

# Exploration of the Potent Bacterial Strain for the Application of Feather Degradation

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## ABSTRACT

Feathers are generated in huge amounts annually as waste products of commercial poultry processing plants. Microbial keratinase have become biotechnologically vital enzyme for hydrolysing the highly rigid, strongly cross-linked structural feather polypeptides, keratin. The present study focuses on the bioconversion of insoluble feather keratin to soluble feather residue that has high nutritional values and can be employed as a supplement for livestock feeds. It explores the effectiveness of using microbial activity for degradation of keratin. It also aims in identifying the novel isolates exhibiting high keratinase activity. The study evaluates various conventional and biotechnological approaches for improving yield and nutritional value of feather meal. It also measures the potential of the bacterial isolates in degrading the feather meal. The results of the study showed that microbial hydrolysis involving keratinolytic activity serves as a cost-effective and potent alternative for improving the nutritional value of animal feed obtained from feather waste. Considering that feather protein serve as an excellent source of metabolisable protein and that microbial keratinases enhance the digestibility of feather keratin, keratinolytic bacterial isolates can be used to produce animal feed protein by degrading poultry waste.

**KEY WORDS:** Protease, Keratinase, Feather Degradation.

## 1. INTRODUCTION

Every day huge quantities of chicken is being utilised by the human society leading to the accumulation of vast amount of feather waste in the poultry industries, reaching millions of tons per year worldwide (Williams, 1991). Chicken feather wastes are considered as a vital source of protein for livestock due to the presence of high protein content (Thys and Brandelli, 2006; Anbu, 2007; Kumar, 2008). The shortage of protein in animal feed industries demanded for a new protein source with rich nutritional value (Odetallah, 2003). In the present scenario, the feather waste is converted into feather meal, a digestible dietary protein for animals using physical and chemical treatments. Chicken feathers comprise more than 90% protein; the dominating one is the beta-keratin, an insoluble and fibrous structural protein (Feughelman, 2002). The physical and chemical treatments are used currently to increase the digestibility of this feather keratin. However, such processes consume large amounts of energy and together destroy certain amino acids, thus yielding products of poor digestibility and inconsistent nutritional quality (Papadopoulos, 1986). Dymatic hydrolysis by microorganisms that involves keratinolytic activity serves as an effective alternative for improving the nutritional value of feather meal (Lin, 1992).

Feather constitutes approximately 5-7% of the total weight of mature chicken. The major constituent, keratin is subjected to high degree of cross-linking by various bonds such as cysteine disulfide bonds, hydrogen bond and hydrophobic interactions (Feughelman, 2002). Keratin is insoluble and not degradable by proteases such as trypsin, pepsin and papain (Williams, 1990). The feather accumulation in the environment can be controlled by degrading keratin with keratinolytic enzyme produced by some microorganisms (Onifade, 1998). Keratinolytic activity has been reported in various bacterial genera such as bacillus (Williams, 1990; Lin, 1999; Suntornsuk, 2003; Zerdani, 2004; Suntornsuk, 2005), flavobacterium (Brandelli and Riffel, 2002; Nam, 2002), thermo anaero bacter (Antranikian and Riessen, 2001), vibrio (Brandelli and Sangali, 2000) and chryseo bacterium (Riffel, 2003). In this regard, keratinolytic enzymes may have important role in biotechnological processes involving keratin-containing wastes from leather and poultry industries through the conception of non-polluting and less expensive treatment (Onifade, 1998). Even though bacterial keratinolytic proteases showed a potential means for feather bioconversion, improvement of enzyme activities and higher yields are required to make these suitable for industrial applications (Kim, 2001).

For the past several decades, many studies have been done in converting features into digestible dietary proteins even though feature meal serves as a poor digestible meal. At present, many efforts are being laid to hydrolyse keratin by microbial activity for obtaining proteins and amino acids (Sangali and Brandeli, 2000; Mortiz and Latshaw, 2001; Riffel, 2007). Diverse groups of microorganisms such as fungi and bacteria have been reported to produce keratinase (Deivasigamani and Alagappan, 2008; Han, 2012). Microorganisms that degrade feathers and their keratinolytic enzymes could be used to enhance the digestibility of feather keratin (Suntornsuk, 2003; Zerdani, 2004; Suntornsuk, 2005). Currently, the major focus of this study is to identify novel isolates with high keratinase

activity and improve the yield using conventional and biotechnological approaches. Lin (1992) have reported that keratinolytic bacteria are found in soil and poultry compost. In the present study, exploration has made through various sequential screening methods to obtain a potent bacterial isolate which possess high potential in degrading feather protein.

## 2. MATERIALS AND METHODS

**2.1. Selection and Collection of Samples:** The sampling area was chosen accordingly to obtain the bacterial isolate which may have exhibit keratinolytic activity. Hence, the samples were collected from waste dumping of different poultry farm and chicken meat centres. Focus was laid on such areas where the poultry wastes were accumulated for a long period. The duff soil samples comprising poultry waste was collected from a depth of around 10 cm, so as to avoid fresh feather litters. The collected samples were packed in a sterile polythene container and subjected to processing within 24 h.

**2.2. Enumeration of Proteolytic Bacterial Frequency:** The enumeration of total viable bacterial counts (TVBC) and total proteolysis bacterial counts (TPBC) was carried out by serial dilution and plating techniques in triplicates. One gram of soil sample was mixed in 100 ml of sterile distilled water and serially diluted upto  $10^{-7}$ . The diluted samples were plated on sterile nutrient agar medium (peptone - 0.5 g, beef extract-0.2 g, yeast -0.3 g, Sodium chloride - 0.5 g, agar -1.7 g, distilled water -100 ml, pH-7.2) for detecting the total viable bacterial count. The diluted samples were also plated on to another series using casein agar plates(skimmed milk powder - 0.1 g, tryptone - 0.5 g, yeast extract - 0.25 g, Sodium chloride - 0.85 g, dextrose - 0.1 g, agar - 1.7 g, distilled water - 100 ml, pH - 7.2) for detecting the total proteolysis bacterial count. Both the plates were incubated at 37°C for 24 h. The number of colonies grown on nutrient agar plates accounted for TVBC and those showing protein utilisation zone around the colony in casein agar medium accounted for TPBC.

The frequency of proteolytic bacteria (FPB) present in the sample was calculated as:

$$\text{Percentage of FPB} = \text{TPBC/TVBC} \times 100$$

**2.3. Isolation and Purification of Proteolytic Bacteria:** The bacterial single colony that showed conspicuous substrate utilisation zone in casein agar was taken into consideration for further study. The colonies were purified by streak plate method using nutrient agar and incubated at 37°C for 24 h. All pure isolates maintained in liquid and solid media were stored in refrigerated condition.

**2.4. Screening of Keratinolytic Activity:** The keratinolytic activity of the proteolytic bacterial population was evaluated by screening the isolates using keratin screening medium. The isolates were streaked into sterile media amended with keratin as protein substrate. The agar medium consisted of ammonium chloride(0.05 g), NaCl (0.05 g), MgCl<sub>2</sub>(0.01 g), K<sub>2</sub>HPO<sub>4</sub>(0.03 g), KH<sub>2</sub>PO<sub>4</sub>(0.04 g), yeast extract(0.1g), keratin powder (0.5 g), agar(1.7g) and distilled water(100 ml) with a pH of 7.5. The isolates which showed proteolytic activity were streaked at the centre of the sterile keratin agar plates and incubated at 37°C for 24 h. The isolates showing keratin utilisation zone were remarked as positive isolates.

**2.5. Screening of Feather Degradation Efficiency:** The screening of feather degradation was carried out in two ways in order to evaluate the potential of isolates capable of degrading feather meal and raw feather.

**2.6. Primary Screening:** The primary screening was performed to check the ability of the isolates for their feather keratin utilisation. The positive isolates obtained in keratinolytic screening step were utilised for primary screening process. The screening was carried out using feather meal agar medium which consisted of NH<sub>3</sub>Cl<sub>2</sub>(0.05 g), NaCl (0.05 g), MgCl<sub>2</sub>(0.01 g), K<sub>2</sub>HPO<sub>4</sub>(0.03 g), KH<sub>2</sub>PO<sub>4</sub>(0.04), yeast extract(0.1g), colloidal feather (0.5 g), agar(1.7 g) and distilled water(100 ml) with pH of 7.5. The isolates showing keratinolytic activity were streaked at the centre of the sterile feather meal agar plate and incubated at 37°C for 24 h. The isolates showing substrate utilisation zone were considered as positive isolates.

**2.7. Secondary Screening:** The secondary screening was carried out to find the efficiency of feather meal utilising bacteria in degrading the raw feather. The degradation was carried out in broth medium consisting of MgSO<sub>4</sub>.7H<sub>2</sub>O (0.2 g), K<sub>2</sub>HPO<sub>4</sub> (0.3 g), KH<sub>2</sub>PO<sub>4</sub> (0.4 g), CaCl<sub>2</sub> (0.22g), yeast extract (0.1g) and chopped raw feather (0.1g). The isolates which showed positivity in feather meal agar medium were inoculated and the tubes were incubated at 37°C for more than 48 h. The degeneration of raw feather was marked as positive. The isolate which showed higher feather degeneration tendency was considered as the potent feather degrading microorganism.

## 3. RESULTS AND DISCUSSIONS

In order to obtain a potent bacterial strain with the ability to degrade the poultry feather wastes, an exploration was done on various samples. Around 10 samples were collected, 5 from the yards of poultry farm waste dumping and 5 from the waste dumped by the commercial chicken meat sale centres. The samples were slightly blackish brown in colour with partial moisture.



**Fig.1.Selected isolate in casein agar medium**

**3.1. Total Viable Count vs. Proteolytic Count:** The total viable count of heterotrophic bacteria showed more or less similar results in both the sample types (Table1). However, the FPB% showing utilisation zone in casein agar plate (Fig.1) was found to be higher in the samples taken from chicken meat centre waste dumping. The waste of poultry farms contains more bird excreta, bedding material, polluted feeds, dead birds, broken eggs and less feather quantity leading to low levels of protein substrate (Lu, 2003). On the other hand the waste dumping of commercial meat centres contains more feather quantity and meat wastes leading to higher level of protein substrates. This may be the reason for the presence of higher level of proteolytic bacteria in the samples taken from the waste dumping of chicken meat centre (Martin and McCann, 1998).

**Table.1.Frequency occurrence of proteolytic bacteria**

| Samples | Poultry Farm Waste Yard |                    |                    |                    |                    | ChickenMeatCentre Waste Dumping |                     |                     |                    |                     |
|---------|-------------------------|--------------------|--------------------|--------------------|--------------------|---------------------------------|---------------------|---------------------|--------------------|---------------------|
|         | S1                      | S2                 | S3                 | S4                 | S5                 | S6                              | S7                  | S8                  | S9                 | S10                 |
| TVBC    | 32x10 <sup>5</sup>      | 38x10 <sup>6</sup> | 56x10 <sup>4</sup> | 89x10 <sup>4</sup> | 76x10 <sup>4</sup> | 89x10 <sup>5</sup>              | 102x10 <sup>4</sup> | 202x10 <sup>4</sup> | 96x10 <sup>4</sup> | 196x10 <sup>5</sup> |
| TVPC    | 65x10 <sup>1</sup>      | 39x10 <sup>2</sup> | 79x10 <sup>1</sup> | 36x10 <sup>1</sup> | 41x10 <sup>1</sup> | 31X10 <sup>2</sup>              | 60X10 <sup>1</sup>  | 74X10 <sup>2</sup>  | 81X10 <sup>1</sup> | 90X10 <sup>2</sup>  |
| FPB (%) | 0.020                   | 0.010              | 0.141              | 0.004              | 0.053              | 0.034                           | 0.058               | 0.366               | 0.084              | 0.045               |

TVC – Total viable count, TVPC- Total proteolytic bacterial count, FPB –Frequency of proteolytic bacteria

\*All the values given in the table are average of triplicates

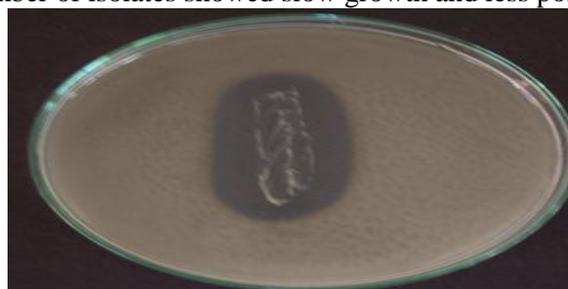
**3.2. Keratinolytic Activity:** The keratinolytic activity among the proteolytic bacterial population was explored using keratin agar medium. The results showed that only around 55% of proteolytic bacterial isolates showed positive keratinolytic activity and rest of them showed negative (fig.2). Least number of isolates showed very less activity.



**Fig. 2.Selected isolate in keratin agar medium**

Research conducted by Matikeviciene (2009), showed that the isolated bacterial strains known to degrade keratin or produce the keratinase primarily composed of Bacillus including *B. subtilis* and *B. licheniformis*. Another study conducted by Samuel Pandian (2012), involving screening of microorganism from the soil sample taken from the dumping site of poultry processing plant, have reported to show the isolation of pseudomonas thermaerum. This bacterium showed proteolytic activity but not keratinolytic activity.

**3.3. Feather Degradation:** The strains which showed keratinolytic activity were screened for feather degradation capacity. As a primary screening, the isolates were provided with well ground colloidal feather meal as the substrate. The results showed that almost all the isolates which showed positivity in the keratin agar also showed positivity in feather meal agar. Very least number of isolates showed slow growth and less positivity (Fig.3).



**Fig.3.Isolate on feather meal agar**

From the research on degradation of feather waste conducted by Vigneshwaran (2010), it was found that *Bacillus licheniformis* was able to grow and produced keratinase in nutrient medium in which feather meal served as an additional carbon and nitrogen source and acted as enzyme inducer. It was also reported that novel keratinolytic isolate, *Bacillus licheniformis* could be a potential candidate for the degradation of feather keratin and can be used as additives in poultry field.

The secondary screening was made by providing raw feather as protein substrate. The results of this screening showed that all the isolates which gave positive result in feather meal agar medium showed positivity in feather degradation (Fig.4). However, the time taken for the degradation process showed differentiation among the isolates.

A study on biodegradation of poultry feather by novel bacterial isolate, *Bacillus altitudinis*, GVC11, conducted by Vijay Kumar (2011), reported that GVC11 was found to grow and degrade chicken feathers in 2 days in distilled water without additional nutrient. There are various reports that indicate different degradation time of different bacterial species, for example, *B. licheniformis* degraded feathers in 10 days (Park and Son, 2009) while *B. pumilus* took 7 days to do the same (Vijay Kumar, 2011).



**Fig.4. Feather degeneration**  
1. Control; 2. Test

#### 4. CONCLUSION

The strategy of isolation of keratinolytic microorganisms utilised in this present study revealed that similar to the isolates of fungi, certain bacterial strains capable of degrading feather keratins are present in the soil dumping. It was found that the selected isolates were able to grow and display keratinolytic activity in diverse keratin wastes. These novel isolates can contribute for potential biotechnological use in processes involving keratin hydrolysis. The study shows that microbial conversion of feather wastes is a potential technique for the degradation of feathers and their utilisation as a feedstuff. Since bacteria can grow faster than fungal species, they can perform effectively in many industrial applications.

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