International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 3, 2024
E-ISSN: 0974-4614
p-ISSN: 0972-0448

Extraction, purification and characterization of lysine from selected clinical isolate of Staphylococcus aureus and examination of its antibacterial activity against pathogenic bacterial species

Sheymaa S. Ismael[1], Entesar H. Ali[2], Iman I. Jabbar[3]

[1,2,3]Department of Science Division of Biotechnology, University of Technology, Baghdad, Iraq
Email:Shaaam.090@gmail.com[1], Entesar.H.Almosawi@uotechnology.edu.iq[2],
Iman.I.Alsaedi@uotechnology.edu.iq[3]

Received: 13.08.2024 Revised: 12.09.2024 Accepted: 18.10.2024

ABSTRACT
Lysine is an α-amino acid that is a precursor to many proteins. Human body cannot synthesize lysine, but there are organisms can produce it. Lysine plays several roles in humans. Therefore, this study aimed to extract and purify lysine from different bacterial pathogens. 20 samples, that collected from wound and burn infections, were obtained from Baghdad hospitals. Bacteria isolated culturally on different media, then examined and identified via microscopic, biochemical tests, the API system, and the Vitek2 system. The predominant bacteria being 2 (33.34%) isolates of Staphylococcus aureus, and 2(33.34%) isolates of Pseudomonas aeruginosa, followed by 1 (16.67%) isolates of Escherichia coli and 1 (16.67%) isolates of Staphylococcus epidermidis. Then, S. aureus, P. aeruginosa and E. coli, that isolated from skin, were utilized to detection of lysine production using qualitative method (ninhydrin reagent) and quantitative analysis (ferric ninhydrin reagent). Only S. aureus was produced lysine at 6.78 mg/ml. To produce lysine, S. aureus was inoculated in Luria broth and produce lysine at 6.621 mg/ml and it reaching 8.45 mg/ml using HCL (1 N). Ion exchange chromatography was utilized for lysine purification, whereas its concentration reaching 9.434 mg/mL. The collected lysine solution was lyophilized and yielded 1.85 grams of a white-yellowish powder. For characterization of lysine, Fourier-transform infrared spectroscopy (FTIR) and Ultraviolet–visible spectroscopy (UV-vis) were utilized. UV-Vis revealed a maximum absorbance (λ_{max}) of lysine at 300.77 nm. Zeta potential measurement of lysine was -46 mV, which determined using electrophoretic light scattering (ELS). Purified Lysine has a melting point at 271°C.

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

INTRODUCTION

Proteins, the most crucial macromolecules for the functioning of organisms including humans and animals, are built from the building blocks of amino acids. Nine of the twenty L-amino acids are required for proper nutrition in humans and other animals, and L-lysine is one of them. Animals can't get enough L-lysine from their food alone; it's a necessary amino acid [1]. The α-amino acid used in protein production is lysine, which may be represented by the symbols Lys or K. It comprises the following: the α-amino group, which is protonated $-NH^{3+}$ under biological circumstances, the α-carboxylic acid group, which is deprotonated $-COO^{-}$ under biological conditions, and a side chain lysis ($(CH_2)_4NH_2$) [2].

There is a wide range of possible uses for amino acids like lysine in the medical, culinary, pharmaceutical, and personal care sectors. Some biomaterials used in wound healing are synthesised using amino acids [3]. Since lysine is often involved in crucial protein structural processes, its most prevalent function is in protein production, also known as proteinogenesis. Additionally, lysine plays a significant role in the metabolism of fatty acids and the synthesis of structural proteins for connective tissues. Additionally, lysine has been associated with calcium homeostasis, renal retention, and intestinal absorption [4].

Lysine can be produced by different microorganisms, including S. aureus. Staphylococci are significant bacteria that have an impact on industries, ecosystems, and animal and human health. Their varied lives encourage their predominance in a variety of habitats, both pathogenic and nonpathogenic, where they may endure for brief or extended periods of time. This makes their removal and spread challenging. Since both species are the primary reservoirs, staphylococci may spread from these sources to food, colonising both human and animal skin, noses, and throats [5]. Significant lysine uptakes were seen during the stationary phase of S. aureus. This amino acid may be used by S. aureus in order to maintain cytoplasmic pH homeostasis, which may arise from a proton influx [6].

Hence, this study aimed to extract lysine and purify lysine from Staphylococcus aureus, in addition to characterize this enzyme using different techniques.

EXPERIMENTAL

Specimens Collection

Twenty swab samples were obtained from several Baghdad City hospitals, including AL-Shahid Ghazi Al-Hariri, which specializes in surgery, Baghdad Teaching Hospital, and others, to test for wound and burn infections. To prepare each swab, five samples were taken from burns and other deep lesions; the samples were then cleaned with 70% ethyl alcohol and slightly moistened with a little quantity of brain heart infusion media.

Isolation and Identification of bacteria

Bacteria isolated culturally on MacConkey agar, Eosin methylene blue agar (EMB), blood agar and mannitol salt agar (MSA), then bacterial isolates were examined and identified by microscopic (gram staining), biochemical tests (catalase, oxidase and coagulase tests), the API system, and the Vitek2 system [7].

.

Detection and screening of lysine production by bacterial isolates

The following procedures were followed in order to find the optimal isolate for lysine synthesis [8]: To activate the bacterial isolates, we took three or four colonies from the first culture and suspended them in a 10-milliliter (ml) test tube with Luria broth. Using a spectrophotometer, the turbidity was corrected to 0.5 at 620 nm according to the McFarland method. For 72 hours, each tube was incubated at 37°C on an orbital shaker set to 160 rpm in order to produce lysine. We used centrifugation at 6,000 rpm for 15 minutes to get rid of the cell pellets. After that, we collected the supernatant in a dry, sterile flask and kept it at 4°C until we needed it for an assay. The general ninhydrin reagent gives a purple colour for a positive result, and the specific ninhydrin-ferric reagent compares the concentration of lysine to a standard. The quantity of lysine generated allowed for the selection of a powerful hyper-producer of the amino acid from among several bacteria that were isolated. Additional research made use of the isolation.

Determine of lysine concentration

Lysine concentrations of the cultures in test tubes were detected spectrophotometrically using the specific ninhydrin-ferric reagent. Unlike the traditional ninhydrin reaction method, which measures the total amino acids, with the specific reagent, the interference of other amino acids could be excluded, and lysine in the fermentation broth could be determined accurately and reliably [9]. Solution of specific ninhydrin-ferric reagent: 7.3gm of ninhydrin was dissolved in 245-mLof methylcellosolve. 01gm of $FeCl_3$ was dissolved in 1 L of citric acid-Na_2HPO_4 buffer solution (21 g/L of citric acid, adjusted to pH 2.2 with 0.1M Na_2HPO_4). Then the solution A and B was mixed. 20µL was transferred from supernatants of Luria broth cultures or purified lysine to new test tubes containing 180µL of specific ninhydrin- ferric reagent in each well. The tubes were sealed and put in the oven at 105 °C for 40 min. The tube were cooled to room temperature, and 200 µL of dimethyl sulfoxide (DMSO) was added to each well. The reaction mixtures were measured spectrophotometrically at 480 nm. The concentrations of lysine were calculated as through a calibration curve obtained with the standard solution of lysine (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/ml) [10].

Extraction of lysine

Lysine is mainly produced by using selected isolates, which comprises the multi-step process including fermentation, cell separation by centrifugation, product separation, and purification [11]. Inoculated Luria broth with bacterial suspension and incubated in the shaker at 110 rpm at 37°C for S. aureus, pH was adjusted to 7 for 72 hours. After incubation, the suitable antibiotic was added and the shaker for 15 minutes, then centrifuged (6000 rpm for 15 minutes). The sediment is neglected, and the supernatant was taken and collected in a dry and sterile flask at 4°C until use in purification [12]. Then added (1N HCL) to decrease pH to 3 and become ready to purified.

Purification of lysine

Purification of lysine by ion exchange chromatography according to Sousa and Queiroz [13]. The preparation of the ion exchange chromatography column has been prepared by the following steps: The exchange resin Amberlite IR 120 was used in this experiment. About 60 gm of the resin was suspended with 500 ml of 1N NaOH, for 10 minutes then suspension was washed several times with distilled water. Re-suspended the resin with 500 ml of 1N HCl, for 10 minutes, and then resin is used for loading the glass column dimension (15×3 cm). The column was fixed to the stand in a vertical position, taking care not to allow air bubbles. Loading the sample (supernatant crude lysine) on to the column, the sample was let lose into the column, so that it slowly penetrates into the ion- exchange resin. After loading the column, it was thoroughly washed with 500 ml of equilibrating buffer so that all the unbound compounds (positive charge compounds) were washed off. Using the

elution phase with some modifications, the compounds of the extract (negative charge compounds) that were bound to the column were eluted begin using (NaOH pH = 8.9) to elute all positive charge amino acids that bound to the column that have PI less than the PI of lysine 9.74. Then eluted for lysine by using 212 mL (1N NaOH pH = 9.814) [14]. The eluent fractions were collected in 5 mL in serially numbered tubes. The flow rate of the column was adjusted to 5 ml/5 minutes.

Characterization of lysine

UV- Visible Spectroscopy, FTIR, and melting point were used to detect the purification of lysine produced by S. aureus.A UV-Visible spectrophotometer (Metertechsp 8001), in the range of 200–800 nm, was used to study the optical properties of lysine [15].The FT-IR 8400S (Shimadzu-FTIR) spectrophotometer was used to analyze lysine in the 4000 cm-1 to 400 cm-1 range [16].The Stuart melting point apparatus (SMP30) was used to measure and record the temperatures of crystalline samples (lysine) held within capillary tubes. These tubes were accommodated in an illuminated chamber within the aluminum block. The tubes were viewed through a magnifying lens on the front of the unit. All controls were via the membrane keypad. The temperature range was from ambient to 400°C. Temperatures on the LCD screen. When the melt cycle was started, once the melt had occurred, they stopped the melt cycle, and the cooling was automatic to ambient temperature [17].

Antibacterial activity

The antibacterial potential of the lysineagainst Gram's negative and Gram's positive bacterial strains using agar well diffusion assay [18, 19]. About 20mL of on Muller–Hinton (MH) agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop (18). After culturing the organisms, 6 mm- diameter wells were bored on the agar plates using of a sterile tip. Into the bored wells, different concentrations of the samples were used. The cultured plates containing lysine and the test organisms were incubated overnight at 37°C before measuring and recording the average the zones of inhibition diameter [20, 21].

RESULTS AND DISCUSSION

Isolation and identification of bacteria

Twentyswab samples from wound and burn infection were collected from different hospitals in Baghdad City including AL-Shahid Ghazi Al-Hariri for specialist surgery, Baghdad Teaching Hospital. Bacterial culture was positive in 6 of 20 cases obtained from swab samples with a percentage of 30%. Whereas, the number of isolates that gave a negative result was 70%. All samples that were primarily obtained by cultured on Nutrient Agar were then sub-cultured on MacConkey Agar, Mannitol Salt Agar, Blood Agar (figure 1).Microscopical inspection of P. aeruginosa isolates using the gram stain revealed cells with gram-negative single rod shapes. In the instance of S. aureus, Gram stain revealed cocci that were organized in pairs or clusters and were non-spore forming . Microscopy of E. coli revealed that it is a gram-negative bacterium that is rod-shaped, aggregates singly or in pairs, and does not produce spores were matched to the microscopic morphology.

The biochemical test results were summarized in Table (1), and indicated that S. aureus were positive for coagulase and catalase, while they were negative for oxidative test. P. aeruginosa were positive for catalase and oxidase. E. coli and S. epidermidis were positive for catalase and negative for oxidase and coagulase.The predominant bacteria being 2 (33.34%) isolates of S. aureus, followed by 2(33.34%) isolates of P. aeruginosa, then 1 (16.67%) isolates of E. coli and 1 (16.67%) isolates of S. epidermidis shown in table (2).

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	33.33%
P. aeruginosa	2	33.33%
E. coli	1	16.67%
A. baumannii	1	16.67%
Total	6	100%

Burns are defined as tissue damage resulting from extended exposure to intense heat, sunlight, radiation, chemicals, or electric currents. Burns may range from mild to potentially fatal. In study conducted by Al-Azzawi et al. [22], Staphylococcus aureus (15%) was the most common bacterium identified, followed by A. baumannii (12%), P. aeruginosa (10%), Klebsiellapneumoniae (8.3%), Escherichia coli (7.6%), Proteus mirabilis (6%), and Burkholderiacepacia (2.3%). A more recent study [23] indicated that P. aeruginosa, E. coli, and Acinetobacter spp. were the most common bacteria isolated from infected burn wounds, with the lowest percentages being recorded for these two species. Additional researchers also found something similar [24, 25]. Al-bayati and Ali[26] indicated that the most prevalent bacterium found in patients was Pseudomonas aeruginosa (48.1%), followed by E. coli (8.7%), and Klebsiella spp. (3.1%).

Of the organisms detected in wounds and burns, Staphylococcus sp. is the most common, accounting for 44.7% of all wound microbes and 31.3% of burn microbes. The results showed that 57 out of 163 (35%) were from wounds, 25 (44%), and 32 (56%). These results were related to Enterobacteriaceae sp. Additionally, out of the total 163 isolates, 14.1% belonged to Pseudomonas sp., 3.1% to Acinetobacter sp., and 1.8% to Corynebacterium sp.; from burns, the corresponding percentages were 52.2%, 40%, and 66.7% [27].

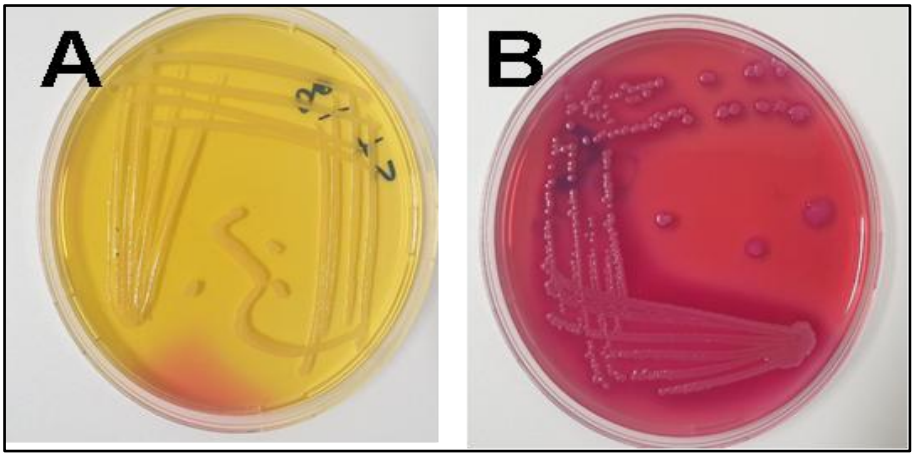

Fig. 1: A: S. aureus in mannitol salt agar; B: E. coli in MacConkey agar.

Detection and screening of lysine production by bacterial isolates

In this study, Staphylococcus aureus, P. aeruginosa and E. coli, that isolated from skin, were used to detect and lysine production. The first method involved qualitative detection using the general ninhydrin reagent after activating the isolates with Luria broth, revealing that only Staphylococcus aureus produced lysine. The second method involved quantitative analysis using ferric ninhydrin reagent, determining that the lysine concentration produced by Staphylococcus aureus was 6.78 and 5.82 mg/ml respectively. The study by Hato [28] indicate the screening results for identifying the most effective bacterial isolates for lysine production indicated that S. aureus isolates were capable of producing lysine more than E. coli Notably, the S. aureus isolates demonstrated a significantly higher yield of lysine, with producing reach to 55.4 mg/mL.

The purple color indicates the presence of lysine, and the colorless indicates the absence of lysine. While yellow color was only ninhydrin reagent. Ninhydrin reacts with amino acids, producing distinguishable colors after heating. The assay's mechanism involves the reaction of two ninhydrin molecules (2, 2-dihydroxyindane-1, 3-Dione) with a free alpha-amino acid from lysine, leading to a purple color upon heating. This process breaks the bond to liberate the free alpha-amino acid from lysine [29]. At elevated temperatures, ninhydrin acts as an oxidizing agent, causing deamination and decarboxylation of the amino acids. The reaction sequence includes the condensation of reduced ninhydrin molecules, released ammonia, and a second ninhydrin molecule, ultimately forming a purple-colored diketohydrin complex [30].

Production of lysine

After inoculation of Luria broth with Staphylococcus aureus suspension for the crude lysine shown concentration reach to 6.621 mg/ml, then centrifuged (6000 rpm for 15 minutes). The sediment is neglected, and the supernatant was 72 hrs. taken then added (1N HCL) to decrease pH to 3 and become ready to purified and increase the concentration to 8.45 mg/ml.

Purification of lysine

Purification was carried out using ion exchange chromatography on an Amberlite IR-120 ion exchange column. A total of 100 fractions, each containing 5 mL of elution, were collected. The presence of lysine in each fraction was determined using both qualitative and quantitative assays. The purification process revealed a single peak in fractions numbered 32 to 51, with the lysine concentration reaching 9.434 mg/mL. This concentration was achieved from the Staphylococcus aureus isolate figure 2 and table 3 .

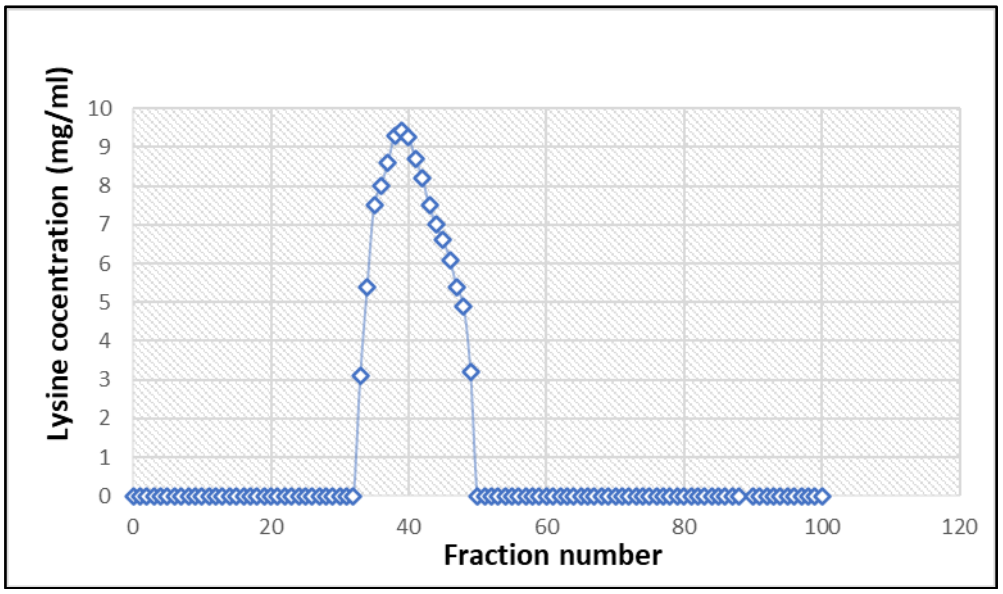

Fig. 2: Ion exchange chromatography of lysine produced by S. aureus using ambrlite IR-120 column (3x5 dimension) by using (NaOH , pH=9.8)for elute lysine from other amino acid.

Table 3: Purification steps of lysine.

The result	OD at 480nm	Concentration
Crude lysne	0,403	6,621 mg/ml
Lysine concentrate wih 1N HCL	0,506	8,45 mg/ml
Purification of lysineby ion exchange chromatography	0,561	9,434 mg/ml

Column chromatography was one of the most significant and used method available for the separation and chemical analysis of complicated organic mixtures. By loading the mixture into the column packed with adsorbent material, the separation and purification process can be considered as the typical polar sorbent. It has been used to separate and purify steroids, lipids, amino acids, and colorants [31].

Purification of lysine was carried out according to [32] by column chromatography using a clear glass column with dimensions of 15x3 cm packed with amberlite IR120 (60g), which is unique for column chromatography. The supernatant from the extraction of lysine was poured into the column, pooled, and passed through the column.

A strong acid cation resin, amberlite IR120, was used to adsorb lysine as a cationic species. Lysine, being a basic amino acid, has an isoelectric point at pH 9.74. During the adsorption stage, the free biomass cultivated medium was contacted with the resin at a pH range of 1.5–6, where lysine carried a positive charge. This allowed the adsorption of lysine onto the strong cationic resin. The separation conditions, including pH, eluent concentration, and lysine adsorption isotherms, were evaluated in the adsorption system. Lysine solutions were contacted with resin in tubes, and the amount of free lysine was then measured using both qualitative and quantitative assays [28].

Lyophilization of lysine

After purifying lysine using an Ambrlite IR-120 ion exchange column, the peak with the highest lysine concentration was collected. The collected lysine solution was then subjected to lyophilization using a lyophilizer device, operating at -80°C for 22 hours. This process yielded 1.85 grams of a white-yellowish powder. The resulting lysine powder used for applications and characterization studies.

Characterization of lysine

The FTIR spectrum of lysine reveals several characteristic peaks corresponding to various functional groups present in the molecule figure 3. Peaks at 512 cm^{-1} and 546 cm^{-1} were attributed to C-C and C-N skeletal vibrations, respectively. The 685 cm^{-1} and 780 cm^{-1} peaks were associated with N-H out-of-plane bending and

wagging vibrations, confirming the presence of primary amines. The peaks at 862 cm^{-1} and 899 cm^{-1} correspond to C-H bending and N-H wagging vibrations, respectively. C-N stretching is observed at 1099 cm^{-1}, while symmetric and asymmetric CH2 bending vibrations are seen at 1336 cm^{-1} and 1420 cm^{-1}. The peak at 1565 cm^{-1} peak related with N-H bending [33], and the peak at 1625 cm^{-1} attributed to C=O stretching in the carboxylate group [34]. Less common peaks at 2114 cm^{-1} and 2219 cm^{-1} might be due to overtones or combination bands involving C-H stretching and C≡N stretching vibrations, respectively. Finally, the broad peak at 3260 cm^{-1} indicates N-H stretching vibrations [35].

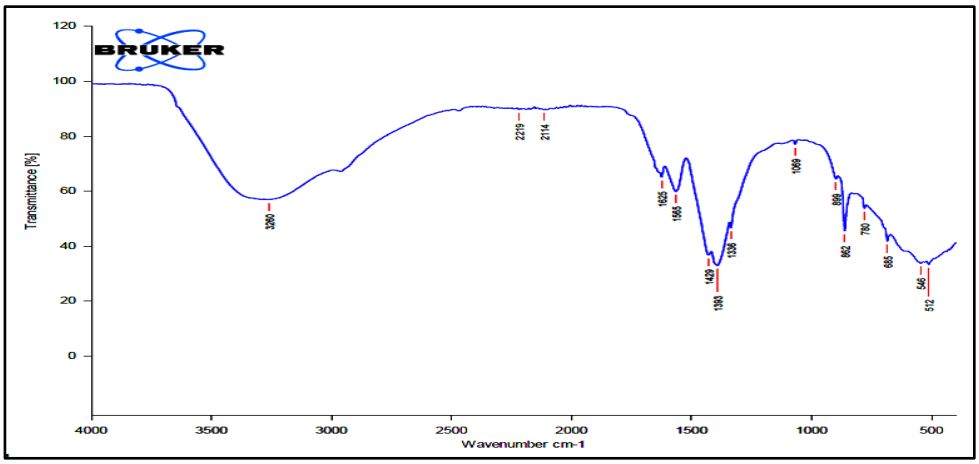

Fig.3: The FTIR analysis of lysine.

The UV-Vis detection spectrophotometry of lysin in this study revealed a maximum absorbance (λmax) at 300.77 nm with an absorbance value of 2.45 ABS shown in figure 4.

The results align with previous studies, the variability occurs may be due to variations in sample preparation and environmental conditions. Stagi and their collagous which demonstrated the absorbance band at 278 nm due to aggregation formation and intermolecular interactions, suggesting comparable interactions in this study [36]. Stagi [37] observed that the UV-Vis spectra showed the presence of two more overlapping absorption bands at higher wavelengths, around 275-300 and 265-310 nm respectively. This result aligns closely with the findings of [38] noted near-UV absorption peaks for lysine residues around 275-300 nm, aligning with our λmax and suggesting similar interactions within lysin molecules. While Ansari [39] observed a characteristic peak at 202 nm for poly-L-lysine, with no absorbance between 300 and 400 nm, indicating no advanced glycation end products, contrasting with study findings possibly due to different structural conformations.

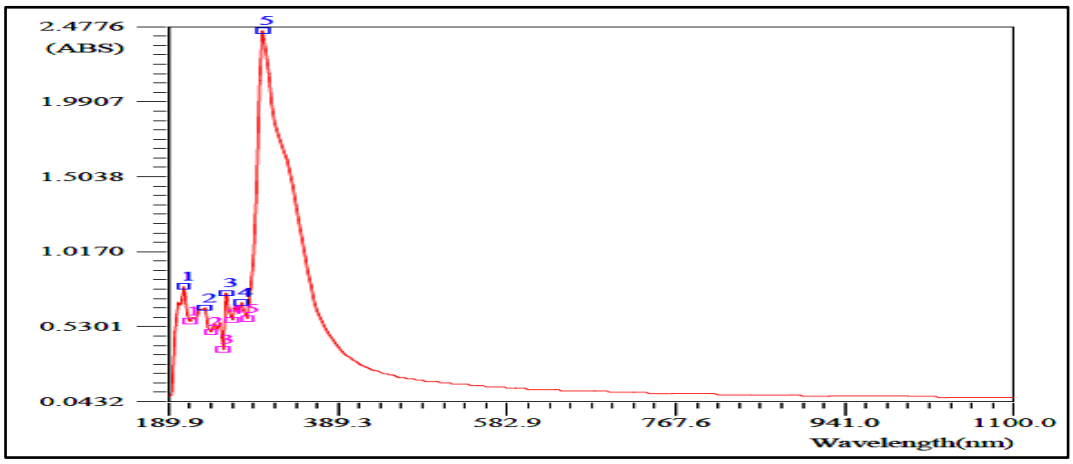

Fig.4: UV-Vis spectra of lysine.

The zeta potential measurement of lysine was determined to be -46 mV using electrophoretic light scattering (ELS) (figure 5). This negative zeta potential value suggests that lysine molecules are negatively charged under the measured conditions, likely due to deprotonation of amino groups at a specific pH level. The observed value of -46 mV indicates that lysine dispersions are highly stable, as zeta potential values greater than +30 mV or less than -30 mV typically denote good colloidal stability due to electrostatic repulsion. This stability is significant for pharmaceutical formulations, where controlling the zeta potential through pH adjustments can enhance

formulation stability, and in protein interaction studies, where the negative charge of lysine can influence its interactions with other positively charged molecules. Wiącek and Chibowski found the zeta potential results varying of lysine between -10 and -85 mV. And have stability at ph 6.8 after 60 min in ethanol with the zeta potential reach to -48 [40]. in studies by [41] where the zeta potential of lysine was measured in solutions of varying pH, the values ranged from positive at lower pH levels to more negative as the pH increased above the isoelectric point. This propensity is unique to amino acids and reflects their amphoteric nature which allows them to operate as both acids and bases depending on the pH of the environment [42].

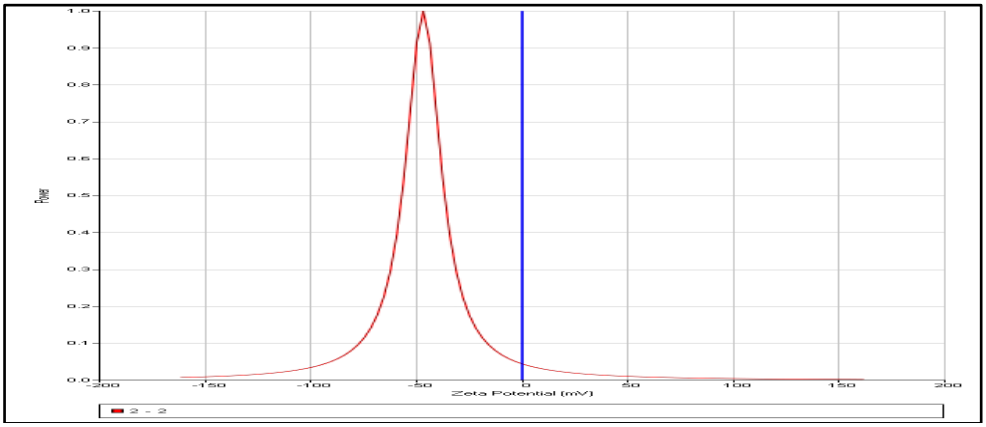

Fig.5: The zeta potential of lysine.

Melting point of lysine result indicate that purified Lysine from S. aureus has a melting point at 271°C. the thermal characteristic is important to understanding stability and activity of lysine within various temperature condition specially when derived from microorganisms. Hato et al. [28]indicate the melting point for purified lysine from Staphylococcus aureus was 268 °C. Do et al. [43]shown that amino acids form crystalline solids with high melting point from 200°C to 300°C. This high melting point is owing to their zwitterionic structure, resulting in strong ionic bonds which require an excessive amount of energy to break down [44] indicate melting points of lysine was reaching to 260.91 °C.

Antibacterial activity of lysine

As shown in figure (6), lysine exhibited moderate activity against S. aureus was 8 mm at 100% and minimal effect on E. coli (4 mm at 50% and 100%), aligning with earlier studies on its efficacy against Gram-positive bacteria. Li et al. [45] reported the diameters of inhibition zones of E. coli (10 ± 0.5 mm) and S. aureus (12 ± 0.1 mm) treated with 200 mg/ml lysine were the minimum inhibition concentration of lysine against both E. coli and S. aureus was determined to be 12.5 mg/ml. Additionally, scanning electron microscopy revealed that lysine caused noticeable damage to the morphology of the tested bacterial cells. [46] suggested that peptidoglycan in cell wall of S. aureus could be dramatically damaged by high concentration of lysine thus causing cell wall more fragile. [47] reported the antibacterial activity of lysine against E. coli and S. aureus with inhibition zone between 7-20 mm.

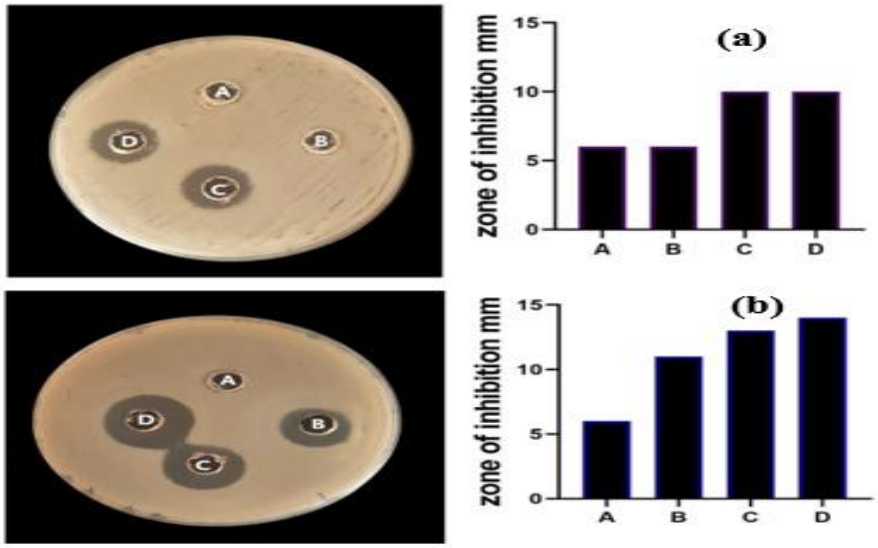

Fig.6: Antibacterial activity of Lysine against (a: E.coli and b: S. aureus). A, Control. B, 25%. C, 50%. D, 100%.

CONCLUSIONS

It is worth noting that lysine can be successfully produced from Staphylococcus aureus, which shows that microbial sources may be used to provide essential amino acids in a sustainable and economical way. This technology is vital for several industrial and medicinal uses, and the purification procedure utilizing ion exchange chromatography shows that it is successful in isolating very pure lysine. The lysine-producing enzyme's structural and functional characteristics may be better understood using FTIR and UV-Vis analysis. This information is vital for figuring out how it reacts in various environments and for improving manufacturing procedures. Research suggests that lysine has different levels of antibacterial action against S. aureus and E. coli.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to all those who contributed to the successful completion of this research.

CONFLICT OF INTEREST

There Are No Conflicts Of Interest Between Authors.

REFERENCES

1. Hou Y, Wu G. Nutritionally essential amino acids. Advances in Nutrition. 2018;9(6):849–51.
2. Lopez MJ, Mohiuddin SS. Biochemistry, essential amino acids. In: StatPearls [Internet]. StatPearls Publishing; 2024.
3. Oliveira C, Sousa D, Teixeira JA, Ferreira-Santos P, Botelho CM. Polymeric biomaterials for wound healing. Front BioengBiotechnol. 2023; 11:1136077.
4. Hall CJ, da Costa TPS. Lysine: biosynthesis, catabolism and roles. WikiJournal of Science. 2018;1(1):1–8.
5. Omran ZH, Hussen AK, Yahya AM, Mohammed DA, Rawdhan HA, Ahmed HM, Sattar RJ, Hussen YA, Jasm BH, Sadoon NM. Copper Nanoparticles Against Two Types of Bacteria Staphylococcus Aureus and Escherichia Coli. Journal of Nanostructures. 2024 Jul 1;14(3):780-8.
6. Alreshidi MM, Dunstan RH, Macdonald MM, Gottfries J, Roberts TK. The uptake and release of amino acids by Staphylococcus aureus at mid-exponential and stationary phases and their corresponding responses to changes in temperature, pH and osmolality. Front Microbiol. 2020;10:3059.
7. Jasim BH, Ali EH. Isolation, extraction, purification, and characterization of fibrinolytic enzyme from Pseudomonas aeruginosa and estimation of the molecular weight of the enzyme. Archives of Razi Institute. 2021 Oct 1;76(4):809-20.
8. MOOSAVI-NASAB, M.; ANSARI, S.; MONTAZER, Z. Fermentative production of lysine by Corynebacterium glutamicum from different carbon sources. Iran Agricultural Research, 2008, 25.1.2: 99-106.
9. Alissianto YR, Sandriani ZA, Rahardja BS. The effect of amino acid lysine and methionine addition on feed toward the growth and retention on mud crab (Scylla serrata). In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2018. p. 012059.

10. Wang J, Yang J, Shi G, Li W, Ju Y, Wei L, et al. Transcriptome profiles of high-lysine adaptation reveal insights into osmotic stress response in Corynebacterium glutamicum. Front BioengBiotechnol. 2022;10:933325.
11. Zhou G, Ruhan A, Ge H, Wang L, Liu M, Wang B, et al. Research on a novel poly (vinyl alcohol)/lysine/vanillin wound dressing: Biocompatibility, bioactivity and antimicrobial activity. Burns. 2014;40(8):1668–78.
12. Isobe K, Matsui D, Asano Y. Comparative review of the recent enzymatic methods used for selective assay of L-lysine. Anal Biochem. 2019;584:113335.
13. Sousa A, Sousa F, Queiroz JA. Impact of lysine-affinity chromatography on supercoiled plasmid DNA purification. Journal of Chromatography B. 2011;879(30):3507–15.
14. Chen Z, Liu G, Zhang J, Bao J. A preliminary study on L-lysine fermentation from lignocellulose feedstock and techno-economic evaluation. Bioresour Technol. 2019;271:196–201.
15. Yang H, Ludewig U. Lysine catabolism, amino acid transport, and systemic acquired resistance: what is the link? Plant Signal Behav. 2014;9(7):e28933.
16. Ahire K, Kumar D, Ali A. Differential glycation of arginine and lysine by glucose and inhibition by acesulfame potassium. Journal of BioScience and Biotechnology. 2018;7(1):11–5.
17. Lukasheva E V, Makletsova MG, Lukashev AN, Babayeva G, Arinbasarova AY, Medentsev AG. Fungal enzyme l-lysine α-oxidase affects the amino acid metabolism in the brain and decreases the polyamine level. Pharmaceuticals. 2020;13(11):398.
18. Abdula AM, Mohsen GL, Jasim BH, Jabir MS, Rushdi AI, Baqi Y. Synthesis, pharmacological evaluation, and in silico study of new 3-furan-1-thiophene-based chalcones as antibacterial and anticancer agents. Heliyon. 2024 Jun 15..
19. Khashan KS, Abdulameer FA, Jabir MS, Hadi AA, Sulaiman GM. Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2020;11(3):035010.
20. Jihad MA, Noori FTM, Jabir MS, Albukhaty S, AlMalki FA, Alyamani AA. Polyethylene glycol functionalized graphene oxide nanoparticles loaded with nigella sativa extract: a smart antibacterial therapeutic drug delivery system. Molecules. 2021;26(11):3067.
21. Mohammed MKA, Mohammad MR, Jabir MS, Ahmed DS. Functionalization, characterization, and antibacterial activity of single wall and multi wall carbon nanotubes. In: IOP Conference Series: Materials Science and Engineering. IOP Publishing; 2020. p. 012028.
22. Al-Azzawi MH, Alkalifawi EJA. Detection of Bacteria Causing Burn Infection Isolated from Several Hospitals in Baghdad. Ibn AL-Haitham Journal For Pure and Applied Sciences. 2023;36(3):1–8.
23. Ghafil JA, Flieh M. Isolation and Identification of Bacterial Burn Wound Infection in Iraqi Patient. Indian Journal of Forensic Medicine & Toxicology. 2021;15(4).
24. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, et al. Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-years retrospective study. Antibiotics. 2021;10(10):1162.
25. Al-Saffar MF, Jarallah EM. Isolation and characterization of Pseudomonas aeruginosa from Babylon province. Biochem Cell Arch. 2019;19(1).
26. Al-bayati LH, Ali HHM. Aerobic pathogenic bacteria pattern of burn infection and their antimicrobial susceptibility (2017 to 2019) in Al-Kut city, Iraq. Biochem Cell Arch. 2020;20(2).
27. Khudhair MK, AlAubydi MA. Determination the prevalence and antimicrobial susceptibility of bacteria isolated from burns and wounds. Iraqi Journal of Agricultural Sciences. 2023;54(1):93–9.
28. Hato AF. Use of purified lysine from local pathogenic isolates of Escherichia coli and Staphylococcus aureus in some biological applications. Mustansiriyah University; 2022.
29. Yemm EW, Cocking EC, Ricketts RE. The determination of amino-acids with ninhydrin. Analyst. 1955;80(948):209–14.
30. Das S. Recent applications of ninhydrin in multicomponent reactions. RSC Adv. 2020;10(32):18875–906.
31. Ali EH, Mohammed KR. Extraction, purification and characterization of peroxidase from pseudomonas aeruginosa and utility as antioxidant and anticancer. Baghdad Sci J. 2019 Oct 1;16(4).
32. Robatjazi SM, Shojaosadati SA, Karbasy SM. Purification of L-lysine in simulated moving bed and fixed-bed chromatography. Se Pu. 2004;22(4):431–4.
33. Gavali, L. V., Mohammed, A. A., Al-Ogaili, M. J., Gaikwad, S. H., Kulkarni, M., Das, R., & Ubale, P. A. (2024). Novel terephthalaldehyde bis (thiosemicarbazone) Schiff base ligand and its transition metal complexes as antibacterial Agents: Synthesis, characterization and biological investigations. Results in Chemistry, 7, 101316.
34. Suzuki M, Yumoto M, Shirai H, Hanabusa K. Supramolecular gels formed by amphiphilic low-molecular-weight gelators of Nα, Nε-Diacyl-L-lysine derivatives. Chemistry–A European Journal. 2008;14(7):2133–44.

35. Rozenberg M, Shoham G. FTIR spectra of solid poly-l-lysine in the stretching NH mode range. Biophys Chem. 2007;125(1):166–71.
36. Stagi L, Farris R, de Villiers Engelbrecht L, Mocci F, Carbonaro CM, Innocenzi P. At the root of l-lysine emission in aqueous solutions. Spectrochim Acta A Mol Biomol Spectrosc. 2022;283:121717.
37. Stagi L, Sini M, Carboni D, Anedda R, Siligardi G, Gianga TM, et al. Modulating the poly-l-lysine structure through the control of the protonation–deprotonation state of l-lysine. Sci Rep. 2022;12(1):19719.
38. Homchaudhuri L, Swaminathan R. Near ultraviolet absorption arising from lysine residues in close proximity: a probe to monitor protein unfolding and aggregation in lysine-rich proteins. Bull Chem Soc Jpn. 2004;77(4):765–9.
39. Ansari NA, Moinuddin, Ali R. Physicochemical analysis of poly-L-lysine: An insight into the changes induced in lysine residues of proteins on modification with glucose. IUBMB Life. 2011;63(1):26–9.
40. Wiącek A, Chibowski E. Stability of oil/water (ethanol, lysozyme or lysine) emulsions. Colloids Surf B Biointerfaces. 2000;17(3):175–90.
41. Smith MC, Crist RM, Clogston JD, McNeil SE. Zeta potential: a case study of cationic, anionic, and neutral liposomes. Anal Bioanal Chem. 2017;409:5779–87.
42. Guckeisen T, Hosseinpour S, Peukert W. Isoelectric points of proteins at the air/liquid interface and in solution. Langmuir. 2019;35(14):5004–12.
43. Kamble, S. A., Barale, S. S., Mohammed, A. A., Paymal, S. B., Naik, N. M., & Sonawane, K. D. (2024). Structural insights into the potential binding sites of Cathepsin D using molecular modelling techniques. Amino Acids, 56(1), 33.
44. Meira RZC, Biscaia IFB, Nogueira C, Murakami FS, Bernardi LS, Oliveira PR. Solid-state characterization and compatibility studies of penciclovir, lysine hydrochloride, and pharmaceutical excipients. Materials. 2019;12(19):3154.
45. Li YQ, Han Q, Feng JL, Tian WL, Mo HZ. Antibacterial characteristics and mechanisms of ε-poly-lysine against Escherichia coli and Staphylococcus aureus. Food Control. 2014;43:22–7.
46. Tan Z, Shi Y, Xing B, Hou Y, Cui J, Jia S. The antimicrobial effects and mechanism of ε-poly-lysine against Staphylococcus aureus. Bioresour Bioprocess. 2019;6(1):1–10.
47. Vibhute A, Nille O, Kolekar G, Rohiwal S, Patil S, Lee S, et al. Fluorescent carbon quantum dots functionalized by poly l-lysine: efficient material for antibacterial, bioimaging and antiangiogenesis applications. J Fluoresc. 2022;32(5):1789–800