Production, purification and characterization of keratinase using chicken feather as a substrate by *Bacillus sp.*

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Production, purification and characterization of keratinase using chicken feather as a substrate by Bacillus sp.

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Abstract:

Chicken feather is recognized as a solid waste generated from poultry farms and is highly resistant to degradation. Microorganisms play a vital role in degradation of insoluble keratins. Thus Bacillus sp. was screened from soil samples of slaughter house and poultry farm area using azokeratin medium. An optimum growth condition of keratinolytic strain was standardized. The physical parameters such as, pH, temperature and enzyme activity were studied. Maximum enzyme activity was obtained within 48 hrs (0.3 U/ml). The optimal conditions for the keratinolytic activity were determined to be pH 8.0 and temperature 30°C. In addition the Bacillus sp. was able to completely degrade with in a period of 48 hrs. The identified protein exhibited an apparent molecular mass of 38 kDa.

Keywords: Chicken feather, insoluble keratin, keratinase, crude protein.

Introduction:

Feather is generated in bulk quantities as a by-product in the poultry industry globally. It is a very rich source of protein with β-keratin constituting 91% of feather protein. The presence of keratin makes feather recalcitrant to most common proteases like trypsin, pepsin, papain, and so forth, thus slowing down its degradation process in nature. (Mc Govern, 2000). The bulk of feather waste is poorly recycled in nature and has limited utility due to the chemically unreactive nature of keratin. Thus, recycling of this by-product is neither profitable nor environmentally friendly. The disposal of this waste is a global environmental issue leading to pollution of both air and underground water resources. (Jeevana Lakshmi, 2007).
Keratin is insoluble proteins from feathers, wool, hooves, scales, hair, nails (hard keratins) and stratum corneum (soft keratins) and show extremely resistant to the action of physical, chemical and biological agents. Mechanical stability and high resistance to proteolytic degradation of keratin are due to their disulfide bonds, hydrogen bonds, salt linkages and cross linkings (Deivasigamani et al., 2008).

Several feather degrading bacteria have been isolated from soil, poultry wastes, hair debris and animal skin, and most of these isolates were confined to genera *Streptomyces* and *Bacillus*. Feather contains over 80% of protein and conversion of feather into feed by keratinolytic microbes is inexpensive. The keratinolytic microorganisms and technologies developed for feather degradation not only remove the waste feathers efficiently from the nature but also to make the by-products as a valuable protein supplement. The protein rich, concentrated feather meal can also be used for organic farming as a nitrogen fertilizer. Use of keratinase enzymes in leather industry was known long back in dehairing process as an alternative to chemical processing. Keratin-containing materials such as feathers, hair and wool can be used as substrates for keratinase production (Gupta and Ramnani, 2006). Feather was the mostly utilized substrate, especially by *Bacillus sp*. The current study is focused on the isolation and characterization of keratinase producing *Bacillus sp.* from chicken feather waste to degrade the poultry feather waste (insoluble protein) to soluble protein.

**Materials and Methods:**

**Isolation of keratinolytic microorganisms:**

Soil samples and Chicken feather were collected from a local slaughter house and poultry processing site at Eachnari, Coimbatore. Feathers were extensively washed in tap water and finally in double distilled water. Feathers were dried under sunlight and in hot air oven at 60°C for 48 h. Keratinase producing bacteria was isolated from casein agar medium and identified as *Bacillus sp.* Keratinase was confirmed using azokeratin medium (Suntornsuk and Suntomsuk, 2003).
Keratinase activity and feather degradation:

Chicken feather 5mg was transferred to 100ml of basal medium (NH₃ Cl-0.5 gl⁻¹, K₂H PO₄-0.4gl⁻¹, MgCl-0.24gl⁻¹) and pH was adjusted to 7 with 1N NaOH. The bacteria (Bacillus sp.) was inoculated into the culture flask and incubated for 4 days at 37° C in shaker with 120rpm. The percentage of feather degradation was determined by calculating the weight of control of feather and treated sample (Geun.Tae park et al.,2009).

Assay for Keratinase:

Keratinolytic activity of culture filtrates was measured spectro-photometrically according to the method of Hamaguchi et al. (2000). Keratin powder(20mg),3.0ml phosphate buffer (28Mm, pH 7.8) and 2.0ml culture filtrate were incubated in a shaker waterbath at 150rpm at 37° C for 1 hour. After the addition of 10%trichloroacetic acid (TCA) and centrifugation at 10,000 g for 15min, the optical absorption of the supernatant was measured at 280nm wavelength using a double-beam spectrophotometer toward the blank. The blank was treated in the same way except for the addition of TCA which done before the initiation of enzyme reaction. The increase of 0.1unit absorption is equal to one unit of enzyme activity.

Effect of pH and temperature on keratinase activity:

The optimal pH for keratinolytic activity of enzyme was measured in the range of pH 6-8. The optimum temperature was determined by incubating reaction mixture at different temperature range from 30C-50C.

Purification of keratinase:

The culture medium was pre-filtered through Whatmann No.1 filter paper to remove the residues. The crude concentrated keratinase solution was precipitated in particular quantity of TrisHCl buffer (pH 6.2) and dialysed against same buffer. The dialysed sample was applied to Sephadex 100 column with 25mM TrisHCl buffer(pH6.2) and column was eluted with the same buffer. The fractions were assayed for keratinase activity.
Characterization and identification of molecular weight of keratinase:

The culture filtrate was centrifuged at 10,000 rpm for 15 min. The supernatant acts as a crude enzyme. The molecular weight determination was carried out by SDS-PAGE using 10% polyacrylamide gel according to Laemmli method and stained with Coomassie brilliant blue R 250. Around 10 µl of enzyme and purified keratinase from dialysis and column chromatography were run on SDS-PAGE with standard marker.

Results and Discussion:

The isolated *Bacillus* sp which grow well upto 50°C indicating that the organism is a thermophilic and halotolerant. It grew optimally at pH 8.0 but showed good growth at neutral pH. *Bacillus* sp can produce keratinase which can degrade the feather keratin to soluble crude protein. The organism degraded the feather more efficiently in much shorter time 48 hours and relatively high keratinase activity (0.3 U/ml). Most other keratin-degrading strains such as *Bacillus licheniformis* degraded chicken feather at 50°C in 10 days (Williams et al., 1990) and Bockle et al., 1995 demonstrated that *Streptomyces pactum* DSM40530 partially degraded native chicken feather at 50°C, the maximum feather activity was at 50°C. For the production of keratinase and degradation of feather, 1% basal medium and 5% feather with inoculums was used and maintained in a shaker at 120 rpm/min at 37°C. Keratinase activity was estimated at different intervals (1, 2, 3 and 4 days) at pH 7.0 in that maximum keratinase activity was found at 48 hours (0.3 U/ml) (Fig.1). On increasing the feather concentration the extent of feather degradation decreases because of decrease in enzyme activity (Avinashi et al., 2011). The percentage of weight loss of feather after 96 hours incubation was above 90 %. The optimal conditions for the enzyme production by *Bacillus* sp was observed at pH 8 (Fig.2) and temperature 30°C (Fig.3). After 4th day of incubation, *Bacillus* sp. can utilize the soluble protein in that medium. *Bacillus* sp. was grown on feather medium, feather barbules were effectively degraded. Appreciable amounts of free amino acids and sulfhydryl groups accumulated in the culture broth because feather keratin has disulfide, hydrogen, salt bonds (Mazotto et al., 2010). This suggests the presence of proteolytic- and disulfide bond-reducing activity. Therefore the culture filtrates was collected after 4th day by centrifugation to remove the bacterial cells and was purified by column chromatography.
The feather keratin was degraded and exhibited an apparent molecular mass of 38 kDa. We can also use this feather as a substrate to produce high amount of keratinase. Keratinase can degrade all the protein molecules (Alexandre et al., 2005), so it may also be used detergent purpose. This enzyme can be an alternative to sodium sulfide, the major pollutant from tanneries, and may completely replace it.

**Conclusion:**

It is evident from the results that *Bacillus sp.* is a good producer of keratinase. The maximum active alkaline keratinase production was achieved after 48 hours of incubation at 30°C and pH 8.0, at 120rpm in a 5% feather medium. The enzymes could be used as additives in animal feed to improve feather meal digestibility. This result shows the significance of enzyme in industrial applications for better removal of hair from sheep wool for leather making.

**References:**


