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Sustainable Management of Agricultural Waste: Focus on Poultry Waste and Feathers

Niharika Singh

Assistant Professor, S.S. Jain Subodh P.G. (Autonomous) College, Jaipur, Rajasthan, India

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ABSTRACT

The poultry industry generates vast amounts of waste, including feathers and litter, which pose significant environmental and health hazards. This study focused on isolating, identifying, and characterizing keratinolytic bacteria from poultry waste with the aim of understanding their potential for waste degradation. Soil samples were collected from various poultry farms in Rajasthan, India, and bacteria were isolated using standard microbiological techniques. Morphological, biochemical, and physiological analyses were performed to characterize the isolated bacteria, and molecular characterization was conducted on selected isolates. A total of 41 bacterial isolates were obtained, and screening for keratinolytic activity revealed promising candidates for further study. These isolates were classified into three groups based on physiological characteristics. Additionally, molecular analysis confirmed the presence of keratinolytic bacteria, including *Xanthomonas maltophilia*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The study found that these bacteria possess keratinolytic activity, making them potential candidates for various industrial applications. Physiological characterization revealed optimal conditions for bacterial growth and keratinolytic activity, with pH and temperature affecting enzyme activity. Furthermore, the study highlighted the potential of these bacteria in biotechnological applications, including the degradation of feather waste for the production of valuable products such as animal feed protein and fertilizers. Overall, the findings suggest that keratinolytic bacteria isolated from poultry waste hold promise for environmentally friendly waste management practices and could contribute to the development of sustainable solutions for poultry waste disposal.

Key words: *Poultry waste, Keratinolytic bacteria, Isolation, Characterization, Feather degradation, Biotechnological applications, Environmental sustainability, Microbial keratinase, Waste management*

Introduction

Agriculture, serving as the foundation of human civilization, fulfills essential needs by cultivating crops, raising livestock, and providing raw materials for various industries. However, the agricultural sector also generates significant amounts of waste, posing environmental and health hazards if not managed properly. This essay focuses on addressing the sustainable management of poultry waste and feathers, two substantial components of agricultural waste, by exploring the challenges they pose, the opportunities they present, and the innovative solutions that can be implemented.

Poultry Waste: A Growing Concern

Poultry farming has experienced remarkable growth globally, with India emerging as a major player in the industry. As one of the leading producers of eggs, chicken, and poultry meat, India's poultry sector faces escalating challenges in managing the waste generated by its operations. Poultry waste

Assistant Professor
Corresponding author's email: niharikas715@gmail.com

encompasses various materials, including bedding material, excreta, feed, feathers, and wastewater, all of which pose significant environmental pollution concerns. The accumulation of poultry waste can emit unpleasant odors, attract pests such as flies and rodents, and contaminate soil and water sources if not managed effectively. Moreover, the excessive application of poultry waste to land near farms can lead to soil degradation and water pollution, further exacerbating environmental concerns.

Despite the availability of various treatment and disposal options for poultry waste, including composting, anaerobic digestion, and incineration, inadequate waste management practices persist in many poultry farms. Proper disposal and management practices are essential to recycle nutrients effectively and mitigate the associated environmental and health risks. Neeraj Sharma *et al*. (2022) have identified a range of treatment strategies for agricultural waste management, emphasizing both physiochemical approaches and biological methods. Additionally, Olexa and Goldfarb (2008) stress the importance of containing waste within sealed containers to prevent leakage during disposal, reducing the risk of environmental contamination.

Feathers: From By-product to Resource

Feathers constitute a significant by-product of the poultry industry and present additional challenges in waste management. With the potential to produce millions of tons of feathers annually, the global poultry sector faces the dilemma of underutilizing this valuable resource. Feathers, primarily composed of keratin, possess inherent resistance to degradation, making their disposal challenging. Improper treatment and disposal of feather waste can lead to environmental pollution and pose risks to human health by releasing pollutants such as nitrous oxide, ammonia, and hydrogen sulfide into the environment.

However, despite their challenges, feathers offer numerous practical applications across various industries. They can be used for decorative purposes, therapeutic products, fertilizers, dusters, bedding materials, and feedstocks. Conventional processing methods, such as chemical treatment and steam pressure cooking, require significant energy inputs and may degrade certain amino acids present in feathers. Hence, there is a pressing need for innovative techniques to efficiently transform feathers into value-added commodities, promoting resource efficiency and environmental sustainability in the poultry industry.

Keratin Protein: The Key to Feather Stability

The exceptional stability and resistance to degradation exhibited by chicken feathers are attributed to the presence of keratin, a stiff protein abundant in cysteine and disulfide linkages within its â-keratin structure. Although keratin is inherently resistant to proteolytic enzymes, microbial degradation can occur through the action of keratinases produced by certain bacteria, fungi, and actinomycetes. Microbial keratinases have gained recognition for their potential in converting keratinous waste, specifically chicken feathers, into valuable products such as biofertilizers and animal feeds.

Scholars such as Gupta and Ramnani (2006), Singh *et al.* (2019), and Brandelli (2008) have highlighted the diverse applications of microbial keratinases, ranging from fertilizers to biodegradable thermoplastics. Additionally, research by Qiu *et al.* (2022) emphasizes the economic potential of microbial processes for converting feathers into valuable products. By harnessing microbial degradation techniques, feathers can be transformed from a waste product into a valuable resource, promoting environmental sustainability and resource efficiency in the poultry industry.

Industrial and Biotechnological Applications

Keratinases offer promising applications in various industrial and biotechnological sectors. They can be utilized in fertilizer production, industrial peptide synthesis, and therapeutic applications, among others. Moreover, various bacteria, including species of Bacillus, exhibit keratinolytic activity, suggesting their potential for biotechnological applications. Despite challenges in feather processing, microbial hydrolysis shows promise for improving feather waste utilization and reducing environmental pollution.

Objectives of the study

The main objectives are to analyze and study the decomposition of the poultry waste. The aim of the study was enumerated as follows:

- To isolate the micro-organisms from the poultry waste dumping ground.
- To study the keratolytic property of the isolated microbes.
- To select the micro-organisms with high poten-

tial of poultry waste degradation.
- To identify and characterize the isolated microbes morphologically and chemically.

Significance of the Study

Poultry is one of the most blooming industries of the era and so is the quantity of waste produced by them. Also, the waste generated by this industry is hazardous and is not easily degradable. The study will enhance the understanding of decomposition process of poultry waste and mitigation strategy thereof. The study forms an elaborated platform for the researchers for understanding the harms caused by the poultry waste both quantitatively and qualitatively, secondly the research enumerates the decomposition constraints of these waste and by-products, thirdly the study enumerates the various alternatives available to deal with them and last but not the least, it enumerates the utilization of the poultry waste in a better productive manner. This research will provide a platform for the scholars to concentrate on the keratolytic property of the isolated microbes which helps in the decomposition of feathers, litter, and other poultry wastes. Further chemical and morphological characteristics of these isolated microbes are analyzed to understand the biological process of breaking the keratinous enzymes contained in the poultry wastes and their scientific utilization for producing other useful products such as cattle fodder or renewable energy generation etc.

Materials and Methods

Sample Collection

Soil samples were collected from different poultry farms in Rajasthan between January 2018 and December 2019 using standard microbiological procedures. Approximately 50 grams of soil were collected from each site in sterile containers, properly labeled, and transported to the Microbiology laboratory for analysis.

Sampling Sites

Samples were collected from eight distinct sites, including locations in Bandikui and Jaipur districts. Specific addresses were provided for each site to ensure accurate sample collection and identification.

Isolation of keratinolytic microorganism

Bacterial isolation pre-enrichment was done by transferring 10 g of soil sample into 90 ml Feather Meal Broth (FMB) (0.3 g/l K2HPO4, 0.5 g/l NaCl, 10 g/l cut feathers, and 0.4 g/L KH2PO4) and afterwards incubated for 48 hrs at 37 °C. Serial dilution of the sample was then done at 10-3, 10-5 and 10-7 dilutions and plated into Feather Meal Agar (FMA) (FMB + 15g/l agar). The plates were then incubated for five days at 37 °C. Separate colonies were streaked into FMA and incubated for two days. Cultural characteristics of isolates were observed. Well isolated colonies were streaked into Luria Bertani (LB) Agar (5g/l yeast extract, 10g/l tryptone, 15 g/l agar and 10g/l NaCl slants) and incubated at 37 °C for 24 hours. These served as culture stocks for the succeeding steps. Isolates were Gram stained and observed under light microscope to check for purity and to determine morphological characteristics.

Screening for keratinolytic bacteria

Initial screening of isolates was done by proteolytic activity assay using Milk Agar plates (3 g/l yeast extract, 5 g/l peptone, 12 g/l agar and 100 ml/l sterile UHT non-fat milk). Bacteria were inoculated onto plates and incubated for 24 hours. Isolates with zones of clearing are protease positive and were used for the succeeding steps.

Assay of keratinase activity

Initial screening of isolates was done by proteolytic activity assay using Milk Agar plates (3 g/l yeast extract, 5 g/l peptone, 12 g/l agar and 100 ml/l sterile UHT non-fat milk). Bacteria were inoculated onto plates and incubated for 24 hours. Isolates with zones of clearing are protease positive and were used for the succeeding steps.

Under the same conditions, one unit (U) of keratinase activity was described as the amount of enzyme that produced a 0.01 increase in absorbance between the sample and its regulation.

Biochemical Characterization

Starch utilization test

Starch agar [(percent w/v) peptic digest of animal tissue, 0.5; yeast extract, 0.15; beef extract, 0.15; soluble starch, 0.2; NaCl, 0.5; agar, 1.5] was used to do this examination. The culture plates were filled with iodine solution after 2-4 days of proper development. A transparent region (in which starch was destroyed) in a blue context (where starch was not depleted) suggested a positive hydrolysis.

Urea hydrolysis

The group morphology of possible keratinolytic isolated bacteria was determined by plating them individually on nutrient agar plates containing – NaCl (5g/l), Peptone (5g/l), and Beef extract (3g/l). Form, height, coloration, transparency, height, and margin are all factors to be considered. Methodologies were reported as defined in Prescott's Microbiology, including the structure of the cultures.

Effect of Temperature and pH

To control the impact of pH on chosen isolates' growth (OD at 600), 20 mM concentrations of glycine-NaOH, phosphate, and acetate buffers with a broad variety of pH (4.0- 11.0) were used. The pH range of each buffer was - glycine-NaOH buffer (pH 6.0-8.0), phosphate buffer (pH 6.0-8.0), and acetate buffer (pH 4.0 and 5.0). Including each buffer, 10 g per litre of poultry feathers were applied. Growth was measured at temperatures varying from 10 ºC to 50 °C in the vicinity of a 20 mM phosphate buffer to assess the optimal temperature. The seven-day growth analysis was checked out. The absence of growth was reported as a '-' while the presence of growth was reported as a '+'.

Molecular Characterization

Genomic DNA isolation and sequencing were performed to identify the isolated bacteria at the molecular level. This involved isolating genomic DNA from bacterial cells, amplifying the 16S rRNA gene via PCR, conducting amplified ribosomal DNA restriction analysis (ARDRA), sequencing the amplified product, and analyzing the sequence data using NCBI tools and software programs.

This comprehensive methodology facilitated the identification and characterization of keratinolytic bacteria isolated from poultry farm soil samples in Rajasthan, providing valuable insights into their potential applications and contribution to sustainable waste management practices.

Results

Isolation of bacteria

In this study, a targeted sampling approach was utilized in order to isolate microbial populations with keratinolytic potential. Soil with degrading feathers was used as microbial source in order to increase the chances of isolating bacteria with keratinase activity. Furthermore, samples were pre-enriched in FMB for two days in order to magnify the population of the target microorganisms from the soil samples. Initial isolation yielded 2 bacterial isolates that were capable of thriving in FMA.

Preliminary screening

Screening is a highly precise tool for separating mechanically important life types. Preliminary screening was done to verify the proteolytic activity. The protease assay was used, and the no. of isolates were narrowed down and then evaluated. The isolates were grown on milk agar. Two isolates were able to form clearing zones indicative of proteolytic activity. Isolate 1 exhibited largest clearing zones whereas the clearing zone of isolate 2 was small. These isolates were recovered and were subjected for further testing.

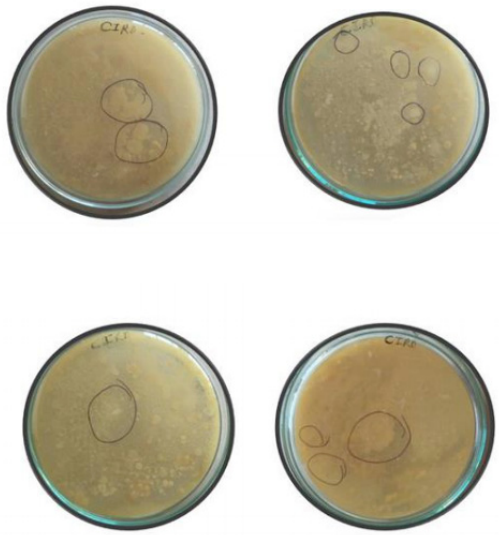
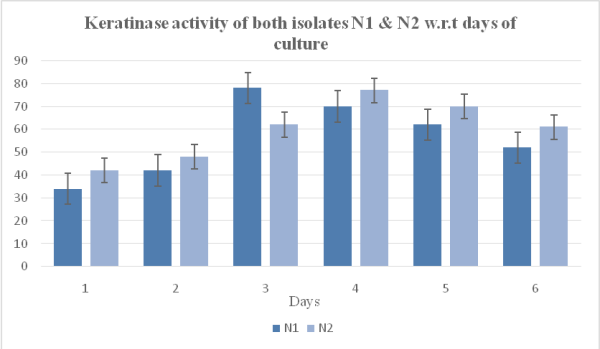


Fig. 1. Showing isolates with clear zones surrounding it.

Keratinase assay

Isolates with highest keratinase activity were identified by further interrogating their capacity to degrade feathers. This was done by using feather broth. It is a medium that consist of feathers and is a sole source of nitrogen and carbon. Whole feathers were supplemented in the media in order to visually inspect feather degradation. This was further supported by the observed highest turbidity in these isolates' indicative of feather utilization for growth. These two isolates (N1 and N2) were recovered and identified.

The keratinase activity of both the isolates were



Graph 1. Depicting keratinase activity of both isolates

calculated by cultivating them on a feather broth. The percentage of degradation of feather in the culture media was recorded as the keratin digesting activity of the bacteria. The maximum activity of N1 was seen on 3rd day of culture. The maximum activity of N2 was seen at 4th day of culture.

Characterization of the Isolates

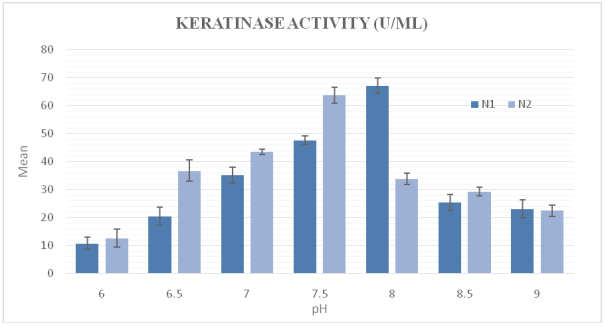
Preliminary morphological and biochemical parameters were used to classify all of the keratinolytic bacterial strains. The comparative morphological, physiological, and biochemical characters of the selected strains were depicted in Table 1.

Table 1. Comparative results of both the isolates

No.	Tests performed	Isolate 1 (N1)	Isolate 2 (N2)
Morphological observation			
1.	Colour	Yellow	Creamy
2.	Colony shape	Round to ovate	Pinpoint
3.	Cell shape	Short rods	Rod
Biochemical tests			
1.	Starch test	Positive	Negative
2.	Urea test	Positive	Negative

Effects of pH and temperature on keratinase enzyme activity of Isolates

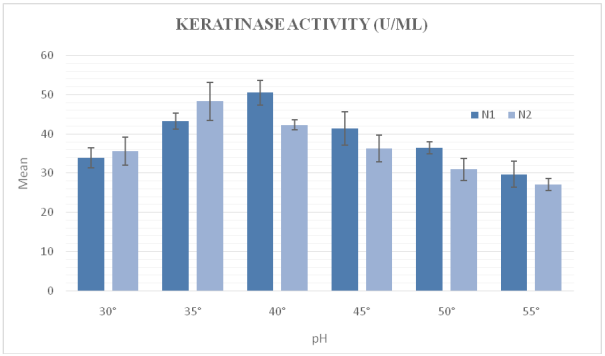
The bacterial growth of isolates N1 & N2 over a pH range between 6.0 and 9.0 was studied. Maximum bacterial growth of N1 was observed at the pH of 7.5. For N2 isolate maximum bacterial growth was observed at the pH of 8, and minimal at the pH of 6.0. The maximum keratinolytic activity of N1 was seen at the pH of 8 (67.17 U/ml), and least at 6 with the activity of 10.80 U/ml and for N2 isolate maximum keratinolytic activity was seen at the pH of 7.5 (63.76 U/ml), followed by 7 with the activity of



Graph 2. Depicting keratinase activity w.r.t pH

43.52 U/ml and least at 6 with the activity of 12.63 U/ml.

In solid media, the growth pattern was measured by observing the media under microscope. The visible growth pattern of N1 isolate was good at the temperature of 35 °C and for N2 isolate at the temperature of 40 °C. Growth of for N1 reduced at 40 °C and no growth was visualized at 55 °C. For N2 growth reduced at 45 °C and no growth was visualized at 50 °C. N1 maximum keratinolytic activity was seen at the temperature of 40 ºC (50.45 U/ml), and least at 55 ºC with the activity of 29.74 U/ml.[2] maximum keratinolytic activity was seen at the temperature of 35 ºC (48.28 U/ml), and least at 55 ºC with the activity of 27.14 U/ml.



Graph 3. Depicting keratinase activity w.r.t temperatures

Discussion

Feathers, which are generated in vast amounts, are one of the by-products of poultry farms. Hundreds of billions of chickens are killed per year, resulting in tonnes of poultry feather by-products. In order to be used as a nutritional substitute for livestock diets, these feathers are now exposed to physical and chemical therapies. However, these methods have the potential to degrade amino acids, resulting in

lower protein content (Kulkarni and Jadhav, 2014). For keratin degradation, another choice is to use micro-organisms with keratinase activity. This concept has sparked a slew of research projects aimed at isolating bacteria that can thrive on keratin-rich substrates. Riffel and Brandelli (2006) were able to separate three Gram negative bacteria (Burkholderia, Chrysobacterium, and Pseudomonas) as well as one Gram positive bacterium (*Microbacterium* sp.) with keratinase activity during development. Kulnarni and Jadhav conducted a similar analysis in 2014 and were able to distinguish keratinolytic Breuvedimonosterrae organisms.

Keratinolytic Bacillus cereus was isolated in this experiment. B. cereus is a Gram-positive, rod-shaped bacteria that forms endospores and is facultatively anaerobic. They are gelatinase and catalase positive (Coia and Cubie, 1995). These physiological characteristics match the isolates found. Bacillus sp. has been shown to dissolve keratin, and other experiments have since been able to separate the same organisms from their samples. The complete nucleotide sequence of the keratinase structural gene's coding and flanking regions has been established in B. licheniformis. The involvement of the kerA gene may explain the presence of keratinase, a serine protease (Lin *et al*., 1995). Any serine proteases, such as subtilisins, are identical in sequence and structure across Bacillus organisms. The same may be said for keratinase, which could open the door to a molecular approach to screening bacteria that degrade keratin. Jeevana Lakshmi *et al.* (2013) identified two native strains of B. cereus and B. subtilis. Furthermore, they had pinpointed the ideal conditions for achieving maximum enzyme efficiency. This adds to the growing body of evidence that B. cereus is a keratinolytic bacterium.

Conclusion

From the results of the present study, it can be concluded that-

- The soil collected from various locations of Rajasthan were rich in various bacteria.
- Keratinophylic bacteria were also found to be present in the soil samples.
- Various morphological, cultural, biochemical and physiological studies showed that all keratiphyllic isolates were divided into 3 groups.
- Molecular characterization confirmed the presence of Xanthomonas maltophilia, Pseudomonas aeruginosa and B. subtilis having keratinophyllic activity.
- These bacteria can be good sources of keratinase enzyme which is utilized for several commercial, scientific, and industrial applications, including in cosmetics, biomedicine, renewable energy production, and biological control etc.
- These bacteria can also been used to destroy feathers β-keratin, a fibrous and insoluble structural protein heavily cross connected by disulfide connections (which makes 90% composition of feathers). This makes them resistant to animal, insect, and protease digestion, causing major disposal issues. Feather waste is also created at a rate of 22 million kg per year (US alone). The use of keratinolytic bacteria to degrade feathers is a cost-effective and environmentally favourable option.
- The digested products of feather waste might be utilised to make fertilisers, animal feed, or natural gas.
- Feathers were utilized as the principal resource of nitrogen, sulphur, 'C', and energy for these bacteria, which was cultivated in basal medium. The isolate with strong keratinolytic activity might be employed for biotechnological applications in the keratin hydrolysis process, such as recycling chicken waste for protecting the environment and bioconversion of feathers into livestock feed protein. The employment of microbial keratinase for keratinous waste management and pollution prevention is a helpful and cost-effective method.
- These keratinolytic isolates might be a good choice for degrading feather keratin and using it to make animal feed protein.

Conflict of Interest - None

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