مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
Journal of the College of Basic Education Vol.32 (NO. 135) 2026, pp. 20-30

# Purification of DNase Enzyme from *Staphylococcus aureus* Isolated from Various Clinical Sources

**Yasser abdulhussein shinbar**[(1)]
yasirabd89@gmail.com
**Khawlah Jebur Khalaf**[(1)]
khawlah.falah@uomustansiriyah.edu.iq
**Hind Hussein Obaid**[(2)]
Hind_3000@yahoo.com
**Raghad Akram Aziz**[(3)]
ragaad.edbs@uomustansiriyah.edu.iq
[(1)] Department of Biology- College of Science- Mustansiriyah University- Baghdad – Iraq
[(2)] Department of Biology- College of Science- University of Baghdad - Baghdad – Iraq
[(3)] Department of Biology- College of Basic education- Mustansiriyah University- Baghdad – Iraq

**Abstract:**
This study designed to isolate and purify (DNase) from *Staphylococcus aureus* that isolated from several clinical source collected at Al-Shaheed Al-Sader Hospital within the period extended from December 2023 to June 2024, it was 200 isolates. The isolates were screened for DNase production by using qualitative and quantitative assay, with 94% were DNase producers. The highest DNase production was detected in isolate S129, which was subjected to purification by precipitation with ammonium sulfate, ion exchange chromatography (IEC), and gel filtration chromatography. DNase activity was optimized at a saturation range of (75-85)% ammonium sulfate, and the maximum purity was achieved by ion exchange chromatography with (0.3 M NaCl), 121 U/mg specific activity of and a overall yield 21.1%. The results confirm the widespread production of DNase in *S. aureus* and demonstrate the effectiveness of the applied purification methods, providing valuable insights for further research on DNase as a virulence factor.

**Keywords.** *Staphylococcus aureus*, DNase, Purification.

## 1. Introduction:

*Staphylococcus aureus* is a greatly adaptable bacterium that causes a widespread range of human's infections, reaching from mild skin situations to severe diseases such as pneumonia, bacteremia, and endocarditis (1). *S. aureus* first described in the 1880s by Sir (Alexander Ogston), is a Gram-positive, spherical bacterium that usually forms clusters resembling grapes (2). It is a facultative anaerobe; it can grow in anaerobic and aerobic

conditions, and exhibits ability to thrive in environments with great salt concentrations. This versatility allows *S. aureus* to colonize a variety of human ecological niches, including the skin, nasal passages, and mucous membranes (3, 4). The *S. aureus* pathogenicity largely attributed to its production of several virulence factors, such as super antigens like Toxic ShockSyndrome Toxin-1 (TSST-1) and exfoliate toxins, that responsible for toxic shock syndrome and staphylococcal (scalded skin syndrome), separately (5, 6). Another worry is the emergence of Methicillinresistant *S. aureus* (MRSA), which is antibiotics resistant, mainly beta-lactams, complicating treatment and leading to higher morbidity and mortality (1). MRSA has become increasingly prevalent in both healthcare and community settings, requiring new approaches to prevention and treatment (7). Resistance to antibiotics, especially *S. aureus*'s capacity to produce beta-lactamase and the existence of the (mecA) gene, which encodes of penicillin-binding protein (PBP2a), continues to complicate clinical management. The spread of MRSA underscores the need for continuous research on the molecular mechanisms behind its resistance and virulence, which is essential for the development of more effective treatments and preventive measures in the face of rising antibiotic resistance (1, 8). Staphylococcal nuclease (DNase), produced by *S. aureus*, is a vital virulence factor that significantly contributes to the pathogen's ability to evade host defenses. DNase catalyzes the hydrolysis of DNA, breaking them down into smaller fragments, which aids in immune evasion by degrading neutrophil extracellular traps (NETs), thereby allowing *S. aureus* to persist in the host (3). Additionally, the enzyme promotes biofilm formation, which increases bacterial resistance to antimicrobial agents and host immune responses (4, 9). Therefore, the aim of this study was to purify and estimate the molecular weight of DNase enzyme from a high-producing *S. aureus* clinical isolate.

**Materials and Methods:**

**Chemicals:** The chemicals used for enzyme production and purification were obtained from BDH (British Drug Houses) Co., England, and Sigma-Aldrich Chemical Co., USA. The culture media were purchased from BiolifeItaliana, Italy.

**Sample collection:** Between December 2023 and June 2024, 200 clinical specimens, including urine, burns, wounds, blood, stool, sputum, and nasal samples, were collected from Al-Shaheed Al-Sader Hospital for the isolation of *S.aureus* bacteria. In the laboratory, in sterile environments, the bacteria were isolated as pure colonies on Mannitol Salt Agar and Blood Agar. The isolates were then examined microscopically using the Gram stain, and their

identification was confirmed through cultural morphology and biochemical tests. (10)

**Detection of DNase Production from *Staphylococcus aureus* isolates:** DNase production was assessed using two methods: the qualitative streaking Method (Jeffries *et al.*, 1957) and the quantitative Optical Density (OD) measurement method (11).

**Qualitative Detection of DNase Production:** To screening for DNase-producing isolates using the streaking culturing technique, *S. aureus* were streaked onto DNase agar media. And incubation for 24 hours at 37°C, the results was interpreted as follows:

- Positive Result: If DNase is produced, the DNA in the medium is hydrolyzed, resulting in a clear zone around the bacterial growth.
- Negative Result: In the absence of DNase production, no clear zone forms, and the DNA remains intact, causing the medium to appear cloudy.

**Quantitative Assay of DNase Production:** The isolates were grown in nutrient broth medium at 37°C for 24 hr. After incubation, cultured broth by centrifugation at (10,000 rpm) for 10 minutes and the supernatant was sterilized using a 0.22 µm Millipore filter. By using DNase assay the enzymatic activity was measured in the supernatant. The isolate showing the highest enzymatic activity was considered the highest producer.

**DNA extraction from strawberries**: The process begins by preparing an extraction solution consisting of 50 ml of water, 1 ml of detergent, and 2g of salt. The dish soap helps break down the cell membranes, while the salt assists in separating the DNA from proteins. Strawberries are then mashed to release the DNA, and the mashed fruit is combined with the extraction solution. After gently mixing for 5-10 minutes, the mixture is strained to separate the liquid from solids. Chilled ethanol is added to precipitate the DNA, which can be collected using a stick. The DNA is washed in 70% ethanol, suspended in a DNA buffer, then the concentration and purity are measured using a Nanodrop instrument, where the ratio of optical densities at 260 and 280 nm (260/ 280) O.D.is used to determine the purity (12).

**DNase assay:** This method was carried out according to Ohsaka *et al.* (1964), by assessing the change in optical density. A 190 µl volume of strawberry DNA and 1 ml of DNase buffer (0.01 M $CaCl_2$ , 0.1 M Tris-HCl, pH 8) were incubated with 70 µl of the sample for 60 minutes at 37°C. adding 320 µl of 0.33 M EDTA (pH 8.0) for terminate reaction. The alteration in absorbance at 260 nm was used to calculate the enzyme activity, expressed as one unit of activity. The DNase activity was calculated using the following formula(13):

$$DNase\ activity = \frac{A \times V}{t \times e \times E}$$

Where: A=Absorbance at 260 nm, V=Total volume of the reaction, t = Incubation time (in minutes), e = Enzyme volume and E = Extinction coefficient for single-stranded DNA (0.027)

**Extraction of DNase from *Staphylococcus aureus* isolate:** The extraction of DNase was described by Ohsaka *et al*. (1964). The highest producer *S. aureus* isolate (129) was cultured in 500 mL of Nutrient Broth at 37°C in a shakingincubator at 100 rpm for 24 hr. After incubation, for 10 minutes the culture was centrifuged at 10,000 rpm, and by using Millipore filter 0.22 µm supernatant was filtered to sterilize it. The resulting supernatant was then used for DNase activity assays, protein concentration measurements, and subsequent enzyme purification (13).

**Purification of DNase**

**A. Ammonium Sulfate Precipitation:** The supernatant was cooled to 4°C, and $(NH_4)_2SO_4$ was added gradually in an ice bath to the crude enzyme with stirring for 60 minutes continuously. This was done to achieve saturation percentages of (55, 65, 75, 85, and 95) %. Afterward, the mixture was centrifuged (10,000 rpm), 15 minutes and 4°C. The precipitate was dissolved in Tris-HCl buffer, and DNase activity was measured to determine the optimal saturation percentage for further processing (14).

**B. Dialysis:** The dialysis process was performed in a dialysis tube with a 10,000 MW cutoff after the precipitation step. The sample was dialyzed by 0.1 M Tris-HCl buffer for 24 hr. at 4°C and pH 8 with stirring. Following dialysis, the sample was concentrated by adding sucrose until the volume reached 15 mL and stored in the refrigerator for further purification (15).

**C. Carboxymethyl Cellulose Chromatography (CMC):** Resin of CMC was prepared, washed, and equilibrated in 0.1 M Tris-HCl buffer pH 8. The CMC was packed into a column (17.5 x 2.5) and equilibrated with the same buffer. Elution was done by using a NaCl gradient (0.1 to 0.5 M). A total of 100 fractions were collected, with 20 fractions corresponding to each concentration. The flow rate was maintained at approximately one drop every 5 seconds, with 3 mL per tube. Each fraction absorbance was measured at (280) nm using a spectrophotometer UV-VIS. Enzyme activity was recorded in each tube (14, 16).

**D. Gel Filtration Chromatography (GFC):** A specific amount of Sephadex G-75 was mixed with 0.1 M Tris-HCl buffer (pH 8) to make suspension, and packed into a purification column 1.5 × 80 cm. The column was equilibrated with the same buffer. The DNase got from the IEC step that equilibrated

previously with same buffer then applied to the matrix. Elution was carried out at a flow rate of three mL per tube, by the equilibration buffer. The absorbance at 280 nm was measured, and DNase activity was calculated in all tubes (14).

**E. Molecular weight Determination of DNase.** By using SDS-PAGE DNase molecular weight was determined; Gels with 5% and 7.5% concentrations were prepared using base and buffer solutions. Electrophoresis was performed in three steps: resolving gel, stacking gel, and sample preparation with sample buffer. Protein samples were loaded with a protein ladder, and electrophoresis was run at 180 V. Gels were stained with (Coomassie Brilliant Blue) and distained. The MW was estimated by measuring the relative mobility (Rm) and constructing a standard curve (15).

**Results and Discussion:**

**Isolation of Bacteria:** Two hundred samples were collected from different clinical sources. 84(42%) isolates were recognized as *S. aureus* by using examination via (culture and microscopic), biochemically and VITEK® 2 Compactsystem.

**Detection of DNase Production by *Staphylococcus aureus*:** The detection of nuclease production in *S. aureus* was performed using dual methods: qualitative and quantitative screening.

**Qualitative Detection of DNase:** In the qualitative method, 84 *S. aureus* isolates were growing on DNase agar by using streaking culturing technique on. The outcomes revealed 79 (94%) of them were DNase producers, indicated by a color change of the agar from blue to pink or rose. In contrast, only 8 (6%) isolates were DNase-negative (Figure 1).

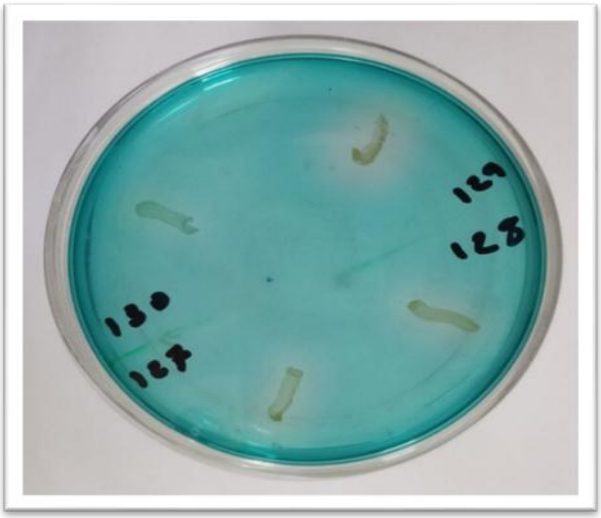



**Figure (1): DNase production by *Staphylococcus aureus* on DNase agar medium at incubation temperature 37°C for 24 hrs.**

**Quantitative Detection of DNase:** The quantitative assay for DNase production detected by using Optical Density (OD). The results indicated that isolate number 129 exhibited the highest level of DNase activity. This isolate will be further studied for DNase extraction and purification. These findings are consistent with previous studies, which reported DNase production rates in *S. aureus* ranging from 66% to 100%, depending on the geographic source and environmental conditions of the isolates. For instance, Khwen *et al.* (2021) reported sixty six percent of *S. aureus* produced DNase as detected using DNase agar medium (17). On the other hand, quantitative detection of DNase activity, measured by optical density (OD), showed that isolate number 129 exhibited the highest DNase activity level. This indicates that quantitative assessment provides a more accurate representation of enzyme activity levels compared to qualitative detection alone. For instance, Ali *et al*. (2022) reported that approximately 87% of *S. aureus* isolates in clinical environments exhibit DNase activity (18). This suggests that DNase production is a common trait among *S. aureus* isolates

**DNase Extraction:** The highest producing isolates *S. aureus* (S129) were cultured in nutrient broth medium by shacking incubation at 32 °C overnight. Supernatant of these isolates culture was collected and filtered by Millipore filter 0.22 µm current study shown that the supernatant had achieving 59 U/ml enzyme activity.

**Purification steps:**

**A.Ammonium sulphate precipitation:** Five different saturation rates of ammonium sulphate (55, 65, 75, 85 and 95) % were used to detect the optimal range for DNase precipitation, the results showed that the optimal range for DNase from S129 was precipitated in the saturation range between (75-85 %) as shown in figure (2). where enzyme activity reached 53 U /ml, and specific activity (16 U/mg protein).

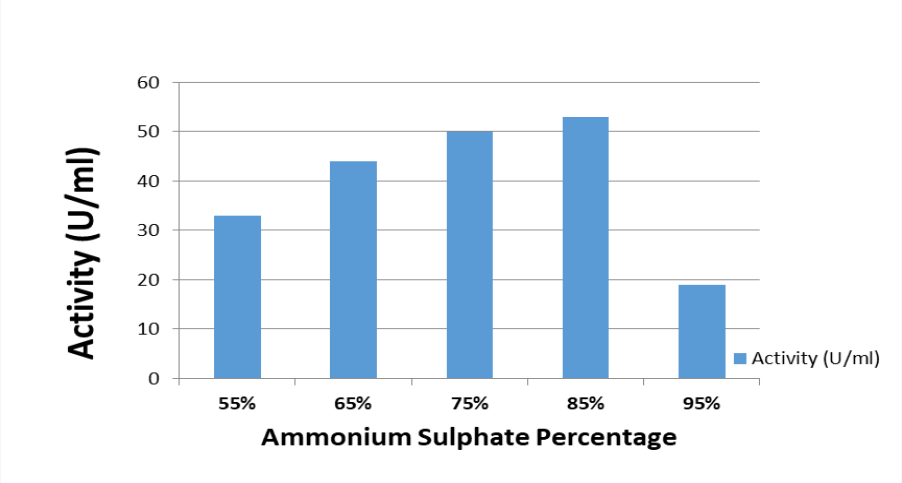



**Figure (2): Precipitation of DNase in different ammonium sulphate percentage.**

**B. Ion Exchange Chromatography:** By using a CMC column. A total of 100 fractions, each containing 4 ml of elution, were collected, and enzyme activity was measured for each fraction. The ion exchange chromatography profile for the isolate S129 revealed a peak during the wash step, but no DNase activity was observed. The protein peak, eluted in 0.2M NaCl, appeared in fractions 60 to 69, showing the highest DNase activity of 64 U/mg Figure (3).

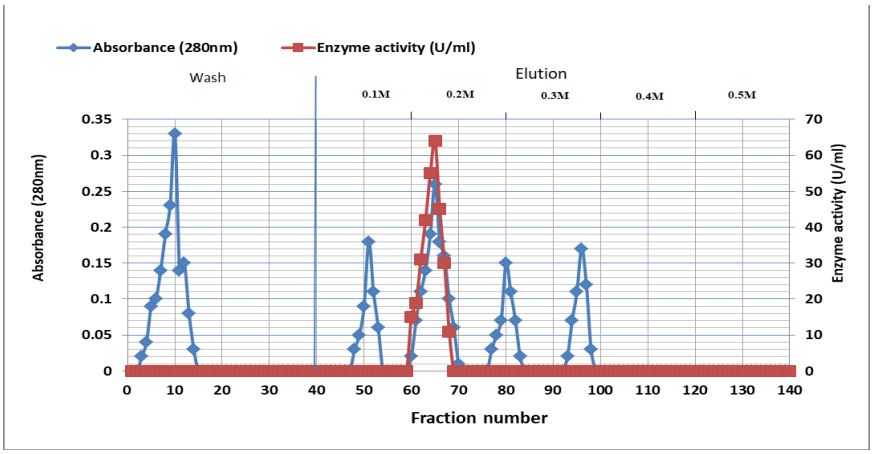



**Figure (3): IEC of DNase produced by *Staphylococcus aureus* (129) using CMC (2.5 × 17.5) with flow rate was 30ml/h. and 4 ml/fraction.**

**C. Gel Filtration Chromatography (GFC):** The next purification step involved gel filtration chromatography. Following ion exchange chromatography, fractions demonstrating DNase activity were collected and subjected to Sephadex G-75, equiliibrated via 0.1 M Tris-HCl at pH 8 as shown in Figure (4).

مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
Journal of the College of Basic Education Vol.32 (NO. 135) 2026, pp. 20-30

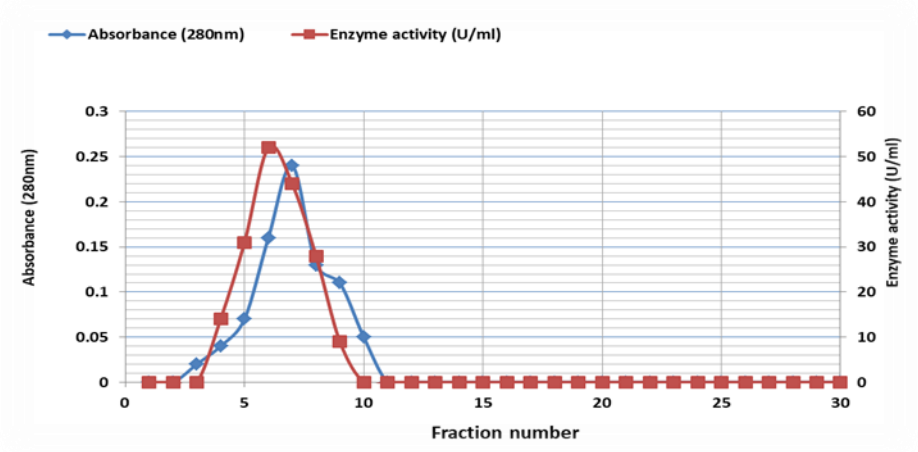



**Figure (4): GFC of DNase through Sephadex G-75 column (1.5x80 cm).The flow rate was 30ml/h. and 4 ml/fraction.**

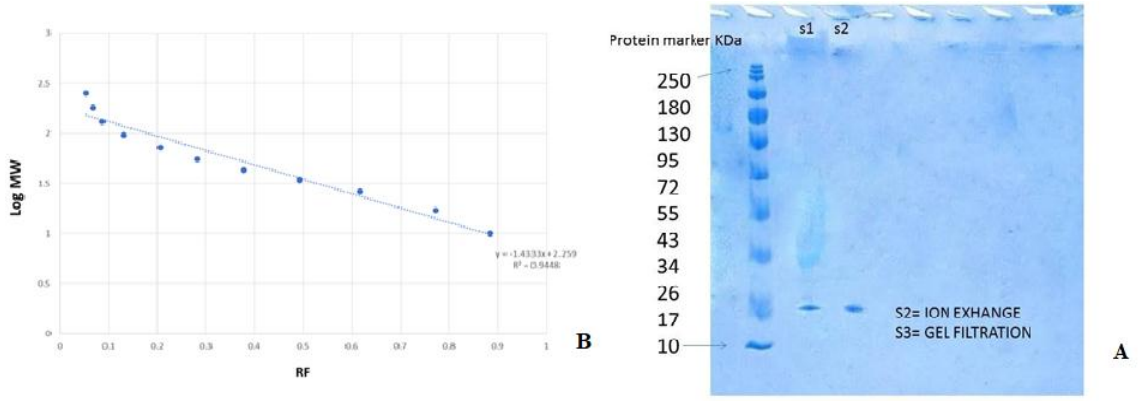
The results showed one absorption peak of grates DNase activity after elution step Figure (4). DNase activity, Protein concentration and specific activity were estimated, as presented in Table (1) specific activity was (120.9 U/mg), purification fold 11.5 and overall yield 21.1%.

**Table (1): purification steps of DNase from *Staphylococcus aureus* (129).**

| Purification stages | Volume (ml) | DNase activity (U/ml) | concentration of Protein (mg/ml) | Specific activity (U/mg) | Total activity (U) | Purification (folds) | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude DNase | 100 | 59 | 5.6 | 10.5 | 5900 | 1 | 100 |
| Ammonium sulfate (85%) | 70 | 53 | 3.3 | 16 | 3710 | 1.5 | 62.8 |
| IEC on CM-cellulose | 28 | 64 | 0.71 | 90.1 | 1792 | 8.5 | 30.3 |
| Gel filtration chromatography on Sephadex G-75 | 24 | 52 | 0.43 | 120.9 | 1248 | 11.5 | 21.1 |

**D.Molecular weight (MW) of DNase:** The MW of DNase produced by *S. aureus*(129)was determined using SDS-PAGE. The results showed a single DNase band for both IEC and GFC Figure (5), indicating the enzyme's purity. The molecular weight was measured using the equation in Figure (5), and it was found to be 17 kDa.

مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
Journal of the College of Basic Education Vol.32 (NO. 135) 2026, pp. 20-30




**Figure(5):**A-**Polyacrylamide gel electrophoresis of purified DNase producing by *Staphylococcus aureus* (129) B- Standard curve for protein standard to estimated molecular weight of pure DNase producing by *Staphylococcus aureus* (129).**

Higher salt concentrations limit the availability of free water molecules, leading to the aggregation and precipitation of proteins, particularly those with hydrophobic regions (19). A study by Khwen *et al.* (17) characterized DNase from *S.aureus*, reporting MW was 19 kDa and a specific activity of 42 U/mg, using a purification method comparable to the one employed in this work. In the current study, the DNase enzyme exhibited an approximate molecular mass of 17 kDa, which aligns with the established molecular weight range of microbial DNases (15–30 kDa). These results parallel to Al-Biyati *et al.* findings (20), who isolate DNase from *Streptomyces sp.* using a two-phase aqueous system followed by GFC on a Sephacryl S-200 column, yielding a 4.33of purification fold and a recovery rate of 28.8%. The integration of IEC and GFC in this study demonstrated strong potential for effective DNase purification. Variations in yield and purification efficiency across studies may be attributed to differences in microbial strains, buffer systems, or chromatographic parameters. SDS-PAGE estimated the MW of the purified DNase to be approximately 17 kDa. DNase activity responds differently to varying levels of ammonium sulfate saturation, largely due to shifts in protein solubility and conformational stability influenced by ionic strength (21).

**Conclusion:** Two-step chromatographic approach for purifying DNase from *Staphylococcus aureus* demonstrated efficiency in balancing enzyme purity and yield. The IEC and subsequent GFC effectively isolated DNase, confirming its molecular characteristics. This method provides a reliable, scalable solution for DNase purification, offering valuable insights for both clinical applications and future research, highlighting its potential for further development in biochemistry and medicine.

**Ethical approval:** This work was conducted in agreement with the ethical values. It was done with an oral and analytical patient approval formerly the sample acquisition. Mustansiriyah University College of Science, a local ethics committee, approved the protocol of study, information of subject, and permission form on 1 Augast 2023, using the document number BCSMU/1221/00037M.

**References:**

1. Kourtis, K. Hatfield, J. Baggs, Y. Mu, I. See, E. Epson, J. Nadle, M. A. Kainer, G. Dumyati, S. Petit, S. M. Ray, Emerging Infections Program MRSA Surveillance Participants, and J. A. Jernigan, *Morbidity and Mortality Weekly Report* 68, 214–219 (2019).
2. Lowy, F. D. *The New England Journal of Medicine* 339, 520–532 (1998).
3. M. M. Alreshidi, R. H. Dunstan, M. M. Macdonald, M. Saeed, S. Elkahoui, and T. Roberts, *Microorganisms* 11, 1–13 (2023).
4. Otto, M. (2014). *Current Opinion in Microbiology* 17, 32–37.
5. Fey, P. D. and Olson M. E., (2010). *Future Microbiology* 5, 917–933.
6. Abo-Ksour,M. F.; Al-Jubori, S. S. and Jawad, H. A. (2018). *Al-Mustansiriyah Journal of Science* 29, 123–128.
7. Tong, S. Y. Davis, J. S. Eichenberger, E. Holland, T. L. and Fowler V. G., *Clinical Microbiology Reviews* 28, 603–661 (2015).
8. Alganady, A. S. (2019). *Al-Mustansiriyah Journal of Science* 30, 43–51.
9. R. S. Najim, M. H. Risan, and D. N. Al-Ugaili, *Al-Mustansiriyah Journal of Science* 35, 74–80 (2024).
10. Carroll, K. C., Butel, J. S., Morse, S. A., Mietzner, T. A., Shors, T., & Nash, A. (2019). Jawetz, Melnick & Adelberg's medical microbiology (28th ed.). McGraw-Hill Education.
11. Schreier, P. H. (1969). DNase production and its determination in bacterial cultures. *Applied Microbiology*, 18(5), 845-849.
12. OpenStax. (2018). Biology 2e. OpenStax, Rice University.
13. Ohsaka, A.; Jun-Ichiro, M. and Laskowski, M. (1964). The Use of Purified Micrococcal Nuclease in Identifying the Nucleotide Terminus Bearing a Free 5'-Monophosphate. *Journal of Biological Chemistry*, 239, pp: 3498-3504.
14. Himmelhoch, S. R. (1971). Chromatographic methods for the purification of DNase. *Journal of Chromatography*, 56(3), 322-329
15. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.
16. Sulkowski, W., Laskowski, M. (1966). Purification and characterization of DNase from *Staphylococcus aureus*. *BiochimicaetBiophysicaActa*, 120(3), 387-393.
17. Khwen, N. N., Authman, S. H., & Al-Marjani, M. F. (2021). Purification and characterization of thermo stable DNase of *Staphylococcus aureus* isolated from different clinical sources. *Medico-Legal Update*, 21(2), 1352–1358.
18. Ali, A. M., & Ahmed, R. H. (2022). Prevalence and characterization of coagulase-positive *Staphylococci* from food products and human specimens in Egypt. *Antibiotics*, 10(1), 75

19. Burgess, R. R. *Refolding of Proteins: A Practical Approach* (Oxford University Press, Oxford, 2009), pp. 259–282.
20. Al-Biyati, A.I.; Al-Jumaily, F. E. & Ibrahim, A. M. (2005). Purification of DNase produced by local *Streptomyces* isolates. *Iraqi Journal of Biotechnology*, 4(1): 90-107.
*21*. F. Gibson, (2006) Protein Purification: Principles and Practice, 3rd ed. *Springer Science & Business Media, New York.*

# تنقية انزيم DNase من *Staphylococcus aureus* المعزولة من مصادر سريرية مختلفة

**ياسر عبد الحسين شنبار [1]، خولة جبر خلف [1]، هند حسين عبيد [2]، رغد أكرم عزيز [3]**
[1]قسم علوم الحياة – كلية العلوم – الجامعة المستنصرية – بغداد – العراق
[2]قسم علوم الحياة – كلية العلوم – جامعة بغداد – بغداد – العراق
[3]قسم علوم الحياة – كلية التربية الأساسية – الجامعة المستنصرية – بغداد – العراق

**مستخلص البحث:**
هذه الدراسة تهدف الى تنقية انزيم DNase من بكتريا *Staphylococcus aureus* بعد عزلها من مصادر سريرية مختلفة و التي جمعت من مستشفى الشهيد الصدر خلال الفترة الممتدة من كانون الاول 2023 و الى حزيران 2024 وكان عددها الكلي 200 عزلة. تم بعدها الكشف عن قدرتها على انتاج انزيم DNase بإستخدام الطريقتين النوعية و الكمية و قد اظهرت النتائج ان 94% من العدد الكلي للعزلات كانت قادرة على انتاج الانزيم اما العزلة الاعلى انتاجاً فكانت 129 و التي استخدمت لاحقا لانتاج و نقية الانزيم بواسطة الترسيب بكبريتات الامونيوم و كروماتوكرافيا التبادل الايوني ثم كروماتوكرافيا الترشيح الهلامي. معدل الترسيب بكبريتات الامونيوم الامثل تم تحديده بنسبة تشبع (75%-85)، اما اعلى نقاوة بكروماتوكرافيا التبادل الايوني فقد تم الحصول عليها باستخدام NaCl 0.3 و بفعالية نوعية 121 وحدة/ مليغرام و حاصل 21.1%. أكدت هذه النتائج الانتشار الواسع لإنتاج إنزيم DNase في بكتيريا *S. aureus*، وتُظهر فعالية طرق التنقية المطبقة، مما يوفر رؤى قيّمة لأبحاثٍ إضافية حول إنزيم DNase كعامل ضراوة.
**الكلمات المفتاحية:** المكورات العنقودية الذهبية، الدناز، التنقية.