

# Isolation and screening of keratinolytic bacteria from the poultry feather dumped soil of ICAR-NEH region, Imphal centre

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## ABSTRACT

Keratinolytic microbiome had ponderous application. Poultry waste degradation, composting and potential cast down of beta-amyloid precursor and prion proteins were major hallmarks of keratin degrading microbe. Certain microbe, fungi having photolytic and hydrolytic action degrade the long chain cross-linking disulfide bond of keratin present in animal hair, nails, hoofs, and feathers. Gram-positive bacteria proved to be major potential degrader as compared to Gram-negative bacteria. The poultry feather dumped soil was collected from the ICAR centre for NEH region, Lamphelpat, Manipur and characterized for the detection of various unicellular prokaryotes and multicellular eukaryotes which prove to have potential biotechnological Mecca. The isolate identified as for Gram-positive Bacillus shows maximum degrading potential. Single Gram-negative bacteria with least relative keratinolytic activity identified as *Stenotrophomonas lamphella* had been isolated. The relative action measuring the keratinase activity of isolate recorded predominant Bacillus the most having greater keratinolytic action as compared to the *Stenotrophomonas*.

## 1. INTRODUCTION

Keratin belongs to the class of fibrous protein present mostly in eukaryote. They were classified as alpha-keratin and beta-keratin. Alpha-keratins were tough, insoluble and show helical confirmation. Protofibril act as a basic unit. Beta-keratin shows antiparallel beta-pleated sheet; soft and flexible and have extended chain conformation. In vertebrate entire alpha-keratin set up the whole dry weight of hair, wool, feathers, nail, claws, scales, horns, and hooves and much of the outer layer of the skin. Proteolytic resistance due to the cross-linking of protein chain by histidine was also one important biological aspects. Feathers were produced in large amounts as a waste by-product of poultry processing plant [1]. Large quantities of feathers are produced, and billions of chicken were killed annually to produce 8–10 billion tons of poultry feathers in the poultry industry. It was reported that two different groups of alkaline serine protease shown to active at a temperature within 20–50°C and 28–90°C [2]. Among all the microbial enzyme, proteinase named keratinase having E.C. No. 34.99.11 had imaginable anatomizing keratin substrate such as hair, feather, and collage. Tanning is an important process in leather industry where

collagen or other keratin fibrils were directly converted into leather [3]. Keratinase had shown to have applications in leather industry, cosmetics, pharmaceuticals, biodegradation of waste, pollution control and its isolation, screening, characterization becomes an important aspect in microbial industry. Soil having much proximity to poultry farm could be the most potent keratinolytic microbiome reservoir. The soil could contain most of the keratinophilic and keratinolytic microbes including some strain of fungi. Soil rich in keratinous material is most conducive for growth and occurrence of keratinophilic fungi. Stability of keratin depends on the disulfide bonds and its resistance to enzymatic degradation. The presence of sulfide was detected on extracellular medium probably participating in the breakdown of sulfide bridges of the feather keratin [4]. Carbon and nitrogen source could be used with enrichment technique to identify potential keratinolytic bacteria [6]. In this study, we recorded the isolation of different strains of microbiome which are keratinolytic and state its important therapeutic, industrial, and waste control applications. Carbon and nitrogen source could be used with enrichment technique to identify potential keratinolytic bacteria [6]. In this study, we recorded the isolation of different strains of microbiome which are keratinolytic and state its important therapeutic, industrial, and waste control applications.

## 2. METHODOLOGY

### 2.1. Soil Sample Collection

Soil sample collected from the three site of poultry farm of ICAR for NEH region, Lamphelpat, Manipur were taken either deeper

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than 20cm or superficial horizon or incubated at agar medium [7]. Decaying feathers were present with the soil sample. The sample was collected in polythene bag marking A1–A5. Soil sample was aged and gray. Soil sample with decayed feathers was incubated and kept for monitoring quality improvement. They were cultured for 5 days at 98.6°F. Necessary salt medium of pH value 7.3 comprising 0.5 g/l NaCl, 0.4 g/l.0.3 g/l  $\text{KH}_2\text{PO}_4$ ,  $\text{MgCl}_2$ , and 4 g/l yeast extract.

## 2.2. Isolation

For the isolation of keratinolytic microbiome, we employed Nutrient agar medium. To check the presence of keratinolytic or keratinophilic fungus we took 15 g of soil with 150 ml of distilled water and diluted on agar medium (300 ml). All the plates were kept for incubation of 6 days. Plate with a clear zone of colonization will indicate the presence of keratinolytic bacterial or fungi. After careful isolation, they were stored in sabouraud dextrose agar medium. The isolate completely degraded feather pieces after liquid culture at 30°C [9]. Keratinolytic fungi could metabolize using carbon, sulfur, and nitrogen as an energy source [14].

## 2.3. Screening and Characterization of Keratinolytic Microbiome

### 2.3.1. Keratinolytic microorganisms

Different colonies were noted on the keratin agar and inoculated on new sterile feather agar plate. They were kept for incubation at 37°C for 2 days [16,17]. The plate showing clearance zone was taken as keratinolytic [18]. In another modified broth media with feathers strain were inoculated and the flask was kept for incubation at 120 rpm for 6 days. We observe the confirm degradation of feathers. For protein and keratinase assay, we took the supernatant. Lowry's method was employed for protein determination [20-22]. Strain showing degradation was identified morphologically, culturally, and biochemically.

### 2.3.2. Evaluation of keratinase action [Tables 1 and 2]

Crude cultured broth was assayed after 6 days of incubation for keratinase activity. 1.5 ml of crude enzyme was diluted for phosphate buffer (0.05M of pH 7). 1 ml of keratin solution was added and stored for 15 min at 50°C. We stop the reaction by adding 2.5 ml of 0.5M trichloroacetic acid [23-28]. The mixture content was centrifuged at 2000 rpm for 30 min, and absorbance was recorded at 280 nm. By adding enzyme solution with 2 ml of trichloroacetic acid avoiding keratin we prepare the control [31]. The potential degradation of keratin substrate was tested on agar medium, and substrate degradation was visually inspected, and aliquots were removed.

Enzyme unit per ml = absorbance \*4 dilution rate/0.01 XT

Where T = incubation time period and 4 denoted the tidal volume used

Lowry's methods determine the protein concentration.

## 2.4. Feather Compost Preparation

Keratinase ability to stable in the presence of detergents, metal ions, and surfactants is the core to industrial approach [20]. Degraded feather compost could be used as potential biofertilizer and serve as a control in pollution standard. Degraded feather and 1 kg of sand were mixed uniformly in black plastic. The compost contains isolated 40% *Bacillus* ( $10^{10}$  cfu/ml) and 6% newly isolated *Stenotrophomonas lamphella* ( $10^6$  cfu/ml). Black plastic bin was covered with moist paper and maintained the moisture content by adding sterilized water. The combined band of degraded poultry feather, sand, inoculums with native isolate serve as a control for the bioconversion of fertilizer. The temperature and moisture content was monitored continuously. We determine the process temperature every 12 days interval using a thermometer in different locations. Based on dry weight, moisture content was expressed giving the percentage of original wet weight sample containing water (Bressollier et al., 1999). pH electrical conductivity, nitrogen, phosphorous, and potassium were also analyzed with the help of ICAR NEH, Lamphelpat, Manipur [30].

## 2.5. Therapeutic Approach

Certain protease or kinase has been reported that their ability to disrupt the beta-amyloid fibrils or amyloid matrix that are assiduity in Alzheimer's disease. Nattokinase is one phenomenal example disrupting the prion disease and another neurodegenerative disease [24,35]. Further research needs to be carried out in the medicinal and therapeutic aspects.

## 3. RESULTS AND DISCUSSION

All the five isolates from the poultry soil collected shows positive bacterial growth. 98% of the isolate was found to be Gram-positive and single isolate having moist consistency, rod, anaerobe, and straight acclivity confirms the Gram-negative one. The morphological characteristics of the A5 isolate and its biochemical analysis characterization mention in Table 3 and most importantly enzymatic cleavage action (beta-galactosidase) confirmed the identity of Gram-negative *Stenotrophomonas*. The isolate shows the maximum degradation at the 3<sup>rd</sup> day as shown in Figure 2, and the keratinase activity decreases from the 4<sup>th</sup> day onward as shown in the graphical representation in Figure 1. The keratinase produced by the isolated activity degrades the feather at pH value 8. The exact identification of the bacterial will be confirmed by 16srRNA sequencing. Depending on its keratinolytic activity, enzyme keratinase producing microbiome was cultured in the basal salt medium. The isolate 1–4 was found to be Gram-positive and is mostly shows the characteristics of *Bacillus*. The specific activity was shown in the 3<sup>rd</sup> isolate, and maximum feathers were degraded at around 68 U/ml and 2 mg/ml protein. The sulfitolysis degradation was observed least as compared to proteolysis. The 3<sup>rd</sup> isolate showing maximum degradation is found to be *Bacillus*, and 5<sup>th</sup> isolate the only Gram-negative having least keratinolytic activity was named after the land source where poultry farm resides, the ICAR complex lamphelpat and the isolate was named as *S. lamphella*. We conclude that the poultry

**Table 1:** Physiobiology and cultural characteristics of isolates

S. No	Size	Shape	Color	Surplus	Opacity	Acclivity	Texture	Gram's character
A1	0.8 mm	Circular	White	Evenness	Opaqueness	Flat	Moist	+ve, rod
A2	1.8 mm	Circular	White	Regularity	Opaqueness	Convex	Moist	+ve, rod
A3	1 mm	Circular	White	Regularity	Semitransparent	Flat	Moist	+ve, cocci
A4	2 mm	Circular	White	Irregularity	Opaqueness	Convex	Moist	+ve, rod
A5	1.1 mm	Circular	White	Regularity	Opaqueness	Straight	Moist	+ve, rod

**Table 2:** Feather degrading microbiome and keratinase action

Number of days	Relative action percentage
1	33
2	43
3	86
4	65
5	61
6	53

**Table 3:** Biochemical analysis of A5 isolates

Test characteristics	Results of the test
Shape	Rod, curve, straight
Indole test	Negative
Methyl red test	Negative
Oxidase test	Positive
Voges-Proskauer test	Negative
Citrate utilization test	Variable
Gram staining	Negative
Capsulated test	Negative
Motility	Positive
Fermentation of glucose	Positive
Fermentation of lactose	Positive
Fermentation of maltose	Positive
ONPG beta-galactosidase	Negative
Salicin test	Positive

ONPG: Ortho-Nitrophenyl-β-galactoside

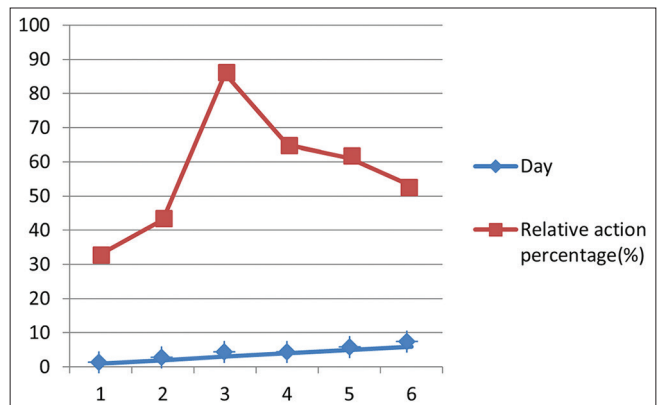


**Figure 1:** Feather degradation by keratinolytic microbiome on several days

soil with a relative activity of degradation was mostly Gram-positive and belongs to *Bacillus*. The multicellular eukaryotes like fungi may be present and needs certain morphological, biochemical analysis, and 18 srRNA sequencing for the exact identification.

#### 4. CONCLUSION

The ecophysical and morphological characteristics of most Gram-positive *Bacillus* and gram lone Gram-negative *Stenotrophomonas* showed the concluding remarks of identification and characterization



**Figure 2:** Percentage of relative action in Y-axis versus number of days in X-axis

of the isolate. The relative action measuring the keratinase activity of isolate recorded predominant *Bacillus* the most having greater keratinolytic action as compared to the *stenotrophomonas*. Based on the physiochemical analysis of feather depicting change in Ph (slightly alkaline) and wide range of temperature (41–55°C) showing the thermophilic range. The moisture content during the composting varied during initial composting (30–50%) and drops drastically afterward. The moisture content 50% and above shows an optimum range of composting. The method used could be the alternative to farm composting might help in removal of recalcitrant feather having valuable land use application. Valued therapeutic approach like natokinase ability to breakdown the amyloid fibrils, beta amyloid protein of most lethal prion and neurodegenerative disorder known as Alzheimers could be a well established trademark of restorative medicine.

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