# Isolation and identification of feather degrading bacteria from feather-dumped soil

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**Abstract:** Feathers contain 90% of protein in the form of keratin. Feathers are generated in large quantity as a by-product in poultry farms leading to environmental pollution. As chemical treatment process is expensive and does not completely degrade the feather wastes, an alternative process would be required. Microbial process is economical compared to chemical process, and it also helps convert feathers into value added products such as nitrogen fertilisers, feedstuffs, films and rare amino acids. The soil samples for the wastes were collected from a poultry dump area in Trichy. They were serially diluted and 50 microorganisms were obtained. The microorganisms obtained from the feather-dumped soil were inoculated on solid agar plates at 30°C for 24 h. Out of those, 10 organisms showed a clear zone of hydrolysis. The collected samples of soil were tested and screened to isolate pure keratinolytic strains. Clear zone of hydrolysis in milk agar medium confirmed the presence of proteolytic activity of organisms. The positive strain 1 which produced keratinase enzyme was identified as *Bacillus cereus* by 16S rRNA analysis.

**Keywords:** *Bacillus cereus*; feather; keratin; keratinase; keratinolytic activity.

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## 1 Introduction

Feathers as a worldwide by-product of poultry farming, amount to millions of tons per year. They contain over 90% crude protein in the form of keratin (Latshaw et al., 1994). They are considered a part of solid waste since their highly rigid structure makes them tough to degrade. Feathers constitute nearly 8.5% of the total weight of a chicken [Gousterova et al., 2005]. Thus the amount of solid waste generated in India from 823.5 million chickens with an individual body weight of 2 kg would be approximately 140 million kg, while the same estimate for the US would be around 1.044 billion kg (McGovern, 2000). Hence, there is a demand for developing biotechnological alternatives for recycling of such wastes. Feather wastes represent a potential alternative to more expensive dietary ingredients for animal supplement for animal feed, but feather meal production is an expensive process, requiring significant amounts of energy. Generally, the feather is steam pressure cooked and chemically treated before use. The protein obtained is not degraded by common proteolytic enzymes such as trypsin, pepsin and papain [Bach et al., 2011]. The chemical processing of feather also causes environmental pollution. Disposal of this wastes results in environmental issues such as ground water contamination and spreading of diseases such as chlorosis, mycoplasmosis and fowl cholera.

Chemical and thermal methods of waste treatment are expensive, while recycling it into useful product is difficult [Farag and Hassan, 2004]. This leads to the necessity of developing an alternative technology to make these wastes into useful products. The microbial degradation of feathers represents an alternative technology to improve the nutritional value of feathers as waste [Suntornsuk and Suntornsuk, 2003]. The bioconversion of poultry feather from a potent pollutant to nutritionally non-degraded protein rich feed stuff for livestock offers considerable opportunities for low energy microbial technology.

Keratinase enzymes are produced by some proteolytic organisms such as bacteria, fungi and streptomyces [Syed et al., 2009]. This enzyme is produced by fungi including the species of *Aspergillus*, *Onygena*, *Absidia and Rhizomucor*, some species of dermatophytes including *Trichophyton mentagrophytes*, *T. rubrum*, *T. gallinae*, *Microsporum canis* and *M. gypseum, a fee actinomycetes such as Streptomyces pactum, S. albus and S. fradiae,* some bacillus species of bacteria and the thermophilic *Fervidobacterium pennovorans*. Increase in keratinolytic activity is also found to be associated with thermophilic organisms which require high energy inputs to achieve maximum growth and the decomposition of keratin wastes (Friedrich et al., 2005).

## 2 Materials and methods

### *2.1 Processing of chicken feathers*

The chicken feathers were washed thoroughly with tap water and air-dried. The dried feathers were defatted by soaking them in diethyl ether for 24 h and then washed again thoroughly with distilled water, air-dried and autoclaved. Finally, the feathers were cut into small pieces without the midrib and stored at room temperature.

### *2.2 Isolation of keratinolytic microorganisms*

Soil consists of three zones: top zone, middle zone and rhizo zone. If the soil sample was collected from top zone, there is an absence of organisms because of the wind which makes the organisms migrate. In the middle zone, there is an abundance of organisms as the soil contains excess nutrients and so their survival rate would be high. The soil samples were collected from a poultry dump area in Trichy. The soil samples were serially diluted and plated on a feather meal medium as shown in Table 1 and the plates were incubated for 2 days at 30°C.

**Table 1** Feather meal media composition

| *Chemical composition* | *Value(g/l)* |
|---|---|
| Sodium chloride (NaCl) | 0.05 |
| Ammonium chloride ($NH_4Cl$) | 0.05 |
| Di-potassium hydrogen orthophosphate ($K_2HPO_4$) | 0.03 |
| Potassium dihydrogen phosphate ($KH_2PO_4$) | 0.04 |
| Magnesium chloride ($MgCl_2$) | 0.024 |
| Yeast extract | 0.01 |
| Feather meal | 1.0 |

### 2.3 Inoculum development

The isolated strains were inoculated into a 50-ml medium containing the above chemical composition at pH 7.5. The culture was grown in a shaker at 150 rpm, incubated for 24 h at 37°C and used as an inoculum.

### *2.4 Serial dilution*

Serial dilution was performed to reduce the concentration of organisms in the soil to obtain single colonies. About 1 g of soil sample was added to 10 ml of distilled water. From that, 1 ml of water was taken and 9 ml of water was added to it to make dilution concentration of $10^{-1}$ .Similarly, a dilution concentration of up to $10^{-7}$ was made.

### *2.5 Screening of proteolytic activity*

Milk agar medium or caseinolytic medium as shown in Table 2 was used as the primary screening of keratinolytic organism. The caseinolytic medium was prepared by mixing

the contents together and boiling them well in order to blend them. This medium was then poured into plates and allowed to solidify. A gel puncher was used to make wells in the milk agar mix in the plates. The inoculum was loaded into these wells, the plates were incubated for 2 days at 30°C and then the clear zone formation was examined.

**Table 2** Milk agar media composition

| *Chemical composition* | *Value* |
|---|---|
| Agar | 0.8(g/100ml) |
| Milk | 15ml |
| Distilled water | 85ml |

## 3 Results and discussion

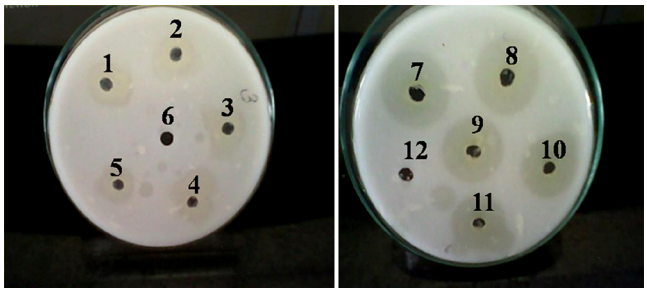
### *3.1 Isolation of keratinolytic bacteria*

Ten microorganisms were obtained when the feather-dumped soil was inoculated on solid agar plates at 30°C for 24 h. From that, eight microorganisms showed clear zone of hydrolysis.

### *3.2 Screening of milk agar plates*

The primary screening was done by milk agar plates. Well diffusion method was carried out to inoculate the organisms. Clear zone of hydrolysis formation confirmed the presence of proteolytic activity (Figure 1).

**Figure 1** Zone of hydrolysis around the bacterial growth on milk agar medium (Well 6, 12 – Control; Strain-7 was selected as maximum hydrolysis) (see online version for colours)




### *3.3 Keratinase assay*

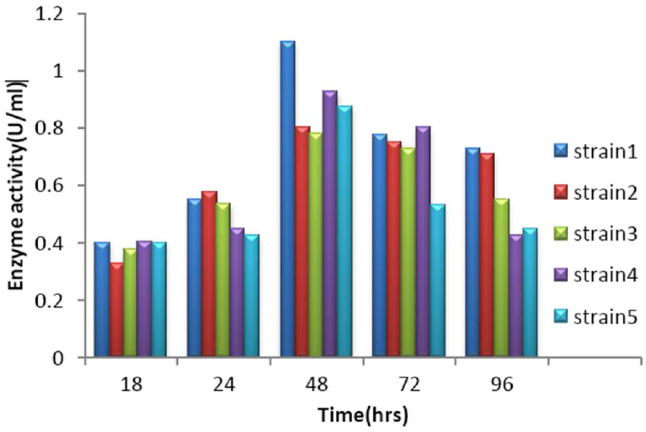
Keratinase activity was determined by modified method of Letourneau et al. (1998) using keratin azure (Sigma Chemicals, USA) as substrate. The keratin azure was suspended in carbonate buffer (10 mM and pH 10) at a concentration of 4 mg/ml. The reaction mixture contained 1 ml of enzyme and 1 ml of keratin azure suspension. The sample was incubated at 37°C, 300 rpm for 1 h. After incubation, the mixture was kept in ice for 15 min followed by centrifugation at 5000*g* for 15 min to remove unutilised substrate. The supernatant was spectrophotometrically measured for the release of the azo dye at

595 nm. A control was kept with enzyme and buffer without substrate. One unit (1U) of keratinase was defined as the amount of enzyme causing an increase of 0.1 absorbance between sample and control at 595 nm in an hour under the given conditions.

### *3.4 Keratinase assay for isolated strains*

The keratinase assay for isolated strains was performed. From that, the maximum keratinase activity was obtained in strain 1 in the 48th h of incubation and minimum keratinase activity was obtained in 18th h in strain 3.

**Figure 2** Graphical representation of comparison of Keratinase activity of various strain (see online version for colours)




### *3.5 16S rRNA analysis*

The positive strain 1 from above data was submitted for 16S rRNA analysis and was identified as *Bacillus cereus* strain. Similar sequences were identified using online BLAST in NCBI nucleotide database.

| *Description* | *Max score* | *Total score* | *Query cover* | *E value* | *Max identity* | *Accession* |
|---|---|---|---|---|---|---|
| *B. cereus* strain JCM 2152 16S ribosomal RNA gene, partial sequence | 1528 | 1528 | 97% | 0.0 | 100% | NR_113266.1 |
| *B. cereus* strain NBRC 15305 16S ribosomal RNA gene, partial sequence | 1526 | 1526 | 97% | 0.0 | 100% | NR_112630.1 |
| *B. cereus* strain ATCC 14579 rRNA operon, partial sequence; and 16S ribosomal RNA gene, complete sequence | 1554 | 1554 | 100% | 0.0 | 99% | NR_074540.1 |

### 3.6 Degradation of feather by Bacillus cereus

The strain of *B. cereus* which was exhibiting maximum zone of hydrolysis was selected and used for degradation of the feathers. It exhibited complete degradation after 3 days of incubation (Figure 3).

**Figure 3** Degradation of feather by *Bacillus cereus* after 3 days of incubation (see online version for colours)

## 4 Conclusion

Keratin is an insoluble fibrous protein present in hair, nails, skin and hooves. Feathers contain 90% of protein in the form of keratin. Degradation of feather by microbes improves the digestibility of feather due to the production of keratinase. The collected samples of soil were tested and screened to isolate pure keratinolytic strains. Clear zone of hydrolysis in milk agar medium confirmed the presence of keratinolytic activity of organisms. The isolated organism was identified as *B. cereus* by 16S rRNA analysis. *B. cereus* exhibited complete degradation of feather after 3 days of incubation at 150 rpm.

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