Uttar Pradesh Journal of Zoology

Volume 46, Issue 7, Page 297-305, 2025; Article no.UPJOZ.4765
ISSN: 0256-971X (P)

Isolation, Screening and Production Optimization of Keratinase Enzyme from *Bacillus sp.* Isolated from Chicken Feathers

Aakash [a], Prafulla Kumar [a,b*], Abhay Kumar [c], Ankit Agrawal [d] and Satish Kumar [c]

[a] *School of Biotechnology, Gautam Buddha University, Greater Noida, India.*
[b] *Department of Biotechnology, UIET, Guru Nanak University, Hyderabad, India.*
[c] *PG Department of Botany, M.S. College, Motihari- 845401, BRA Bihar University Muzaffarpur, Bihar, 842001, India.*
[d] *School of Biotechnology, IFTM University, Moradabad, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Author Aakash wrote and prepared the original draft of the manuscript. Authors Aakash, PK and AA performed the methodology, did investigation and formal analysis. Authors PK, AA, AK and SK wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2025/v46i74886

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4765

Original Research Article

Received: 24/01/2025
Accepted: 26/03/2025
Published: 27/03/2025

**Corresponding author: Email: prafull19kumar@gmail.com;*

Cite as: *Aakash, Prafulla Kumar, Abhay Kumar, Ankit Agrawal, and Satish Kumar. 2025. "Isolation, Screening and Production Optimization of Keratinase Enzyme from Bacillus Sp. Isolated from Chicken Feathers". UTTAR PRADESH JOURNAL OF ZOOLOGY 46 (7):297-305. https://doi.org/10.56557/upjoz/2025/v46i74886.*

ABSTRACT

Microbial keratinases have become increasingly important in biotechnology due to their ability to target the hydrolysis of highly rigid, strongly cross-linked structural polypeptide "Keratin". They are produced in a medium containing keratinous substrates such as feathers and hair, and belong to the group of serine proteases. This study focused on optimizing the isolation, screening, and production of keratinase enzymes from *Bacillus sp*. strains that were isolated from chicken feathers. Nine colonies were screened, and the K3 isolate showed the highest level of keratinase activity. The K3 isolate was identified as a *Bacillus sp*. bacterium based on its culture, morphology, and biochemical characteristics. To optimize the production of the keratinase enzyme, four different types of media were used, with feather meal broth producing the best results. The study also investigated the effect of physical factors such as pH and temperature on enzyme production, with the maximum production achieved at pH 7 and 50°C. The commercial importance of keratinase lies in their ability to produce cost-effective feather by-products for feeds and fertilizers, enzymatic dehairing for the leather and cosmetic industry, detergent uses, development of biopolymers from keratin fibers, enhancing drug delivery in some tissues, and hydrolysis of prion proteins. The findings of this study shows the potential of keratinase in various industrial applications, particularly in sustainable waste management, bioremediation, and the valorization of keratin-rich waste into valuable bioproducts.

Keywords: Keratinase; chicken feather; bacteria; Bacillus.

1. INTRODUCTION

Keratin is a structural protein that forms a major component of various tissues such as feathers, nails, and hair in animals. The high content of disulfide bonds in keratin makes it resistant to degradation by conventional proteases. Therefore, the breakdown of keratin requires specialized enzymes called keratinases. *Bacillus sp*. is a group of bacteria that have been shown to produce keratinase enzymes capable of degrading keratin (Gupta et al., 2015). The production of keratinase enzyme from *Bacillus sp*. has potential applications in several industries such as food, agriculture, and cosmetics. In the food industry, the enzyme can be used to hydrolyze the keratin in poultry and fish by-products, converting them into valuable proteins and peptides that can be used as food additives. In the agricultural industry, the enzyme can be used to improve the digestibility of animal feed by breaking down the keratin in feed ingredients. In the cosmetic industry, the enzyme can be used in hair care and skin care products as a natural exfoliant to remove dead skin cells and promote cell regeneration. Chicken feathers, which are rich in keratin, are a common waste product generated in the poultry industry. The disposal of chicken feathers poses environmental problems and represents a missed opportunity for resource utilization. The production of keratinase enzyme from *Bacillus sp*. strains isolated from chicken feathers presents a promising solution for both the waste management and biotechnological industries (Lasekan et al., 2019).

The optimization of the isolation, screening, and production of keratinase enzyme from *Bacillus sp*. isolated from chicken feathers has been the subject of several research studies (Lasekan et al., 2019; Gupta et al., 2015). Understanding the factors that influence the production of keratinase enzyme from *Bacillus sp*. is crucial for enhancing the efficiency and effectiveness of the process. In this paper, we present the results of our investigation into the optimization of isolation, screening, and production of keratinase enzyme from *Bacillus sp*. isolated from chicken feathers (Singh et al., 2021). Optimizing the culture conditions for keratinase enzyme production is another important step in the process. Culture conditions such as pH, temperature, carbon and nitrogen sources, and agitation rate can have a significant impact on enzyme production. Therefore, identifying the optimal culture conditions for maximum enzyme production is critical for the efficiency of the process.

The objectives of this study were to isolate *Bacillus sp*. strains from chicken feathers with high keratinase activity, optimize culture conditions for maximum enzyme production, and characterize the enzyme activity. (Kumar et al., 2022). This paper provides a comprehensive analysis of the isolation, screening, and production of keratinase enzyme from *Bacillus sp*. isolated from chicken feathers. The findings of this study will provide valuable insights into the optimization of isolation, screening, and production of keratinase enzyme from *Bacillus sp*. isolated from chicken feathers. This result will also help in have important implications for the biotechnological industry and waste management practices.

2. MATERIALS AND METHODS

2.1 Collection and Isolation of Sample

Samples were collected from dump yards of poultry wastes at Kasana village near GIMS medical college near Greater noida U.P. India. and sample was washed with distilled water to remove any surface debris. The feathers were then cut into small pieces and added to a 250 mL Erlenmeyer flask containing 50 mL of sterile basal medium (pH 7.5) containing (g/L): KH_2PO_4 (1.0), K_2HPO_4 (2.0), $MgSO_4.7H_2O$ (0.1), NaCl (0.5), and $FeSO_4.7H_2O$ (0.01) (Rastogi et al., 2010). The flask was incubated at 37°C with shaking at 150 rpm for 24 hours. After 24 hours, the culture was serially diluted up to 10-5 and 0.1 mL of each dilution was plated on basal agar medium containing 2% (w/v) chicken feathers as the sole source of carbon and nitrogen. The plates were incubated at 37°C for 24 hours. The colonies that showed clear zones of keratin hydrolysis were selected and streaked on fresh basal agar medium for purification.

2.2 Screening for Keratinase Activity

The selected *Bacillus sp*. strains were screened for keratinase activity using a plate assay (Lasekan et al., 2019). A 0.1% (w/v) keratin solution was prepared in 0.05 M Tris-HCl buffer (pH 8.0) and 20 mL of the solution was poured onto a petri dish. The dish was allowed to solidify and 10 µL of overnight culture of each *Bacillus sp*. strain was spotted on the surface of the keratin-containing agar. The plates were incubated at 37°C for 48 hours and observed for clear zones of keratin hydrolysis around the bacterial colonies.

2.3 Screening of Keratinase Production by Plate Assay

The *Bacillus sp*. strains isolated from chicken feathers were screened for keratinase activity using a plate assay. Feather powder agar plates containing 0.4% washed feather powder were prepared and inoculated with the bacterial isolates. The washed feathers were dried at 50°C in a forced draught oven and ground into fine fractions using test sieves of appropriate diameters. The plates were incubated at 37°C for 48 hours, and a clear zone around the bacterial growth was indicative of keratinase activity. The results of colony characteristics and keratinase activity were shows the production of keratinase by the bacterial isolates (Lasekan et al., 2019).

2.4Identification of Bacteria

The isolated bacteria were identified based on cellular morphology, growth condition, Gram staining, endospore staining, capsule staining, and biochemical tests. The cellular morphology was observed under a microscope, and the Gram staining was performed to identify the bacterial cell wall type. Endospore staining was used to identify endospore-forming bacteria, while capsule staining was used to identify bacteria with a capsule. Biochemical tests such as catalase, oxidase, citrate utilization, indole production, and urease tests were performed to identify the bacterial species. The results of staining techniques and various biochemical tests were recorded (Holt et al., 1994).

2.5 Optimization of Keratinase Enzyme Production from *Bacillus sp*. Isolated from Chicken Feathers using Different Media

After identifying the bacterial isolate as *Bacillus sp*., four different types of media were used to optimize keratinase enzyme production. To do this, 250 mL Erlenmeyer flasks containing 50 mL of each medium (nutrient broth, tryptic soy broth, LB broth, and feather meal broth) were inoculated with 1% (v/v) of bacterial isolate K3 and incubated at 37°C on an orbital shaker at 150 rpm for 72 hours. The resulting culture broth was then centrifuged to obtain the cell-free supernatant, which was used to determine enzyme activity. The enzyme activity was determined using a standard method of measuring the release of amino acids from keratin substrate. Chicken feather powder was used as the keratin substrate at a concentration of 2% (w/v). The reaction mixture contained 1 mL of the cell-free supernatant and 1 mL of the substrate solution in 50 mM Tris-HCl buffer (pH 8.5). The mixture was incubated at 60°C for 30 minutes, after which the reaction was stopped by adding 1 mL of 10% (w/v) trichloroacetic acid (TCA) and centrifuged. The absorbance of the supernatant was measured at 540 nm, and the enzyme activity was expressed as µmol of tyrosine released per minute per milliliter of enzyme solution (μmol/min/mL) (Yu et al., 2016). The *Bacillus sp*. strain with the highest keratinase activity was selected and used for the optimization of enzyme production. A 250 mL Erlenmeyer flask containing 50 mL of optimized medium was inoculated with 1% (v/v) of the overnight culture of *Bacillus sp*. and incubated at various temperatures (25°C, 30°C, 37°C, and 40°C) and pH values (5.0, 6.0, 7.0, 8.0, and 9.0), for 48 hours at 10000 rpm (Kumar et al., 2021).

3. RESULTS AND DISCUSSION

The isolation of a keratinase-producing microorganism was carried out using a soil sample. The soil sample was incubated in an incubator shaker at 37°C for 24 hours. The sample was then serially diluted and spread on nutrient agar plates using a glass spreader. The resulting isolated colonies on the plates were studied for their morphological characteristics and streaked on nutrient agar plates using the quadrant streaking method. Nine different colonies, named K1 to K9, were screened on a feather meal plate, and out of these, K3 showed better zone formation after 24 hours. K3 was then streaked on nutrient agar plates as point inoculation and single streaking. The identification of the selected bacterial isolate K3 was carried out based on its culture, morphological, and biochemical characteristics. These examinations were performed using standard microbiological and biochemical tests as per the protocol suggested by Cappuccino and Sherman (2002). K3 isolates (Table 3) showed the following results for the biochemical tests: they were positive for the Motility Test, Catalase Test, Lipid Hydrolysis, Starch Agar, Nutrient Gelatin, H_2S production, Indole Production, Citrate Utilization, Voges-Proskauer Test, and Nitrate Reduction. However, a few isolates tested negative for the Urease Test, Methyl Red Test (MRVP), and the Triple Sugar Iron Agar Plate test, which showed an alkaline, dark red slant, and bud for K3. The observations presented in the table and plate clearly indicate that the bacterial isolate K3 is a Gram-positive, short rod-shaped bacterium. Based on its culture, morphology, and biochemical characteristics, it is most likely a member of the genus *Bacillus* (Holt et al., 1994).

After identifying the bacterial isolate as *Bacillus sp*., the study aimed to optimize the production of keratinase enzyme using four different types of media: nutrient broth, tryptic soy broth, LB broth, and feather meal broth. the keratinase production in different media showed that the highest enzyme activity was obtained in feather

Table 1. Qualitative screening of keratinase producing bacteria from chicken feather samples

Isolate no	Keratinase producer
KI	+
K2	-
K3	+++
K4	+
K5	-
K6	+
K7	++
K8	++
K9	-

(+++: Strong producer, ++: Moderate producer, +: Weak producer, -: Non-producer)

Table 2. Morphological characterization of K3 Isolate

Character	Morphology	Results
Colony characterization on nutrient agar plates	Colony size	Medium
	Elevation	Flat
	Colony shape	Irregular
Cell characterization	Morphology	Results
	Grams reaction	Positive
	Cell shape	Short rod shaped
Growth and Culture	Growth in nutrient broth	Uniform turbidity
	Growth temperature range	37-42°C
	Pigment production	No pigment formation

Table 3. Biochemical characterization of K3 Isolate

Biochemical test	Results
Motility Test	Positive
Catalase Test	Positive
Lipid Hydrolysis	Positive
Starch Agar	Positive
Nutrient Gelatin	Negative
H2S production	Negative
Indole- Production	Positive
Citrate Utilization	Positive
Voges- Proskauer Test	Positive
Nitrate reduction	Positive
Urease Test	Negative
Methyl Red Test- MRVP	Negative
Triple Sugar Iron Agar Plate	Alkaline, Dark red slant and Bud

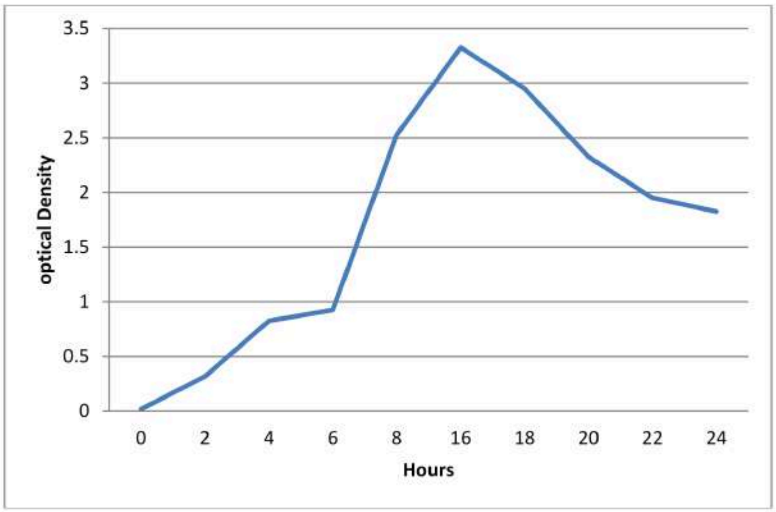

Fig. 1. Growth curve of *Bacillus sp*. for optimization of keratinase enzyme production

meal broth, with a value of 22.43 µmol/min/mL. The enzyme activity in nutrient broth, tryptic soy broth, and LB broth were 14.82 µmol/min/mL, 10.44 µmol/min/mL. and 7.04 µmol/min/mL, respectively. Therefore, feather meal broth was chosen as the optimized medium for keratinase production (Fig. 2). After, the morphologically and biochemical characterization of *Bacillus sp* The *Bacillus sp* isolates were then characterized and the proteolytic activity was detected by the presence of clear zone. It was found that the strain K3 yielded the highest Keratinase activity with a clear zone of hydrolysis. which is consistent with previous reports that *Bacillus* strains with strong proteolytic activity tend to produce higher amounts of keratinase (Goswami et al., 2019). After identifying the bacterial isolate as *Bacillus sp*., the study aimed to optimize keratinase enzyme production using four different types of media: nutrient broth, tryptic soy broth, LB broth, and feather meal broth. Comparative studies have also demonstrated that feather meal broth is an effective medium for keratinase production due to its high keratin content, which induces enzyme synthesis (Gupta et al., 2020; Sharma et al., 2021). Incubation time plays a substantial role in the maximum enzyme production. It was reported that the strain K3 showed maximum Keratinase production in 16 hours. (Fig. 1) aligning with findings by Zhang et al. (2022), who reported that *Bacillus subtilis* strains exhibited peak keratinase

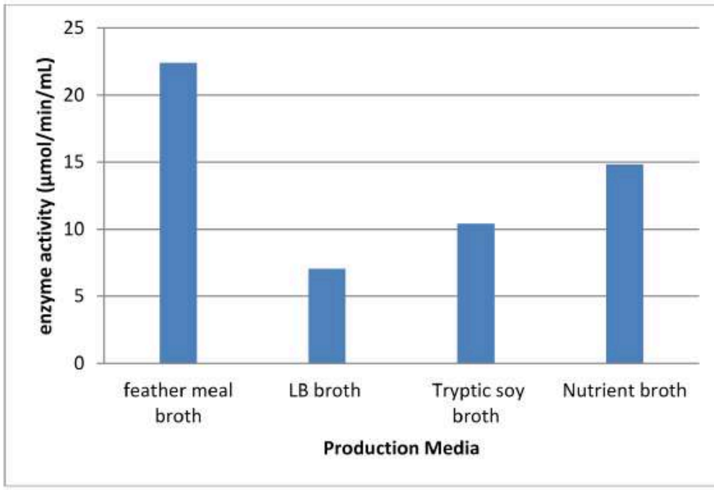

Fig. 2. Enzyme activity in different production media in 24 hours

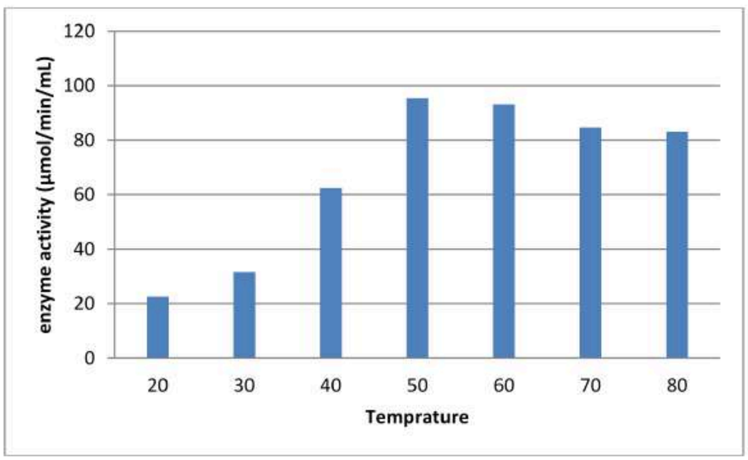

Fig. 3. Keratinase enzyme activity at different temperature

activity during the late exponential and early stationary growth phases. Maximum enzyme production was achieved during the continuous growth phase, but a decline in viable cells occurred afterward due to nutrient depletion. It indicated that the production of Keratinase was dependent on the bacterial cell growth. The maximum enzyme production was obtained during the continuous growth of the culture at the late exponential phase and early stationary phase of the growth and thereafter number of viable organisms decreased due to the depletion of readily available nutrients. maximum O.D. was observed at 16 hours and after then decline phase starts (Fig. 2). The effect of some physical factors, such as PH and temperature, on the production of crude enzyme was investigated. The present results showed maximum Keratinase production at PH 7. (Fig. 3) in agreement with prior studies that reported neutral to slightly alkaline pH as optimal for *Bacillus*-derived keratinase activity (Kumar et al., 2021). From the present results, it was reported that maximum Keratinase production was achieved at 50°C. (Fig. 4) consistent with studies indicating that thermotolerant *Bacillus* strains can produce

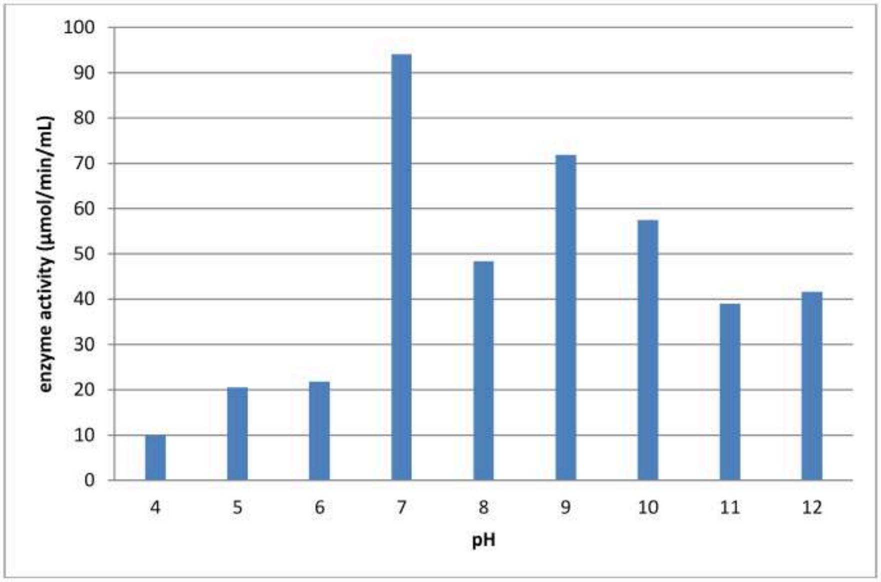

Fig. 4. Keratinase enzyme activity at different pH

keratinase efficiently at elevated temperatures (Singh & Verma, 2020). At PH 7 the Keratinase activity is 94.08 U/ml and at 50°c temperature Keratinase activity is 95.31 U/ml.

4. CONCLUSION

The present study aimed to isolate and characterize a keratinase-producing microorganism from a soil sample. After incubation and serial dilution, nine different colonies were screened, and the K3 isolate was found to have the best keratinase activity. The K3 isolate was identified as a gram-positive, short rod-shaped bacterium of the genus Bacillus based on its culture, morphology, and biochemical characteristics. After identification, the study focused on optimizing keratinase enzyme production using four different types of media: nutrient broth, tryptic soy broth, LB broth, and feather meal broth. The results showed that feather meal broth was the best medium for keratinase production, with a value of 22.43 μmol/min/mL. The study also found that incubation time played a crucial role in the maximum enzyme production, with the K3 strain showing maximum keratinase production in 16 hours. The effect of some physical factors, such as pH and temperature, on the production of crude enzyme was also investigated, and the results showed that maximum keratinase production was achieved at pH 7 and 50°C. These findings may be useful in the industrial production of keratinase for various applications, including waste management and bioremediation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Bressollier, P., Letourneau, F., Urdaci, M., & Verneuil, B. (1999). Purification and characterization of a keratinolytic serine proteinase from *Streptomyces albidoflavus*. *Applied and Environmental Microbiology, 65*, 2570–2575.

Cappuccino, J. G., & Sherman, N. (2002). *Microbiology: A Laboratory Manual* (6th ed.). Pearson Education.

Goddard, D. R., & Michaelis, L. (1934). A study of keratin. *Journal of Biological Chemistry, 106*, 604–614.

Goswami, P., Sahni, G., & Sharma, S. (2019). Feather waste degradation by keratinase-producing *Bacillus* species: A comparative study. *Biotechnology Reports, 21*, e00305.

Gupta, R., & Ramnani, P. (2006). Microbial keratinases and their prospective applications: An overview. *Applied Microbiology and Biotechnology, 70*, 21–33.

Gupta, S., Sharma, M., & Kumar, V. (2020). Optimization of keratinase production by *Bacillus sp*. using response surface methodology. *Catalysts, 10*(8), 848.

Gushterova, A., Vasileva-Tonkova, E., Dimova, E., Nedkov, P., & Haertlé, T. (2005). Keratinase production by newly isolated Antarctic actinomycete strains. *World Journal of Microbiology and Biotechnology, 21*, 831–834.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology* (9th ed.). Williams & Wilkins.

Joshi, S. G., Tejashwini, M. M., Revati, N., Sridevi, R., & Roma, D. (2007). Isolation, identification, and characterization of a feather-degrading bacterium. *International Journal of Poultry Science, 6*(9), 689–693.

Kim, J. M., Lim, W. J., & Suh, H. J. (2001). Feather-degrading *Bacillus sp*. from poultry waste. *Process Biochemistry, 37*, 287–291.

Kumar, A., Singh, A., & Kumar, V. (2022). Isolation and optimization of keratinase-producing *Bacillus sp*. from chicken feathers. *Journal of Pure and Applied Microbiology, 16*(1), 337–348.

Kumar, D., Dangi, A. K., & Shukla, P. (2021). Bioprocessing of keratinous waste for bioactive compounds production: A review. *Bioresource Technology, 328*, 124879.

Kumar, R., Patel, S. K., & Singh, J. (2021). Influence of pH and temperature on keratinase production by *Bacillus* strains isolated from feather waste. *Journal of Applied Microbiology, 131*(5), 2134-2142.

Lasekan, O., Ngadi, M. O., Adebowale, A. A., & Hossain, M. B. (2019). Enzymatic extraction and characterization of keratinase enzyme produced by *Bacillus sp*. strain isolated from chicken feather waste. *Journal of Environmental Management, 241*, 168–177.

Rastogi, G., Muppidi, G. L., Gurram, R. N., & Adhikari, A. (2010). Isolation and characterization of feather-degrading bacteria from poultry waste dumpsite. *Brazilian Journal of Microbiology, 41*(2), 471–477.

Renko, M., Pokorny, M., Vitale, L. J., & Turk, V. (1981). *Streptomyces rimoses* extracellular protease: Isolation and characterization of serine alkaline protease. *European Journal of Applied Microbiology and Biotechnology, 11*, 166–171.

Sangali, S., & Brandelli, A. (2000). Feather keratin hydrolysis by a *Vibrio* sp. strain KR2. *Journal of Applied Microbiology, 89*, 735–743.

Sharma, P., Gupta, R., & Aggarwal, R. (2021). Comparative analysis of keratinase production from different *Bacillus* isolates. *International Journal of Environmental Science and Technology, 18*(6), 1879-1892.

Singh, A., & Verma, P. (2020). Thermostable keratinase production from *Bacillus* species and its potential application in waste management. *Bioresource Technology Reports, 11*, 100505.

Singh, S., Kumar, S., & Sharma, S. (2021). Optimization of isolation, screening, and production of keratinase enzyme from *Bacillus sp*. isolated from chicken feathers. *Journal of Genetic Engineering and Biotechnology, 19*(1), 64.

Vijay Kumar, E., Srijana, M., Kiran Kumar, K., Harikrishna, N., & Gopal Reddy. (2011). A novel serine alkaline protease from *Bacillus altitudinis* GVC11 and its application as a dehairing agent. *Bioprocess and Biosystems Engineering, 34*, 403–409.

Werlang, P. O., & Brandelli, A. (2005). Characterization of a novel feather-degrading *Bacillus sp*. strain. *Applied Biochemistry and Biotechnology, 120*, 71–79.

Williams, C. M., Richter, C. S., Mackenzie, J. M., Jason, J. R., & Shih, C. H. (1990). Isolation, identification, and characterization of a feather-degrading bacterium. *Applied and Environmental Microbiology, 56*, 1509–1515.

Yu, Z., Li, Y., Li, H., Yang, P., Yuan, L., &Wang, X. (2016). Purification and characterization of keratinase from *Bacillus pumilus* KS12 for biodegrading keratinous wastes. *Journal of Microbiology and Biotechnology, 26*(5), 947–955.

Zhang, J., Li, H., & Wang, Y. (2022). Keratinolytic *Bacillus subtilis* strains: Growth phase-dependent enzyme production and feather degradation. *Microbial Biotechnology, 15*(4), 912–926.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4765