact on ADI was rarely discussed. The presence of Fe^{3+} metal ions improved the enzymatic activity. In general, Fe^{3+} is involved in the pathways that carry electrons away from enzyme active sites.[36] On the other hand, Unissa *et al.*[37] reported the inhibition of ADI by 10 mM EDTA. Also, Henningham *et al.*[38] found that the super fluous Zn^{+2} and Co^{+2} inhibited ADI activity. ADI from *Pseudomonas putida* inhibited almost quantitatively by Hg^{+2}, Al^{+3}, Cu^{+2}, and Ag^{+2} ions.[39]

Conclusions

Klebsiella pneumoniae was identified through amplification of its 16S rRNA gene. The *K. pneumoniae* N1 isolate from your area can be a reliable starting point for your arginine deiminase manufacturing needs. The minimal salt medium supplemented with 0.5% lactose as a carbon source and 0.5% yeast extract as a nitrogen source, adjusted to pH 7 and incubated at 37ºC, provided the ideal nutritional and environmental conditions for ADI production by *K. pneumoniae* N1 isolate. After three stages of purification—ammonium sulfate precipitation (70%), ion exchange chromatography using DEAE-cellulose, and gel filtration using sepharose-6B—ADI was obtained from *K. pneumoniae* N1 with a high specific activity. The purified ADI stable in a pH ranging from 5 to 9, temperatures (37ºC–50ºC) enabling its use in the future for therapeutic study. EDTA is a metal chelator inhibits enzyme activity suggesting that the ADI belong to a metalloenzyme.

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Conflicts of interest
The others declare no conflicts of interest.

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