National Journal of Advanced Research
Online ISSN: 2455-216X; Impact Factor: RJIF 5.12
www.allnationaljournal.com
Volume 2; Issue 4; July 2016; Page No. 21-22

Degradation of keratin waste products by *Kocuria rosea*

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Abstract
Feathers are rich in keratin which is an insoluble protein and are introduced naturally into the environment as a waste product in large quantities worldwide. These feathers are mechanically or chemically treated to form feather meal, a dietry protein supplement for animals. Alternatively, keratin can be biodegraded by some microorganisms. The keratinase enzyme produced can be used in dehairing in leather industry. In the present study a strain of *Kocuria rosea* was used to degrade feathers at varying pH and temperature. This gram positive spherical shaped bacterium degraded feather within 5 days of incubation. The optimum pH and temperature for feather degradation and keratinase secretion was at 8.5 and 37° C. Feather degradation reached upto 88.62 % and the enzymatic activity was found to be 81.8 U/mL.

Keywords: Feathers, keratinase, *Kocuria rosea*

1. Introduction
Feathers are rich in keratin and are generated as waste products in large quantities in the poultry Industry. These feathers constitute a sizable waste disposal problem. Feathers can be treated and can be used as protein supplements in the poultry foodstuffs. Current production of feather meal involves a treatment at elevated temperature and high pressure resulting in the loss of some essential amino acids [1]. Thus as an alternative method the nutritional value might be improved by microbial action by keratinase enzyme. These keratinase produced can also be used in the dehairing stage of leather processing. Several microorganisms have been identified that has been able to produce keratinase. They are various species of fungi, actinomycetes and bacteria viz., *Doromyces microspores*, *Aspergillus sp*, *Alternaria radicina*, *Trichurus spiralis*, *Stachybotrys atra*, *Onygena sp*, *Absidia sp*, *Rhizomucor sp* [2], *Scopulariopsis brevicaulis* [3], Crysosporium *keratinophilum* [4], *Streptomyces thermoviolaceus*, *Streptomyces pactum* [5], *Flavobacterium pennavorans*, *Bacillus licheniformis* [6], *B. pumilus* [7] and *Vibrio sp* [8]. *Kocuria rosea* was found as a new feather degrading organism [9].

2. Materials and Method
2.1 Selection of strain and culture media
The feather degrading orange colour pigment producing strain of *Kocuria rosea* was procured from the culture collection centre at Department of Biotechnology, Veer Narmad South Gujarat University, Surat.
Nutrient broth (Hi media, Mumbai) was used for the activation of culture and for further preservation.

2.2 Collection and processing of feathers
Feathers (*Gallus gallus)* were collected and washed with distilled water. Washed feathers were dried at 80° C and stored at 4° C until used.

2.3 Optimization of Fermentation medium
100 mL nutrient broth supplemented with glycerol using feather as substrate was inoculated with 2% Inoculum. A total of 9 sets were made with three varying pH (8, 8.5, and 9) and temperature (30° C, 37° C and 45° C). The fermentation was carried out for 5 days at 120 rpm.

2.4 Estimation of protein
The protein estimation was done by Folin-lowry method using BSA (1 mg/mL) [10].

2.5 Determination of degree of feather degradation (DFD)
The residual feather was washed, dried and scaled to calculate DFD by using following equation [11].
DFD (%) = (TF-RF) × 100/TF
Where, TF is the total feather and RF is the residual feather

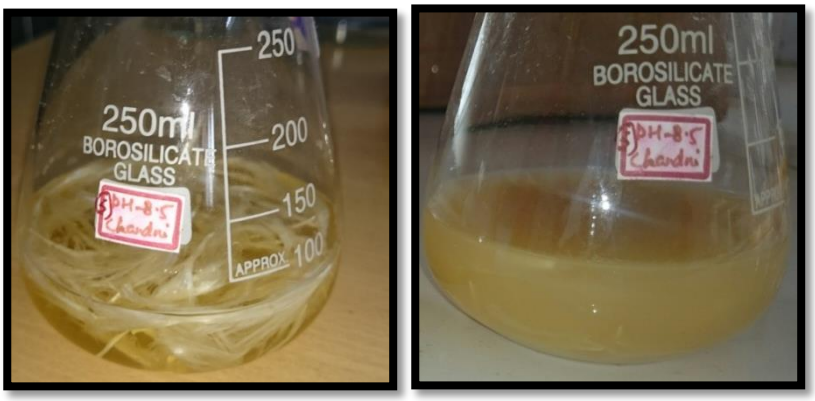
2.6 Determination of Keratinase activity
The keratinase activity was measured after 5 days of incubation the cultured broth was filtered and centrifuged at 4°C, 10000 rpm for 10 minutes to obtain crude enzyme. The reaction mixture contained 1 mL of enzyme and 10 mg of feathers in 2 mL 0.05 M Tris HCl pH 8.5. The reaction mixture was incubated at 37° C for 1 h and the reaction was terminated by adding 1 mL of 10 % trichloroacetic acid. The debris was removed by filtration and the absorbance was taken at 660 nm [12]. The L- Tyrosine (1 mg/mL) was used as standard. The amount of tyrosine released into the filtrate was measured at 660 nm absorbance. One unit of keratinase activity is expressed as the amount of enzyme which converts 1µg of tyrosine per minutes at 37° C.

3. Results and Discussion
Biodegradation of feathers were studied at varying pH and temperature. The optimum pH and temperature for feather degradation was at 8.5 and 37 °C. Amongst the literature cited for keratinase production on 5th day (fig-1b) of incubation we obtained the maximum activity. The highest degree of degradation was 88.62% which is shown in table-1 and keratinase activity was 81.8 U/mL.
The protein estimation done as per Folin-Lowry and the protein liberation reached upto 0.66 mg/mL.

4. Tables and Figures

Table 1: Degree of feather degradation and keratinase activity

Set	Temperature (°C)	pH	DFD (%)	Enzyme activity (U/mL)
1	30	8	9.7	9.1
2	37	8	5.4	5.5
3	45	8	18.27	37.1
4	30	8.5	15.39	32.1
5	37	8.5	88.62	81.8
6	45	8.5	17.44	34.9
7	30	9	13.2	14.7
8	37	9	71.52	59.7
9	45	9	19.52	41.3



(a) Day 1 (b) Day 5

Fig 1: (a) Before degradation and (b) After degradation

5. Conclusion

Utilization of these potential keratin degraders will definitely find biotechnological use in various industrial processes involving keratin hydrolysis. The keratinase enzyme produced by the organism under study can be used in leather industry and in cosmetic for dehairing etc. The protein liberated can be used as animal feed having high nutritive value. It would be able to solve the waste disposal problem of poultry waste and with limited resources recyclying of keratinacious waste would be beneficial both financially and environmentally.

6. References

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