INTERNATIONAL JOURNAL OF RESEARCH CULTURE SOCIETY
Monthly Peer-Reviewed, Refereed, Indexed Journal
Volume - 10, Issue - 6, June - 2026
ISSN(O): 2456-6683
[Impact Factor: 9.241]
IJRCS

DOIs:10.2017/IJRCS/202606009 --:-- Research Paper / Article / Review

PRODUCTION OF KERATINASE BY USING *Bacillus* sp ISOLATED FROM POULTRY WASTE

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Abstract: *Poultry feather waste is a major environmental concern due to its high keratin content and resistance to degradation. This study investigated the use of keratinolytic Bacillus spp. for converting raw chicken feathers into nutrient-rich livestock feed. Soil samples from poultry processing plants were screened for feather-degrading bacteria. The isolates showed effective growth and feather degradation in feather meal medium at pH 7 and room temperature within 1–5 days. Based on microscopic and biochemical analyses, the isolates were identified as Bacillus spp. Keratinase activity and soluble protein production were evaluated using culture filtrates and the Bradford assay. The results demonstrated efficient feather degradation and protein release, indicating the potential of keratinolytic Bacillus spp. for sustainable feather waste bioconversion into valuable products such as animal feed and biofertilizers.*

Key Words: *Poultry feathers, Keratinolytic Bacillus, Keratinase, Feather degradation, Livestock feed, and Bioconversion.*

1. INTRODUCTION:

Chicken feather waste is a major by-product of the poultry industry, generated in large quantities worldwide. A mature chicken produces about 125 g of feathers, representing 5–7% of its body weight, and global poultry processing results in millions of tons of feather waste annually. Improper disposal methods such as landfilling and burning cause serious environmental problems, including air and water pollution (Gupta & Ramnani, 2006). Chicken feathers are rich in Keratin, an insoluble and highly stable protein that constitutes about 90% of feather content. Keratin contains α-helix and β-sheet structures stabilized by disulfide bonds, making it resistant to degradation by common proteolytic enzymes (Brandelli et al., 2010).

Conventional degradation methods are energy-intensive and destroy essential amino acids, reducing the nutritional value of feather protein. In contrast, biodegradation using keratinolytic microorganisms is an efficient and eco-friendly approach. Among these, Gram-positive bacteria such as Bacillus and Streptomyces are widely reported for their keratin-degrading ability (Onifade et al., 1998). These organisms produce Keratinase, a proteolytic enzyme that breaks down keratin by cleaving peptide and disulfide bonds, converting it into peptides and amino acids.

Keratinase-mediated biodegradation offers a sustainable method for converting feather waste into valuable products such as animal feed, fertilizers, and bioactive compounds. In this study, keratinase-producing *Bacillus* species were isolated from soil and poultry waste, and their potential for feather degradation and production of protein-rich feed was investigated, contributing to eco-friendly waste management (Gupta & Sharma, 2011).

2. LITERATURE REVIEW:

Samuel Pandian and Jawahar Sundaram (2012) isolated and characterized a feather-degrading keratinolytic bacterium from feather-dumped soil, identified as *Bacillus* sp. The isolate showed high keratinase production, with

optimum enzyme activity at pH 7–7.5, 40°C, using 2% feather meal after 96 hours of incubation. SDS-PAGE analysis revealed a single protein band corresponding to keratinase activity, while FTIR analysis confirmed changes in functional groups during feather degradation. The study also suggested that the keratinase produced by *Bacillus* sp. is an alkaline protease, and the keratin-degrading gene was further analyzed for characterization.

P. Jeevan Lakshmi et al. (2013) characterized two native keratin-degrading bacteria, *Bacillus subtilis* (BF11) and *Bacillus cereus* (BF21), capable of completely degrading poultry feathers. Strain improvement and optimization of culture conditions increased keratinase production nearly 50-fold, achieving yields of about 518–520 KU/mL. Maximum enzyme production was obtained using starch as the carbon source, soybean meal as the nitrogen source, and an optimum pH of 8.5. The study also revealed increased levels of sulfur-containing amino acids, indicating effective hydrolysis of feather keratin disulfide bonds.

3. OBJECTIVES

- To isolate, identify, and characterize keratinase-producing bacteria from soil and feather waste samples.
- To produce and characterize keratinase enzyme, and evaluate its activity and protein content using keratinase assay and Bradford method.
- To convert feather digest obtained through keratin degradation into a value-added chicken livestock feed.

4. METHODOLOGY:

Sample collection

Soil and rotten feathers waste were collected aseptically in sterile plastic bags from poultry processing plants and brought to the laboratory.

Isolation of keratinolytic *Bacillus* Sp.

Keratinolytic *Bacillus* sp. was isolated from soil samples using the spread plate method. Distinct colonies were selected and screened for keratin degradation activity.

Identification of keratinolytic *Bacillus* sp.

The organisms isolated were identified by macroscopic observation Microscopic observation and Biochemical observation.

Maintenance of culture and Inoculum preparation

Nutrient agar slants of *Bacillus* species were refrigerated and used for further methods. Bacterial culture grown in nutrient broth at 37° C, and incubated for 24 hours was used as inoculums.

Production of keratinase using feather basal medium

Feather degradation by the test isolate, *Bacillus* species was carried out in the Erlenmeyer flask containing 99 ml feather meal broth. The flask was inoculated using 1 ml of selected strain as inoculums. The bacterial strain was cultured at room temperature on a rotary shaker (110 rpm). Controls for white feather degradation without inoculums were also prepared.

Estimation of Protein by Bradford Method

Protein estimation was carried out using the Bradford assay. The Bradford reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol, followed by mixing with 100 mL of 85% phosphoric acid and making up the volume to 1 L with distilled water. The solution was filtered through Whatman No. 1 filter paper and stored in an amber bottle. Bovine Serum Albumin (1 mg/mL) was used as the standard protein solution. Protein standards (10–100 µg) were prepared in the range of 0.1–1 mL and made up to 1 mL with distilled water. A blank containing 1 mL of distilled water was also prepared. For the unknown sample, 0.5 mL was taken and made up to 1 mL with distilled water. To all tubes, 5 mL of Bradford reagent was added, mixed gently, and incubated briefly. The absorbance was measured at 595 nm using a spectrophotometer. A standard calibration curve was plotted, and the protein (keratinase) concentration in the sample was determined from the curve.

Application of Feather Digest

Feather digest can be utilized as a protein-rich chicken livestock feed. The process involves the conversion of feather meal into a usable feed supplement through enzymatic hydrolysis. In this method, a flask containing feather meal diluted in phosphate buffer along with keratinase was incubated in a 50 °C water bath for two hours. After incubation, the mixture was boiled and gently simmered until complete evaporation of the liquid, resulting in the formation of a dry powder. This feather-derived powder can be further used as an economical and nutrient-rich feed ingredient for poultry and livestock.

5. RESULTS :

The microscopic identification of *Bacillus* species was carried out using Gram staining, Hanging drop method, and Endospore staining to observe cell morphology, motility, and spore formation. Further characterization was performed using biochemical tests including the Indole test, Methyl red test, Voges–Proskauer test, and Citrate utilization test. Enzymatic activities were determined by the Catalase test, Oxidase test, and Urease test. Additional tests such as the Nitrate reduction test, Hydrogen sulfide production test, and Casein hydrolysis test were also performed. The results obtained from all these tests were systematically recorded and tabulated.

Table 1: Microscopic Identification and Biochemical test

S No	Details of experiment	Result
1	Gram staining	Gram positive rod shaped Bacilli
2	Endospore staining	Spore forming
3	Motility Test	Motile
4	Indole test	Negative
5	MR	Negative
6	Voges proskauer	Positive
7	Citrate utilization test	Positive
8	Catalase test	Positive
9	Oxidase test	Negative
10	Urease test	Negative
11	Nitrate Reduction test	Positive
12	H_2S Production test	Negative
13	Casein hydrolysis test	Positive

Characterization of keratinase enzyme

The keratinase enzyme was identified after keratinase activity assay by using bovine serum albumin as standard protein and value were calculated.

From the Bradford test graph, the amount of keratinase present in the sample was 3 mg/ml So, Keratinase activity = (ΔDP/T)/V × DF) =1.4 mg/ml

Table 2: Keratinase activity assay by Bradford method

	Standard Volume(ml)	Concentration(mg)	Distilled water(ml)	Bradford reagent(ml)	OD at 595nm
B	0	0	1	5	0
S1	0.2	1	0.8	5	0.06
S2	0.4	2	0.6	5	0.15
S3	0.6	3	0.4	5	0.18
S4	0.8	4	0.2	5	0.21
S5	1.0	5	-	5	0.30
T	0.5	-	0.5	5	0.09

From graph:

Unknown concentration T = Concentration of test from graph / Volume of test

= 1.5/ 0.5

= 3 mg/ml

Estimation of protein by Bradford method

For the known concentration of bovine serum albumin, the protein content was estimated, tabulated and standard graph was plotted. Similarly appropriate volume of purified sample extracted from feather was analysed for protein content in the spectrophotometric value with the standard graph plotted was 5.3mg/ml.

Table 3: Estimation of protein by Bradford method

	Standard Volume(ml)	Concentration(mg)	Distilled water(ml)	Bradford reagent(ml)	OD at 595nm
B	0	0	1	5	0
S1	0.2	1	0.8	5	0.06
S2	0.4	2	0.6	5	0.15
S3	0.6	3	0.4	5	0.16
S4	0.8	4	0.2	5	0.24
S5	1.0	5	-	5	0.30
T	0.5	-	0.5	5	0.16

From graph:
Unknown concentration T = Concentration of test from graph / Volume of test
= 2.6/ 0.5
= 5.2 mg/ml

Application of feather hydrolysate
Keratinase attacks disulphide bonds to degrade keratin present in the feather. Biodegradation of keratin using keratinase produce peptide and rare amino acids such as serine cysteine and proline and essential amino acids such as threonine, valine, methionine, isoleucine, leucine, lysine, histidine and tyrosine. So it can be applied in the production of chicken livestock feed that are cheap and rich in nutrients.

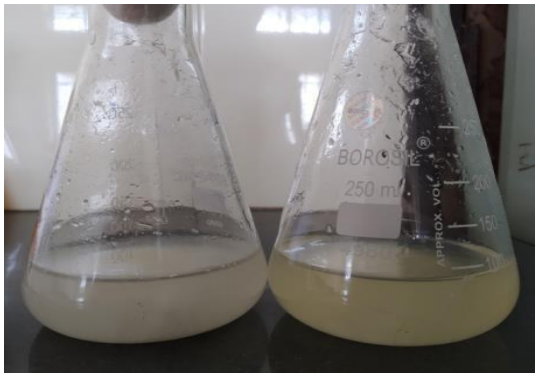
LIST OF FIGURES

1. White chicken feathers

2. Feather meal

3.1 Control : cultivation of *Bacillus* sp in meal media at 0 hour incubation

3.2 Inoculation: Cultivation of *Bacillus* sp in meal media at 24 hours Incubation



6. DISCUSSION

Keratin is a tough structural protein present in skin, hair, nails, and feathers, with chicken feathers containing about 90% keratin. Its strength comes from α-helix and β-sheet structures stabilized by disulfide and hydrogen bonds, making it resistant to degradation. However, certain microorganisms can break down keratin by producing extracellular enzymes called keratinases (Brandelli et al., 2010; Gupta & Ramnani, 2006).

In this study, bacteria isolated from poultry soil samples were screened for keratin-degrading ability using feather meal agar and skim milk agar. Clear zones indicated keratinolytic activity, and the identified organism was *Bacillus* species, known for such capabilities. The isolate showed keratinase activity of 1.4 (mg/s)/ml and protein production of 5.2 mg/ml (Lateef et al., 2010; Friedrich et al., 1999).

Keratinase breaks down keratin into soluble proteins, peptides, and amino acids, improving digestibility. This process highlights the potential of using feather waste as a low-cost, nutrient-rich protein source, especially in animal feed production (Onifade et al., 1998; Tesfaye et al., 2017).

7. SUMMARY:

An attempt was made to convert raw chicken feathers into nutrient-rich livestock feed using keratinolytic *Bacillus* sp. Soil microorganisms from poultry processing plants were screened for feather degradation. The isolates grew well in feather meal medium at pH 7 and room temperature within 1–5 days, and based on microscopic and biochemical tests, were identified as *Bacillus* sp.The isolate was cultured in feather meal medium on a rotary shaker, and the filtrate was used to assess keratinase activity and soluble protein concentration using the Bradford method.The results demonstrate that feathers can be effectively degraded by keratinolytic microbes, producing high-protein feed, amino acids for industrial use, or nitrogen-rich fertilizer. This bioconversion offers an eco-friendly and economical solution for feather waste management while generating valuable by-products for agriculture and industry.

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