



## Biotechnological Valorization of Chicken Feather Waste: Extraction and Microbial Degradation of Keratin

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### Abstract:

*The poultry industry produces large amounts of chicken feather waste each year. This waste creates serious environmental and disposal issues due to its tough nature. Chicken feathers mainly consist of keratin, a strong and insoluble protein known for its extensive disulfide bonds, which makes it difficult to break down naturally. This study sought to extract keratin from chicken feathers using chemical treatment and to test microbial degradation as a sustainable waste management method. Cleaned and sterilized feathers underwent alkaline hydrolysis with sodium hydroxide (NaOH) to dissolve keratin. Then, acetic acid was used for acid precipitation to obtain keratin protein. Qualitative protein analysis confirmed the existence of peptide bonds in the extracted keratin, thus ensuring the successful extraction of protein from poultry feather waste. Isolates of keratinase-producing bacteria were then obtained and screened for their ability to degrade feathers. Among the isolates screened, one strain showed high keratinase activity, thus indicating significant feather degradation capability under laboratory settings. The findings indicate that a green and efficient method of converting poultry feather waste into useful products is to combine chemical extraction with microbial degradation. Cosmetics, biomedical materials, and other industries can use recovered keratin. The results show that by combining chemical extraction with microbial degradation, poultry feather waste can be effectively and sustainably transformed into useful products. The recovered keratin could be used in biomedical materials, cosmetic formulations, and other industrial fields. This work demonstrates that it is possible to valorise poultry waste through biotechnological interventions, which supports sustainable waste management and resource recovery.*

### Introduction: Globalization and the Transformation of Statecraft:

The food industry generates substantial keratin waste, particularly chicken feathers, which are rich in amino acids and essential nutrients (3. Moktip, 2025). Keratin is a fibrous and insoluble structural protein, which is widely cross-linked with disulfide, hydrogen and hydrophobic bond (1. Ma, 2024). Keratin found in feathers is known to have excellent resistance to natural degradation. The mechanical stability of keratin and its resistance to microbial degradation depend on the tight packing of the protein chain in  $\alpha$ -helix ( $\alpha$ -keratin) and  $\beta$ -sheet ( $\beta$ -keratin) structures and their linkage by cysbridges due to

high degree of cross-linkage by disulfide bonds, hydrogen bonding and hydrophobic interactions (4. Chauhan, 2023). These structural features of keratin contribute to the high rigidity of the structure, rendering it insoluble and inaccessible to many forms of proteolytic enzymes used in traditional methods for degrading keratin waste. As such, conventional degradation methods frequently have limited efficacy. In poultry processing industries, feathers are typically disposed of by incineration or subjected to chemical hydrolysis. The feather keratin contains a higher concentration of amino

acids, i.e., glycine, alanine, serine, cysteine, and valine, but less lysine, methionine, and tryptophan (Shyam Dev Maurya\*, 2023). While these methods reduce waste volume, they are energy-intensive and may contribute to environmental pollution. One potential solution to the problem of microbial degradation is to use microorganisms (bacteria and fungi). These microorganisms have two major types of microbes that produce an enzyme called keratinase that specifically degrades or acts upon the tough keratin matrix. Keratinase converts the complex keratin structure into soluble peptides and amino acids. Among reported keratinolytic microorganisms, members of the genus *Bacillus* are frequently highlighted due to their strong enzymatic activity, adaptability, and relevance in industrial processes.

By chemically treating the keratin before using microbes to degrade it, you create a partially ruptured keratin network, which makes it easy for the microbes to access the keratin and works better than just using one process alone to aid in breaking down the keratin. The resulting product, keratin hydrolysate, made from both chemical and microbial degradation of the keratin will be rich in amino acids and compatible with biological systems. In addition, keratinases produced by these microorganisms have a wide range of applications in the food, detergent, leather and cosmetic industries (2. Isha Sharma, 2021) The present study therefore focused on isolating and characterizing keratinase-producing bacterial strains, assessing their feather-degrading capability, and extracting keratin protein powder through a combined chemical and microbial treatment strategy.



**Figure 1: Photograph of Chicken Feather**

#### **Material and Method:**

**1. Chemicals:** All Chemicals required for experimental work were of analytical grade, pure and purchased from Hi-media laboratory.

**2 Sample collection:** Soil samples were collected from feather dumping site of local poultry farms located at post Dahi wadi Maan-Satara district area. Sample was collected and labelled in sterile bags and keep until Cultivation. Chicken Feathers were collected, transferred and labelled into sterile bags for further degradation and extraction process.

**3. Cultivation of Keratinase Producing Bacteria:** The cultivation of microorganism was done in FBM (Feather Basal Medium containing  $\text{KH}_2\text{PO}_4$ - 0.7g ;  $\text{K}_2\text{HPO}_4$ - 1.4g ;  $\text{MgSO}_4$  – 0.1g ;  $\text{NaCl}$  – 0.5g and pH-7). The Stalk media was autoclaved for 15 mins at  $121^\circ\text{C}$ . The collected soil from the local poultry farm was weighed 1g and added to the sterile stock media. The flask containing stalk media was incubated for 7 to 8 days at temeperture  $37^\circ\text{C}$ .

**4. Isolation and screening of keratinolytic bacteria:** After the incubation period, the microorganism were isolated from the stock culture media (FBM) by the plating method on nutrient agar. A loopful culture from the stock culture media (FBM) was streaked on Nutrient agar Sterile plate. The plate were kept for incubation for 3 days at  $37^\circ\text{C}$ , then the colonies were purify using streak plate method. The isolates were characterized for colony characteristics, morphological characteristics and biochemical characteristics and identified with

Bergey's manual of determinative bacteriology (Godbole\*, 2017).

**5. Assay for keratinase activity:** The keratin assay was done to find out the feather degradation capability of identified isolates. The collected feathers from the poultry farm dumping site was washed with detergent to remove excess of material present on the feather. The feathers were divided into 2 batch, batch 1 feathers were washed with ethanol, batch 2 feathers were washed with warm water. For keratin assay, nutrient broth was prepared, autoclave for 15 min for 121°C, broth was divided for 2 batch of feathers washed with ethanol and warm water also inoculated with identified isolate. The isolates use feathers as a single carbon and nitrogen source. The two nutrient broth enriched with feathers, identified isolate were kept at room temperature and observed for every six days until the feathers were degraded.

#### **6. Extraction of Protein Keratin from Chicken feathers:**

##### **Step 1- Pre-treatment process:**

The waste chicken feather were collected from Dahi-wadi, Satara district. In this step wise process the feathers were washed with detergent and also with soapy water to get rid of oil grease and most important to kill microorganisms present on the surface of feathers. The feathers were air dried under proper condition.

##### **Step 2- Extraction and precipitation process:**

For keratin extraction, prepare 1N NaOH solution for 100ml, weigh 1.5g of feathers and

add into the prepared NaOH solution. Stir the solution continue for 5-10 min. After the mentioned time, observe for any color change. The solution will change from white to pale yellow. Filter the solution using a funnel and what-man filter paper. Add drop wise acetic acid to the filtered solution, let the solution be stable for 30min. A white precipitation forms at the bottom of the flask.

##### **Step 3 Purification of the extract:**

Add 1.5 ml of solution to sterile vials and proceed for centrifugation for 5,000 rpm for 5min. Collect the pellet discard the supernatant, the pellets were placed in petri plate and then was air dried with the help of hot air oven for 1hr and was observed between for every 10 min. After drying by hot air oven, dried.

##### **Results:**

Soil samples were inoculated in feather meal broth to obtain bacterial isolates which are feathers degrading and were capable of producing extra cellular keratinase, using feather (keratin) as sole carbon source. After a week of incubation the flasks showed turbidity and disintegration of feathers were observed.



**Figure 2: Feather Meal Broth**

#### **1. Colony Characteristics:**

Size	Shape	Color	Margin	Elevation	Opacity	Gram Nature	Motility
<1mm	Circular	Pale yellow	Entire	Elevated	Opaque	Gram Positive	Motile



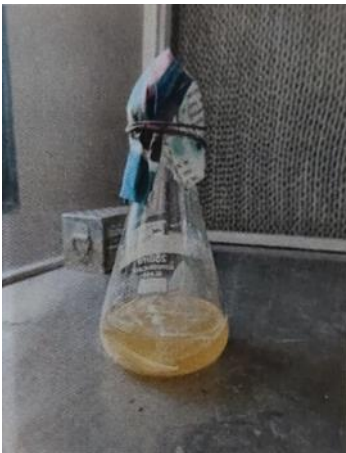
**Figure 3: Nutrient Agar plate Containing Bacterial Colonies**

## 2. Biochemical Characteristics:

Sr.no.	Biochemical Test	Result
1	Catalase test	+
2	Oxidase test	+
3	Methyl red test	-
4	Voges-Proskauer test	+
5	Indole test	-
6	Sugar Fermentation test	
	a. Glucose	+
	b. Lactose	+
	c. Mannitol	+

**Table 1: Biochemical test**

## Keratin Assay:



**Figure 4: Feathers on day 0**



**Figure 5: Feathers on day 12**



**Figure 6: Feathers on day 31**

The Keratin assay showed the degradation of feathers done by isolated microorganism present in Nutrient Broth Media after 31 days.

## Yield Calculation percentage for Extracted Keratin Powder:

Total weight of Obtained Keratin = 0.45 g

Total weight of waste chicken feathers = 1.5g

$$\begin{aligned} \text{Yield Calculation (\%)} &= \frac{\text{Total weight of obtained keratin}}{\text{Total weight of waste chicken feather}} \times 100 \\ &= \frac{0.45}{1.5} \times 100 \\ &= 30\% \end{aligned}$$

Therefore, 30% yield of keratin from Chicken feather weighed 1.5g was obtained respectively.



**Figure 7: Extracted Keratin Powder from Chicken Feather**

### Conclusion:

The findings of this study highlight the significant role of *Bacillus subtilis* in the biodegradation of keratin obtained from chicken feathers. Being a Gram-positive, rod-shaped bacterium, it demonstrated strong keratin-degrading ability and proved to be an effective biological agent for feather waste management. An interesting observation was made regarding the washing methods used before degradation. Feathers treated with ethanol showed a noticeably higher degradation rate compared to those washed with warm water, suggesting that ethanol pretreatment may improve keratin breakdown efficiency.

The extracted keratin was further analyzed to understand its properties. The yield was found to be approximately 30%, with a low ash content of 1%, indicating minimal impurities. The measured density (0.235 g/ml) reflects its lightweight nature. Chemical analysis confirmed the presence of peptide bonds and aromatic amino acids, supporting the structural complexity of keratin. Overall, these results enhance our understanding of keratin composition and support environmentally friendly approaches for feather waste utilization.

### Discussion:

This study explored the potential of keratinase-producing bacteria isolated from poultry waste soil in Dahiwadi, Satara, for the degradation of chicken feathers. Since feathers are composed of tough, highly stable keratin, their natural breakdown is slow and challenging, making microbial treatment a more sustainable alternative to conventional disposal practices. The isolated strain was identified as a Gram-positive, rod-shaped *Bacillus* species, most closely related to *Bacillus subtilis*, based on morphological and biochemical characteristics. The culture was preserved in glycerol for future use. After 31 days of incubation, the bacterium showed clear keratin-degrading ability, particularly affecting the feather barbs and partially the rachis. Although complete degradation was not observed, the results aligned with earlier findings.

### Acknowledgements:

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### References:

1. Isolation and identification of Feather-Degrading bacteria and cloning of their keratinase gene. *Preprints.org*. <https://doi.org/10.20944/preprints202401.0930.v1>
2. Sharma, I., & Kango, N. (2021). Production and characterization of keratinase by *Ochrobactrum intermedium* for feather keratin utilization. *International journal of biological macromolecules*, 166, 1046–1056. <https://doi.org/10.1016/j.ijbiomac.2020.10.260>
3. Moktip, T., Salaipeh, L., Cope, A. E., Taherzadeh, M. J., Watanabe, T., & Phitsuwan, P. (2025). Current

- Understanding of Feather Keratin and Keratinase and Their Applications in Biotechnology. *Biochemistry research international*, 2025, 6619273. <https://doi.org/10.1155/bri/6619273>
4. Chauhan, Aishwarya & Sharma, Ruchi & Devi, Sunita. (2023). Identification and Characterization of Keratinolytic Bacterium Isolated from Poultry Waste.
  5. Maurya, S. D., & Singh, A. (2023). Extraction and Characterization of Keratin from Waste Broiler Chicken Feathers. *Environmental Protection Research*, 3(2), 372–381. <https://doi.org/10.37256/epr.3220233579>
  6. Alamoudi SA, Khalel AF, Alghamdi MA, et al. Isolation, Identification and Characterization of Keratin-Degrading *Streptomyces rochei* AM8. *J Pure Appl Microbiol.* 2022;16(3):2045-2054. doi: 10.22207/JPAM.16.3.58
  7. Sunil Chhimpa, Chandra Shekhar Yadav, P.J. John, 2016. Isolation and Identification of Keratin Degrading (keratinolytic) Bacteria from Poultry Feather Dumping Sites. *J. Biodiv. Environ. Sci.*, 8(6), 109-119.
  8. Godbole, S., Pattan, J., Gaikwad, S., & Jha, T. (2017). Isolation, Identification and Characterization of Keratin degrading microorganisms from Poultry soil and their Feather degradation Potential. *International Journal of Environment, Agriculture and Biotechnology*, 2(4), 2060–2068. <https://doi.org/10.22161/ijeab/2.4.64>
  9. Areeb Inamdar, SaheraNasreen and Rashiqua Siddiqui, Screening And Production Of Extra Cellular Feather Degrading Enzyme From Bacterial Isolates, *Indian J.L. Sci.* 2012,vol 1(2),pp 19-24
  10. Sharma, Kratika. (2025). Isolation, Identification and Characterization of Keratinolytic Bacteria from Poultry Waste. *International Journal of Science and Research (IJSR)*. 14. 1133-1140. [10.21275/MR25125221856](https://doi.org/10.21275/MR25125221856).