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The effect of purified lysine from *Staphylococcus aureus* and their effected on human blood clotting

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ABSTRACT— L-lysine is one of nine necessary amino acids for human and animal nutrition. In animals, Llysine is considered necessary and must be supplied from dietary sources. Two hundred and fifty clinical specimens were collected from different clinical sources. Twenty-two isolates (8.8%) were identified as *Staphylococcus aureus*. The ability of *S. aureus* isolates to produce lysine was examined qualitatively and quantitatively. The results showed that all isolates under study had the ability to produce lysine at different concentrations. The optimal conditions for the production of lysine were tested, in which 37 °C was the best temperature. The optimum pH was 7.4. The optimum incubation period for lysine production was 72 hours. The purification was done using affinity ion exchange chromatography on amberlite IR-120. The total amount of purified lysine was 473 mg/L. The effect of lysine on human blood clotting (13 coagulation factors) may be on coagulation factors (XII, XI, IX, VIII, and X) of the intrinsic coagulation pathway. Also, lysine may be on extrinsic coagulation factors (VII) and common coagulation factors (X, V, 1, and II), inferred by PT, INR, PTT, and fibrinogen tests.

KEYWORDS: purified lysine, blood clotting, nutrition

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) has a spherical shape and is Gram-positive. It is salt tolerant and facultatively anaerobic. It may grow in mannitol-salt agar medium with 7.5% of sodium chloride and give positive hemolysis, coagulase, and catalase reactions but negative oxidase reactions. It is non-spore-forming, non-motile, and rarely capsulated [29].

Amino acids are the building blocks of proteins, which are the most critical macromolecules for human and animal function. L-lysine is one of the 9 necessary amino acids for human and animal nutrition. L-lysine is one of the 20 L-amino acids present ecumenically in most living species [8]. L-lysine is used as a drug, a chemical agent, a food ingredient, and a feed additive. Its popularity has risen significantly in recent years, as well as several hundred thousand tons of L-lysine are generated each year, virtually completely by microbial fermentation. Animals require L-lysine, which must be supplied from dietary sources [1].

Synthetic polypeptides were studied in vitro and in vivo for their impact on rabbit blood coagulation. The basic L-lysine polypeptides increased the time it took for whole blood to coagulate increased the time it took for prothrombin to clot, and negated the action of heparin [25].

2. Materials and methods

2.1 Isolation of bacteria

Based on cultural, morphological, and biochemical tests, 22 *S. aureus* isolates (8.8) were isolated from 250 clinical specimens. These characteristics include colonial morphology and the size of the colony. The

bacterial isolates were then analyzed and identified using a variety of methods, including culture, microscopic examination, biochemical testing, the API system, and the Vitek2 system [15].

2.2 Detection of lysine production by isolated bacteria

Bacterial isolates were activated by picking 3 colonies of each isolate from the original culture and suspending them into a test tube containing 10ml of Luria broth (For preparation of Luria broth medium was done according to [23]. Fermentation medium had the following composition: 100g of glucose, 5g of CaCO3, 1g of KH2PO4, 1g of 9 (NH4) in 950ml of distilled water, 0.4g of MgSO4.7H2O, 0.002g of MnSO4.H2O, 0.002g of FeSO4.7H2O, 0.0005g of biotin, and 0.0005g of calcium pantothenic acid were dissolved, and the volume was completed to one litter with distilled water and then sterilized by autoclave). The turbidity was adjusted to McFarland at 620 nm and measured by using a spectrophotometer. All tubes incubated at 37°C in a 130 rpm orbital shaker for 72 hours to observe the production of lysine. The centrifugation was done at 6,000 rpm for 15 minutes to remove the cell pellets and the supernatant was taken and collected in a dry and sterile flask at 4 °C until used for the assay method of detection of lysine.

2.2.1 Qualitative detection of lysine

A) General ninhydrin assay

General amino acid detection was accomplished by adding one ml of supernatants to lysine production in a tube with 200 ul of ninhydrin reagent (ninhydrin reagent was prepared according to [15], by dissolving 0.2 grams of ninhydrin in 10 ml of acetone).

B) Thin layer chromatography (TLC)

According to [22], the TLC plates of silica gel 20×20cm were used. The preparing solvent, which contained N-butanol, acetic acid and water (5:3:2, v/v/v), was standardized and poured into the chromatography tank, which was saturated with a mobile phase. The sample was spotted about 1 cm from the margin of the silica sheet, and left for about 5 minutes at room temperature to dry, and then immersed the silica gel (about 1 cm) in the prepared solvent. After that, Ninhydrin is sprayed over the silica gel and placed in the oven at 105 °C for five minutes of spot vision. The Rf value of chromatography was observed in the TLC plates. The isolated lysine was estimated using the following equation:

$$\mathbf{Rf=}\ \frac{\boldsymbol{Distance\ of\ sample}}{\boldsymbol{Distance\ of\ mobile\ phase}}$$

2.2.2 Determine of lysine concentration (quantitative analysis)

Description	Solutions	Procedure
Using a special ninhydrin-ferric reagent, the lysine contents of a culture in 96 well plates have been measured spectrophotometrically. Dissimilar a standard ninhydrin method that measures total amino acids, the particular reagent allowed for the elimination of other amino acids, allowing for the accurate	**A)** 3.7g of ninhydrin was dissolved in 245-mLof methylcellosolve. **B)** 10g of $FeCl_3$ was dissolved in 1000 ml of citric acid- Na_2HPO_4 buffer solution. Then a solution A and B was mixed.	**A)** Luria broth cultures or pure lysine supernatants (20µl per well) were transferred to fresh 96 well plates containing 180 L of particular ninhydrin-ferric reagent for each well. **B)** A plates have been sealed and baked at 106°C for 45 minutes. **C)** The plates were chilled to room temperature before each well was filled with 200 mL dimethyl sulfoxide. **D)** At 480nm, reaction mixtures have been spectrophotometrically quantified. **E)** Lysine concentrations were estimated

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Volume 140, Issue 01, April, 2022

and reliable determination of lysine in fermentation broth [31].		using a calibration curve generated using a standard lysine solution (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/ml) as previously described

2.2.2.1 Prepared of Standard Curve for lysine

- Serial concentrations of lysine (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/ml) were prepared by dissolving lysine powder with PBS.
- The absorbency of each concentration was measured by the specific ninhydrin-ferric reagent prepared (in section 2.2.2).
- The absorbance was plotted against the corresponding concentrations of lysine as shown in figure (2.1).

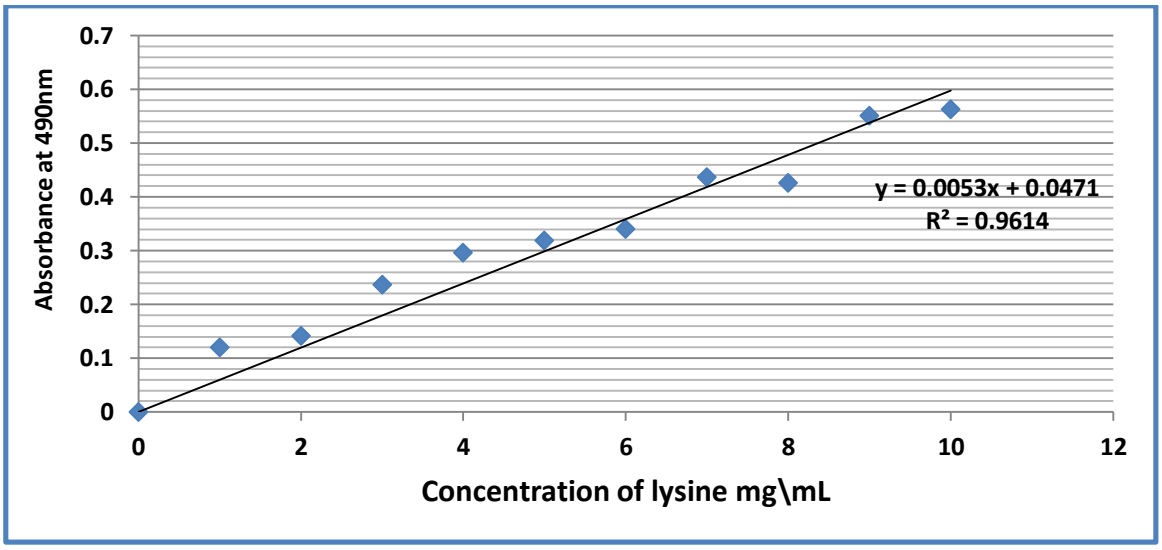


Figure (2.1) Standard Curve for lysine

2.3 Optimization of lysine production

A- Inoculum preparation

The selected isolate of S. aureus was activated by picking 3-4 colonies from the original culture and suspending them into 5ml of Luria broth (2.2). Using a spectrophotometer, the turbidity was adjusted to obtain approximately 0.5 McFarland standard suspensions at 620nm.

2.3.1 Optimal incubation temperature

In section A, a bacterial suspension was prepared and incubated for 72 hours at various temperatures (28, 31, 34, 37, 40, and 43) to determine the lysine concentration.

2.3.2 Optimal incubation periods

Staphylococcus aureus was inoculated with bacterial suspension (prepared in section A) and incubated at 37°C. Bacterial suspensions were incubated in different incubation periods (24, 48, 72, and 96 h) to determine the optimum incubation period for lysine production. A lysine concentration was determined.

2.3.3 Optimal pH values

Luria broth was prepared with different pH values (5.4, 6.4, 7.4, 8.4, and 9.4). pH was adjusted by using an electronic pH-meter and 1N of NaOH solution or 1N of HCl solution. The media were prepared, autoclaved and inoculated with the inoculums (prepared in section A), and then incubated at 37°C at 72 hrs. A lysine

concentration was determined.

2.4 Extraction of lysine

Lysine is mostly produced by fermenting selected isolates, which is a multi-step process that includes fermentation, cell separation via centrifugation, product separation, and purification. Inoculated with bacterial suspension (prepared in section A 2.3) and incubated for 72 hours in a shaker at 110 rpm at 34°C for E. coli pH 7.4. After incubation, the antibiotic was added and the shaker for 15 minutes, then centrifuged (6000 rpm for 15 minutes). The sediment is neglected, and the supernatant is taken and collected in a dry and sterile flask at 4°C until used in purification.

2.5 Purification of lysine

Affinity ion exchange chromatography was prepared according to the method described by [18] in table (2.1).

Table (2.1): Steps of preparation affinity ion exchange chromatography

Steps	Description
Solutions	A solution of sodium hydroxide (NaOH 1N, pH=14). It was prepared by dissolving 20 grams of sodium hydroxide in 500 milliliters of distilled water. B. A solution of hydrochloride acid (HCl 1N, pH=2). The solution was prepared by diluting 41 mL HCl in 500 mL distilled water.
Column preparation	**A)** Amberlite IR 120, a cation exchange resin, was employed in this experiment. With 500 ml of 1N NaOH, 60 g of resin was suspended, for 30 minutes and, then suspension was washed several times with distilled water. **B)** Re-suspended the resin with 500 ml of 1N HCl, for 30 minutes, then washed several times with D.W., and then resin is used for loading the glass column dimension (15×3 cm). In a vertical position, the column is fastened to a stand. The resin in a loading buffer has been poured into column, with care taken to avoid air bubbles.
Sample preparation	One liter of supernatant (prepared in section 2.4), which was adjusted to pH=3, was used in this experiment.
Loading the Column	The column cock was progressively opened and HCl above a resin bed had been allowed to drain before placing a sample onto a column. The sample was released loose into the column once the buffer level reached the column bed, allowing the sample to gently penetrate into ion-exchange resin. The column's flow rate was changed to 5 ml/5 minute. Following the loading of a column, it was carefully washed with 500 mL of water to remove all unbound compounds.
Elution	The compounds of the extract that were bound to the column were eluted by using NaOH (prepared in section 2.5.1. A). The eluant fractions were collected in 5 ml aliquots in serially numbered tubes.

2.6 Characterization of lysine

UV- Visible Spectroscopy and, powder XRD were used to detect the purification of lysine produced by *S. aureus*.

2.6.1 Scanning UV-VIS Spectroscopy

Scanning UV- Visible spectrophotometer (Metertech sp 8001), in a range of 200-800 nm was used to study optical properties for lysine.

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

2.6.2 Powder X-Ray Diffraction (XRD)

Powder X-ray diffraction (XRD-7000 Shimadzu Maxima-a) 40 KV volume and a current of 20 Ma is used to identify the crystalline phases and estimate the crystalline size. The XRD patterns were recorded in 2 s in the range of 10r–60r by step scanning, employing a Cu tube with a wavelength of Cu 1.54 A [7].

2.7 Study the effect of purified lysine on blood clotting

2.7.1 Blood sample collection

I) Blood samples from various groups were collected from healthy volunteers among graduate students at Mustansiriyah University's College of Science.

II) Blood specimens were collected in sodium citrate tubes (for coagulation factor tests) and also in lysine tubes for coagulation factor tests.

3. Results and discussion

3.1 Isolation and Identification of Staphylococcus aureus

Twenty-two of the *S. aureus* (8.8) isolates were isolated from different clinical sources, including: urinary tract infections, diarrhea, burn swabs, and wound swabs, as shown in table (3.1), from a total of 250 specimens.

Table (3.1): Number and percentage of bacterial isolates

Source of isolated	NO. of *S. aureus* isolates	% according to the total NO. of isolates *S. aureus*
Urine	10	45.45
diarrhea	4	18.18
Burn swab	5	22.73
wound swab	3	13.64
Total	22	100

3.2 Detection of lysine production by Staphylococcus aureus

3.2.1 Qualitative detection of lysine

A) General Ninhydrin Test

After final heating, ninhydrin produces a variety of distinct colors with amino acids, as shown in figure (3.1). The mechanism of this assay was based on the fact that after heating, two molecules of ninhydrin react with free alpha-amino acid (form lysine) to produce the purple after heating [4].

B) Thin layer chromatography (TLC)

During lysine purification from the S. aureus isolate, Rf was determined and the result was approximately 0.19. This matches with standard lysine, in figure (3.2).

The identification of amino acids is critical for determining the presence of amino acids in a variety of natural products. There are several methods for the determination of amino acids in biological samples. Thin layer chromatography was an important method for the identification of amino acids by various spray reagents. One of these reagents, ninhydrin, was the most popular because of its remarkably high sensitivity. However, ninhydrin produces the same purple/violet color as most amino acids [26].

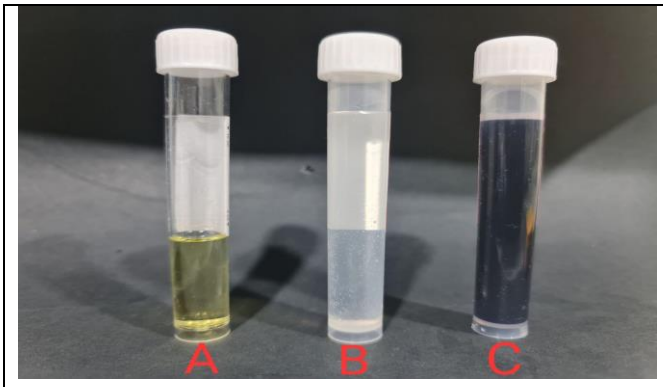


Figure (3.1): General Ninhydrin Test
A) Ninhydrin reagent
B) Negative result
C) Positive result

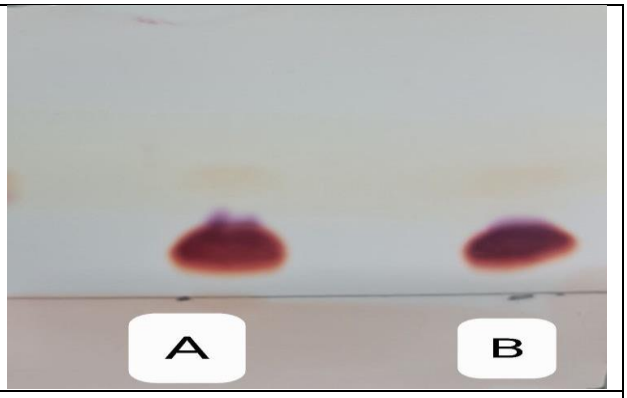


Figure (3.2): The separation and detection of lysine purified from *S. aureus* isolate by thin layer chromatography
A (purified lysine), B (standard lysine)

3.2.2 Quantitative lysine detection by ninhydrin-ferric reagent

Figure (3.7) shows the result of lysine infractions. The yellow color indicates the absence of lysine, while the dark-purple color indicates the presence of lysine in that fraction. The concentrations of lysine were calculated against the lysine standard curve, as shown in figure (3.3). The highest purified lysine was 41.5 mg/mL, the highest 89 for *S. aureus*.

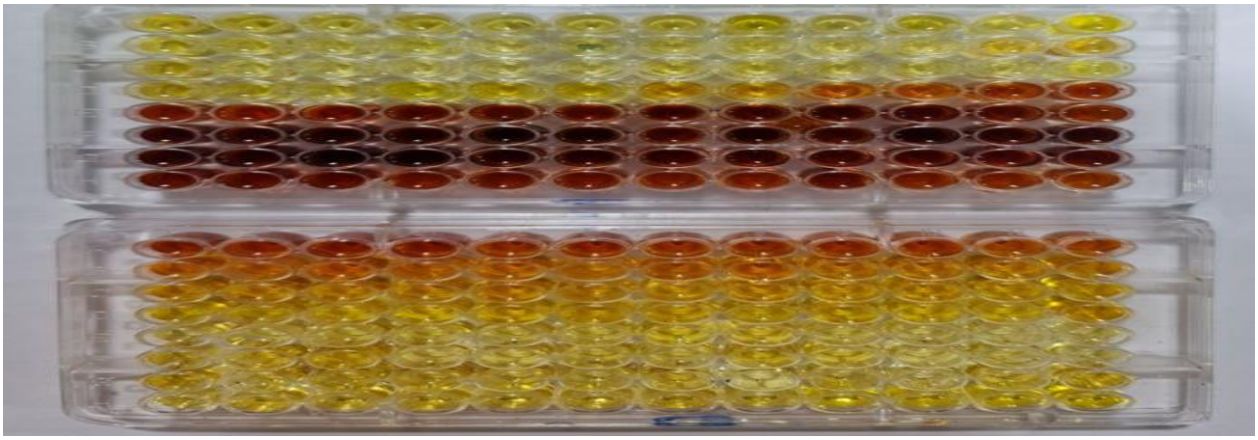


Figure (3.3): Concentration of lysine by using Ninhydrin-ferric reagent

In quantitative and qualitative amino acid analysis, the reaction of ninhydrin with the main amino group to create the colored molecule has been widely used [28]. Ninhydrin-ferric reagent reacts highly and specifically with lysine at pH values of between (1- 2.2). At pH values between 7.0 and 8.0, the ninhydrin-ferric reagent interacts strongly and selectively with lysine (1 - 2.2). A reaction of ninhydrin with ornithine, proline, arginine, glycine, & histidine is inhibited by ferric ion. Without dilution, this approach can be used to quantify the content of lysine in culture broth [10]. The ninhydrin color method was a widely used amino acid determination method that can perform qualitative and quantitative analysis of free amino acids. By enhancing the classic ninhydrin method, the specific detection of lysine was obtained. The ninhydrin ferric lysine complex is dissolved by adding DMSO, which also increases the linearity of the ninhydrin ferric reagent to lysine. This approach is unaffected by sugar, urea, or ammonia [9].

3.3 Optimization of lysine production

3.3.1 Temperature

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

Different temperature grades were used for the production of lysine and it was between 28 and 43 °C, as shown in figure (3.4), The optimum temperature for the production of lysine was 37 °C (55.4 mg\mL).

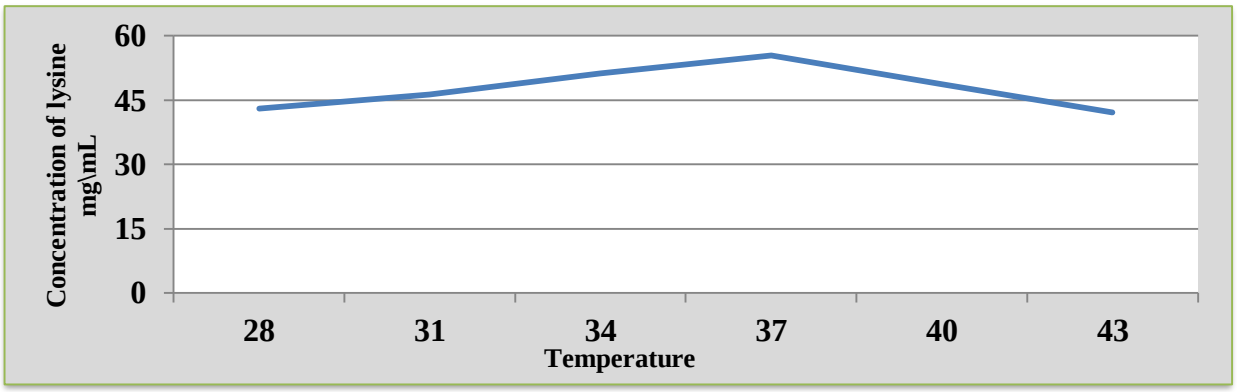


Figure (3.4): Production of lysine from *S. aureus* grown at various temperatures

Acidity (pH), temperatures, and other environmental conditions affect the rate of bacterial development. Bacteria have a lot of similarities in their environments. Bacteria flourish under ideal development circumstances, but if those factors are disrupted, stress can result in decreased or halted growth, dormancy, or death. An important idea in food preservation is to maintain sub-optimal growing conditions [27].

Two variables determine when an organism's development ends at low temperatures: 1) reaction rates for specific enzymes in the organism drop dramatically, and 2) low temperatures limit a fluidity of a cytoplasmic membrane, interfering with transport systems [17]. High temperatures cause structural cell components to denature and heat-sensitive enzymes to inactivate. While the rate of growth increases as the temperature rises, it tends to fall fast after that, until the temperature reaches its maximum [3].

[6] reported that the optimal temperature for lysine production was 30˚C, for C. glutamicum. [14] reported that C. glutamicum grew at an optimal temperature of 30 ◦C for the production of lysine.

3.3.2 pH

A wide variety of media pH values from 5.4 to 9.4 were used to determine the best pH for the production of lysine. It appears that the highest productivity at pH=7.4 was 51.2 mg/mL, as shown in figure (3.5).

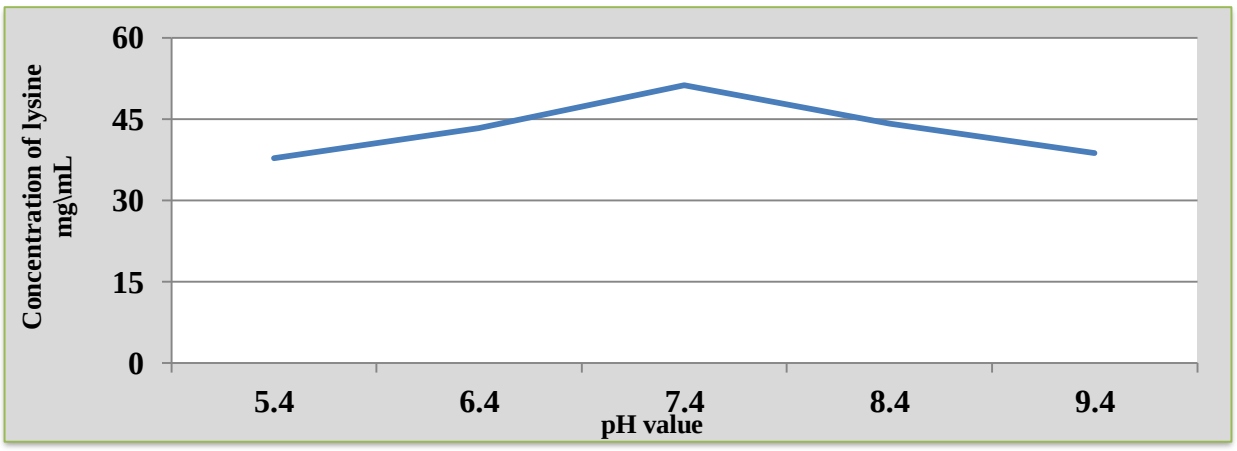


Figure (3.5): Production of lysine from S. aureus grown at different pH value

Changes in medium pH have a significant impact on nutrients uptake, which aids in growth & metabolite production [20]. *S. aureus* can grow in pH ranges ranging from 4.2 to 9.3, with an optimum pH of 7-7.5 [11]. [6] found a pH=7.6, which was an optimized value for lysine production from C.glutamicum. These results could be attributed to the fact that these isolates favored this pH for metabolism. Furthermore; the optimal pH for production is not necessarily the same as the optimum growth pH. Within 48 and 72 hours of incubation, a decrease in production was observed. This may occur due to reduced microbial growth and cell death, which is associated with the depletion of available resources required for growth [13].

3.3.3 The time of incubation

The incubation period of S. aureus for a period of 72 hours at 37°C was given. The optimum incubation period for lysine production was 53.2 mg/mL, as shown in figure (3.6).

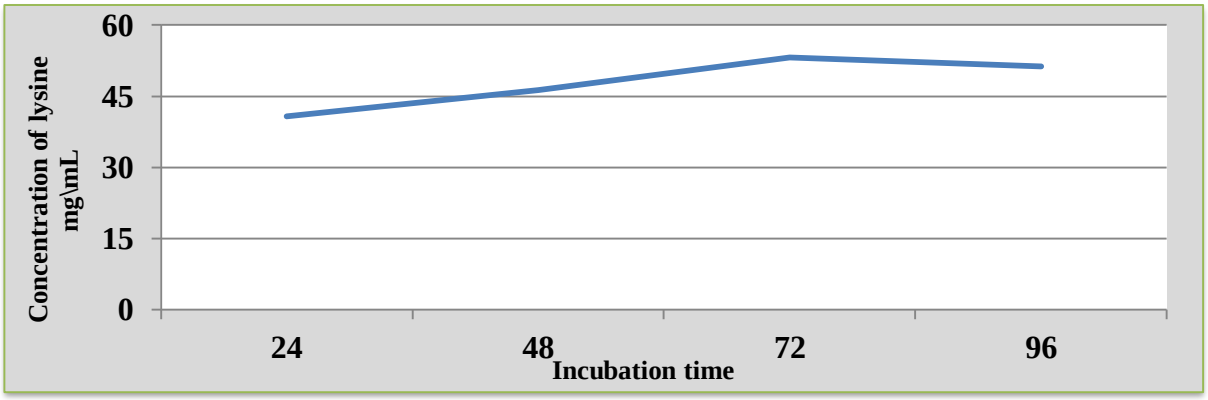


Figure (3.6): Production of lysine from *S. aureus* grown at different incubation period

Biosurfactant-producing bacteria [16] can be affected by factors such as incubation period, temperature, and pH. [5] mentioned lysine-producing strains that produce large amounts of the amino acid. The bacterium identified as B.megaterium (SP14) accumulated a lysine yield of 3.56 mg/ml in a broth culture in 96 h.

[6], C. glutamicum showing the highest production of lysine (8.2 mg/mL) was selected for optimization of nutritional and physical parameters for the highest production of lysine in a shake flask. The optimized values of physical requirements were an initial pH of 7.6, a 30 C temperature, 300 rpm, and a 60-h incubation time.

3.4 Extraction of lysine

Staphylococcus aureus isolates (SA8) were grown in a shaking incubator in 1000 ml of Luria broth prepared as described in Section (2.2) and incubated at 37 °C for 72 hours with pH 7.4. Bacterial cell debris was removed by centrifugation at 6,000 rpm for 15 min. Then the supernatant (which contained crud lysine) was collected for the purification step.

3.5 Purification of lysine by affinity ion exchange chromatography

After purification by ion exchange chromatography on an Ambrlite IR-120 ion exchange column, 100 fractions were collected, each of which contained 5 ml of elution. The presence of lysine was calculated for each collected fraction by qualitative and quantitative assay. Fractions numbered 85 to 90 showed the highest lysine levels, reaching between 40.28 to 41.5 mg/ml. Moreover, fractions numbered under 74 and above 94 don't have lysine, so it was neglected as shown in figure (3.7). The total amount of purified lysine was 473 mg/L

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

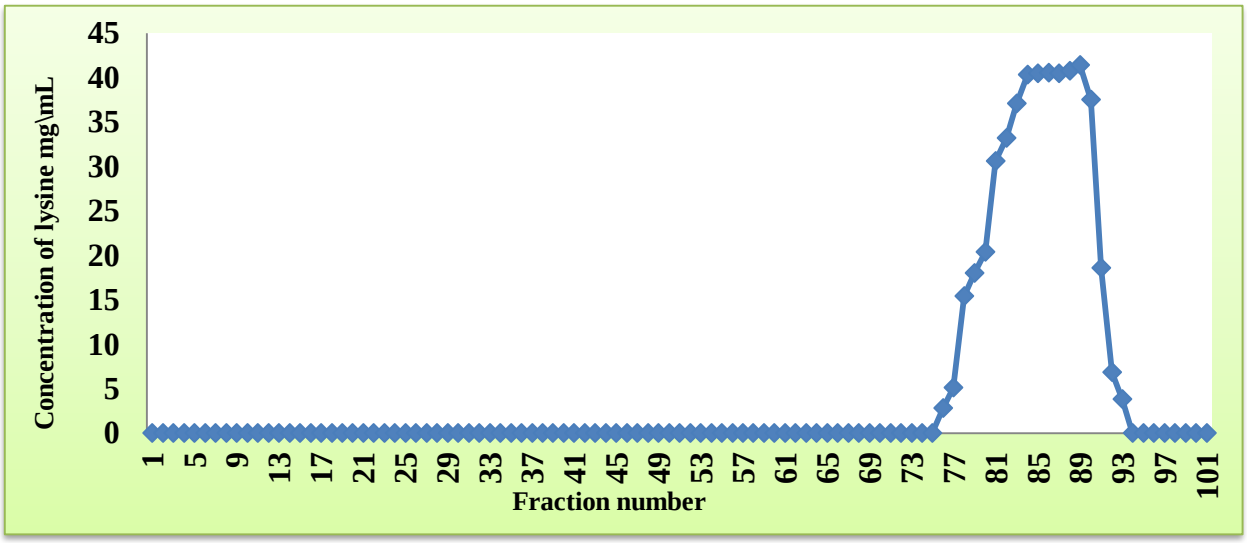


Figure (3.7): Affinity ion exchange chromatography of lysine produced by S. aureus using ambrlite IR-120 column (3 × 15). The flow rate was a 60 mL / hrs, 5 mL per tube; at temperature 25°C

One of the most significant and commonly used procedures for separation and chemical analysis of complex organic mixtures was column chromatography. A separation and purification procedure can be compared to that of a standard polar sorbent by feeding the mixture onto a column filled with adsorbent material. Steroids, lipids, amino acids, and colorants have all been separated and purified using it [21].

The bacteria that create lysine through fermentation in a bioreactor will be used to purify the lysine bacteria, which will involve bio-separations and ion-exchange chromatography columns. When a buffer alters the pH of the column, converting the charge on lysine to a neutral charge, the lysine will attach to a negatively charged column and release from it [34].

Purification of lysine was carried out according to [23] using a column chromatography utilizing clear column glass with dimensions (15x3 cm) packed in amberlite IR120 (60g), which is unique for column chromatography, and then supernatant (from lysine extraction) was poured in the column, pooled, and passed through the column.

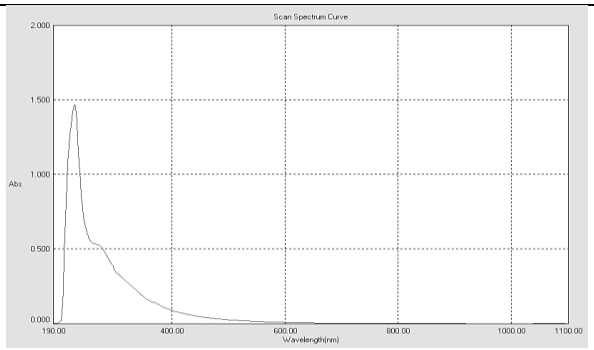
Amberlite IR 120, a strong acid cation resin, was employed for adsorb lysine as cationic species at low pH. Lysine is just a basic amino acid with a pH of 9.74 as its isoelectric point. During the adsorption step, resin was connected with free biomass grown medium at pH 1.5 – 6, where lysine had a positive charge. The adsorption of lysine onto to the strong cationic resin was enabled as a result of this. In an adsorption system, the separation conditions were investigated, including pH, eluent concentration, and lysine adsorption isotherms. The amount of free lysine was tested after the lysine solutions were treated with resin in tubes (Qualitative and Quantitative assay). The current results showed that the optimal pH for adsorption was in the range of 1 to 4. Desorption can be achieved by changing the pH to 14 by using NaOH solution as an eluent.

3.6 Characterization of lysine

3.6.1 Scanning Ultraviolet-visible (UV-Vis) spectroscopy

To confirm the diagnosis of the purification of lysine produced by *S. aureus*, purified lysine and standard lysine used as controls were examined by a UV spectrometer with a wavelength of 228 nm, as shown in

figure (3.8).



S. aureus **Standard lysine**

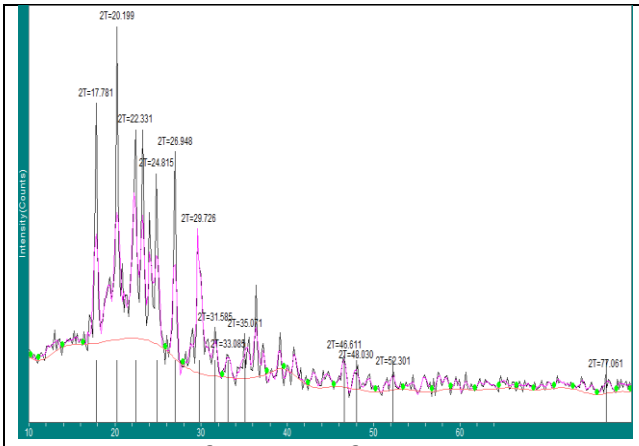
Figure (3.8): UV-VIS spectra of lysine produced by *S. aureus* at 0.1 mg\mL in D.W, detected at 228 nm

For diverse sorts of compounds, UV-VS spectroscopy is simple, quick, rapid, sensitive, and selective. For particle characterisation of colloidal suspensions, just a brief length of time is necessary for measurement, and calibration is not required [30]. Thermal research by [12] demonstrated that the produced crystals of L-lysine hydrochloride dihydrate are thermally stable. With a UV cut-off wavelength of 228 nm, an optical transmittance spectrum reveals that the material has high optical transparency across the visible range. The poly-lysine, on the other hand, has random coil-like far-UV absorption spectra with a peak at 202 nm [2].

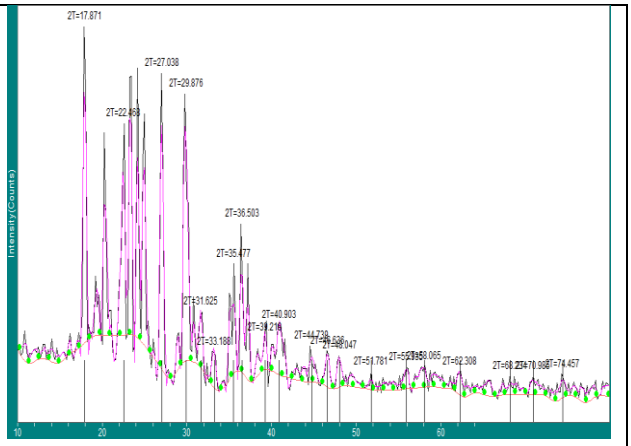
3.6.3 Powder X-ray diffraction (XRD)

Examined the purified lysine using powder X-ray diffraction (XRD), the obtained XRD pattern of the purified lysine was further confirmed by the presence of a characteristic peak in the XRD image Figure (3.9). XRD spectra showed that the structure of lysine has 17 distinct diffraction peaks at 12.105°, 20.269°, 24.827°, 26.738°, 29.738°, 31.567°, 35.129°, 36.441°, 40.854°, 43.168°, 44.819°, 46.481°, 47.954°, 49.573°, 51.611°, 55.935°, and 58.385° (2 range, 12–60°) to lysine face centered cubic (FCC) planes (020), (120), (112), (022), (122), (131), (131), (122), (233), (233), (233), (241), (204), (204), 251), (251), and (342), respectively for purified lysine from S. aureus. Compared with standard lysine with 20 distinct diffraction peaks at 17.871°, 22.468°, 27.038°, 29.876°, 31.625°, 33.188°, 35.477°, 36.503°, 39.219°, 40.903°, 44.739°, 46.636°, 48.047°, 51.781°, 55.935°, 58.065°, 62.308°, 68.234°, 70.985°, and 74.457° (2 range, 16–75°) to lysine FCC planes (111), (200), (112), (022), (122), (131), (131), (131), (131), (122), (223), (241), (112), (204), (251), (342), (342), (334), (334) and (334), respectively.

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022



Purified lysine from S. aureus



Standard lysine (control)

Figure (3.9): Powder XRD spectra of purified lysine produced by bacteria isolates

Because crystalline L-lysine has a strong proclivity for incorporating water to form the hydrate phase under ambient atmospheric conditions, the only way to obtain a pure (non-hydrate) crystalline phase is to dehydrate it under strict anhydrous conditions, resulting in the microcrystalline powder sample. As a result, powder X-ray diffraction techniques have been used to identify the crystal structure of L-lysine [32].

L-lysine crystal structure was discovered directly from powder XRD data, making it the latest of a 20 genetically encoded proteinogenic amino acids to have its enantiomerically pure form crystallized. Due to the difficulties of creating single crystals of appropriate size and quality for crystal structure determination from single crystal XRD data, crystal structures of both L-lysine hemihydrate and L-lysine monohydrate have indeed been established directly from powder XRD data. Shows suite cell dimensions and metric symmetries, on the other hand.: L-lysine hemihydrate, monoclinic a = 9.54 Å, b = 5.22 Å, c = 17.61 Å, β = 101.1°, (V = 860.6 Å 3); L-lysine monohydrate, orthorhombic a = 5.94 Å, b= 20.61 Å, c = 6.95 Å, (V = 850.3 Å 3) [33].

3.7 Study the effect of lysine on human blood clotting

Lysine has broad biomedical applications, though little is known about its hemocompatibility. Table (3.1) depicts the effect of lysine on human blood clotting (13 coagulation factors), revealing a non-significant difference (P ≥0.01) between standard lysine and purified lysine groups, but a highly significant difference (P≥ 0.01) in both standard lysine and purified lysine groups compared to the control group in PT, PTT, INR, and fibrinogen tests.

The optical coagulation analyzer instrument stops reading at the highest value within the normal range (over 90 for PT, over 120 for PTT, and over 9 for INR) and this value was determined in the statistical analysis.

Table (3.1): Effect of purified lysine on human blood clotting

Tested groups	**Tested factors**			
Normal (control)	PT (second)	PTT (second)	INR	Fibrinogen (g\L)
Mean ± SE	B 12.51 ± 0.28	B 34.10 ± 1.06	B 1.11 ± 0.03	A 2.25 ± 0.06
Standard lysine	PT (second)	PTT (second)	INR	Fibrinogen (g\L)

Mean ± SE	A 90.00 ± 0.00	A 120.00 ± 0.00	A 9.00 ± 0.00	B 1.67 ± 0.00
Purified lysine	PT (second)	PTT (second)	INR	Fibrinogen (g\L)
Mean ± SE	A 90.00 ± 0.00	A 120.00 ± 0.00	A 9.00 ± 0.00	B 1.59
P value	0.001	0.001	0.001	0.01

PT: Prothrombin Time, INR: International Normalized Ratio, PTT: Partial Thromboplastin time, SE: Stander Error of Mean, g\L: Gram per Liter

According to the results, lysine may have an effect on coagulation factors (XII, XI, IX, VIII, and X) of the intrinsic coagulation pathway as a similar mechanism to heparin's effect on the intrinsic pathway. Also, lysine may have an effect on extrinsic coagulation factors (VII) and common coagulation factors (X, V, 1, and II) as a similar mechanism to warfarin's effect on the extrinsic pathway, inferred by PT, INR, PTT, and fibrinogen tests.

After an addition of lysine-containing peptides, thromboelastographic studies (in vitro series) demonstrated a 17–100% increase in the total period of plasma coagulation. Under the effect of these peptides, there is a reduction in the generation of thrombin and factor Xa, as well as a suppression of their action. Lysine-containing peptides demonstrated the greatest anticoagulant potential in in vivo settings. Those who increased the time of reaction corresponding to phase I of blood coagulation, which reflects a formation of thromboplastin, thrombin, and factor Xa, a time of clot formation (phase II of blood coagulation) from the appearance of first fibrin filaments to the formation of a clot, and the total time of coagulation [24]. The characteristics of thromboelastography under action of test peptides (containing lysine) suggest hypocoagulation and inhibition of thrombin activity, as well as an increase in blood anticoagulant activity and just a drop in fibrinogen concentration, according to [19].

Zhang and Liu (2017) revealed that plasma coagulation in the presence of poly-lysine was evaluated by measuring the prothrombin time (PT) and activated partial thromboplastin time (APTT). Human whole blood coagulation in the presence of poly-lysine was evaluated with the thromboelastograph (TEG). They discovered that poly-lysine at 0.001 mg/mL did not result in a significantly different APTT from a control, whereas poly-lysine at 0.01 mg/mL resulted in a significantly higher APTT than the control, and poly-lysine at 0.1 mg/mL did not result in a significantly different PT from a control, whereas poly-lysine at 1 mg/mL resulted in a significantly higher PT than the presence of 0.01 mg/mL of poly-lysine, TEG parameters for whole blood coagulation were within the normal range; while poly-lysine ≥ 0.1 mg/mL caused one or more abnormal TEG parameters.

[25], Synthetic polypeptides were investigated for their effects on rabbit blood coagulation in vitro and in vivo. The basic L-lysine polypeptides prolonged whole blood clotting time, increased prothrombin time, and neutralized the effect of heparin.

4. Conclusions

Isolates of S. aureus obtained from different clinical sources could produce lysine in high percentages. The optimum temperature, pH, and time of incubation for lysine production were 37°C, 72, 72 h, and 130 rpm/minute. Purified lysine has anticoagulant activity (prevents blood clotting formation). There may be an

effect on coagulation factors (XII, XI, IX, VIII, and X, VII, X, V, 1 and II), and fibrinogen.

5. References

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