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Biodegradation of Keratin from Chicken Feathers by Fungal Species as a Means of Sustainable Development

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Abstract

Keratinolytic microorganisms have a great importance in feather waste degradation and its use for improvement of livestock feed and production of protein hydrolysates. Annually several thousand tons of feather wastes are discharged into the surrounding environment as a by-product of commercial poultry processing. Microorganisms could minimize regulatory problems of uncontrolled accumulation of waste feathers. This residue is almost pure keratin, which is not easily degradable by common proteolytic enzymes.

The present study deals with identification of fungi that play a significant role in the degradation of chicken feather and keratin degradation ability of the isolated fungi. Feathers of broiler chicken were collected from Jaggi poultry farm, Mandir Hasaud, Raipur. Fungi were isolated by feather baiting technique. Feathers were inoculated in Sobouraud Dextrose Agar (SDA) medium and their pure culture was prepared. Fungus were identified by Lacto phenol cotton blue staining method as *Trichoderma*, *Gliocladium*, *Fusarium*, *Syncephalastrum*, *Mucor*, *Aspergillus Flavus*. The pure culture were grown in mineral media with 500 mg of feathers as a sole source of Nitrogen and Carbon and incubated for the period of 25 days. At 5 days intervals, the biochemical changes associated with biodegradation was evaluated by analyzing the culture filtrate. The release of Nitrate, Cystine, Cysteine and methionine components during the process of biodegradation was studied which proved the efficient degradation of keratin. There was also a change in pH of the medium towards alkalinity. *Mucor* and *Aspergillus Flavus* were the most powerful bio remedial fungus in the current study. With an increasing world-wide concern for the environment it is possible to use these six fungus for the degradation of enormous quantity of waste feathers. Biodegradation leads to recycle the wastes and thus maintaining the environmental quantity of the biosphere.

Keywords: Fungus; Bioremediation; Poultry processing; Keratin; Feather baiting; Lacto phenol cotton blue staining

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 [1]. Typically, each bird has up to 125 gm of feather and with more than 400 million chickens being processed every week worldwide, the daily accumulation of feather waste reaches five million tons [2]. The bulk of feather waste is poorly recycled in nature and has limited utility due to the chemically unreactive nature of keratin. Conventionally, this waste has been converted into feed supplement, resulting in feed of poor quality which is nonviable economically [3]. 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 [4]. In recent years, feather treated with microbial keratinase is attracting wide attention with several applications. Keratinase-treated feather is increasingly considered as a viable source of dietary protein in food and feed supplements, as the enzyme-treated end product retained high nutritive value. Keratinases are projected to generate a potential worldwide market similar to other proteases. Diverse groups of microorganisms are reported to produce keratinase like fungi (*Doratomyces microsporus, Alternaria radicina, Trichurus spiralis, Aspergillus sp., Rhizomucor sp., Absidia sp., Stachybotrys alba,* etc.), *actinomycetes* (*Streptomycespactum, S. alvs, S. thermoviolaceus, S. fradiae, Thermoactinomyces candidus* etc.), and several bacterial species (*Fervidobacteriumislandicum, Pseudomonas aeruginosa, Microbacterium sp.*, and many species of *Bacillus* including *Bacilluslicheni formis* and *B. pumilus*) earlier [2,5-8]. However, the full commercial potential of keratinases is yet to be realized. Major component of feather is keratin which is insoluble fibrous protein. Keratin is highly resistance to hydrolysis by week acids, alkalies, ethanol or salt solution [9] and also to enzymatic digestion [10]. The durability of Keratin is due to cross binding of closely packed polypeptide chain in which cystine molecules are held together by disulphide bonds(S-S). However, the keratinophilic fungi have been frequently isolated from soil, where they colonize various keratinous substrates, degrade them and add the mineral content to the soil [11].

Feathers of birds are most suitable substrate for the survival of much fungus in nature [12]. Ramesh [13] isolated a number of pathogenic and non-pathogenic fungi from keratin substrates and studied their intensity and the type of hair degradation. However, there is no detailed investigation on the degradation of feathers. Therefore, the present investigation was envisaged to study the biodegradation of feathers. Currently, almost all the habitats of the

world have been surveyed for the presence of keratinophilic fungi [14]. Most of these fungi belong to families Arthrodermataceae and Onygenaceae, order Onygenales in Ascomycetes [15].

Methodology

Method of sample collection and isolation of potential fungal colonies

Feathers of broiler chicken were collected from Jaggi poultry farm, Mandir Hasaud, Raipur. Fungi were isolated by feather baiting technique. Feathers of hens were cut and finely powdered and sterilised by using 70% sodium hypochlorite solution for 5 minutes. These sterilised feathers were then inoculated on Sabouraud Dextrose Agar (SDA) media to obtain culture of fungal colonies. Six different colonies obtained were inoculated in 50 ml mineral media (Sodium Nitrate, 3 g; Dipotassium Hydrogen phosphate, 1 g; Potassium Chloride, 0.5 g; Magnesium Sulphate 0.5 g, Ferrous Sulphate 0.01 g) along with 500 mg of feathers as a sole source of Nitrogen and Carbon [16] in 250 ml flasks and incubated for observation.

Identification of isolates

Fungus was identified by Lacto phenol cotton blue staining method. A drop of Lacto phenol cotton blue was taken on a slide. Fungal hyphae was picked from plate with the help of a needle and placed on the slide containing Lacto phenol. Then the Slide was covered with a cover slip and observed under the microscope (Figures 1-6).

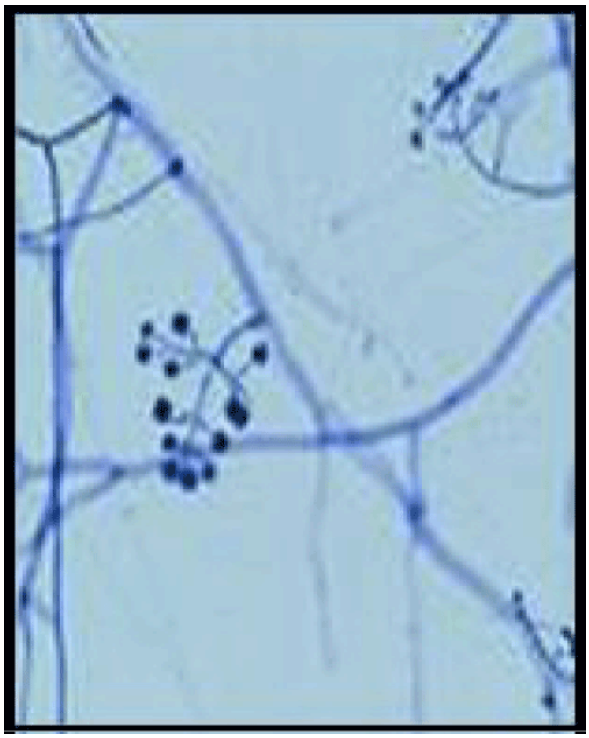

Figure 1: Trichoderma

Figure 2: Gliocladium

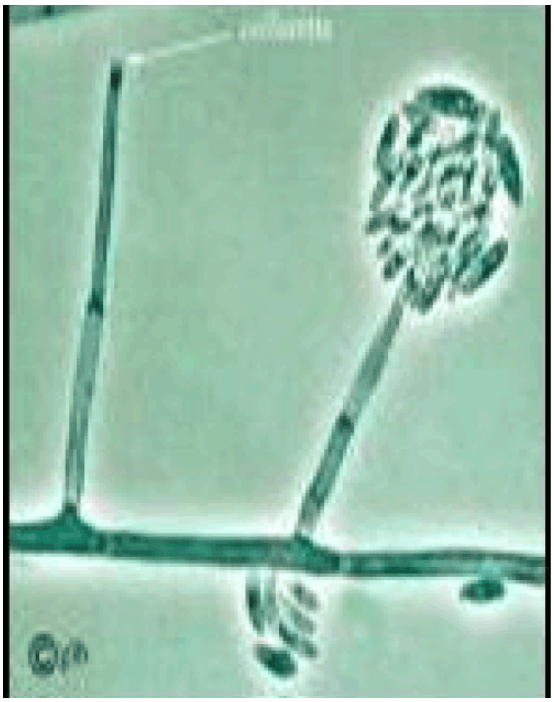

Figure 3: Fusarium

Figure 4: Syncephalastrum

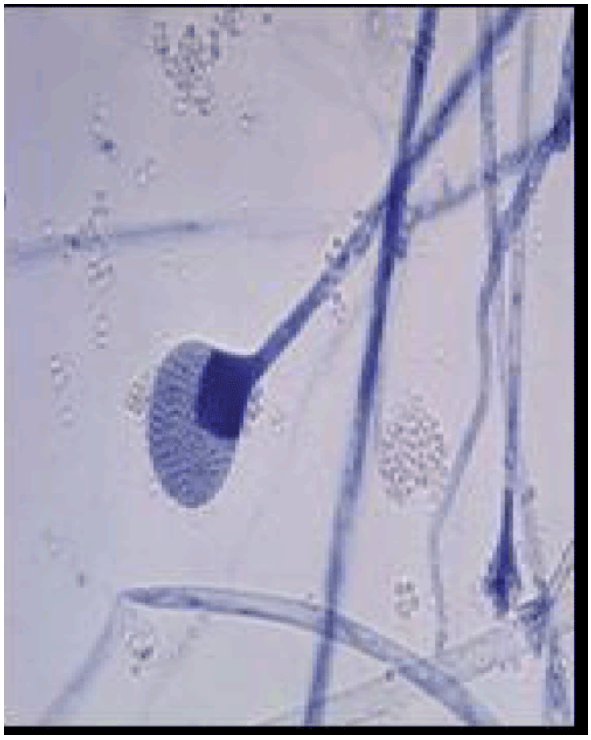

Figure 5: Aspergilus

Estimation of keratin degradation

Filtrates were collected in an interval of 5 days starting from the 10th day of incubation. Filtrate was collected by filtering the incubated media by arranging 2-3 Wattman filter paper on one above another. This filtrate does not contain any feathers, fungal culture and mineral media (Table 1). Change in pH was measured by using digital pH meter with a glass electrode. Determination of nitrate release (NO_3) was done by the method of Goldsmith [17]. Determination of cysteine and cystine was done by the method of Ramakrishna [18]. Determination of methionine was done by the method of Timothy [19].

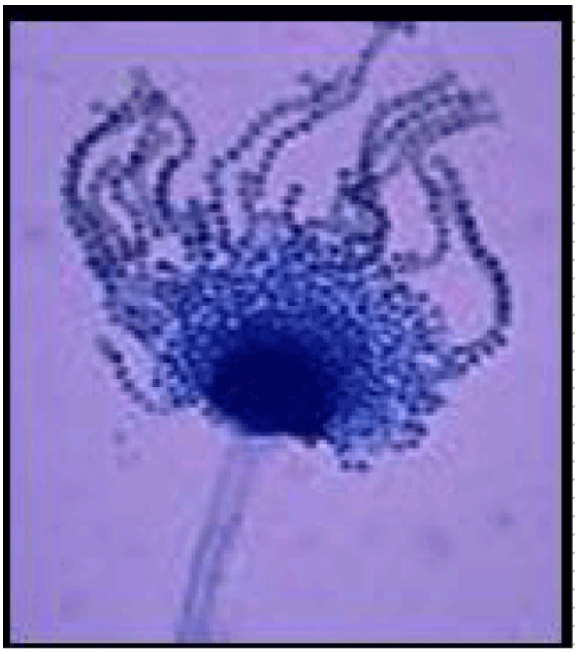

Figure 6: Mucor

Filtrate	Microorganism
Filtrate 1	*Trichoderma*
Filtrate 2	*Gliocladium*
Filtrate 3	*Fusarium*
Filtrate 4	*Syncephalastrum*
Filtrate 5	*Aspergillus*
Filtrate 6	*Mucor*

Table 1: No. of filtrates

Result and Discussion

Increase in pH

During the process of biodegradation there was a gradual increase of pH in to the alkaline phase for feathers till 25 day incubation. However, the pH increases from 10th day, 20th day for 1st to 4th culture and increases continuously from 10th to 25th day in case of 5th and 6th

culture (Figure 7). Such an alkalinisation of the medium may be due to excretion of excess nitrogen via deamination and ammonium excretion. Keratin degradation involves rupturing the disulphide linkage between the peptide chain of keratin molecules by some extra and intra cellular enzymes collectively called keratinise.

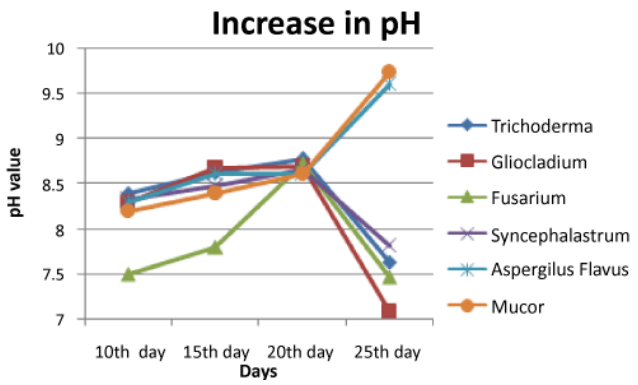

Figure 7: Increase in pH

Increase in Nitrate

The liberation of nitrogenous compound gradually increases from 10th day to 20th day for 1st to 4th culture and increases continuously from 10th day to 25th day in case of 5th and 6th culture (Figure 8).

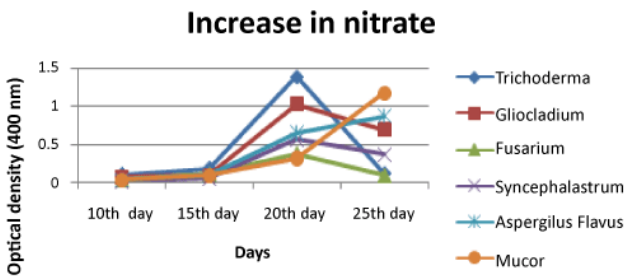

Figure 8: Increase in Nitrate

Increase in Methionine

Methionine release in the filtrate increases from 10th to 20th day in 1st to 5th culture and increases continuously from 10th to 25th day way in case of 6th culture (Figure 9).

Increase in Cystine

Accumulation of cystine may be direct reduction of disulphide bridges of keratin [20]. Chicken feathers, a type of eukeratin which connected histidine, lysine, and arginine in a definite proportion of 1:4:12 and 3-5% of sulphur nearly all of which is in the form of cystine [21]. In filtrate 1, 2, 3 and 6 cystine release increases from 10th day to 25th day whereas increases continuously in 4th and 5th culture (Figure 10).

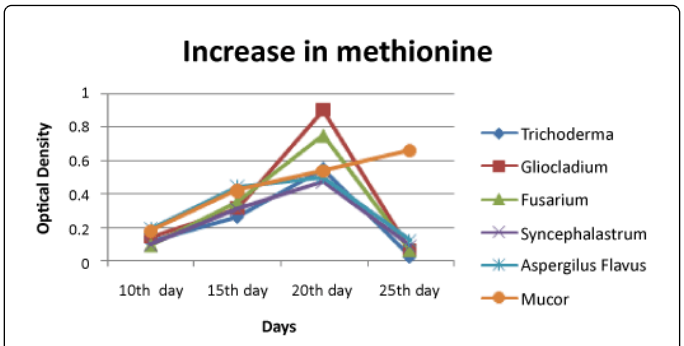

Figure 9: Increase in Methionine

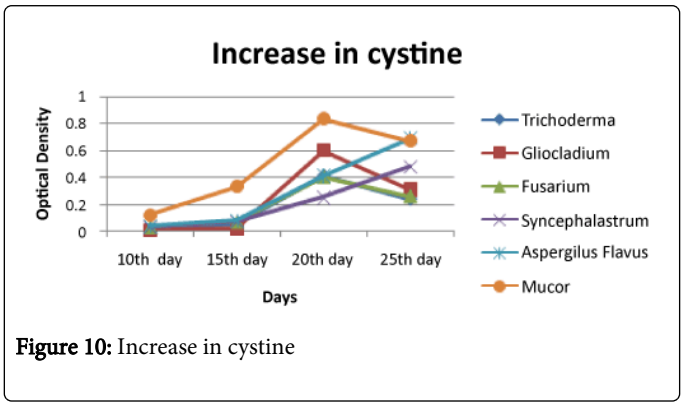

Figure 10: Increase in cystine

Increase in Cysteine

The release of sulphydryl compounds namely, cysteine release increases from 10th day to 20th day and decreases in 25th day in all the culture except in 5th culture in which cysteine release increases continuously (Figure 11). During the process of degradation the -S-sulfo groups (-S.SO3H) and Sulfhydryl groups were formed by Sulfitolysis [22].

The fungus degrades this highly resistant Keratin of feather. With an increasing world-wide concern for the environment it is possible to use these 6 fungi for the degradation of enormous quantity of waste feathers. Biodegradation leads to recycle the wastes and thus maintaining the environmental quantity of the biosphere.

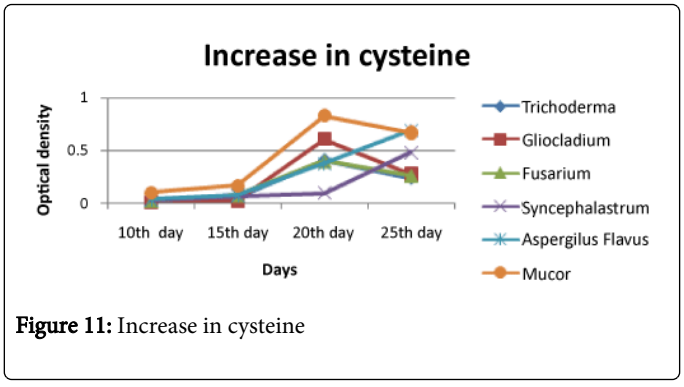

Figure 11: Increase in cysteine

Current and future use

Wastage of protein-rich reserve is ultimately converted into feather meal using keratinolytic fungi [23,24]. The addition of microbial digested feather meal to the animal feed improves digestibility and bolstered growth of poultry. Nutritional enhancement can also be achieved by hydrolysis of raw feathers using these keratinolytic fungi. Microbial-digested feather meal is also used as slow nitrogen releasing fertilizer. Keratinophilic fungi are used for the production of biodegradable films, coatings and glue from keratinous waste. Keratinases of these fungi are utilized in enzyme-based detergents which are used in the removal of keratinous soils, common in the laundry, on collars of shirts, etc. These enzymes are also used for cleaning up of drains clogged with keratin waste. These keratinases are also employed in the leather industry in hair saving dehairing in place of chemical based dehairing. Recently, these keratinases have been found to degrade prion protein leading to the prevention/cure of mad cow disease [25]. Further, keratinases are applied in the modification of silk and wool fibers, for acne or psoriasis, for making vaccines of dermatophytosis and has addictives in skin-lightening agents. In addition to the keratinases, these fungi have the potential to generate natural gas for fuel from poultry-waste degradation.

Waste material, hen feather, a bio sorbent, was successfully utilized in removing a water-soluble hazardous triphenyl methane dye, Brilliant Blue FCF from wastewater. Chicken feathers could help save trees by taking the place of wood pulp in air filters, paper products, and other uses, according to chemist Walter Schmidt of the U.S. Agricultural Research Service. Replacing half the wood-pulp content of composite paper with chicken feathers means only half as many trees. Chicken feathers can also to be use for the production of fuel.

References

1. Mabrouk MEM (2008) "Feather degradation by a new keratinolytic Streptomyces sp. MS-2." World Journal of Microbiology and Biotechnology 24: 2331-2338.
2. Han M, Luo W, Gu Q, Yu X (2012) "Isolation and characterization of a keratinolytic protease from a feather-degrading bacterium Pseudomonas aeruginosa C11," African Journal of Microbiology Research 6: 2211-2222.
3. AcdaMN (2010) "Waste chicken feather as reinforcement in cement-bonded composites." Philippine Journal of Science 139: 161-166.
4. Lin X, Lee CG, Casale ES, Shih JCH (1992) "Purification and characterization of a keratinase from a feather-degrading Bacillus licheniformis strain." Applied and Environmental Microbiology 58: 3271-3275.
5. Deivasigamani B, Alagappan KM (2008) "Industrial application of keratinase and soluble proteins from feather keratins." Journal of Environmental Biology 29: 933-936.
6. Mukhopadhyay RP, Chandra AL (1993) "Protease of a keratinolyticstreptomycete to unhair goat skin." Indian Journal of Experimental Biology 31: 557-558.
7. Nam GW, Lee DW, Lee HS (2002) "Native-feather degradation by Fervidobacteriumislandicum AW-1, a newly isolated keratinase-producing thermophilic anaerobe." Archives of Microbiology 178: 538-547.
8. Suneetha V, Lakshmi VV (2005) "Optimisation of parameters for fermentative production of keratinase by feather degrading microorganisms." World Journal of Microbiology and Biotechnology 7: 106-115.
9. Page RM (1950) Observations on keratin digestion by Microsporumgypseum. Mycopathologia 42: 591-602.
10. Weary PE, Canby CM, Cowley EP (1965) Keratinolytic activity of Microsporumcanis and Microsporumgypseum. J Invest Derma 44: 300-310.
11. Kunert J (1989) Biochemical mechanism of keratin degradation by the Actinomycete Streptomyces fradiae and the fungus Microsporumgypseum: A comparison. J Basic Microbiol 29: 597-604.
12. Sarangi SM, Ghosh GR (1990) Episoochory transmission of keratinophilic fungi by the Eusynancthropic common house sparrow in Orissa. Kavaka 18: 14-18.
13. Ramesh VM (1996) Studies on keratinophilic fungi characterization of keratinolytic, potential and fungitoxic evaluation of some plant extracts. Ph.D. thesis, University of Madras.
14. Kushwaha RKS (2000) In Biology of Dermatophytes and other Keratinophilic Fungi (edsKushwaha, R. K. S. and Guarro, J.), Bilbao, RevistaIberoamericana de Micol 86-92.
15. Currah RS (1985) Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Myxotrichaceae and Onygenaceae. Mycotaxon, 1985, 24, 1-216.
16. Day WC, Tonic P, Stratman SL, Leeman U, Harman SR (1968) Isolation and properties of an extracellular protease of Trichophytongranulosum. biochim BiophysActa 167: 597-606.
17. Goldsmith J, Livoni P, Noaberg CL, Segal IH (1973) Regulation of nitrate uptake in Penicilliumchrysogenum by ammonium ion. Plant physiology 52: 362-367.
18. Ramakrishna R, Siraj P, PrakasaSastry CS (1979) Spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Current Science 48: 815-816.
19. Timothy E McCarthy, Sullivan MX (1941) A New and highly specific colorimetric test for methionine. J BiolChem 141: 871-876.
20. Noval JJ, Nickerson WJ (1959) Decomposition of native keratin by Streptomyces fradiae. J Bacteriol 77: 251-263.
21. Singh CJ, Geetha Singh B, Sundara Singh B (1996) Biodegradation of some keratin substrates invitro by some keratinophilic fungi. Advances in Plant Sciences 8: 271-276.
22. Kunert J (1976) Keratin decomposition by dermatophytes. II. Presence of S- sulfocysteine and cysteic acid in soluble decomposition products. Z AllgMikrobiol 16: 97-105.
23. Shih JCH (1993) Recent Development in Poultry Waste Dizgestion and Feather Utilization—A Review. Poultry Science 72: 1617-1620.
24. Bertsch A, Colleo N (2005) A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. 96: 1703-1708.
25. Wang JJ, Borwornpinyo R, Odetallah N, Shih JCH (2005) Enzymatic degradation of a prion-like protein, Sup35NM-His6. 36: 758-765.