Antibacterial Activity of Arginine that Extraction and purification from *Staphylococcus aureus*

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Abstract

Arginine, a semi-essential α-amino acid involved in various metabolic pathways, including protein synthesis, nitric oxide production, and urea cycle function, is conditionally essential in humans, meaning it can become necessary under certain physiological conditions. This study investigated the extraction and purification of L-arginine from bacterial pathogens isolated from clinical samples. Twenty samples were collected from wound and burn infections at hospitals in Baghdad, Iraq. Bacterial isolates were cultured on selective media and identified using a combination of microscopic examination, biochemical tests (including catalase, coagulase, oxidase, and urease), and the Vitek 2 automated system (bioMérieux). The predominant isolates identified were Staphylococcus epidermidis (60%), Pseudomonas aeruginosa (20%), Escherichia coli (15%), and Staphylococcus aureus (5%). Subsequently, S. aureus, P. aeruginosa, and E. coli isolates were screened for L-arginine production. Initial screening employed a qualitative method using a suitable indicator for arginine or its metabolic products (e.g., pH change due to ammonia production from arginine deamination). At the same time, quantitative analysis was performed using high-performance liquid chromatography (HPLC) with appropriate derivatisation or another suitable method like enzymatic assays specific for arginine. In initial cultures, S. aureus was confirmed as an L-arginine producer, yielding 6.78 mg/mL. Optimisation studies using Luria-Bertani (LB) broth and supplementation with 1 N HCl increased L-arginine production to 8.45 mg/mL. L-Arginine purification was achieved using ion-exchange chromatography, resulting in a final concentration of 9.434 mg/mL. Lyophilisation of the purified L-arginine solution yielded 1.85 g of a white-yellowish powder. The melting point of the purified L-arginine was determined to be 223–228°C.

Keywords: Antibacterial activity, Arginine production, *E. coli*, Burns, Ninhydrin reagent, Protein.

INTRODUCTION

Proteins, essential macromolecules for life, are constructed from amino acids. Nine proteinogenic L-amino acids are necessary for human and animal nutrition, with L-arginine

being conditionally crucial. This means that while usually synthesised by the body, L-arginine becomes essential under specific physiological stresses or developmental stages [1]. L-arginine (Arg or R) possesses a unique structure comprising an α-amino group (protonated $-NH^{3+}$ at physiological pH), an α-carboxylic acid group (deprotonated $-COO^{-}$ at physiological pH), and a guanidino side chain ($(CH_2)_4NH_2$)) [2]. L-arginine finds diverse applications in medicine, nutrition, pharmaceuticals, and biotechnological processes. Its involvement in nitric oxide (NO) synthesis makes it crucial for cardiovascular health, immune function, and neurotransmission [3]. Furthermore, L-arginine plays a key role in the urea cycle, facilitating the removal of ammonia, and is involved in the synthesis of creatine, polyamines, and other essential biomolecules [4]. Various microorganisms, including certain strains of staphylococci, can synthesise L-arginine. Staphylococci are a diverse group of bacteria inhabiting multiple environments, including human and animal skin and mucous membranes. While some species are commensal, others, like Staphylococcus aureus, are opportunistic pathogens. The ability of staphylococci to thrive in diverse niches contributes to their persistence and transmission [5]. Certain staphylococcal species utilise L-arginine through the arginine deiminase (ADI) pathway, converting it to ornithine and ammonia. This pathway contributes to pH homeostasis and biofilm formation, which is essential for bacterial survival and pathogenicity [6].

MATERIAL AND METHODS

Specimens Collection

Thirty-two swab samples were obtained from several Baghdad City hospitals. Five samples were taken from burns and other deep lesions to prepare each swab; the samples were then cleaned with 70% ethyl alcohol and slightly moistened with a small quantity of brain heart infusion media.

Isolation and Identification of Bacteria

The bacterial isolates were cultured on MacConkey agar, Eosin methylene blue agar (EMB), blood agar, and mannitol salt agar (MSA). They were then examined and identified using microscopy, biochemical tests, and the Vitek 2 system [7].

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Detection and screening of arginine production by bacterial isolates

The following procedure was employed to identify optimal arginine-producing isolates: Colonies inoculated into 10 mL of Luria broth and incubated at 37°C for 72 hours with shaking at 160 rpm on an orbital shaker. Bacterial growth was standardised to a 0.5 McFarland turbidity (equivalent to an optical density of approximately 0.08-0.13 at 620 nm) using a spectrophotometer before incubation. Following incubation, cultures were centrifuged at 6,000 rpm for 15 minutes to pellet cells. The resulting supernatant, containing the produced arginine, was collected in sterile flasks and stored at 4°C until further analysis. Arginine production was assessed using ninhydrin assays. A general ninhydrin reagent was used for initial detection

(purple indicates a positive result). A specific ninhydrin-ferric reagent was then used for quantitative analysis, comparing the arginine concentration in the samples to a standard curve. A high-producing isolate was selected for further characterisation based on the quantity of arginine produced. This isolate was subsequently used in additional experiments [8].

Determine arginine concentration

Arginine concentrations in the culture supernatants were determined spectrophotometrically using a specific ninhydrin-ferric reagent. This method offers improved specificity for arginine quantification compared to traditional ninhydrin assays, minimising interference from other amino acids and enabling accurate and reliable measurement of arginine in the fermentation broth [9]. The specific ninhydrin-ferric reagent was prepared as follows: Solution A was prepared by dissolving 7.3 g of ninhydrin in 245 mL of methylcellosolve. Solution B was prepared by dissolving 0.1 g of $FeCl_3$ in 1 L of citric acid- Na_2HPO_4 buffer (21 g/L citric acid, adjusted to pH 2.2 with 0. 0.1M Na_2HPO_4). Solutions A and B were then mixed. For the assay, 20 µL of supernatant from the Luria broth cultures or purified arginine standards were transferred to individual microplate wells (or test tubes). Each well received 180 µL of the prepared ninhydrin-ferric reagent. The plate (or tubes) was sealed and incubated at 105 °C for 40 minutes. After cooling to room temperature, 200 µL of dimethyl sulfoxide (DMSO) was added to each well. The absorbance of the reaction mixtures was measured spectrophotometrically at 480 nm. Arginine concentrations in the samples were determined using a calibration curve generated with standard arginine solutions (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/mL) [10].

Extraction of arginine

Arginine production typically involves a multi-step process, including fermentation, cell separation (usually by centrifugation), product separation, and purification using selected microbial isolates [11]. In this study, Luria broth was inoculated with the bacterial suspension and incubated at 37°C with shaking at 110 rpm for 72 hours (specifically for *S. aureus*, the pH was maintained at 7). After incubation, a suitable antibiotic was added, and the culture was shaken for an additional 15 minutes before centrifugation at 6,000 rpm for 15 minutes. The cell pellet was discarded, and the supernatant was collected in a sterile flask and stored at 4°C until purification [12]. Before purification, the pH of the supernatant was adjusted to 3 by adding 1 N HCl.

Purification of arginine

Arginine was purified by ion exchange chromatography, adapted from the method described by Wallace and Rochfort [13]. The ion exchange column was prepared: Approximately 60 g of Amberlite IR 120 resin was suspended in 500 mL of 1 N NaOH for 10

minutes, followed by repeated washing with distilled water. The resin was then resuspended in 500 mL of 1 N HCl for 10 minutes before being packed into a glass column (15 × 3 cm). The column was mounted vertically, ensuring no air bubbles were trapped within the resin bed. The crude arginine-containing supernatant was carefully loaded onto the column, allowing it to permeate the resin. After loading, the column was washed thoroughly with 500 mL of equilibration buffer to remove unbound positively charged compounds. Elution of bound compounds (negatively charged compounds) was performed with modifications to the original protocol. Initially, NaOH at pH 8.9 was used to elute positively charged amino acids with a pI lower than that of arginine (10.76). Arginine was then eluted using 212 mL of 1 N NaOH at pH 9.814 [14]. Eluted fractions of 5 mL were collected in serially numbered tubes at a flow rate of 5 mL per 5 minutes.

Antibacterial activity

The antibacterial activity of the purified arginine against Gram-negative and Gram-positive bacterial strains was assessed using the agar well diffusion assay [15, 16]. Approximately 20 mL of Mueller-Hinton (MH) agar was aseptically poured into sterile Petri dishes. Bacterial cultures were retrieved from stock cultures using a sterile loop [15]. After preparing bacterial lawns, wells (6 mm diameter) were created in the agar plates using a sterile pipette tip. Different concentrations of the purified arginine were then added to the wells. The inoculated plates containing the arginine samples and the test organisms were incubated overnight at 37°C. Following incubation, the diameters of the inhibition zones were measured and recorded. [17, 18].

RESULTS AND DISCUSSION

Isolation and identification of bacteria

Thirty-two swab samples from wound and burn infections were collected from various hospitals in Baghdad City. Bacterial cultures were positive in 6 of the 32 samples (18.75%), while 26 (81.25%) were negative. Primary cultures of Nutrient Agar were subcultured onto MacConkey Agar, Mannitol Salt Agar, and Blood Agar (Figure 1). Microscopic examination revealed that P. aeruginosa isolates were Gram-negative rods, S. aureus isolates were Gram-positive cocci in pairs or clusters (non-spore-forming), and E. coli isolates were Gram-negative rods occurring singly or in pairs (non-spore-forming). Biochemical tests (Table 1) showed that S. aureus was catalase- and coagulase-positive but oxidase-negative; P. aeruginosa was catalase- and oxidase-positive; and E. coli and S. epidermidis were catalase-positive but oxidase- and coagulase-negative. The predominant isolates were S. aureus (2 isolates, 33.34%), P. aeruginosa (2 isolates, 33.34%), E. coli (1 isolate, 16.67%), and S. epidermidis (1 isolate, 16.67%) (Table 2). Burns, defined as tissue damage from exposure to heat, sunlight, radiation, chemicals, or electricity, can range from mild to fatal. Previous studies have reported varying

distributions of bacterial species in burn infections. Al-Azzawi et al. [19] found S. aureus (15%) to be the most common, followed by A. baumannii (12%), P. aeruginosa (10%), K. pneumoniae (8.3%), E. coli (7.6%), P. mirabilis (6%), and B. cepacia (2.3%). Other studies [20–22] have also identified P. aeruginosa, E. coli, and Acinetobacter spp. as frequent isolates from burn wounds. [23] reported P. aeruginosa (48.1%) as the most prevalent, followed by E. coli (8.7%) and Klebsiella spp. (3.1%). In broader analyses, Staphylococcus spp. is commonly found in wounds (44.7%) and burns (31.3%). In one study of 163 isolates, 35% were from wounds, with Enterobacteriaceae sp. accounting for 44% and 56% of those isolates. Of the total 163 isolates, 14.1% were Pseudomonas sp., 3.1% were Acinetobacter sp., and 1.8% were Corynebacterium sp., with corresponding percentages from burn isolates being 52.2%, 40%, and 66.7% [24].

Detection and screening of arginine production by bacterial isolates

This study screened Staphylococcus aureus, P. aeruginosa, and E. coli isolated from wound and burn infections for arginine production. Initial screening employed a qualitative method using a general ninhydrin reagent to detect the presence of amino acids and/or their metabolic products (e.g., pH change due to ammonia production from arginine deamination), revealing that S. aureus showed a positive reaction. Quantitative analysis of arginine production by S. aureus was performed using a specific enzymatic assay. This analysis determined the arginine concentration produced by S. aureus. While [25] reported higher arginine yields in S. aureus, reaching 55.4 mg/mL, it is essential to note that differences in bacterial strains, growth media, and culture conditions can significantly influence amino acid production. The specific ninhydrin-ferric reagent used in this study minimises interference from other amino acids, allowing for more accurate quantification of arginine [30].

Production of arginine

After inoculation of Luria broth with Staphylococcus aureus suspension, the crude arginine concentration reached 6.621 mg/mL. The culture was then centrifuged at 6,000 rpm for 15 minutes. The supernatant, collected after 72 hours of incubation, was treated with 1 N HCl to decrease the pH to 3, preparing it for purification and resulting in an increased arginine concentration of 8.45 mg/mL[31].

Purification of arginine

Arginine was purified using ion exchange chromatography on an Amberlite IR-120 cation exchange column (15 x 3 cm) packed with 60 g of resin [32]. The supernatant containing crude arginine was loaded onto the column. Arginine, an essential amino acid with an isoelectric point (pI) of 10.76, was adsorbed onto the resin at a pH range of 1.5–6, carrying a positive charge. After washing the column to remove unbound substances, arginine was eluted using [specify the eluent used, e.g., NaOH at a specific concentration and pH, or a gradient elution method].

One hundred fractions (5 mL each) were collected, and the presence of arginine in each fraction was determined using qualitative (ninhydrin reagent) and quantitative assays. Analysis of the fractions revealed a single peak of purified arginine in fractions 32 to 51, with the highest concentration reaching 9.434 mg/mL (Figure 2). This purified arginine was obtained from the Staphylococcus aureus isolate. The collected arginine solution from these fractions was then lyophilised at -80°C for 22 hours, yielding 1.85 g of a white-yellowish powder, which was used for further characterisation studies [33].

Antibacterial activity of arginine

The antibacterial activity of purified arginine against S. aureus and E. coli was assessed using an agar well diffusion assay (Figure 3). Arginine exhibited moderate activity against S. aureus, with a zone of inhibition of 8 mm at 100 mg/mL, and minimal activity against E. coli, with zones of inhibition of 4 mm at both 50 and 100 mg/mL. While [34] reported higher inhibition zones for arginine (10 ± 0.5 mm for E. coli and 12 ± 0.1 mm for S. aureus) at a much higher concentration (200 mg/mL), direct comparison is difficult due to the different concentrations used in this study. While the exact mechanism of arginine's antibacterial activity is not fully understood, it may involve similar mechanisms to those reported for arginine, such as disruption of the bacterial cell wall [35, 36].

CONCLUSIONS

This study demonstrates arginine production from Staphylococcus aureus, highlighting the potential of microbial sources for producing this conditionally essential amino acid. The purification procedure utilising ion exchange chromatography proved effective in isolating arginine with high purity. The antibacterial assays demonstrated varying degrees of activity of purified arginine against S. aureus and E. coli.

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CONFLICT OF INTEREST

No conflicts of interest

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TABLES

Table (1): the biochemical result of bacteria isolates

Biochemical test	***S. aureus***	***P. aeruginosa***	***S. epidermidis***	***E. coli***
Mannitol salt agar	Yellow colonies	Non-growth	Red colonies	Non-growth
MacConkey agar	Non-growth	Pink colonies	Non-growth	Pale colonies
Gram stain	G+ve cocci	G-ve rod	G+ve cocci	G-ve cocci
Coagulase test	+	-	-	-
Catalase test	+	+	+	+
Oxidase test	-	+	-	-

Table (2): percentage of bacterial isolates

Type of isolates	**No. of isolates**	**Percentages of isolates**
S. aureus	2	5%
P. aeruginosa	6	20%
E. coli	5	15%
S. epidermidis	19	60%
Total	32	100%

Table (3): Purification steps of arginine.

The result	**OD at**	**Concentration**

	480nm	
Crude arginine	0,416	7,673 mg/ml
Arginine concentrate with 1N HCL	0,498	9,67 mg/ml
Ion exchange chromatography	0,577	10,632 mg/ml

FIGURES

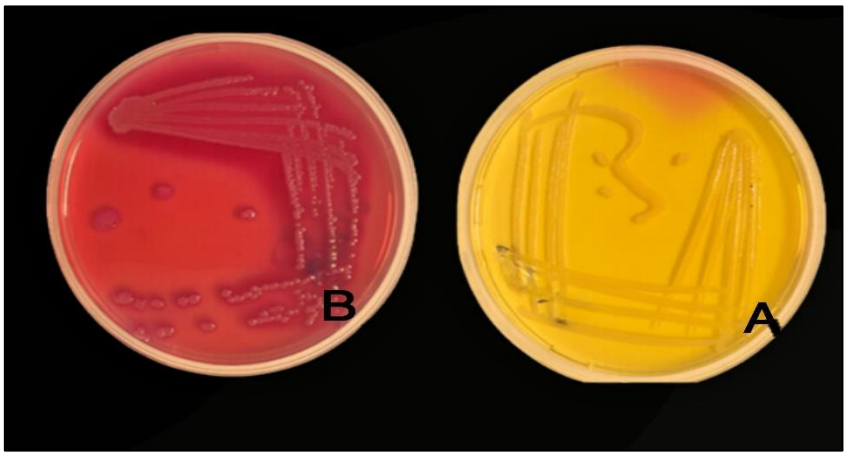

Fig. 1: A: *S. aureus* in mannitol salt agar; B: *E. coli* in MacConkey agar.

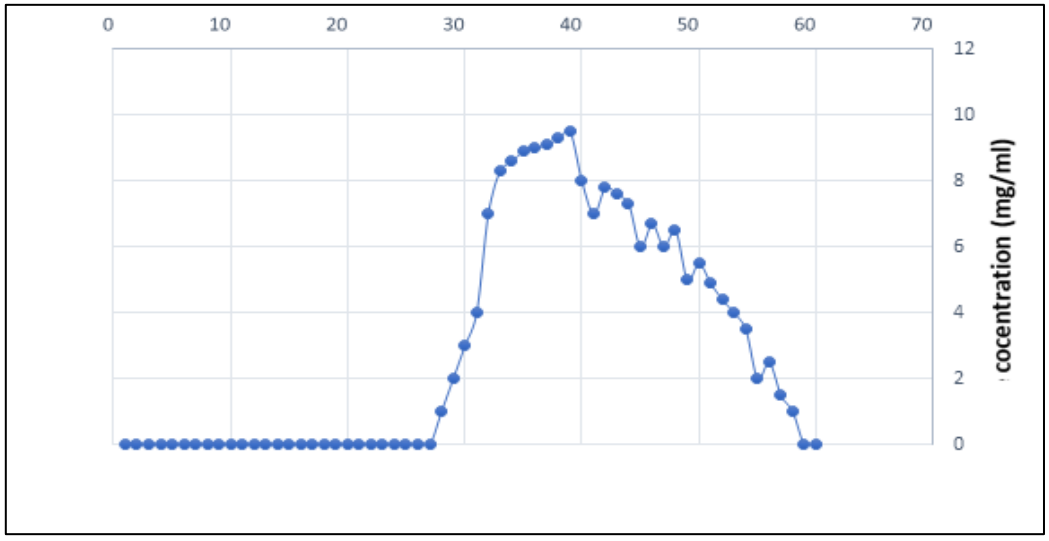

Fig. 2: Ion exchange chromatography of arginine produced by *S. aureus* using ambulate IR-120 column (3x5 dimension) using (NaOH, pH=9.8)for eluting arginine from another amino acid.

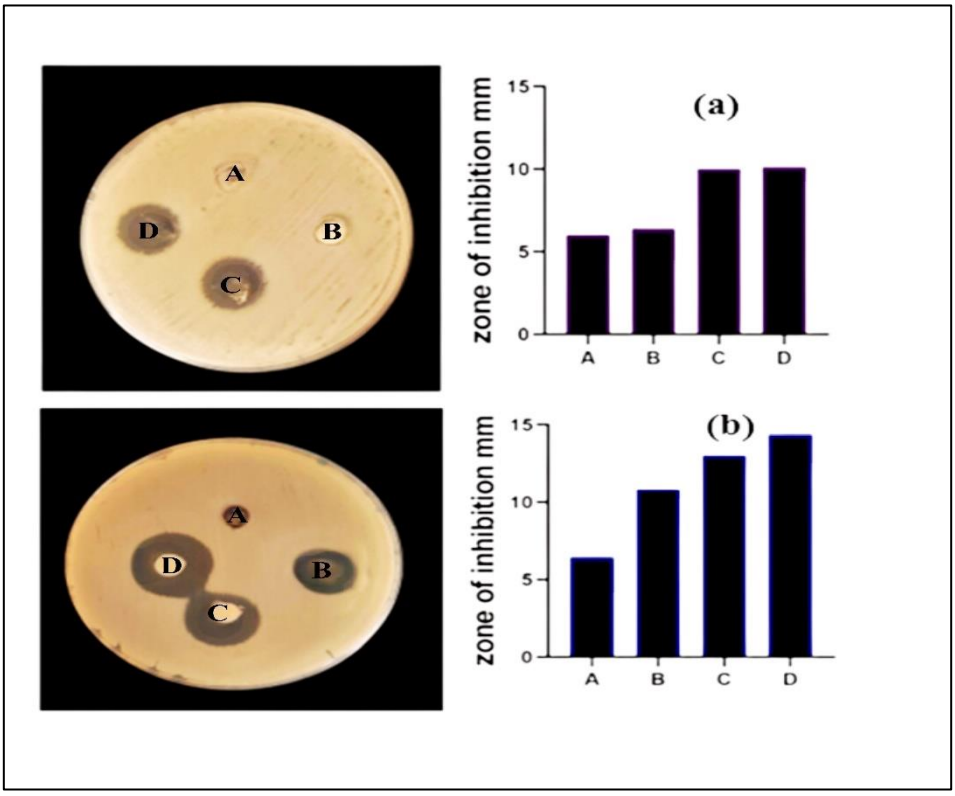

Fig.3: Antibacterial activity of arginine against (a: *E.coli* and b: *S. aureus*). A, Control. B, 25%. C, 50%. D, 100%.