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Study of keratinolytic properties of bacteria isolated from poultry wastes

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Abstract

Keratin is a complex protein that constitutes a major component of feathers, hair and nails. The keratin rich waste materials, such as poultry wastes pose a significant environmental challenge. The aim of this study is to isolate and screen keratinolytic bacteria from soil where poultry wastes are dumped, with the potential for applications in bioremediation.

Soil samples were collected from areas surrounding poultry farms where feather waste was excessively discarded. The samples were first processed, serially diluted and then plated onto nutrient agar plates. Then the colonies were cultured on skimmed milk agar plates and were observed for the proteolytic activity in the form of clear zones. The isolates which showed clear zones on the skimmed agar plates were then processed and inoculated into modified liquid basal medium supplemented with chicken feather. A total of 5 bacterial isolates were capable of degrading the feathers in 20 days of incubation. These isolates which were able to degrade the feather were then inoculated onto selective media (feather meal agar) containing keratin as the sole carbon source to confirm their keratinolytic activity.

Biochemical characterization was carried out for the 5 bacterial isolates. The results of these tests indicated that the 5 bacterial isolates might belong to *Bacillus spp, Psuedomonas spp* and *Microbacterium spp.*

Thus, these novel keratinolytic bacterial isolates have potential use in processes involving keratin hydrolysis.

Keywords: Poultry wastes; Keratinolytic bacteria; Keratinases; Feather meal agar; Bioremediation

1. Introduction

Human civilization, with its numerous activities, results in the accumulation of huge amounts of solid wastes in the environment. With the expansion of human population, disposal and management of solid waste is becoming one of the major alarms faced by the environment[4]. Even though various methods of disposal such as burying, incineration or disposing it in the specified locations have been devised to reduce the quantity of solid waste generated annually, a significant amount of it is still created globally[18].

Feathers, hair, horns, nails and hooves are examples of keratin wastes that are mostly produced from animal body parts and as wastes from industrial processes, primarily from butcher shops, animal farms and leather industries[17]. These wastes accumulate in the ecosystem and are regarded as pollutants which increase the likelihood of environmental danger[1]. For instance, an estimated 40 million tonnes of these waste feathers are burnt annually spewing out sulphur dioxide and carbon dioxide in the process[6].

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Under mild circumstances, keratin proteins are difficult to solubilize because they are chemically inert biomaterials[10]. This is because keratin components have a high number of disulphide bonds between cysteine amino acids. Consequently, it is challenging for typical proteolytic enzymes (trypsin, pepsin) which are primarily derived from plant sources to fully break down keratin into smaller components[3]. Most reports suggest that bacteria and their enzymes are responsible for the whole breakdown of keratin wastes. Biodegradation of keratin wastes by keratinolytic microorganisms and their enzymes (keratinases) overcome the drawback observed by chemical and thermal treatments[19]. Keratinases are extracellular enzymes. Many microorganisms capable of degrading keratin by producing keratinases belong to the domain bacteria[21]. These organisms are typically found in a setting that has accumulated keratinous materials such as soil and dump-yards containing decomposing feathers[5].

2. Material and methods

2.1. Collection of samples

Disposed poultry feathers and soil containing dumped feathers were collected from poultry farms in and around Bengaluru. The collected samples were serially diluted, and each dilution was plated onto nutrient agar plates. Colonies grown were studied for their morphological characteristics. Gram staining was performed.

2.2. Primary screening for proteolytic bacteria

All the isolates obtained were plated on skimmed milk agar plates[2]. The plates were incubated at 37°C for 48 hours and then were examined for the formation of clear zones[11]. Among all the isolates screened, the ones which were able to produce clear zones on skim milk agar were thus taken forward for secondary screening[2].

2.3. Secondary screening for keratinolytic bacteria

The proteolytic isolates obtained from primary screening were subjected to secondary screening in order to specifically identify keratinolytic bacteria using modified basal liquid medium supplemented with chicken feather[7]. 25mL of modified basal liquid medium was taken in boiling tubes and one surface-sterilized, dried, medium sized chicken feather was added to each tube followed by inoculation of selected isolates[20]. To further confirm the feather degrading ability of these isolates, they were plated on feather meal agar[16].

2.4. Identification and Biochemical Characterization of the isolates

A series of different biochemical tests were performed to identify the above obtained keratinolytic isolates[14]. These biochemical tests categorize bacteria using various properties such as production of hydrolytic enzymes (Indole test), ability to ferment glucose (Methyl red test and Voges Proskauer test), and ability to use citrate as the sole carbon source (Citrate test) etc[11].

3. Results

Disposed poultry feathers and soil containing dumped feathers were collected from poultry farms in and around Bengaluru. Serial dilutions of the collected samples were performed to reduce the dense cell culture to a more usable concentration. Through this process, a total of 17 colonies were obtained, which were designated as A_1 to A_{17}. Upon morphological characterization and Gram staining, it was observed that 6 isolates were Gram positive rods, 1 isolate was Gram positive endospore forming rod, 1 isolate was Gram positive cocci in clusters, 1 isolate was Gram positive coccobacilli and 8 isolates were Gram negative rods (Fig 1).

The 17 isolates obtained from above were taken forward for screening of proteolytic activity. These isolates were plated on the skimmed milk agar and incubated for 48 hours. 13 isolates showed the formation of clear zones around them that indicates the ability of these isolates to hydrolyze the milk protein, casein through the production of proteolytic enzymes such as proteases (Fig 2). These 13 colonies which showed proteolytic activity were A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8, A_9, A_{10}, A_{11}, A_{12}, and A_{13}.

Selected through the primary screening, these 13 isolates were subjected to whole feather degradation in modified basal liquid medium. It was observed that only 5 out of the13 isolates were able to degrade the feathers effectively within a span of 20 -25 days. Additionally, to confirm the keratinolytic abilities, the isolates were further subjected to growth on feather meal agar plates and all the 5 bacterial isolates were able to grow by utilizing keratin containing feather meal as the sole source of carbon indicating that they are indeed keratinolytic (Fig 3A & 3B). These 5 isolates were A_1, A_3, A_5, A_{10}, A_{13}.

In an attempt to identify the above obtained 5 isolates to the genus level, certain biochemical tests were performed such as IMViC, starch hydrolysis, carbohydrate fermentation, catalase and oxidase tests. Through biochemical characterization, the keratinolytic bacteria were found to belong to the genera *Bacillus, Microbacterium* and *Pseudomonas* (tables 1 & 2).

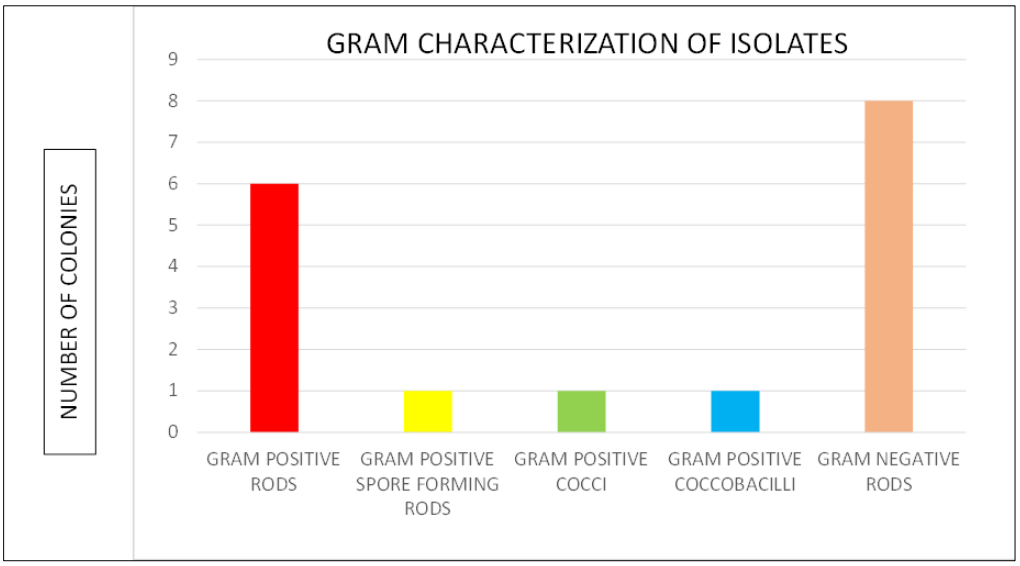
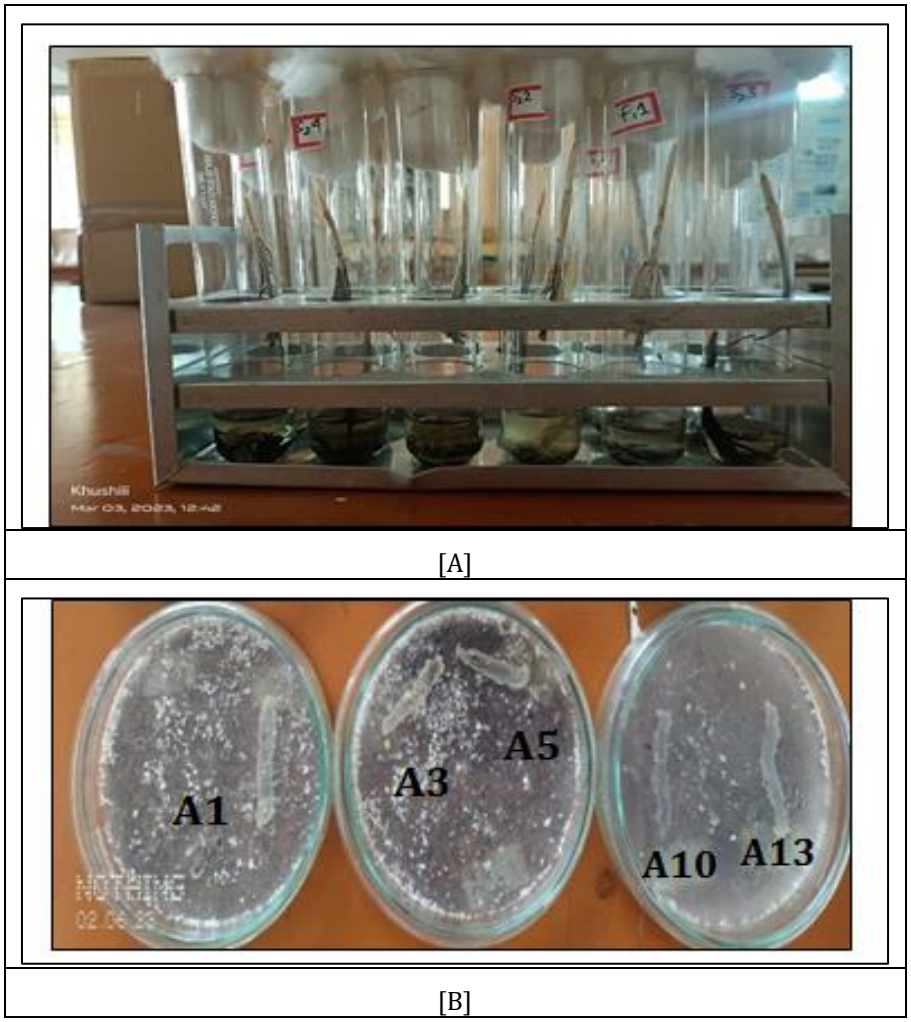


Figure 1 Gram Characterization of the 17 colonies isolated from poultry wastes and soil dumped with poultry wastes

Figure 2 Primary screening of the isolates for proteolytic activity on skimmed milk agar



[A]

[B]

Figure 3 Secondary screening of isolates for Keratinolytic activity. (A) Growth of isolates in Modified Basal Liquid Medium for whole feather degradation. (B) Growth of isolates in Feather meal agar for confirmation

Table 1 Biochemical tests for identification of bacteria

Isolates	Indole	Methyl red	Voges Proskauer	Oxidase	Catalase	Starch	Mannitol	Citrate
A_1	+	-	+	-	+	+	-	+
A_3	-	-	+	-	+	+	-	-
A_5	-	-	+	-	+	+	-	+
A_{10}	-	-	+	-	+	-	-	+
A_{13}	-	-	+	-	+	+	-	-

'+' indicates a positive result ' - ' indicates a negative result

Table 2 Carbohydrate fermentation tests

Isolates	Glucose		Lactose		Maltose		Sucrose	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
A_1	-	-	-	-	-	-	-	-
A_3	-	-	-	-	-	-	-	-
A_5	-	-	-	-	+	-	-	-
A_{10}	-	-	-	-	-	-	-	-
A_{13}	+	-	+	-	-	-	-	-

'+' indicates a positive result ' - ' indicates a negative result

4. Discussion

In conclusion, this research offers valuable insights into the isolation and screening of keratinolytic bacteria from the poultry feather dumped soil. The bacterial isolates that were capable of degrading the feather and exhibiting growth in the feather meal agar, thus showing promising keratinolytic activity might be *Bacillus spp, Psuedomonas spp* and *Microbacterium spp*. This opens avenues for their use in the sustainable waste management practices. These bacteria can break down the keratin rich wastes into simpler compounds. This not only reduces the waste but also potentially transforms it into valuable byproducts, such as biofertilizers or animal feed additives.

Moreover, this study contributes to our understanding of microbial ecology of environments contaminated with keratin rich materials. From an industrial perspective, the discovery of these keratinolytic bacteria opens doors for the development of biotechnological applications. Enzymes produced by these bacteria such as keratinases are of interest in various industries including textiles, cosmetics, and medicine. Understanding the enzymatic mechanism involved in keratin degradation would lead to the production of more efficient and specific enzymes for these industries.

5. Conclusion

This study identified *Bacillus, Pseudomonas*, and *Microbacterium* species with keratinolytic activity, offering a sustainable approach to managing poultry waste. These bacteria can convert keratinous waste into valuable byproducts like biofertilizers, benefiting waste management and industrial applications. Further research can enhance enzymatic efficiency for broader environmental and commercial use.

Compliance with ethical standards

Acknowledgments

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Supplementary information

Materials used [1], Media composition [2], and morphological and Gram characteristics of the 17 isolates [Table S1].

Disclosure of conflict of interest

The authors show no conflict of interest.

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