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ORIGINAL ARTICLE

# Determining the Optimal Conditions for Collagenase Production by a Local Isolate of *Entrobacter Cloacae* Using Local Organic Waste

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***Key words:***
***Metalloprotease group, Collagenase, Chicken feet, Collagen-degrading bacteria***

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## ABSTRACT

***Background:*** *Collagenases are proteolytic enzymes with applications in leather processing, food industries, and biomedicine. Utilizing locally sourced organic wastes as fermentation substrates offers a cost-effective and sustainable method for enzyme production.* ***Aim:*** *To identify the optimal physicochemical and nutritional conditions for maximal collagenase production by a native Enterobacter cloacae isolate using organic waste substrates.* ***Methodology:*** *Forty-eight bacterial isolates from soil, animal, and poultry wastes were screened on gelatin and collagen media to select six active strains, then narrowed to three based on performance on various organic wastes. The two highest producers were identified via cultural, microscopic, biochemical tests and the Compact VITEK2 system. One-factor-at-a-time experiments optimized pH (5.0–10.0), temperature (25–45 °C), substrate type (e.g., chicken foot waste), inoculum size (0.5–2.0 ml/100 ml), inoculum age (18–30 h), nitrogen source (peptone, yeast extract, ammonium salts), incubation mode (static vs. shaking), and incubation time (24–72 h). Collagenase activity was quantified in U/ml.* ***Results****: Both isolates were confirmed as E. cloacae. Maximum enzyme yield occurred at pH 8.0 and 35 °C using chicken foot waste. The optimal inoculum was 1 ml per 100 ml medium (1.1×10⁹ cells/ml, 24 h age). Peptone under static incubation yielded the highest activity, reaching 20.232 U/ml after 48 h.* ***Conclusion:*** *A local E. cloacae isolate efficiently produces collagenase on chicken foot waste under optimized conditions, demonstrating a scalable, low-cost, and eco-friendly strategy for industrial enzyme production.*

## INTRODUCTION

The use of enzymes in industry is of great importance because they are effective, highly specialized, and precise catalysts. Biotechnology studies and research are leading to the discovery of new enzymes and their potential use and exploitation. Proteolytic enzymes (proteases) are the most important in this field[1]. Enzymes are biocatalysts involved in almost all biological reactions within living systems. Many enzymes, such as lipases, proteases, cellulases, and others, have been used in various industrial applications. However, proteases remain the dominant type due to their significant value through their wide-ranging applications in various fields, including dairy, food, paper, detergents, and pharmaceutical industries. Proteases account for approximately 60% of total commercial enzyme sales each year[1].

Collagenase belongs to the metalloprotease group. Collagenase is a specialized enzyme that acts on the substrate collagen[2]. Collagenase was first discovered in 1949 by Mandl, having been isolated from the mammalian pancreas[3].

Collagenase is an important enzyme that plays a fundamental role in the breakdown of collagen, the main protein in connective tissues. This enzyme is of great importance in medical, industrial, and research fields. It is used in wound treatment, removing damaged tissue, and stimulating skin healing. It is also used in the pharmaceutical and cosmetic industries[4,5]. Sources of collagenase production vary. However, bacteria are among the most efficient organisms for its production due to their ease of cultivation and their ability to secrete the enzyme with high efficiency. Among the bacterial strains that produce this enzyme are species belonging to the genera *Clostridium*, *Bacillus*, *Pseudomonas*, and *Enterob7acter*[6].

The idea of using environmental waste to stimulate the production of enzymes has emerged with the growing global trend toward reducing environmental pollution and utilizing natural resources efficiently. Collagen-rich waste, such as fish remains, hides, and bones, can serve as an ideal environment for the growth of collagenase-producing bacteria[6].

## METHODOLOGY

**Preparation of the Isolation Medium**

Isolation medium was prepared consisting of 1 g of gelatin powder, 0.5 g of glucose ($C_6H_{12}O_6$), 0.2 g of potassium dihydrogen phosphate ($KH_2PO_4$), 0.7 g of dipotassium phosphate ($K_2H_2PO_4$), 0.02 g of magnesium sulfate ($MgSO_4.7H_2O$), 0.02 g of calcium chloride dihydrate ($CaCl_2.2H_2O$), and 0.1 g of yeast extract. 1.5 g of agar was added to 100 ml of distilled water. The pH was adjusted to 7, and the medium was autoclaved at 121°C for 15 minutes under a pressure of 1 atmosphere.

***Isolation of Collagenase-Producing Bacteria***

Thirty samples of soil, poultry, and sheep manure from various sites in Anbar were each cleaned, and 1 g was suspended in distilled water for serial ($10^{-1}$–$10^{-5}$) dilutions. The $10^{-4}$ and $10^{-5}$ dilutions (1 ml) were pour-plated on the gelatin-based isolation medium and incubated at 30 °C for 24 h. Colony growth and density were recorded, and individual colonies were repeatedly subcultured on the same medium to obtain pure isolates. Each pure isolate was then spot-inoculated (1 cm diameter) onto collagen agar (collagen replacing gelatin), incubated under the same conditions, and colony diameters measured to gauge collagenase activity. The two isolates exhibiting the largest colony zones were chosen for further study.

**Identification of the selected bacterial isolate**

The selected bacterial isolate was identified based on cultured characteristics and microscopic characteristics. Biochemical tests were also performed on the selected isolate to identify the isolate according to MacFaddin [7]. The diagnosis was then confirmed using the Compact VITEK device.

**Measuring Collagenase Enzyme Activity**

Collagenase enzyme activity was assessed using a liquid culture technique, according to the method of Savita and Arachana [8]. This was done using chicken foot powder as a source of carbon and energy

**Improving Environmental Conditions for Collagenase Enzyme Production**

Liquid production medium (100 ml) was prepared in 250 ml flasks with 1 g waste, 2 g glucose, 0.5 g $K_2HPO_4$, 0.5 g $KH_2PO_4$, and 0.05 g $CaCl_2$. After autoclaving (121°C, 15 min), flasks were cooled and inoculated with 1 ml bacterial culture per 100 ml medium. All treatments were run in triplicate and incubated under the designated conditions.

**pH**

A set of conical flasks containing 100 ml of production medium was prepared, and the pH was adjusted to 11, 10, 9, 8, 7, and 6. Inoculum was added to each flask at a ratio of 1 ml per 100 ml of medium. The flasks were incubated at 30°C for 24 hours. Enzyme activity was then measured to determine the optimal pH.

**Temperature**

The production tanks were prepared as in the previous experiment and incubated at temperatures (45, 40, 35, 30, 25, 20) to obtain the optimum temperature for production.

**Type of carbon source**

Three types of carbon sources (calfskin, fish skin, and chicken feet) were prepared to determine the best carbon source for collagenase production.

**Carbon Source Concentration**

Chicken feet were found to produce the highest concentration of collagenase enzyme, making them the preferred local carbon source for enzyme production. They were added at concentrations of 5, 4, 3, 2, 1, and 0.5g/100 ml of medium to determine the optimal concentration for collagenase production.

**Inoculum Size**

To test the effect of inoculum size on collagenase production, the liquid medium was inoculated with several sizes of bacterial inoculum (5, 4, 3, 2, 1, and 0.5) ml per 100 ml of medium to achieve optimal enzyme production.

**Ventilation**

The ability of the bacterial isolate to produce the enzyme was tested using two types of incubation methods: a fixed incubator and a shaking incubator.

**Nitrogen Source Type**

The effect of organic nitrogen sources, such as peptone, yeast, and urea, and inorganic sources, such as sodium nitrate, ammonium sulfate, and ammonium chloride, on optimum enzyme production was tested.

**Inoculum age**

The vaccine was prepared from the bacteria used in the study at an age of (72-48-24) hours and inoculated into the production medium.

**Incubation Period**

To determine the optimal incubation period for collagenase production, the selected isolate was tested on a production medium. The incubation period was 24, 48, or 72 hours, according to the parameters adopted in previous experiments.

## RESULTS

**Isolation of Collagenase-Producing Bacteria**

Table 1. shows considerable variation in gelatin-hydrolyzing activity among the 17 isolates. Colony diameters ranged from 1.6 cm (K1) to 2.5 cm (U1, X1), with the majority (10/17) exceeding 2.0 cm. The mean zone size was approximately 2.18 cm**.**

**Table 1: Growth rate of bacterial isolates on isolation medium (cm)**

| No. | Isolate symbol | Average diameter of growth zone (cm) |
|---|---|---|
| **1** | A1 | 2.1 |
| **2** | A2 | 2.4 |
| **3** | B1 | 1.8 |
| **4** | C1 | 2.4 |
| **5** | D1 | 2.4 |
| **6** | E1 | 2 |
| **7** | E2 | 1.9 |
| **8** | F1 | 2.4 |
| **9** | F2 | 1.9 |
| **10** | G1 | 2.2 |
| **11** | H1 | 1.9 |
| **12** | Z1 | 2.3 |
| **13** | J1 | 2.2 |
| **14** | K1 | 1.6 |
| **15** | U1 | 2.5 |
| **16** | U2 | 2 |
| **17** | X1 | 2.5 |

Table 2. shows that, after re-culturing on gelatin medium, isolate F1 produced the largest hydrolysis zone (2.3 cm), while C1 produced the smallest (1.6 cm). The other four isolates fell between 1.7 cm (U1) and 1.9 cm (D1, X1).

**Table 2: Average growth diameters of bacterial isolates on gelatin medium (cm)**

| No. | Isolate symbol | Average diameter of growth zone (cm) |
|---|---|---|
| **1** | C1 | 1.6 |
| **2** | D1 | 1.9 |
| **3** | A2 | 1.8 |
| **4** | U1 | 1.7 |
| **5** | F1 | 2.3 |
| **6** | X1 | 1.9 |

Table 3. demonstrates that collagen medium hydrolysis varied among the six isolates, with zone diameters ranging from 1.6 cm (U1) to 2.2 cm (F1). Isolate F1 showed the greatest collagenase activity (2.2 cm), followed by X1 (2.0 cm) and D1 (1.9 cm).

**Table 3: Average growth diameters of bacterial isolates in collagen medium (cm).**

| No. | Isolate symbol | Average diameter of growth zone (cm) |
|---|---|---|
| **1** | C1 | 1.8 |
| **2** | D1 | 1.9 |
| **3** | A2 | 1.8 |
| **4** | U1 | 1.6 |
| **5** | F1 | 2.2 |
| **6** | X1 | 2 |

Table 4. indicates that calf skin supported the largest hydrolysis zones for all three isolates (D1 and X1 each 2.5 cm; F1 2.4 cm), whereas chicken feet and fish skin yielded smaller diameters (2.3 cm and 2.2–2.3 cm, respectively).

**Table 4: Average colony diameters of bacterial isolates on the waste (cm).**

| No. | Isolate symbol | Type of Carbon Source | Average diameter of growth zone (cm) |
|---|---|---|---|
| **1** | **F1** | **Fish skin** | **2.3** |
| | | **Calf skin** | **2.4** |
| | | **chicken feet** | **2.3** |
| **2** | **D1** | **Fish skin** | **2.2** |
| | | **Calf skin** | **2.5** |
| | | **chicken feet** | **2.4** |
| **3** | **X1** | **Fish skin** | **2.2** |
| | | **Calf skin** | **2.5** |
| | | **chicken feet** | **2.4** |

**Bacterial Isolation Identification**

Table 5. shows that both D1 and X1 are Gram-negative, mucous, yellowish-white, convex bacilli occurring singly or in pairs, with colonies ranging from translucent to opaque.

Table 6. reveals identical biochemical profiles for D1 and X1: positive for catalase, Voges-Proskauer, motility, urease, and citrate utilization; negative for oxidase, indole, methyl red, and starch hydrolysis.

**Table 5: Cultural and microscopic characteristics of the bacterial isolate with the local code D1, X1**

| Gram dye | cell aggregation | Bacteria shape | Nature of colonies on solid nutrient medium | | | | | |
|---|---|---|---|---|---|---|---|---|
| Negative | Single or pairs | Bacillus | Hight | Transparency | Edge | Texture | Color | **Shape** |
| | | | Convex | Translucent to opaque | Soft | Mucous | Yellowish White | **Circular** |

**Table 6: Results of biochemical tests for bacterial isolates with local code D1, X1**

| No. | Type of test | Result X1 | Result D1 |
|---|---|---|---|
| 1 | Catalase | + | + |
| 2 | **Oxidase** | - | - |
| 3 | **Indole** | - | - |
| 4 | **Methyl red** | - | - |
| 5 | **Voges-Proskauer** | + | + |
| 6 | **Motility** | + | + |
| 7 | **Urease** | + | + |
| 8 | **Starch hydrolysis** | - | - |
| 9 | **Citrate Utilization Test** | + | + |

**Determining the optimal conditions for the production of collagenase enzyme from *Enterobacter cloacae* bacteria.**

Figure 1. shows a clear pH-dependent pattern: collagenase activity rises from 5.42 U/ml at pH 6 to a maximum of 9.94 U/ml at pH 8, then falls off sharply to 1.67 U/ml by pH 11. Activity at pH 8 is nearly twice that at pH 6 and about three times that at pH 9 (5.32 U/ml).

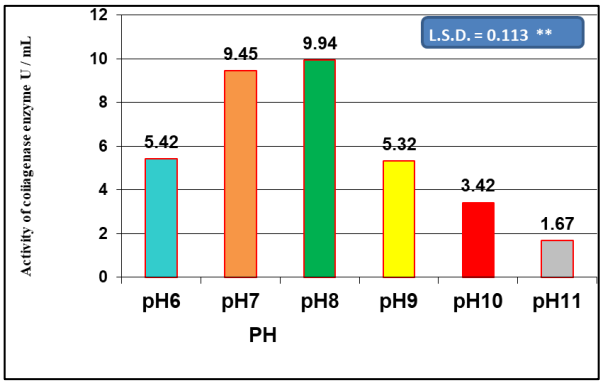


**Fig. 1:** Effect of pH on the production of collagenase enzyme

Figure 2. shows that collagenase activity rises sharply from 1.91 U/ml at 20 °C to a peak of 12.32 U/ml at 35 °C, then declines to 1.27 U/ml by 45 °C. This confirms 35 °C as the optimal incubation temperature for enzyme production.

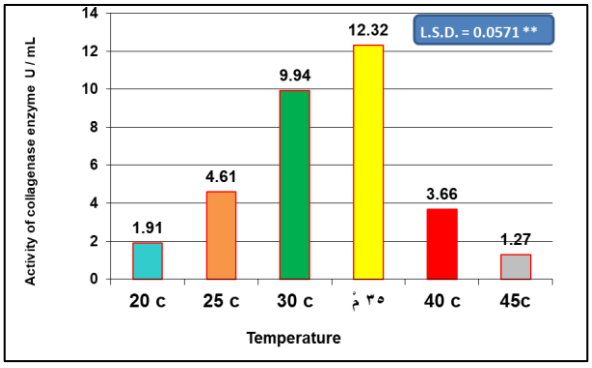


**Fig. 2.** Effect of temperature on the production of collagenase enzyme

Figure 3. illustrates that chicken feet supported the highest collagenase activity (19.73 U/ml), followed by calf skin (18.89 U/ml) and fish skin (17.85 U/ml). The 1.88 U/ml difference between chicken feet and fish skin underscores chicken feet as the superior carbon source.

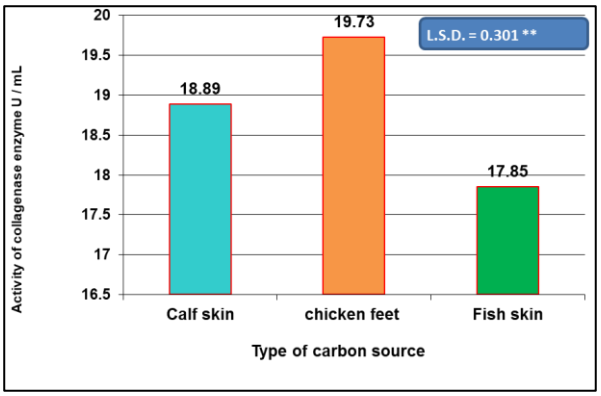


**Fig. 3.** Effect of Type of Carbon Source on the production of collagenase enzyme

Figure 4. shows a bell-shaped response to chicken-feet concentration: activity rises from 6.92 U/ml at 0.5 g/100 ml to a maximum of 19.86 U/ml at 2 g/100 ml, then declines to 13.02 U/ml at 3 g, 8.32 U/ml at 4 g, and 5.61 U/ml at 5 g.

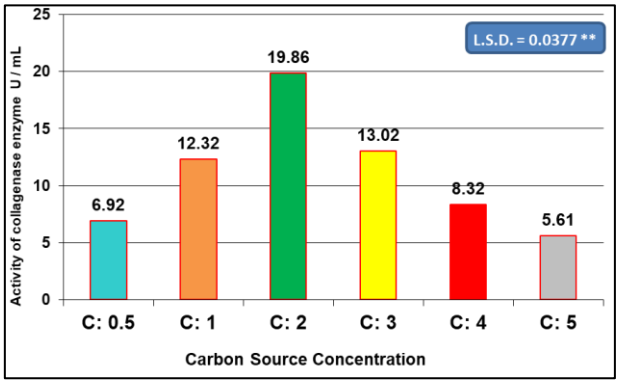


**Fig. 4:** Effect of Carbon Source Concentration on the production of collagenase enzyme

Figure 5. demonstrates that collagenase activity rises sharply from 9.51 U/ml at 0.5 ml inoculum to a peak of 19.79 U/ml at 1 ml/100 ml, then declines steadily to 13.85 U/ml (2 ml), 8.02 U/ml (3 ml), and 3.59 U/ml (5 ml). Thus, 1 ml per 100 ml is the optimal inoculum size for maximal enzyme production.

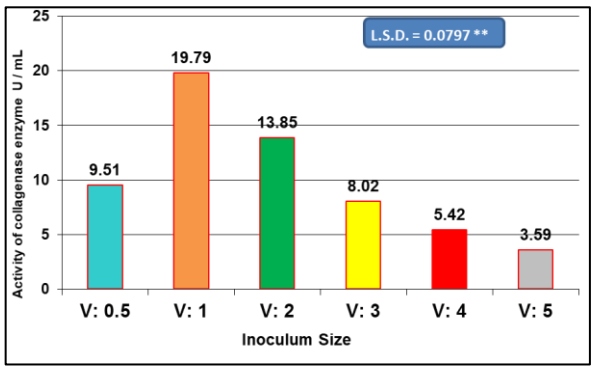


**Fig. 5:** Effect of Inoculum Size on the production of collagenase enzyme

Figure 6. demonstrates that static incubation yields significantly higher collagenase activity (19.68 U/ml) than shaking (16.38 U/ml), representing a ~20% increase under fixed conditions.

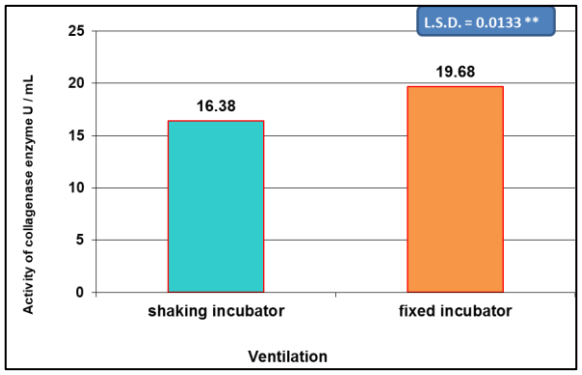


**Fig. 6:** Effect of Ventilation on the production of collagenase enzyme

Figure 7. illustrates that organic nitrogen sources markedly outperform inorganic salts: peptone yields the highest collagenase activity (19.87 U/ml), closely followed by urea (18.86 U/ml) and yeast extract (14.92 U/ml). In contrast, inorganic sources ammonium chloride (9.73 U/ml), ammonium sulfate (8.24 U/ml), and especially sodium nitrate (3.56 U/ml) support substantially lower enzyme production.

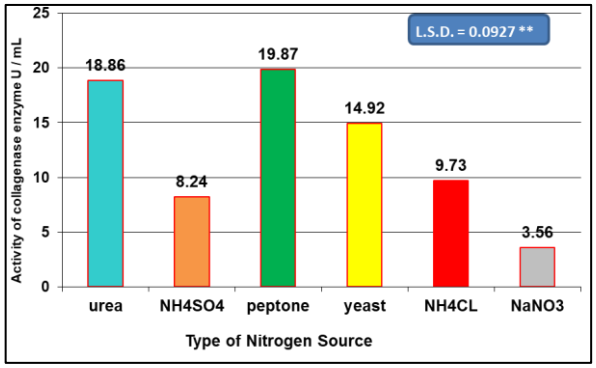


**Fig. 7:** Effect of Type of Nitrogen Source on the production of collagenase enzyme

Figure 8. demonstrates a clear decline in collagenase yield with increasing inoculum age: activity peaks at 19.89 U/ml for a 24 h inoculum, then drops to 16.21 U/ml at 48 h and 13.87 U/ml at 72 h, confirming 24 h as the optimal inoculum age.

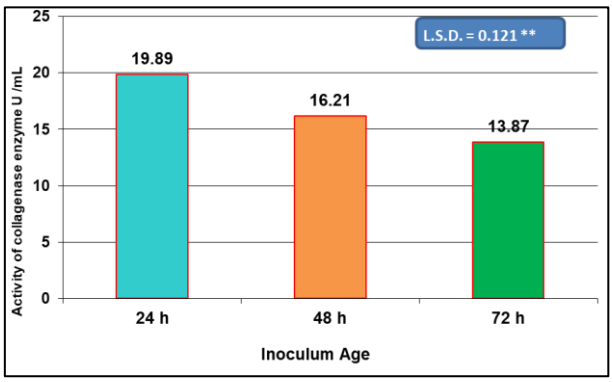


**Fig. 8:** Effect of Inoculum Age on the production of collagenase enzyme

Figure 9. shows collagenase activity peaking at 20.23 U/ml after 48 h, a modest 2.3% increase over the 24 h value (19.77 U/ml), before declining by ~13.5% to 17.54 U/ml at 72 h, indicating 48 h as the optimal incubation duration.

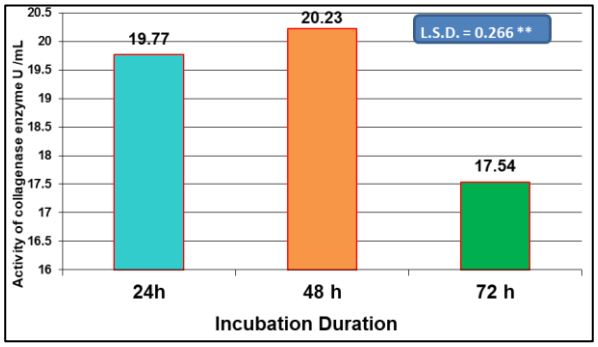


**Fig. 9**: Effect of Incubation Duration on the production of collagenase enzyme

## DISCUSSION

Enzyme yield was highly sensitive to pH, with the optimum at pH 8, likely due to enhanced nutrient solubility and membrane transport at this pH, aligning with previous reports on collagenase producers by Moradi, *et al.*[9], Abdulameer, *et al.*[10] and consistent with Rochima, *et al.* [11] .

Temperature influences both cell growth and enzyme stability; production peaked at 35 °C before denaturation effects dominated, as described by Moore, *et al.*[12] and Uyar, *et al.*[13] and mirrors findings from *Streptomyces spp*. and *C. histolyticum*[14,15] . Chicken feet, rich in type I collagen and low in fat, proved the superior carbon source, in agreement with Jayaprakash,

*et al.* [16] and Radhakrishnan, *et al.* [17] , whereas fish-skin utility has been noted in *Bacillus spp*.[18].

Substrate overload (≥ 5 g/100 ml) inhibited enzyme synthesis, underscoring the importance of balanced carbon-to-nitrogen ratios, with deviations reported for keratinase systems[19-21]. An inoculum of 1 ml/100 ml optimized cell density and minimized lag, matching observations in *S. aureus* by Abbood and Awda [22] and Salih and Hussein [23], though other species prefer higher volumes[24]. Static incubation favored collagenase over shaking, possibly due to mechanical stress or oxygen sensitivity of facultative anaerobes[24,25].

Organic nitrogen (peptone) enhanced biomass and enzyme output relative to inorganic sources, as seen in *Bacillus sp*.[26,27]. A 24 h inoculum ensured cells were in an active growth phase, maximizing metabolic capacity; older cultures exhibited senescence and waste accumulation that depressed production[28]. Finally, a 48 h harvest balanced peak secondary-metabolite synthesis against nutrient depletion, toxin buildup, and autolysis that occur in prolonged cultures[29,30], consistent with *B. subtilis* and *B. licheniformis* systems[24,27].

The optimized collagenase could be further evaluated for debridement in chronic wound care or tissue dissociation in cell isolation protocols, offering a low-cost alternative to commercial enzymes. However, its safety, specificity, and stability must be validated in preclinical models before any therapeutic application.

Strengths include the use of inexpensive, locally sourced wastes and systematic one-factor-at-a-time optimization. Limitations comprise the lack of factorial experimental design, absence of scale-up trials, and no detailed enzyme characterization (e.g., kinetics, stability), which constrain direct comparison to commercial preparations.

Future work should employ statistical designs (e.g., RSM) for multifactor optimization, pilot-scale fermentations, and downstream purification. Comprehensive biochemical and stability profiling and testing in relevant biomedical or industrial assays will be essential to assess commercial viability.

## CONCLUSION

Under optimized conditions of pH 8.0, 35 °C, static incubation with chicken foot waste (2 g/100 ml) and peptone as nitrogen source, inoculated at 1 ml/100 ml (24 h culture) and harvested after 48 h a local *Enterobacter cloacae* isolate produced up to ~20 U/ml of collagenase. These findings demonstrate the feasibility of using readily available organic waste for cost-effective enzyme production. Further work should focus on process scale-up and downstream purification to assess industrial applicability.

**Declarations:**
**Consent for publication**: Not applicable
**Availability of data and material:** Data are available upon request.
**Competing interests:** The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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## REFERENCES

1. Naveed M, Nadeem F, Mehmood T, Bilal M, Anwar Z, Amjad F. Protease—a versatile and ecofriendly biocatalyst with multi-industrial applications: an updated review. Catalysis Letters. 2021;151:307-323.
2. Wu S, Zhou X, Jin Z, Cheng H. Collagenases and their inhibitors: a review. Collagen and Leather. 2023;5(1):19.
3. Luchian I, Goriuc A, Sandu D, Covasa M. The role of matrix metalloproteinases (MMP-8, MMP-9, MMP-13) in periodontal and peri-implant pathological processes. International journal of molecular sciences. 2022;23(3):1806.
4. Herman IM, Niranjan P, Grover K. Protease technology in wound repair. Wound Healing, Tissue Repair, and Regeneration in Diabetes. Elsevier; 2020:357-391.
5. Liu X, Kokare C. Microbial enzymes of use in industry. Biotechnology of microbial enzymes. Elsevier; 2023:405-444.
6. Ghahfarokhi SM, Hosseini F, Ghadam P, Barati F. Isolation and characterization of a collagenase-producing bacterium from the soil contaminated with slaughterhouse waste. Biocatalysis and Agricultural Biotechnology. 2024;61:103407.
7. MacFaddin J. Biochemical tests for identification of medical bacteria, williams and wilkins. Philadelphia, PA. 2000;113(7)
8. Savita K, Arachana P. Production of collagenase by Bacillus KM369985 isolated from leather sample. Int J Biosci IJB. 2015;4:81-87.
9. Moradi M, Kousheh SA, Almasi H, et al. Postbiotics produced by lactic acid bacteria: The next frontier in food safety. Comprehensive reviews in food science and food safety. 2020;19(6):3390-3415.
10. Abdulameer A, Al-Rawi DF, Al-Ani MQ. Production of keratinase enzyme from a local isolate of Kocuria rosea using environmental waste.

Agricultural Biotechnology Journal. 2024; 16(4):391-412.

11. Rochima E, Sekar N, Buwono ID, Afrianto E, Pratama RI. Isolation and characterization of collagenase from Bacillus subtilis (Ehrenberg, 1835); ATCC 6633 for degrading fish skin collagen waste from Cirata Reservoir, Indonesia. Aquatic Procedia. 2016;7:76-84.
12. Moore CE, Meacham-Hensold K, Lemonnier P, et al. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. Journal of experimental botany. 2021;72(8):2822-2844.
13. Uyar F, Porsuk I, Kizil G, Yilmaz EI. Optimal conditions for production of extracellular protease from newly isolated Bacillus cereus strain CA15. EurAsian Journal of BioSciences. 2011;5
14. Shekhter AB, Balakireva AV, Kuznetsova NV, Vukolova MN, Litvitsky PF, Zamyatnin Jr AA. Collagenolytic enzymes and their applications in biomedicine. Current Medicinal Chemistry. 2019;26(3):487-505.
15. Klomklao S, Kuepethkaew S, Benjakul S, Zhang Y, Simpson BK. Enzymes from fish processing waste materials and their commercial applications. Fish Waste to Valuable Products. Springer; 2024:147-194.
16. Jayaprakash S, Razeen ZMA, Kumar RN, et al. Enriched characteristics of poultry collagen over other sources of collagen and its extraction methods: A review. International Journal of Biological Macromolecules. 2024:133004.
17. Radhakrishnan R, Ghosh P, Selvakumar T, Shanmugavel M, Gnanamani A. Poultry spent wastes: An emerging trend in collagen mining. Adv Tissue Eng Regen Med Open Access. 2020;6(2):26-35.
18. Bhuimbar MV, Jalkute CB, Bhagwat PK, Dandge PB. Purification, characterization and application of collagenolytic protease from Bacillus subtilis strain MPK. Journal of bioscience and bioengineering. 2024;138(1):21-28.
19. Izadi P, Fontmorin J-M, Godain A, Yu EH, Head IM. Parameters influencing the development of highly conductive and efficient biofilm during microbial electrosynthesis: the importance of applied potential and inorganic carbon source. npj Biofilms and Microbiomes. 2020;6(1):40.
20. Moonnee YA, Foysal MJ, Hashem A, Miah MF. Keratinolytic protease from Pseudomonas aeruginosa for leather skin processing. Journal of Genetic Engineering and Biotechnology. 2021;19:1-9.
21. Al-Bedak OA-HM, Moharram AM, Hussein NA-G, Taha DM, Stephenson SL, Ameen F. Microbial exploitation of feather wastes for sustainable production of keratinase and collagenase enzymes by Didymella keratinophila AUMC 15399 in submerged fermentation. Fermentation. 2023;9(6):507.
22. Abbood ZH, Awda JM. Study of different factors effected in production of dextranase enzyme from a local isolate of B. subtilis Z2 bacteria. Plant Archives (09725210). 2020;20(1)
23. Salih HN, Hussein S. ASSESSEMENT OF PURIFIED COLLAGENASE INHIBITION ACTIVITY FROM STAPHYLLOCOCCUS AUREUS HN77 BY SOME LOCAL PLANTS EXTRACT. Iraqi Journal of Agricultural Sciences. 2022;53(5):1035-1047.
24. Al-Temimi WK, Aziz SN, Khalaf AA. Production of partially purified collagenase from Bacillus licheniformis and it's use to tenderize aged Buffalo Meat. Basrah Journal of Agricultural Sciences. 2023;36(1):75-89.
25. Gao B, Tan C, Roshani D, et al. Microbial collagenases: an updated review on their characterization, degradation mechanisms, and current applications. Critical Reviews in Food Science and Nutrition. 2024:1-25.
26. AnanadhiPadmanabhan M, Renita A, Stanley SA. Studies on the effect of nitrogen source and the growth of Marine microalgae algae. IEEE; 2010:350-352.
27. Zhu Y, Wang L, Zheng K, et al. Optimized recombinant expression and characterization of collagenase in Bacillus subtilis WB600. Fermentation. 2022;8(9):449.
28. Putatunda C, Behl M, Solanki P, et al. Current challenges and future technology in photofermentation-driven biohydrogen production by utilizing algae and bacteria. International Journal of Hydrogen Energy. 2023;48(55):21088-21109.
29. Salah O, Al-Rawi D, Buniya H. Production of pepsin by bacteria (Staphylococcus sciuri) and fungi (Pythium sp) from soil and rumens cultured in waste whey and brans. 2018;
30. Balakrishnan M, Jeevarathinam G, Kumar SKS, Muniraj I, Uthandi S. Optimization and scale-up of α-amylase production by Aspergillus oryzae using solid-state fermentation of edible oil cakes. BMC biotechnology. 2021;21(1):33.