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Short Communication

Sustainability of Poultry Waste: Bacterial Isolates for Efficient Biodegradation of Chicken Feathers

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Abstract: Chicken feathers are the by-product of the poultry industry, where chickens are raised and processed for meat and eggs. This condition contributes to a major waste problem due to a lack of efficient recycling methods. The conventional disposing method of poultry waste includes burning and chemical treatment, which contributes to environmental problems. Almost 90% of the keratin content in chicken feathers can be beneficial if these wastes are recycled effectively. Hence, the aim of the study is to assess the local bacterial isolates that have higher potential in the biodegradation of chicken feathers. Soil samples with bacterial culture from 3 different locations (dumpsite area, poultry area and compost house) were collected to isolate the potential degrading bacteria. The highest feather-degrading bacterial culture was isolated using milk agar and screened for the keratinase enzyme activity and feather degradation rate using basal salt media. Then, the Gram staining procedure was further carried out to categorise bacteria as Gram-positive or Gram-negative. The finding shows there was a total of 17 bacterial isolates from the soil of three different locations with the highest chicken feather degrading capability. It was found that bacterial isolate 16 (isolated from chicken coop) has the highest keratinase enzyme activity, which was 3.8 U/Ml with the highest degradation rate of 42.8% of 10 g/L chicken feathers in 96 h, and it is Gram-positive. An optimisation of the bacterial culture condition would increase the rate of chicken feather degradation and keratinase enzyme activity. In conclusion, locally isolated bacteria are proven to have the capability to degrade chicken feathers and able to have a good impact on sustainable poultry waste management.

Keywords: Poultry waste; Chicken feather degradation; Keratinase; Biodegradation

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1. Introduction

Chicken feathers are the waste product in the poultry industry, leading to significant dumping challenges. As poultry production grows, so does feather production, resulting in pollutants that can harm the environment and public health if left unaddressed. Traditional methods like burning and chemical treatment are neither environmentally friendly nor cost-effective (Musikoyo *et al*., 2021). However, the discovery and utilisation of microorganisms with the ability to degrade keratin offers a promising alternative. In recent years, researchers have focused on a keratinous protein discovered in chicken feathers. This protein constitutes up to 90% of the feather's protein content and is primarily composed of β-keratin. β-keratin is a fibrous and insoluble structural protein that forms strong cross-links through disulfide bonds. Its abundance and prominence make it a subject of interest for various applications, including biofertilizers, bioactive peptides, and livestock feed (Aktayeva *et al*., 2022; Bhari *et al*., 2021).

Keratin, a fibrous structural protein abundant in feathers, poses significant environmental concerns due to its resilience and slow degradation rate. Microorganisms with keratinolytic activity offer an alternative approach for biodegradation, thereby enhancing the nutritional value of feather waste (Anbesaw, 2022). Recent studies have been reported that a huge number of microorganisms that produce keratinases, with bacteria being the most promising (Aktayeva *et al*., 2022; Almahasheer *et al*., 2022). Feather-degrading bacteria have been found in a variety of environments, including soil. In this study, soil samples were collected from a chicken coop, a dumping site of chicken feather waste, and a vermicompost house where the possibility of isolating chicken feathers is high due to the presence of chicken feathers where biodegradation takes place. These bacteria differ in their capacity to produce keratinase enzyme and break down keratin in feathers (Ahmed *et al*., 2020).

Keratinases represent a unique class of proteases designed to break down resilient, insoluble keratin substrates. Distinguished by their resilience and biochemical versatility, these enzymes exhibit a remarkable diversity in their functional attributes. Unlike conventional proteases, keratinases possess a broad substrate specificity, enabling them to effectively target various insoluble, keratin-rich materials such as feathers, wool, nails, and hair (Subugade *et al*., 2019). The degradation of keratin is a longstanding process linked with dermatomycosis, and specific fungi, including Aspergillus, Actinomyces, and Streptomyces, have been identified as producers of keratinase (Gupta *et al*., 2012; Lakshmipathy & Kannabiran, 2010). Nevertheless, the biotechnological and environmental significance of keratinase came to prominence following the initial report on the isolation and

characterisation of a feather-degrading bacterium, *Bacillus licheniformis* PWD-1. The research primarily centred on feather recycling and the production of feather meal, during which the researchers identified Ker-A enzyme from *B. licheniformis* as a promising keratinase candidate (Gupta *et al.*, 2012).

This study aims to evaluate locally isolated bacterial strains for their potential in biodegrading chicken feathers, thus offering a sustainable solution to the poultry waste problem while harnessing the inherent value of keratin-rich materials. Through this study, the capabilities of bacterial isolates from soil samples in decomposing chicken feathers can be gained, for the development of efficient and environmentally friendly waste management strategies within the poultry industry.

2. Materials and Methods

2.1. Sample Collection and Isolation of Feather-Degrading Bacteria

A total of 30 soil samples were collected from 3 different locations in the northern region of Malaysia (chicken feather dumpsite, chicken coop and vermicompost house), using an auger (Naveen & Madhukar, 2022). The soil samples were sealed in a zipper bag and stored in an ice box before further analysis was done in the laboratory. Chicken feather waste was collected from a local poultry farm, washed several times with tap water and sun-dried. The feathers were then cut into smaller pieces (2 cm) and kept in a zipper bag for further analysis.

The soil samples were incubated in nutrient broth (Oxoid, UK) for 2 days at 30°C and 150 rpm in an orbital shaker incubator (Biosan, USA). The culture was sub-cultured a few times and incubated in an orbital shaker incubator under the conditions described. A 50 µL volume of a 24-hour-old culture was plated on milk agar plates and incubated at 37°C for 24 h for colony formation (Patrawala *et al*., 2017). The plates were observed after 24 h, and selected bacterial colonies were then purified by subculturing on fresh milk agar plates. The single colony obtained was then confirmed by microscopic identification in the following analysis.

2.2. Identification of Bacterial Isolates

Bacterial isolates were observed according to the characteristics of the colony (colour, shape, form, elevation) and microscopic examination, which included cell shape and Gram staining using a Gram staining set (Chemiz, UK). A clean glass slide was passed through a Bunsen flame twice and allowed to cool. A loopful of distilled water was dropped in the

middle of the slide and mixed with a loopful of sample from a single colony. The suspension was then smeared over the central area of the slide and allowed to dry at room temperature. The smear was then heat-fixed by passing through quickly over a Bunsen flame. The heat-fixed smear was then flooded with crystal violet and left for 1 min before rinsing with distilled water. It was then flooded with Gram's iodine and left for 1 min before rinsing with distilled water. It was then decolourised with acetone for a couple seconds, and immediately rinsed with distilled water, followed by counterstain and rinsed with distilled water. The smear was then dried using absorbent paper by gentle blotting. It was examined using an oil immersion objective at a total magnification of 1,000x.

2.3. Screening of Chicken Feather Degrading Bacteria

The screening of chicken feather degradation rate and keratinase enzyme activity was carried out according to Subugade *et al*. (2019) with slight modifications. A 250 mL Erlenmeyer flask containing 50 mL of basal salt medium (HiMedia, India) was supplemented with 0.5 g of chicken feather. A 1 mL volume of inoculum from a 24-hour-old bacterial culture was transferred into the flask and incubated at 37°C for 96 h under shaking conditions (150 rpm).

2.3.1. Keratinase enzyme assay

After 96 h, the hydrolysate was filtered through Whatman No. 1 filter paper, followed by centrifugation of the filtrate at 10,000 rpm for 15 min and decantation of supernatant. The keratinase enzyme activity was determined according to Almahasheer *et al*. (2022) with slight modifications using 5 mg keratin azure (Sigma-Aldrich, Germany) in 0.8 mL of 50 mM Tris-HCl (RPI, USA) buffer as substrate, followed by constant agitation until keratin azure was fully suspended. A 0.2 mL volume of crude enzyme obtained from the extracted enzyme from the filtered hydrolysate was added to the substrate solution and incubated for 15 min in a 50 ℃-water bath. The reaction was terminated by the addition of 0.2 mL of 0.4 M trichloroacetic acid (1MalaysiaBioLab, Malaysia) and centrifuged at 3,000 rpm for 20 min. The absorbance of the supernatant was measured at 450 nm with a spectrophotometer. The unit of keratinase activity was defined as a 0.01 unit increase in the absorbance at 450 nm as compared to the control. A control sample was prepared by adding 0.2 mL of TCA to a reaction mixture before the addition of crude enzyme.

2.3.2. Chicken feather degradation rate

The filtered chicken feather was rinsed with distilled water to remove any leftover soluble material and bacteria. The feather was then oven-dried at 60℃ for 48 h. The chicken feather degradation rate was calculated using Equation 1 below (Yusuf, 2016):

$$Chicken\ Feather\ Degradation\ Rate\ (\%) = \frac{Final\ weight\ of\ feather}{Initial\ weight\ of\ feather} \times 100 \quad (1)$$

2.3.3. Statistical analysis

All statistical analysis was conducted using SPSS software. Double-factor analysis of variance (ANOVA) and LSD multiple comparison test were performed ($p < 0.05$).

3. Results and Discussions

3.1. Identification of Bacterial Isolates

A total of 17 bacterial isolates were isolated from 30 soil samples. These isolates were observed through morphological and biochemical characterisation (Table 1).

Table 1. Morphological characteristics of bacterial isolates.

Bacterial Isolates	Colony Colour	Margin	Form	Elevation	Gram Stain	Cell Shape	Soil Location
1	White	Wavy	Irregular	Flat	-	Bacilli	Vermicompost House
2	White	Even	Circular	Convex	+	Bacilli	Vermicompost House
3	White	Wavy	Irregular	Flat	+	Cocci	Vermicompost House
4	White	Even	Punctiform	Convex	-	Bacilli	Vermicompost House
5	White	Even	Irregular	Flat	-	Bacilli	Vermicompost House
6	White	Even	Circular	Flat	+	Bacilli	Vermicompost House
7	White	Even	Punctiform	Convex	+	Cocci	Vermicompost House
8	White	Even	Punctiform	Convex	+	Cocci	Chicken Coop
9	White	Even	Circular	Convex	+	Cocci	Dumping Site
10	Red	Even	Circular	Convex	+	Cocci	Chicken Coop
11	Red	Even	Circular	Convex	-	Bacilli	Dumping Site
12	Red	Even	Punctiform	Convex	-	Bacilli	Dumping Site
13	Red	Even	Circular	Convex	-	Bacilli	Dumping Site
14	Yellow	Even	Circular	Flat	+	Bacilli	Chicken Coop
15	Yellow	Even	Punctiform	Convex	-	Cocci	Chicken Coop
16	Yellow	Even	Circular	Flat	+	Cocci	Chicken Coop
17	Yellow	Even	Punctiform	Convex	+	Cocci	Dumping Site

1. Colony Characteristics:

   • A total of 9 bacterial isolates were observed to display colonies that were white in colour with even margins, which suggests dominant bacterial strains within the samples. However, 4 isolates exhibit yellow colony colour and 4 isolates with red colony colour.

   • Colony formations varied among isolates, including irregular, circular, and punctiform shapes observed, which also suggests a variety of bacterial strains within the soil samples.

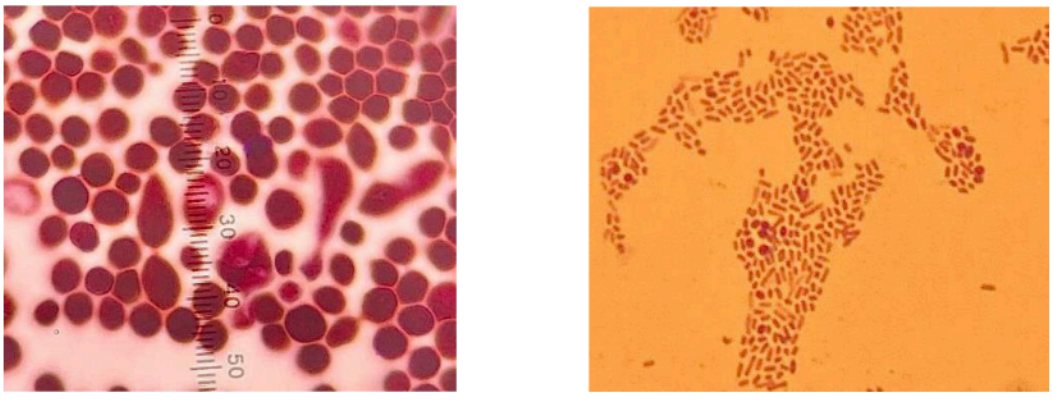
2. Elevation of Colonies:

   • Most isolates showed colonies with convex elevations, which typically indicate robust bacterial growth. Some isolates observed with flat elevations, which suggests a slower growth rate compared to convex elevation colonies.

3. Microscopic Examination:

   • Microscopic examination revealed diverse cell shapes among the isolates, including rod-shaped (bacilli), cocci (spherical), occurring singly, in clusters, or in chains.

   • A total of 10 isolates were identified as gram-positive, including isolate 16 (Figure 1), and 7 isolates were identified as gram-negative, including isolate 2 (Figure 1). Gram-positive bacteria retain the crystal violet stain due to their thick peptidoglycan layer, which is an important characteristic in bacterial classification and identification.



(a) (b)

Figure 1. Microscopic examination of (a) Isolate 16 shows Gram-positive cocci in cluster. (b) Isolate 2 shows Gram-positive bacilli.

3.2. Screening of Chicken Feather Degrading Bacteria

3.2.1. Chicken feather degradation

In this study, isolate 16 exhibited the highest degradation rate of chicken feathers, reaching 42.8% degradation of 10 g/L chicken feathers, although there is no significant difference between bacterial isolates. This surpasses the degradation rate reported in the previous study by Peng *et al*. (2019), indicating that isolate 16 has even greater potential for chicken feather degradation under the conditions tested in this study. Isolate 16 was obtained from the chicken coop area, which has the possibility to isolate feather-degrading bacteria due to the presence of chicken feathers in the area. Conversely, isolate 11 demonstrated the lowest degradation rate among the isolates, with only 26.2% degradation of chicken feathers observed. This indicates that isolate 11 may be less effective or efficient in degrading chicken feathers compared to other isolates tested in the study (Figure 2). Isolate 11 was obtained from the dumping site of chicken feathers, which has the potential of isolating feather-degrading bacteria; however, it might not be the best degrader due to the isolated location, as the dumping site is not solely for chicken feather waste.

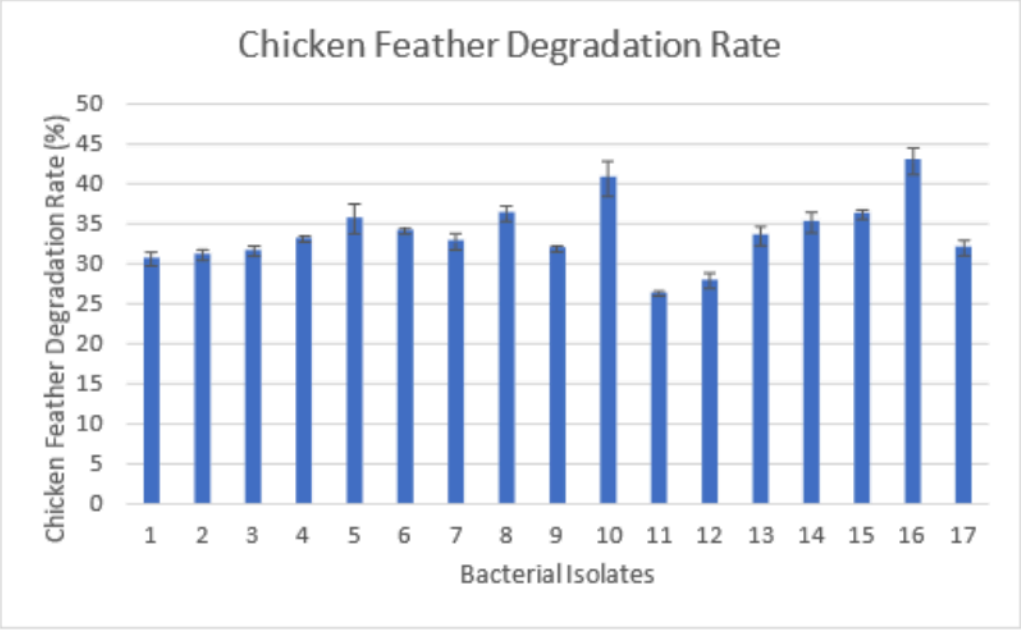


Figure 2. Chicken feather degradation rate. The error bar represents SE of different bacterial isolates.

Future research might focus on determining the processes driving the different degradation rates reported among the isolates. Understanding these bacteria's genetic, biochemical, and physiological properties might help develop techniques to improve their efficacy in feather degradation. Furthermore, investigating the optimum environmental condition that promotes feather degradation by these isolates, such as temperature, pH, and

nutrient availability, may increase their potential for use in waste management procedures. These findings contribute to the growing body of knowledge on degrading bacteria of poultry waste and provide insights for future research and applications in this area.

3.2.2. Keratinase enzyme activity

The study assessed the keratinase enzyme activity of the bacterial isolates in basal salt medium. Keratinase enzymes are known for their ability to degrade keratin, a fibrous protein found in feathers, hair, and other keratinase enzymes. Notably, isolate 16 exhibited the highest keratinase enzyme activity, measuring 3.8 U/mL (Figure 3). This indicates that isolate 16 possesses a significant capacity to produce keratinase enzymes, suggesting its potential effectiveness in degrading feather keratin. Conversely, isolate 5 displayed the lowest enzyme activity among the tested isolates, with a measurement of 2.1 U/mL. This suggests that isolate 5 may have lower efficiency or capacity in producing keratinase enzymes compared to other isolates tested in the study.

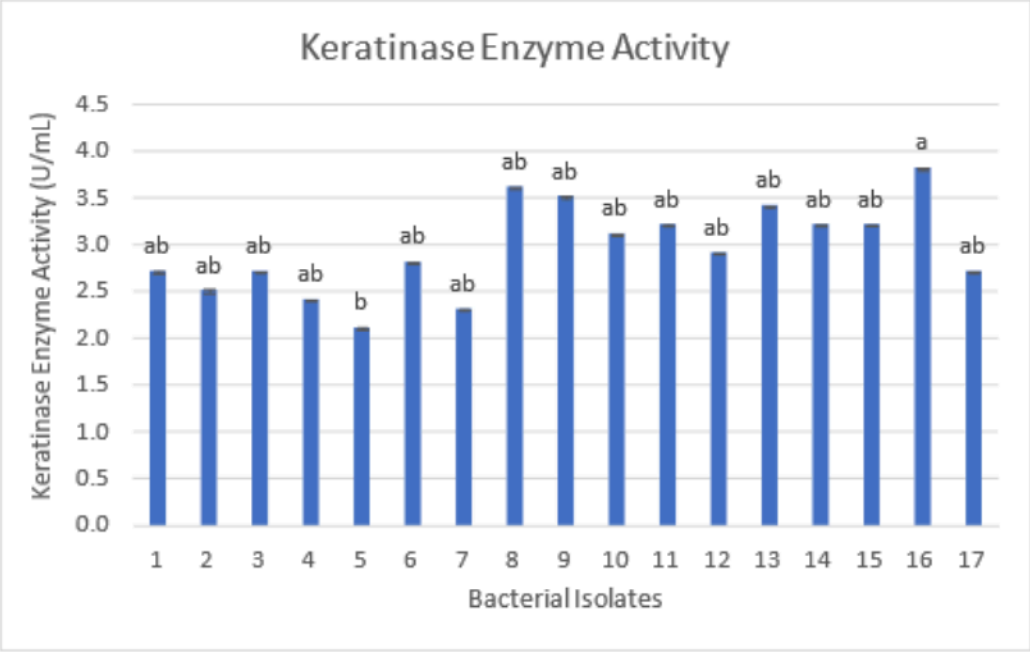


Figure 3. Keratinase enzyme activity. Error bar represents SE of different keratinase enzyme activity. Different letters indicate significantly different levels of keratinase enzyme activity between bacterial isolates.

The finding of high keratinase enzyme activity in isolate 16 suggests its suitability for applications requiring efficient degradation of keratin, such as chicken feathers. Further research could focus on interpreting the genetic and biochemical basis of keratinase enzyme production in bacterial isolates, as well as exploring strategies to enhance enzyme activity.

Additionally, investigating the potential effects of bacteria on keratin degradation could provide insights into optimising enzymatic processes for practical applications

4. Conclusions

The study characterised 17 bacterial isolates obtained from soil samples, focusing on their morphological, biochemical, and enzymatic properties. The isolates predominantly displayed white colonies with even margins, with some variations observed in colour and colony formation. Microscopic examination revealed diverse cell shapes, with most isolates being gram-positive. Notably, isolate 16 exhibited the highest degradation rate of chicken feathers, surpassing the performance reported in a previous study, highlighting its considerable potential in this regard. Additionally, isolate 16 demonstrated the highest keratinase enzyme activity and chicken feather degradation rate among the tested isolates, emphasising its effectiveness in degrading feather keratin. Conversely, isolate 11 shows the lowest degradation rate while isolate 5 shows the lowest keratinase enzyme activity, suggesting differences in effectiveness among the isolates. The variation in keratinase enzyme activity across the isolates underscores the importance of understanding their enzymatic capabilities for potential applications in waste management and bioremediation efforts. These findings contribute to our understanding of microbial diversity and their potential roles in environmental processes. Further research could delve into the mechanisms underlying the observed variations in degradation rates and enzyme activities, as well as explore optimisation strategies for enhancing the performance of these isolates in practical applications.

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Conflicts of Interest: The authors declare no conflict of interest.

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