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Sustainable Biodegradation of Chicken Feather Waste (CFW) by Keratinolytic Bacteria: Temperature and pH Influence on Degradation Efficiency

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Abstract

This study focuses on the sustainable biodegradation of chicken feather waste (CFW) using keratinolytic bacteria. Soil samples containing degrading feathers were targeted for bacterial isolation with keratinase activity. Pre-enrichment in Feather Meal Broth (FMB) yielded 41 bacterial isolates, with 26 Gram-positive and 15 Gram-negative bacteria. All isolates exhibited protease and 14 showed gelatinase activity, indicating their potential for keratin-rich substrate degradation. Keratinase activity was confirmed in all 25 isolates, making them suitable candidates for CFW biodegradation. The isolates demonstrated diverse metabolic capabilities, including catalase activity, H_2S production, citrate utilization, and urea hydrolysis. Optimal pH and temperature conditions for keratinase activity were determined for different isolate groups. Molecular characterization identified Xanthomonas maltophilia, Pseudomonas aeruginosa, and Bacillus subtilis, with B. subtilis being a promising Gram-positive bacterium for CFW biodegradation. This study provides valuable insights into eco-friendly CFW biodegradation, contributing to sustainable waste management in the poultry industry and transforming CFW into valuable resources.

Keywords: chicken feather waste, keratinolytic bacteria, biodegradation, keratinase activity, pH, temperature.

1. Introduction

The poultry industry is a major contributor to the global food supply, producing billions of chickens annually. However, this growth in commercial production has led to a significant increase in poultry waste generation, including feathers, litter, blood, bones, and more. Improper disposal of these wastes poses serious health and environmental concerns, as they can harbor harmful microorganisms, produce offensive odors, and contribute to air and water pollution.

To address this issue, the present research focuses on the microbial degradation of poultry waste as a sustainable and environmentally friendly solution. The study aims to isolate and identify keratinolytic microorganisms capable of breaking down the tough keratin protein present in feathers and other waste products. By harnessing the keratinase enzymes produced by these microbes, it is possible to transform poultry waste into valuable resources like cattle fodder or renewable energy.

The significance of this research lies in its potential to not only mitigate the harmful impact of poultry waste but also to provide alternative uses for the waste products. By understanding the biological processes involved in keratin degradation, the study opens up opportunities for waste management strategies that are economically viable and ecologically responsible. Moreover, this research may pave the way for developing countries to utilize poultry waste for agricultural fertilizers, construction materials, and even energy generation.

This study aims to shed light on the decomposition of poultry waste and the keratinolytic properties of isolated microorganisms. The findings hold promise for transforming the poultry industry's waste management practices and contributing to a more sustainable future for both the industry and the environment.

2. Material and Methods

2.1. Collection and Preparation of Soil Samples

Soil samples were collected from Poultry farms in Dausa and Jaipur districts, Rajasthan, between January 2018 and December 2019, following standard procedures. Different sites were sampled, with approximately 50 grams of soil collected using a sterile spatula and stored in labeled, airtight containers. Samples were promptly transported to the Microbiology laboratory for further analysis.

Figure 1: Collected soil sample

2.2. Isolation of Keratinolytic Bacteria

2.2.1 Processing of Chicken Feathers and Feather Meal Broth Preparation

Chicken feathers were washed, dried, and defatted using Chloroform and Methanol (1:1) for 4 hours. After extensive washing and air-drying, the feathers were cut and autoclaved to prepare Feather Meal.

For keratinolytic bacteria isolation, soil samples were mixed with 0.9% saline to create soil suspensions, which were then inoculated into Feather Meal Broth. The medium contained NaCl, K_2HPO_4, KH_2PO_4, $MgCl_2.6H_2O$, and Feathers, with a pH of 7.0 ± 0.2. Cultures were incubated on a rotary shaker at room temperature until visible turbidity indicated bacterial growth.

2.2.2 Selection of Protease-Producing Bacteria

After the incubation period, serial dilutions (10^{-2} and 10^{-4}) of the culture suspensions were prepared. These dilutions were then spread onto Feather Meal Agar plates for the selection of protease-producing bacteria. The composition of the Feather Meal Agar per liter included NaCl 5 g, K_2HPO_4 1 g, KH_2PO_4 1 g, $(NH_4)2SO_4$ 0.1 g, MgSO4 0.2 g, and Feathers 10 g. Agar was added to a concentration of 15 g per liter, and the pH of the medium was adjusted to 7.0 ± 0.2. The petri plates were incubated at 30°C for 24 hours. Bacterial colonies showing a zone of clearance on the Feather Meal Agar were selected for further studies.

2.3 Screening and Characterization of Bacteria

2.3.1 Morphological Characterization

Bacterial isolates were subjected to three morphological tests. First, Gram's staining was performed on bacterial smears to determine cell morphology and Gram reaction. Second, the isolates were individually plated on nutrient agar to observe colony morphology, including form, height, color, transparency, and margin characteristics. Lastly, the motility test was conducted using Triphenyl Tetrazolium Chloride (TTC) method to assess the motility of bacterial strains. Incubation at 37°C for 24 hours allowed the observation of bacterial motility.

2.3.2 Biochemical Characterization

2.3.2.1 Preliminary/Protease Test

Proteolytic activity of the isolates was determined using Milk Agar plates. Bacteria were inoculated on plates and incubated at 37°C for 24 hours.

2.3.2.2 Gelatinase Assay

Pre-screened isolates were stab inoculated into Nutrient Gelatin (NG) medium and incubated at room temperature for 48 hours to observe gelatinase activity.

2.3.2.3 Keratinase Activity Assay

Feather broth was prepared, and each broth was added with a single whole feather before sterilization. Loopful of each isolate was inoculated into separate feather broth, and cultures were incubated for 14 days at 37°C. Isolates showing feather utilization signs were selected for further studies.

2.3.2.4 Catalase Test
Presence of catalase enzyme was tested by adding loopfuls of colonies into separate glass slides and pouring hydrogen peroxide into the cells.

2.3.2.5 H_2S Production
The development of the strains on Kligler's iron agar slants was used to search for H_2S production.

2.3.2.6 Citrate Utilization
Simmon's Citrate Agar was used to verify citrate use, and plates were observed for change in color of the medium from greenish to yellow.

2.3.2.7 Indole Production
The development of indole was identified by applying Kovac's reagent and examining the formation of a red circle.

2.3.2.8 Starch Hydrolysis
Starch agar was used to examine starch hydrolysis by the bacterial isolates, and culture plates were filled with iodine solution after 2-4 days of proper development.

2.3.2.9 Urea Hydrolysis
Inoculated cultures were tested for urea hydrolysis in urea broth containing K_2HPO_4, yeast extract, phenol red, urea, and other components.

2.3.3 Molecular Characterization
For molecular characterization, genomic DNA was extracted from bacterial cultures using a standard procedure. The extracted DNA was then analyzed using agarose gel electrophoresis to assess its quality and quantity. The 16S rRNA gene of the bacterial isolates was amplified through PCR using universal primers. The resulting PCR products were subjected to Amplified Ribosomal DNA Restriction Analysis (ARDRA) by digesting them with three restriction enzymes (HaeIII, HhaI, MspI) and analyzing the fragments on an agarose gel. Additionally, four bacterial isolates were selected for 16S rRNA gene sequencing, which was performed at a sequencing facility. The obtained 16S rRNA sequences were analyzed using Blastn on NCBI to identify the bacterial strains. A phylogenetic tree was constructed using the neighbor-joining method with the Kimura 2 parameter model to determine the evolutionary relationships among the isolates.

3. Results

3.1 Isolation of Keratinolytic Bacteria
In this study, the isolated keratin-utilizing bacteria from soil samples containing degrading feathers. They employed a targeted sampling approach to increase the chances of isolating bacteria with keratinase activity. Soil samples were pre-enriched in Feather Meal Broth (FMB) to amplify the target microorganism's population. Initial isolation yielded 41 bacterial isolates that were capable of thriving in Skimmed Milk Agar (SMA) medium, indicating the presence of protease activity.

3.2 Morphological Characterization of the Isolates
The study conducted Gram staining to differentiate between Gram-positive and Gram-negative bacteria. Out of the 41 isolates, 26 were Gram-positive (purple-colored), while 15 were Gram-negative (orange-colored). The colony morphology of Gram-negative stains appeared as rod-shaped, and Gram-positive stains appeared as short rod-shaped or cocci.

3.3 Biochemical Characterization
The biochemical characterization of the isolates included the following tests:

Table 1: A summary of all isolates.

Name of test	Positive reaction	Negative reaction
Gram's staining	19	6
Motility test	25	None
Protease	25	None
Gelatinase	14	11
Keratinase	25	None

Catalase	5	None
H_2S productionc	14	11
Citrate utilization	25	None
Indole production	11	14
Starch hydrolysis	19	6
Urea hydrolysis	17	8

Based on these biochemical tests, the isolates were further categorized into three groups:
Group 1: 8 isolates
Group 2: 6 isolates
Group 3: 11 isolates

3.4 Physiological Characterization

The researchers conducted physiological characterization of the isolates from each group based on pH and temperature conditions. They observed the bacterial growth at various pH levels and temperatures to determine the optimal conditions for keratinase activity.

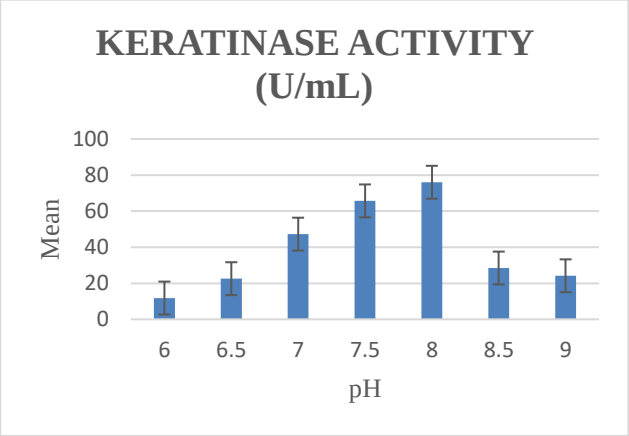
3.4.1 Physiological characterisation of isolates of Group 1:

3.4.1.1 pH

The bacterial growth over a pH range between 6.0 and 9 was studied. Maximum bacterial growth was observed at the pH of 8.0. At the pH of 6.0 the growth was minimal.

Table 2 – Keratinase activity w.r.t pH

pH	Keratinase activity (U/mL)	
	Mean	SD
6	11.80	2.11
6.5	22.54	3.35
7	47.24	2.5
7.5	65.68	1.52
8	76.12	2.87
8.5	28.45	2.10
9	24.15	3.10



Graph 1 – Depicting keratinase activity w.r.t pH

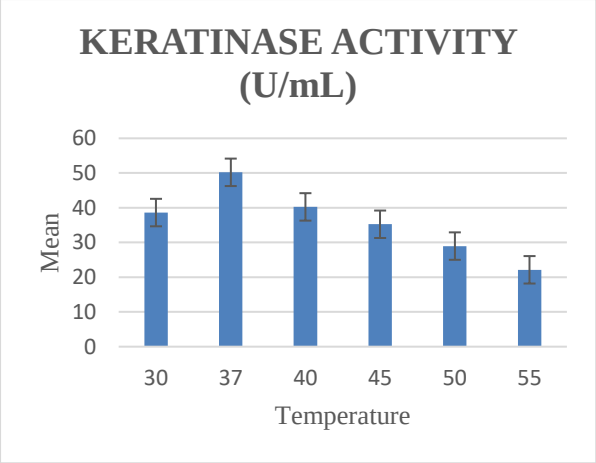
The maximum keratinolytic activity was seen at the pH of 8.0 (76.12 U/mL), followed by 7.5 with the activity of 65.58 U/mL and least at 6 with the activity of 11.80 U/mL.

3.4.1.2 Temperature

In solid media, the growth pattern was measured by observing the media under microscope. The visible growth pattern was good at the temperature of 37°C. Growth reduced at 45°C and no growth was visualized at 55°C.

Table 3- Keratinase activity w.r.t temperature

Temperature	Keratinase activity (U/mL)	
	Mean	SD
30°C	38.62	3.8
37°C	50.21	5.0
40°C	40.25	1.21
45°C	35.25	3.2
50°C	28.97	2.4
55°C	22.14	1.36



Graph 2– Depicting keratinase activity w.r.t temperature.

The maximum keratinolytic activity was seen at the temperature of 37ºC (48.28 U/mL), followed by 40ºC with the activity of 42.25 U/mL and least at 55ºC with the activity of 27.14 U/mL.

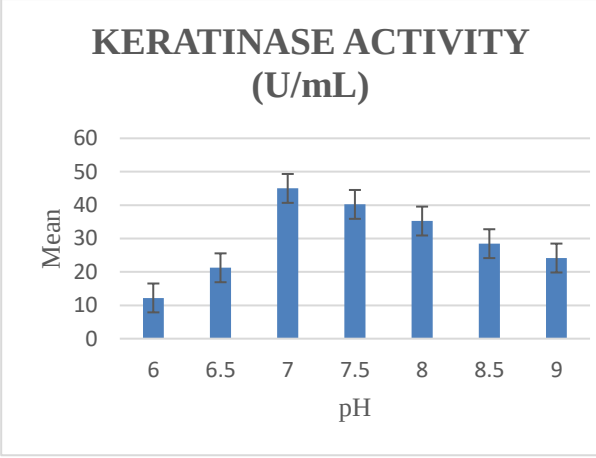
3.4.2 Physiological characterization of Group 2 isolates:

3.4.2.1 pH

The bacterial growth over a pH range between 6.0 and 9 was studied. Maximum bacterial growth was observed at the pH of 7.0. At the pH of 6.0 the growth was minimal.

Table 4 – Keratinase activity w.r.t pH

pH	Keratinase activity (U/mL)	
	Mean	SD
6	12.20	2.11
6.5	21.23	3.35
7	45.0	2.5
7.5	40.21	1.52
8	35.23	2.87
8.5	28.45	2.10
9	24.15	3.10



Graph 3– Depicting keratinase activity w.r.t pH

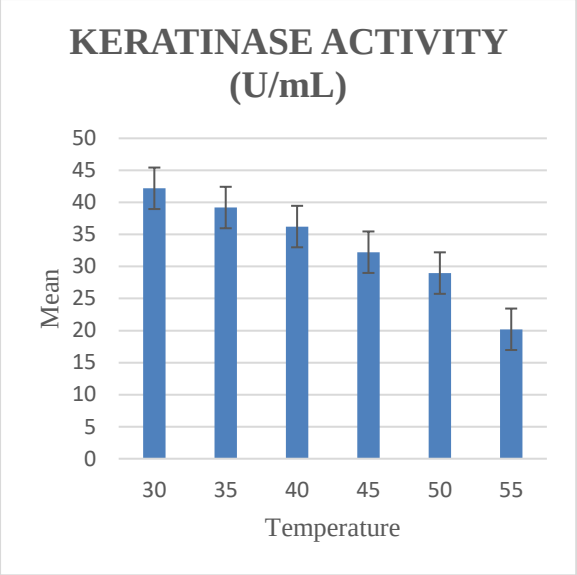
The maximum keratinolytic activity was seen at the pH of 7.0 (45.0 U/mL), followed by 7.5 with the activity of 40.21 U/mL and least at 6 with the activity of 12.20 U/mL.

3.4.2.2 Temperature

In solid media, the growth pattern was measured by observing the media under microscope. The visible growth pattern was good at the temperature of 30°C. Growth reduced at 45°C and no growth was visualized at 55°C.

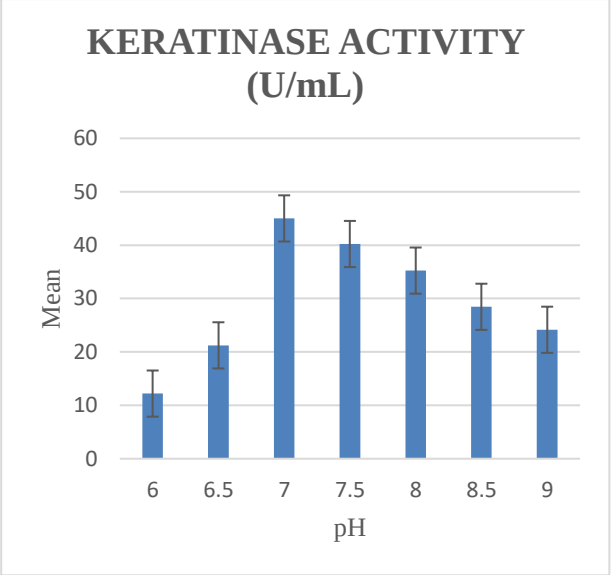
Table 5 - Keratinase activity w.r.t temperature

Temperature	Keratinase activity (U/mL)	
	Mean	SD
30°C	42.20	4.2
35°C	39.2	3.9
40°C	36.23	2.22
45°C	32.23	3.2
50°C	28.97	2.12
55°C	20.20	1.26



Graph 4– Depicting keratinase activity w.r.t temperature

The maximum keratinolytic activity was seen at the temperature of 30ºC (42.20 U/mL).



Graph 5– Depicting keratinase activity w.r.t pH

The maximum keratinolytic activity was seen at the pH of 7.0 (44.0 U/mL), followed by 7.5 with the activity of 42.21 U/mL.

3.4.3 Physiological characterization of Group 3 isolates:

3.4.3.1 pH

The bacterial growth over a pH range between 6.0 and 9 was studied. Maximum bacterial growth was observed at the pH of 7.0. At the pH of 6.0 the growth was minimal.

3.4.3.2 Temperature

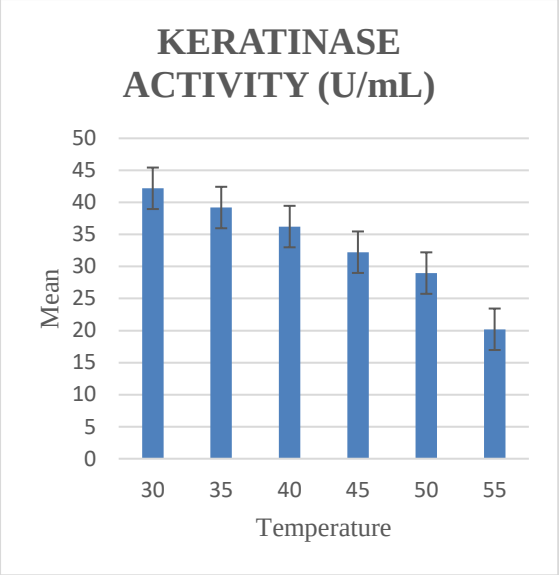
In solid media, the growth pattern was measured by observing the media under microscope. The visible growth pattern was good at the temperature of 30°C. Growth reduced at 45°C and no growth was visualized at 55°C.

Table 6 – Keratinase activity w.r.t pH

pH	Keratinase activity (U/mL)	
	Mean	SD
6	12.25	2.10
6.5	20.13	3.32
7	44.0	2.57
7.5	42.21	1.41
8	36.23	2.76
8.5	29.45	2.11
9	26.15	3.15

Table 7 - Keratinase activity w.r.t temperature

Temperature	Keratinase activity (U/mL)	
	Mean	SD
30°C	41.20	4.26
35°C	39.28	3.91
40°C	36.27	2.28
45°C	30.57	3.25
50°C	25.90	2.15
55°C	18.20	1.06



Graph 6– Depicting keratinase activity w.r.t temperature

The maximum keratinolytic activity was seen at the temperature of 30ºC (41.20 U/mL).

3.5 Molecular Characterization

For molecular characterization, three isolates (1, 3, and 4) were selected from each group. Their 16S rRNA sequences were analyzed, and the results confirmed their identity as Xanthomonas maltophilia, Pseudomonas aeruginosa, and Bacillus subtilis, respectively.

4. Discussion

This study demonstrate the successful isolation and characterization of keratinolytic bacteria from soil samples containing degrading feathers. The targeted sampling approach, along with pre-enrichment in Feather Meal Broth (FMB), resulted in the identification of 41 bacterial isolates with protease activity, as evidenced by their growth on Skimmed Milk Agar (SMA) medium. Further morphological and biochemical characterization revealed that 26 isolates belonged to the Gram-positive bacteria group, while 15 were classified as Gram-negative. Importantly, all isolates displayed protease activity, with 14 of them also showing gelatinase activity, indicating their potential for degrading keratin-rich substrates.

The presence of keratinase activity in all 25 isolates confirmed their ability to degrade feathers, making them promising candidates for the biodegradation of chicken feather waste (CFW). Additionally, all isolates exhibited catalase activity, H_2S production, citrate utilization, and urea hydrolysis, showcasing their diverse metabolic capabilities.

Physiological characterization of the isolates based on pH and temperature conditions provided valuable insights into the optimal conditions for keratinase activity. Group 1 isolates showed maximum keratinolytic activity at pH 8.0 and a temperature of 37°C, while Group 2 and Group 3 isolates displayed optimal activity at pH 7.0 and a temperature of 30°C. These findings are essential for optimizing the biodegradation process and maximizing the utilization of feather waste.

The molecular characterization of selected isolates using 16S rRNA sequencing confirmed their identity as Xanthomonas maltophilia, Pseudomonas aeruginosa, and Bacillus subtilis. Notably, Bacillus subtilis is well-known for its keratinase activity and has been extensively studied for its potential in keratin degradation.

Feathers are a significant waste product globally, and improper disposal can lead to additional costs for producers and environmental challenges. Therefore, there is a growing interest in exploring biotechnological processes to effectively utilize agro-industrial waste. One potential value-added application for feathers is their conversion into feather meal, a digestible dietary protein used in animal feed, achieved through physical and chemical treatments (Ko et al., 2010; Lateef et al., 2010; De Siqueira et al., 2010). However, the presence of keratin, a highly resistant class of structural proteins, makes feather degradation challenging (Ichida et al., 2001; Riffel et al., 2003; Ramnani et al., 2005). Conventional feather-meal production involves energy-intensive steam pressure-cooking, which can destroy certain amino acids and yield non-nutritive amino acids (Wang and Parson, 1997).

Microbial action offers a potential solution for improving the digestibility and amino acid balance of feather meal. Several keratinolytic microorganisms, including species of Bacillus,

Streptomyces, Arthrobacter sp., Microbacterium sp., Vibrio sp., Xanthomonas maltophila, and Chryseobacterium sp., have been reported (Kim et al., 2001; Daroit et al., 2009, 2011; Lucas et al., 2003; Thys et al., 2004; Kocuria rosea, Bernal et al., 2006; Sangali and Brandelli, 2000; De Toni et al., 2002; Riffel et al., 2003; Wang et al., 2008). Screening for non-pathogenic microorganisms with keratinolytic activity could eliminate the need for enzyme isolation and purification, as their biomass might contribute to the protein and amino acid content of fermented feather meal. Enhancing the nutritional value of feathers can lead to an improved protein feedstuff that reduces the reliance on soybean and fish meal in livestock diets.

This study provides valuable insights into the isolation and characterization of keratinolytic bacteria from poultry waste. These findings hold promise for developing sustainable and cost-effective biodegradation processes for chicken feather waste, contributing to improved waste management practices and the utilization of valuable resources from poultry industry by-products.

Conclusion

The study successfully isolated and characterized keratinolytic bacteria from soil samples containing chicken feather waste. The isolates demonstrated protease, gelatinase, and keratinase activities, indicating their potential for feather waste biodegradation. Physiological characterization revealed optimal conditions for keratinase activity in different groups of isolates. Molecular characterization identified the isolates as Xanthomonas maltophilia, Pseudomonas aeruginosa, and Bacillus subtilis, with Bacillus subtilis showing promising keratinase activity. The study highlights the potential of keratinolytic bacteria in sustainable feather waste management and offers insights for future research and optimization of biodegradation processes in the poultry industry.

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