Evaluation of the Nutritional and Environmental Parameters for Enhancing the Activity of Keratinase-Producing Bacteria

Noor Ahmed Al-Amery, Nassir Abdullah Alyousif *

Department of Ecology, College of Science, University of Basrah, Basrah, Iraq; pgs.noor.mahmood@uobasrah.edu.iq (N.A.A.)

* Correspondence: nassir.hillo@uobasrah.edu.iq

Abstract

The production of the keratinase enzyme is one of the most promising methods for the bioremediation of feathers and has attracted increasing attention in the field of biotechnology. This study aims to enhance the production of keratinase enzymes from bacteria isolated from the soil of poultry farms in various areas of Basrah Province by optimizing nutritional and environmental parameters. In the current study, among seventy-four isolates that showed positive results in the primary screening, four bacterial isolates demonstrated the highest activity in the secondary screening. These isolates were identified as *Bacillus subtilis* subsp. stercoris strain EGI18, *Bacillus licheniformis* strain PP1, *Bacillus subtilis* strain MML5328.1U, and *Bacillus pumilus* strain LX11, with keratinase activity values of 20.1 U/mL, 28.8 U/mL, 28.1 U/mL, and 28.1 U/mL, respectively, under standard cultivation conditions. The key nutritional and environmental parameters investigated in this study included carbon sources, nitrogen sources, pH, temperature, and inoculum size. The results showed that the optimal conditions for increasing keratinase enzyme production were: a temperature of 37°C, pH 8, glucose as the best carbon source, tryptone as the most effective nitrogen source, and an inoculum size of 1 mL. When these optimal parameters were applied in combination, maximum keratinase production was achieved from all four bacterial isolates. The keratinase activity values under optimal conditions were 139.0 U/mL for *B. subtilis* subsp. stercoris strain EGI18, 127.0 U/mL for *B. licheniformis* strain PP1, 138.0 U/mL for *B. subtilis* strain MML5328, and 147.0 U/mL for *B. pumilus* strain LX11. These findings indicate that various environmental and nutritional parameters significantly influence the production of bacterial keratinase enzymes, highlighting their importance in optimizing enzyme yield for biotechnological applications.

Keywords: feather degradation; keratin; keratinase; keratinolytic bacteria; optimization

Type: Original Article

Received: 26 December 2024; Revised: 07 January 2025; Accepted for publication: 13 April 2025; Published online: 08 June 2025

1. Introduction

The poultry industry generates large quantities of keratinous waste, primarily in the form of feathers, which constitute approximately 5–7% of the total weight of an adult chicken [1]. Disposing of these feathers poses a significant challenge due to their resistance to natural degradation, leading to considerable waste accumulation. Moreover, conventional disposal methods—such as incineration, landfilling, and thermochemical treatments—are both environmentally damaging and economically inefficient [2].

Bioremediation of keratinous waste, particularly feathers, using microbial keratinase enzymes, presents a promising, eco-friendly, and cost-effective alternative. Microorganisms play a crucial role in biogeochemical processes, helping to maintain ecological balance—even under extreme conditions—by utilizing a diverse range of compounds and elements as energy sources or building blocks for cellular functions. This is largely due to the complexity and diversity of microbial expression systems [3].

Keratinases are a specialized group of protease enzymes capable of withstanding a wide range of temperatures and pH levels. Their adaptability enables them to biodegrade complex, recalcitrant proteins such as keratin by binding to and breaking down insoluble structures like feathers, collagen, elastin, horns, hair, and nails [4].

Numerous studies have reported various bacterial strains from different genera with the ability to produce keratinase enzymes. These include *Geobacillus*, *Pseudomonas*, *Rhodococcus erythropolis*, *Bacillus licheniformis*, *Bacillus pumilus* [5,6], *Aeromonas hydrophila*, *Bacillus brevis*, *Bacillus cereus*, and *Sphingomonas paucimobilis* [7,8], many of which were isolated from feathers or poultry farm soils. Effective bioremediation often results from the synergistic activity of multiple microorganisms operating under specific environmental conditions, such as controlled temperature, humidity, and pH. While

bioremediation generally occurs in aerobic environments, it may also take place under anaerobic conditions [9,10].

The optimal temperature range for keratinase-producing bacteria is generally between 30°C and 80°C [11]. However, many isolates, particularly those from the *Bacillus* genus, have been reported to exhibit maximum keratinase activity at temperatures between 30°C and 45°C [12,13]. As for pH, most keratinase-producing bacteria thrive in neutral to alkaline conditions [14]. These enzymes degrade keratin substrates to obtain energy and have been shown to preferentially cleave hydrophobic and aromatic amino acids [15].

Typically, bacteria produce biocompounds such as keratinase in limited quantities under natural conditions. However, production can be significantly enhanced by modifying nutritional variables—such as carbon and nitrogen sources—and optimizing culture conditions, including temperature, pH, and inoculum size [10,16]. Improving keratinase enzyme production is therefore achievable by fine-tuning medium composition and environmental parameters to create optimal growth conditions for the producing bacteria.

This study aims to evaluate various nutritional and environmental factors to enhance keratinase activity in bacterial strains isolated from keratin-rich, contaminated poultry farm soils in Basrah Province, Iraq.

2. Methodology

2.1 Isolation and Characterization of Keratinolytic Bacteria

The bacterial isolates used in this study were previously obtained from soil samples containing poultry feathers collected from various locations in Basrah Province [17]. Serial dilutions were prepared from 10^{-1} to 10^{-6}, and 0.1 mL of each dilution was spread onto nutrient agar plates. The bacterial isolates were identified using molecular techniques based on 16S ribosomal DNA gene sequence analysis. Genomic DNA was extracted, and PCR amplification was performed using universal primers as described previously [18]. The amplified products were sent to Macrogen Company (South Korea) for sequencing. The resulting sequences were analyzed using Chromas software and compared to known sequences in the NCBI database using BLAST tools.

2.2 Preparation of Feathers

Chicken feathers were collected from a poultry farm in Basrah Province. The feathers were thoroughly washed with tap water, then defatted by soaking them in a 1:1 (v/v) mixture of chloroform and methanol for two days. This was followed by treatment with a 4:1:3 (v/v/v) mixture of chloroform, acetone, and methanol for another two days, with the solvents replaced daily. The feathers were then washed several times with tap water to remove any residual solvents and dried at 50°C for three days [19].

2.3 Primary and Secondary Screening of Keratinolytic Bacteria

Primary screening to assess keratinolytic activity was conducted by streaking bacterial isolates onto skim milk agar. Colonies that formed clear zones around them were considered positive for protease activity. Isolates that tested positive were further purified on nutrient agar for secondary screening. Active cultures were inoculated into nutrient broth, and 1 mL of each activated culture was added to 20 mL of modified mineral salt medium (MSM), consisting of: NaCl (0.5 g/L), KH_2PO_4 (0.7 g/L), K_2HPO_4 (1.4 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L) and chicken feather (2.5 g/L). Cultures were incubated at 37°C for 72 h [20].

2.4 Evaluation of Keratinase Activity

Keratinase activity was assessed by incubating a reaction mixture containing 4 mL of pH 10 buffer (50 mM glycine–NaOH), 20 mg of feather, and 1 mL of diluted keratinase enzyme. The mixture was incubated at 60°C for 60 min in a water bath. The reaction was terminated by adding 4 mL of 5% trichloroacetic acid (TCA). A control sample, lacking enzyme, was also prepared and treated similarly with 1 mL of 5% TCA. After the reaction, the mixtures were held at room temperature and centrifuged at 4,000 rpm for 20 min. The absorbance of the supernatant was measured at 280 nm using a UV–VIS spectrophotometer. One unit (U/mL) of keratinase activity was defined as 0.01 of the absorbance value at 280 nm [21].

2.5 Optimization of Keratinase Production

To enhance keratinase production, various cultural conditions were optimized, including the effects of temperature, pH, carbon and nitrogen sources, and inoculum size [20].

2.5.1 Effect of Temperature

To determine the optimal temperature for keratinase production, cultures were incubated at 25°C, 37°C, and 40°C for 72 h at pH 7 [20].

2.5.2 Effect of pH

The optimal pH for keratinase production was assessed by adjusting the initial pH of the culture medium from pH 6 to pH 10 (in increments of 1) using 0.1 N HCl or 0.1 N NaOH. Cultures were incubated at 37°C for 72 h [5].

2.5.3 Effect of Nitrogen Sources

To evaluate the impact of nitrogen sources, both organic (tryptone, yeast extract) and inorganic (NH_4Cl, $(NH_4)_2SO_4$) nitrogen sources were tested at a concentration of 0.1% (w/v). Cultures were incubated at 37°C and pH 7 for 72 h [20].

2.5.4 Effect of Carbon Sources

The effect of different carbon sources (glucose, sucrose, and starch) was tested by adding each to the production medium at a concentration of 0.1% (w/v). Incubation was carried out at 37°C and pH 7 for 72 h [5].

2.5.5 Effect of Inoculum Size

Different inoculum volumes (0.5, 1, 3, and 5 mL) were evaluated to determine the optimal volume for maximum keratinase production. All cultures were incubated at 37°C and pH 7 for 72 h [5].

2.6 Statistical Analysis

All experiments were performed in duplicate, and results are expressed as mean ± standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) in SPSS to determine significant differences among treatments. A p-value of less than 0.01 was considered statistically significant.

Figure 1: The clear zones around bacterial colonies on the skim milk agar are used to screen for keratinase enzyme-producing bacteria.

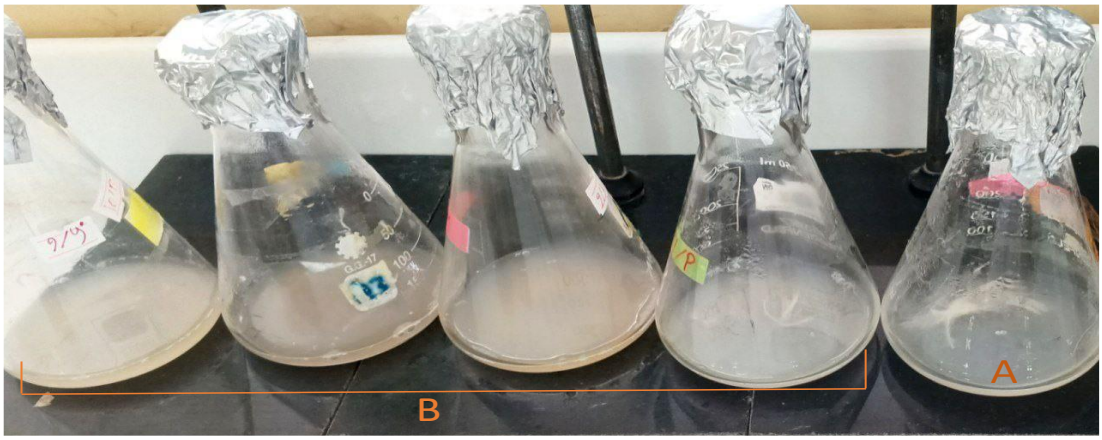

Figure 2: Screening of bacterial isolates for keratinase enzyme production. A: Flask containing MSM without bacterial inoculation (control). B: Flasks containing MSM inoculated with selected bacterial isolates that showed positive results on skim milk agar.

3. Results

3.1 Isolation and Characterization of Keratinolytic Bacteria

Ninety-seven bacterial isolates were obtained from soils containing poultry residues collected from ten different sites across Basrah Province. The isolates were identified using PCR amplification and sequence analysis of the 16S rDNA gene. Most of the isolated bacteria were Gram-positive, spore-forming bacilli. The most frequently identified genera were *Bacillus*, *Paenibacillus*, and *Rummeliibacillus*, as reported in a previous study [17]. Among these, *Bacillus subtilis* was the most frequently occurring and widely distributed species in the sampled habitats.

3.2 Primary and Secondary Screening of Keratinolytic Bacteria

Seventy-four bacterial isolates exhibited positive results in the primary screening for keratinolytic activity, forming clear zones around their colonies, as shown in Figure 1. These positive isolates were then subjected to secondary screening to evaluate their ability to produce keratinase using modified mineral salt medium (MSM), in which feathers served as the sole source of carbon and nitrogen (Figure 2). The results of the secondary screening identified four isolates with the highest keratinase activity: *Bacillus subtilis* subsp. stercoris strain EGI18 (20.1 U/mL), *Bacillus licheniformis* strain PP1 (28.8 U/mL), *Bacillus subtilis* strain MML5328.1U (28.1 U/mL), and *Bacillus pumilus* strain LX11 (28.1 U/mL).

3.3 Optimization of Keratinase Production

In the present study, various factors were evaluated to determine the optimal conditions for keratinase enzyme production. Different temperatures (25°C, 37°C, and 40°C) were tested. Statistical analysis revealed significant differences ($P < 0.01$) in enzyme activity across the four selected isolates at the tested temperatures. The results indicated that the optimal temperature for keratinase production was 37°C, with activity values of 20.0 U/mL, 28.8 U/mL, 29.1 U/mL, and 28.1 U/mL for *B. subtilis* subsp.

stercoris strain EGI18, *B. licheniformis* strain PP1, *B. subtilis* strain MML5328, and *B. pumilus* strain LX11, respectively (Table 1).

A range of pH values (6, 7, 8, 9, and 10) was evaluated for keratinase production. The optimal pH was found to be 8 for *B. subtilis* subsp. stercoris strain EGI18 and *B. subtilis* strain MML5328, yielding enzyme activities of 31.5 U/mL and 28.1 U/mL, respectively. In contrast, pH 7 was optimal for *B. licheniformis* strain PP1 and *B. pumilus* strain LX11, with activity values of 28.8 U/mL and 28.1 U/mL, respectively. pH 6 resulted in the lowest keratinase activity for all four isolates (Table 2). Statistical analysis confirmed significant differences ($P < 0.01$) among the pH treatments.

As shown in Table 3, tryptone was identified as the most effective nitrogen source, resulting in keratinase activities of 42.0 U/mL, 41.7 U/mL, 38.9 U/mL, and 41.7 U/mL for *B. subtilis* subsp. stercoris strain EGI18, *B. licheniformis* strain PP1, *B. subtilis* strain MML5328, and *B. pumilus* strain LX11, respectively. In contrast, NH_4Cl was the least effective nitrogen source for the first three strains, with activity values of 15.0 U/mL, 11.2 U/mL, and 10.8 U/mL, respectively. For *B. pumilus* strain LX11, $(NH_4)_2SO_4$ was the poorest nitrogen source, yielding 12.8 U/mL. These differences were statistically significant ($P < 0.01$).

Various carbon sources were also examined for their impact on keratinase production. As presented in Table 4, glucose was the most effective carbon source, producing activities of 34.3 U/mL, 25.7 U/mL, 40.3 U/mL, and 32.7 U/mL for *B. subtilis* subsp. stercoris strain EGI18, *B. licheniformis* strain PP1, *B. subtilis* strain MML5328, and *B. pumilus* strain LX11, respectively. While starch and sucrose were generally good carbon sources for all isolates, sucrose was the least effective for *B. licheniformis* strain PP1, with only 13.0 U/mL of keratinase activity. Statistical analysis confirmed significant differences ($P < 0.01$) across carbon sources.

Four different inoculum volumes (0.5 mL, 1 mL, 3 mL, and 5 mL) were tested to determine the optimal amount for keratinase production. As shown in Table 5, an inoculum size of 1 mL yielded the highest enzyme activity for all tested strains, while the lowest activity was observed with a 0.5 mL inoculum. These differences were also statistically significant ($P < 0.01$).

After optimizing the media components and culture conditions—including temperature (37°C), pH (8), tryptone as the nitrogen source, glucose as the carbon source, and an inoculum size of 1 mL—the highest keratinase activity was achieved for each of the four selected isolates (Table 6). Statistical analysis showed significant differences in keratinase activity before and after optimizing the environmental and nutritional conditions of the growth medium.

4. Discussion

The current study aimed to optimize the nutritional and environmental parameters to enhance the activity of keratinase enzymes produced by bacteria isolated from poultry farm soils containing feathers in various areas of Basrah Province, Iraq. Although large amounts of feathers are produced as a significant protein-rich byproduct, they remain underutilized despite technological advancements that enable their effective recycling and exploitation [22]. Feather keratin, due to its stable structure supported by hydrogen and disulfide bonds, is highly resistant to proteolytic hydrolysis [23]. However, certain microorganisms can produce proteolytic enzymes capable of degrading insoluble and fibrous proteins such as keratin [24].

Protease enzyme production in microorganisms is influenced by physical factors and culture components. Enhancing these factors can improve microbial growth, feather degradation, and the production of proteolytic enzymes [25]. Dada and Wakil [26] demonstrated that keratinase produced by *Bacillus licheniformis*-K51 (EZYKer-51) could fully degrade 10 g of white chicken feathers within 48 h when the pH was adjusted to 8, temperature to 50°C, and the culture was shaken at 150 rpm. Similarly, Alahyaribeik et al. [6] assessed keratinase production at different temperatures and pH levels by four bacterial species (*Rhodococcus erythropolis*, *Geobacillus stearothermophilus*, *Bacillus pumilus*, and *Bacillus licheniformis*). They found that optimal keratinase production occurred at 37°C, 55°C, 37°C, and 40°C respectively, and at pH values of 7.0, 7.0–8.0, 7.0, and 10.0, respectively.

In the present study, the optimal temperature for keratinase production by the selected species was found to be 37°C. High temperatures are known to promote disulfide bond reduction, thereby facilitating feather degradation [27]. This finding aligns with the results of Alahyaribeik et al. [6], who also reported 37°C as the optimal temperature for keratinase production by *Bacillus pumilus* and *Rhodococcus erythropolis*.

The pH of the medium plays a critical role in metabolic reactions and nutrient transport across bacterial cell membranes [20]. In this study, extracellular keratinase activity peaked at pH 7 and 8 for the four bacterial species. This is consistent with findings by Mukhtar et al. [12], who reported maximum keratinase production by *Bacillus* species at pH 7 and 8. Alkaline pH conditions favor keratin degradation because they convert cysteine residues to lanthionine, enhancing substrate availability for keratinase activity [28]. Changes in environmental pH can also influence enzyme activity by altering the structure of the enzyme's active site and the charges on its amino acids.

Organic nitrogen sources, particularly tryptone and yeast extract, significantly enhanced keratinase production in this study. Nitrogen is crucial for synthesizing microbial enzymes and supporting growth. Tryptone was the most effective nitrogen source, while yeast extract had a lesser but still positive effect. In contrast, inorganic nitrogen sources such as NH_4Cl and $(NH_4)_2SO_4$ had minimal or inhibitory effects. These results align with a study by Akhtar et al. [5], in which yeast extract significantly enhanced keratinase production by *B. cereus* and *Pseudomonas* sp., whereas tryptone had a moderate effect and ammonium sulfate was inhibitory. The inhibitory effect may stem from the acidic nature of ammonium salts, which lower the pH and thus hinder keratinase production, which favors neutral to alkaline conditions.

Carbon sources are vital for microbial growth, energy production, and the biosynthesis of bioproducts. Akhtar et al. [5] reported that starch and maltose enhanced keratinase production by *B. cereus* and *Pseudomonas* sp., while lactose suppressed it. In the current study, three carbon sources—glucose (monosaccharide), sucrose (disaccharide), and starch (polysaccharide)—were evaluated. Glucose was found to be the most effective in promoting keratinase activity, followed by sucrose, while starch had a limited effect. The variation in carbon source efficacy may relate to the chemical complexity of the sugars: monosaccharides are simple and more

Table 1: The effect of different temperatures on keratinase activity.

Variable		Keratinase activity (U/mL)			
		B. subtilis subsp. stercoris strain EGI18	*B. licheniformis* strain PP1	*B. subtilis* strain MML5328	*B. pumilus* strain LX11
Temperature	25°C	18.2	8.0	13.5	14.9
	37°C	20.0	28.8	28.1	28.1
	40°C	12.5	22.3	22.9	26.6

Table 2: The effect of different pH values on keratinase activity.

Variable		Keratinase activity (U/mL)			
		B. subtilis subsp. stercoris strain EGI18	*B. licheniformis* strain PP1	*B. subtilis* strain MML5328	*B. pumilus* strain LX11
pH	6	4.5	4.1	5.3	2.2
	7	20.0	28.8	28.1	28.1
	8	31.5	19.0	28.9	14.8
	9	18.4	8.2	13.9	19.7
	10	19.2	15.7	21.9	13.2

Table 3: The effect the different nitrogen sources on keratinase activity.

Variable		Keratinase activity (U/mL)			
		B. subtilis subsp. stercoris strain EGI18	*B. licheniformis* strain PP1	*B. subtilis* strain MML5328	*B. pumilus* strain LX11
Nitrogen sources	Tryptone	42.0	41.7	38.9	41.7
	Yeast extract	38.3	39.4	38.2	43.5
	NH_4Cl	15.0	11.2	10.8	19.6
	$(NH_4)_2SO_4$	27.4	12.4	14.9	12.8

Table 4: The effect of different carbon sources on keratinase activity.

Variable		Keratinase activity (U/mL)			
		B. subtilis subsp. stercoris strain EGI18	*B. licheniformis* strain PP1	*B. subtilis* strain MML5328	*B. pumilus* strain LX11
Carbon sources	Glucose	34.3	25.7	40.3	32.7
	Sucrose	23.5	25.3	35.2	31.0
	starch	34.8	13.8	21.9	16.4

Table 5: The effect of different inoculum sizes on keratinase activity.

Variable		Keratinase activity (U/mL)			
		B. subtilis subsp. stercoris strain EGI18	*B. licheniformis* strain PP1	*B. subtilis* strain MML5328	*B. pumilus* strain LX11
Inoculum sizes	0.5 mL	9.0	7.0	9.8	19.0
	1 mL	20.0	28.8	28.1	28.1
	3 mL	23.8	28.6	22.0	17.3
	5 mL	30.6	20.5	12.6	20.0

Table 6: Keratinase activity (U/mL) before and after the optimization.

Keratinolytic bacteria	Before the optimization	After the optimization
B. subtilis subsp. stercoris strain EGI18	20.0	139.0
B. licheniformis strain PP1	28.8	127.0
B. subtilis strain MML5328	28.1	138.0
B. pumilus strain LX11	28.1	147.0

readily utilized by bacteria, while disaccharides and polysaccharides require more energy and enzymatic activity to metabolize.

Inoculum size also influenced keratinase production. Four inoculum volumes (0.5 mL, 1 mL, 3 mL, and 5 mL) were tested. For *Bacillus subtilis* subsp. stercoris strain EGI18, keratinase production increased with larger inoculum sizes, consistent with the findings of Sivakumar et al. [29]. However, for the other three isolates (*Bacillus licheniformis* strain PP1, *Bacillus subtilis* strain MML5328, and *Bacillus pumilus* strain LX11), 1 mL was the optimal inoculum size. Larger inocula in these cases led to decreased enzyme activity, potentially due to nutrient depletion and rapid bacterial growth during the lag phase, which can suppress keratinase production [20,30].

5. Conclusion

Based on the results obtained in this study, various environmental and nutritional parameters significantly influence the production of bacterial keratinase enzymes. The findings demonstrated that the optimal conditions for maximizing keratinase production were a temperature of 37°C, pH 8, glucose as the most effective carbon source, tryptone as the best nitrogen source, and an inoculum size of mL. When these optimal parameters were combined, they resulted in the highest keratinase enzyme production from the selected bacterial strains. The highest keratinase activities recorded were 139.0 U/mL, 127.0 U/mL, 138.0 U/mL, and 147.0 U/mL for *B. subtilis* subsp. stercoris strain EGI18, *B. licheniformis* strain PP1, *B. subtilis* strain MML5328, and *B. pumilus* strain LX11, respectively. Further research is needed to determine the chemical structure of the produced keratinase enzymes and to assess their potential applications in various industries, thereby providing direction for future studies.

Acknowledgement

The Department of Ecology, College of Science, University of Basrah, is acknowledged for providing the necessary facilities during the study. Gratitude is directed to the two anonymous reviewers for the valuable suggestions that greatly improved this manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Both authors have contributed equally. They have read and agreed to the published version of the manuscript.

References

[1] Kumar, M.; Bhatia, D.; Khatak, S.; Kumar. R.; Sharma, A.; Malik, D.K. Optimization and purification of keratinase from *Bacillus anthracis* with dehairing application. *J Pure Applied Microbiol* **2019**, *13*, 585-590.

[2] Bagewadi, Z.K.; Mulla, S.I., Ninnekar, H.Z. Response surface methodology-based optimization of keratinase production from *Trichoderma harzianum* isolate HZN12 using chicken feather waste and its application in dehairing of hide. *J Environ Chem Engin* **2018**, *6*, 4828-4839.

[3] Francino, M.P. The ecology of bacterial genes and the survival of the new. *Intern J Evol Biol* **2012**, *1*, 394026.

[4] Gupta, R.; Ramnani, P. Microbial keratinases and their prospective applications: An overview. *Appl Microbiol Biotechnol* **2006**, *70*, 21-33.

[5] Akhter, M.; Marzan, L.W.; Akter, Y.; Shimizu, K. Microbial bioremediation of feather waste for keratinase production: An outstanding solution for leather dehairing in tanneries. *Microbiol Insights* **2020**, *13*,1-12.

[6] Alahyaribeik, S.; Sharifi, S.D.; Tabandeh, F.; Honarbakhsh, S.; Ghazanfari, S. Bioconversion of chicken feather wastes by keratinolytic bacteria. *Process Safety Environ Protec* **2020**, *135*, 171-178.

[7] Jadhav, P.; Pathade, G. Degradation of feathers by bacterial consortium and its application in seed germination. *Intern Res J Biol Sci* **2019**, *8*, 20-23.

[8] Almahasheer, A.A.; Mahmoud, A.; El-Komy, H.; Alqosaibi, A.I.; Aktar, S.; AbdulAzeez, S., Borgio, J.F. Novel feather degrading keratinases from Bacillus cereus group: Biochemical, genetic and bioinformatics analysis. *Microorganisms* **2022**, *10*, 93.

[9] Adams, G.O.; Fufeyin, P.T.; Okoro, S.E.; Ehinomen, I. Bioremediation, biostimulation and bioaugmention: A review. *Intern J Environ Bioremed Biodegrad* **2015**, *3*, 28-39.

[10] Alyousif, N.A.; Al-Tamimi, W.H.; Al-sahib, M.A.A. Evaluation of the effect of various nutritional and environmental factors on biosurfactant production by *Staphylococcus epidermidis*. *Biodiversitas* **2022**, *23*, 3533-3538.

[11] Nam, G.W.; Lee, D.W.; Lee, H.S.; Lee, N.J.; Kim, B.C.; Choe, E.A.; Pyun, Y.R. Native-feather degradation by *Fervidobacterium islandicum* AW-1, a newly isolated keratinase-producing thermophilic anaerobe. *Arch Microbiol* **2002**, *178*, 538-547.

[12] Mukhtar, H.; Ahmad, M.; Arshad, Y. Isolation and screening of keratinase producing bacteria from soil. *Biologia (Pakistan)* **2019**, *65*, 1-6.

[13] Huang, Y.; Łężyk, M.; Herbst, F.A.; Busk, P.K.; Lange, L. Novel keratinolytic enzymes, discovered from a talented and efficient bacterial keratin degrader. *Scientific Rep* **2020**, *10*, 10033.

[14] Mitsuiki, S.; Ichikawa, M.; Oka, T.; Sakai, M.; Moriyama, Y.; Sameshima, Y.; Furukawa, K. Molecular characterization of a keratinolytic enzyme from an alkaliphilic Nocardiopsis sp. TOA-1. *Enzyme and Microbial Technology* **2004**, *34*, 482-489.

[15] Evans, K.L.; Crowder, J.; Miller, E.S. Subtilisins of *Bacillus* spp. hydrolyze keratin and allow growth on feathers. *Canad J Microbiol* **2000**, *46*, 1004-1011.

[16] Abbas, S.Z.; Yong, Y; Khan, M. A.; Siddiqui, M.R.; Hakami, A.A.H.; Alshareef, S.A.; Otero, M.; Rafatullah, M. Bioflocculants produced by bacterial strains isolated from palm oil mill effluent for application in the removal of Eriochrome Black T dye from water. *Polymers* **2020**, *12*, 1545.

[17] Al-Amery N.M.A; Alyousif, N.A. Isolation, screening and molecular characterization of feather degrading bacteria isolated from poultry soil in Basrah province. *Biodiversitas* **2024**, *25*, 2560-2569.

[18] Miyoshi, T.; Iwatsuki, T.; Naganuma, T. Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-size filters. *Appl Environ Microbiol* **2005**, *71*, 1084-1088.

[19] Tork, S.E.; Aly, M.; Nawar L. Biochemical and molecular characterization of a new local keratinase producing *Pseudomonas* sp., MS21. *Asian J Biotechnol* **2020**, *2*, 1-13.

[20] Barman, N.C.; Zohora, F.T.; Das, K.C.; Mowla, M.G.; Banu, N.A.; Salimullah, M.; Hashem, A. Production, partial optimization and characterization of keratinase enzyme by *Arthrobacter* sp. NFH5 isolated from soil samples. *AMB Express* **2017**, *7*, 181.

[21] Tiwary. E.; Gupta, R. Rapid conversion of chicken feather to feather meal using dimeric keratinase from *Bacillus licheniformis* ER-15. *J Bioprocess Biotech* **2012**, *2*, 1000123.

[22] Donner, M.W.; Arshad, M.; Ullah, A.; Siddique, T. Unraveled keratin-derived biopolymers as novel biosorbents for the simultaneous removal of multiple trace metals from industrial wastewater. *Sci Total Environ* **2019**, *647*, 1539-1546.

[23] Eslahi, N.; Hemmatinejad, N.; Dadashian, F. From feather waste to valuable nanoparticles. *Particulate Sci Tech* **2014**, *32*, 242-250.

[24] Valeika, V.; Širvaitytė, J.; Bridžiuvienė, D.; Švedienė, J. An application of advanced hair-save processes in leather industry as the reason of formation of keratinous waste: few peculiarities of its utilisation. *Environ Sci Poll Res* **2019**, *26*, 6223-6233.

[25] Okoroma, E.A.; Garelick, H.; Abiola, O.O.; Purchase, D. Identification and characterisation of a *Bacillus licheniformis* strain with profound keratinase activity for degradation of melanised feather. *Intern Biodeter Biodegrad* **2012**, *74*, 54-60.

[26] Dada, M.; Wakil, S. Conversion of feather to potential feed supplement using keratinase from *Bacillus licheniformis*-K51. *J Appl Sci Environ Sustain* **2021**, *13*, 10-31.

[27] Onifade, A.A.; Al-Sane, N.A.; Al-Musallam, A.A.; Al-Zarban, S. A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. *Biores Technol* **1998**, *66*, 1-11.

[28] Friedrich, A.B.; Antranikian, G. Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order Thermotogales. *Appl Environ Microbiol* **1996**, *62*, 2875-2882.

[29] Sivakumar, T.; Shankar, T.; Thangapand, V.; Ramasubram, V. Optimization of cultural condition for keratinase production using *Bacillus cereus* TS1. *Insight Microbiol* **2013**, *3*, 1-8.

[30] Alyousif, N.A.; AL-Luaibi, Y.Y.Y.; Hussein, W. Distribution and molecular characterization of biosurfactant-producing bacteria. *Biodiversitas* **2020**, *21*, 4034-4040.