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ISOLATION KERATENOLYTIC BACTERIA FROM FEATHER DUMPING SITES OF SATANA

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ABSTRACT

The research work is carried out to find solution for natural degradation of feathers and other poultry waste by the use of suitable microorganisms. Soil sample is collected from the feather dumping sites of various poultry farms located in Satana and its periphery. Soil sample is enriched in minimal medium containing keratin and feathers as a sole source of keratin on a rotary shaker for 15 days at room temprature. Organism was isolated from this broth by cultivating on minimal agar medium containing keratin as a sole source. Degradation study was carried out by using these characterized isolates by shake flask culture method.

Keywords: Keratin, Keratinase, Enrichment, Isolation, Degradation

INTRODUCTION:

Poultry farming is the raising of domesticated birds such as chickens, ducks, turkeys and geese, for the purpose of farming meat or eggs for food. Poultry are farmed in great numbers with chickens being the most numerous. More than 50 billion chickens are raised annually as a source of food, for both their meat and their eggs. According to the World Watch Institute, 74 percent of the world's poultry meat, and 68 percent of eggs are produced in ways that are described as 'intensive'. Worldwide poultry processing plant produced millions of tons of feathers as well as waste products annually, which consist of approximately 10% keratin. Feather represents 5-7% of total weight of mature chicken.
Feathers are the main waste byproduct of poultry processing industry and gradually increase day by day.. The United States and the European countries mainly the United Kingdom generate about respectively 4 billion pounds and 150,000 tons of feather waste per annum respectively. The poultry waste generated from processing industries in India is 350 million tons per year.
The keratin included in chicken feathers is a very inconvenient and troublesome waste product of the poultry farming industry. These poultry feathers are dumped, used for land filling, incinerated or buried, which involves problems in storage, handling, emissions control and ash disposal. Discarded feather also causes various human ailments including chlorosis, mycoplasmosis and fowl cholera etc. Also the feather flour obtained after baking and milling of feathers is the carrier of the unconventional infectious agent, the prion protein, the causative agent of a group of diseases called transmissible spongiform encephalopathies (TSE) that include mad cow disease, scrapie, kuru and Creutzfeld- Jakob disease .
Different approaches have been used for disposing of feather waste including land filling, burning, natural gas production and treatment of animal feed. Most feather waste is landfilled or got burned which involves expense and can cause contamination of air, soil, water.Feather is composed of over 90% (w/w) protein, the main component being β- keratin, a fibrous and insoluble protein highly cross- linked with disulfide bonds. Considering its high protein content, this waste could have a great potential as a source of food and energy.
Keratin is most abundant protein in epithelial cell of vertebrates and represent the major constituent of skin and its appendages such as nails, hairs, feathers and wool. The protein chain are packed tightly either in Alfa helix (Alfa keratin) or in β shape (β- keratin structure)

Keratins are grouped into hard keratin feather, hair, wool and nails and soft keratin (skin and callus). According to one main characteristics of keratin is that they have high mechanical stability and resistant to proteolytic degradation which depends on the di-sulphate and hydrogen bond. Salt linkage and other cross linking keratin material is water insoluble and extremely resistant to egradation by proteol.

Keratin is an insoluble protein and has a stable structure. The mechanical stability of keratin and its resistance to biochemical degradation depend on tight packing of the protein chains in alpha -helix (a-keratin) or b-sheet (b-keratin) structures and linkage of these structures by disulfide bonds. Several keratinophillic fungi are present as common inhabitant of the feathers of different animals.Keratin are divided into two types. α-Keratin: It present in wool, hair, and horn. It is in the form of folded chain. β-Keratin: It present in feather in the form of polypeptide chain.

The production of keratinases has been a domain of saprophytic and dermatophytic fungi,actinomycetes and some *Bacillus species*. Many of the current studies are focusing on potential use of keratinases of bacterial origin for the industrial treatment of keratin containing wool, hair, and horn. It is in the form of folded chain. B-Keratin. Keratinolytic enzymes have found important utilities in biotechnological processes involving keratin-containing wastes from poultry and leather industries, through the development of non-polluting processes.

MATERIALS AND METHODS:

Collection of sample: The feather, hair and soil sample, was collected from feathers dumping site at Satana, Soil, feather, and hair samples were also collected from poultry farm waste, municipal land filling sides and from saloons. Soil sample was collected using sterile scalpel at 4cm depth and transfer to sterile polyethen bag.

Enrichment and isolation: Pre washed feathers and hairs and soil sample were inoculated in to Davis's minimal broth containing feather and hair extracts, Dextrose 1.0 gm, Diapotassium Phosphate 7.0gm, Monopotassium Phosphate 2.0 gm, Sodium Citrate 0.5 gm, Magnesium Sulfate 1.0 gm, Agar 15.0 , distilled water 1 liter, P^H7.0

Media were incubated in orbital shaker incubator at room temperature for 7 to 8 days. Loop full cultures were streaked on Davis Minimal agar containing Skimmed milk, feathers and hairs. Plates were incubated at room temperature for 8 days. Colonies producing keratinizes identified by zone of clearance around it. Isolated colonies were picked up and preserved on minimal agar slant containing keratin after incubation.

Checking efficiency of degradation: For this five tubes containing 25 ml of Davis minimal broth with washed feathers were added as carbon and nitrogen sources. In this 2 ml of 24 hours old culture of screened isolate was added. Flasks were incubated on rotary shaker at 30^{0}c.At the end of every third day incubated broth was filtered through Whatman filterpaper, filtrate was dried in hot air oven at 50 degree till to get constant weight.(It was read by digital weighing balance) and bacterial growth was estimated by taking 5 ml aliquot of same broth centrifuged at 3000 rpm for 20 min pellet was dried in oven and dry weight was measured same as above.

RESULTS:

Screenig and isolation: After being incubated up to 4 days, a plate showed the growth of several colonies. The strains produced clearing zone is characterized by proteolytic activity. Only four isolates designated as kb1, kb2, kb3, kb4 were proteolytic activity. The largest clearing zone was observed for isolate kb1. This strain was selected and used for keratinolytic activity assay.

Fig1 ; Isolated colonies showing zone of clearance around them on skimmed milk agar plate after incubation

Degradation studies:

Observations Table1: Dry weight measurement

Time of Incubation (days)	Dry weight of feathers (gms)	Dry weight of biomass(gms)
3 4.	8. 0	0.02
6	4.4	0.02
9	4.2	0.04
12	3.9	0.08
15	3.8	0.15
18	3.7	0.3
21	3.6	0.34
24	3.5	0.59
27	3.4	0.59
30	3.4	0.59

Fig 2 : Graph showing reduction in dry weight and increase in biomass with respect to time of incubation

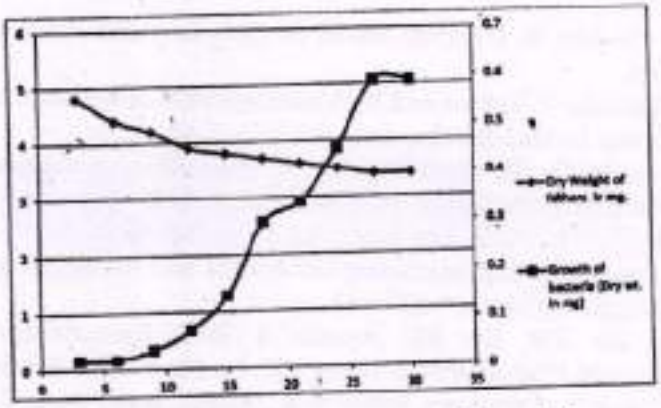

Fig 3 : Set up Before the start of degradation

Fig 4 : set up of Degradation on 30th day

DISCUSSION:

Animal feed typically includes a carbohydrate source and a protein source. Common protein sources use in animal feed include different vegetable proteins from corn meal, soybean meal, and from anim sources such as fish meal, meat and poultry by products. But these are very expensive ,animal protein difficult to digest. Feather waste too is high in protein and very inexpensive, but cannot be used direct in animal feed, as it is difficult for animals to digest. Typical treatments to form feather meal a expensive. These treatments also tend to destroy some amino acids, which are heat-sensitive amino acid This lowers the quality of the protein in the feed. Due to these problems, feather meal is not extensively used in feed, despite the expense of other sources of dietary protein. It is reported that keratinolyt bacteria can degrade feathers. We attempted to isolate feather degrading bacteria from soil samples which were collected from dumping site of poultry wastes. Micro-organisms who have shown keratinolyt activity in the form of zone was further maintained in skimmed milk agar. Degradation studies showe that there is almost complete degradation of feathers up to thirty days.

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REFERENCES:

Alexander, P. and Hudson, R. F.(1954). *Wool: Its Chemistry and Physics*, Chapter 5. London: Chapman and Hall Ltd.

Arai, K. M., R.Takahashi, Y.Yokote and K. Akahane,(1983). *Aminoacid sequence of feather keratin from fowl*. European Journal Biochemistry, 32, 501–510.

Allpress JD, Mountain G, Gowland PC (2002) Production, purification and characterization of an extracellular keratinase from *Lysobacter NCIMB 9497*. Lett Appl Microbiol. 34:337-342.

Anbu P, Gopinath SCB, Hilda A, Lakshmipriya T, Annadurai G (2007) Purification of keratinase from poultry farm isolate *Scopulariopsis brevicaulis* and statistical optimization of enzyme activity. Enzyme Microb Technol. 36:639-647.

Anbu P, Hilda A, Sur HW, Hur BK, Jayanthi S (2008) Extracellular keratinase from Trichophyton sp.HA-2 isolated from feather dumping soil. Int Biodeterior Biodegrad 62:287-292.

Bach Evelise, Fabiana S. Cannavan, Fábio R.S. Duarte, Jaslin A.S. Taffarel, Siu Mui Tsai, Adriano Brandelli (2011) Characterization of feather-degrading bacteria from Brazilian soils, Bioresource Technology. 65: 102–107.

Balaji S, Kumar MS, Karthikeyan R, Kumar R, Kirubanandan S, Sridhar R, Sehgal PK (2008) Purification and characterization of an extracellular keratinase from a hornmeal-degrading *Bacillus subtilis MTCC (9102)*. World J Microbiol Biotechnol. 24(11):2741-2745.

Bernal C, Cairo J, Coello N (2006) Purification and characterization of a novel exocellular keratinase from *Kocuria rosea*. Enzyme Microbial Technol. 38(1-2):49-54. Blyskal B (2009) Fungi utilizing keratinous substrates. Int Biodeterior Biodegrad 63:631-653.

Bockle B, Muller R (1997) Reduction of disulfide bonds by *Streptomyces pactum* during growth on chicken feathers. Applied and Environmental Microbiology. 63: 790–792.

Brandelli A (2005) Hydrolysis of native proteins by a keratinolytic strain of *Chryseobacterium sp.* Ann Microbiol 55:47-50.

Brandelli A (2010) Bacterial keratinases: useful enzymes for bio processing agroindustrial wastes and beyond. Food Bioprocess Technol. 1:105-116.

Brandelli A, Daroit DJ, Riffel A (2008) Biochemical features of microbial keratinases and their production and applications, Appl Microbiol Biotechnol. 85(6), 1735-50.

Cappuccino JG, Sherman N (1996) Microbiology A laboratory manual, Addison- Wesley, 4thedn, England.

Chao YP, Xie FH, Yang J, Lu JH, Qian SJ (2007) Screening for a new *Streptomyces* strain capable of efficient keratin degradation, J Environ Sci 19:1125-1128.

Cheng SW, Hu HM, Shen SW, Takagi H, Asano M, Tsai YC (1995) Production and characterization of keratinase of a feather degrading *Bacillus licheniformis PWD-1*. Biosci Biotechnol Biochem. 59:2239-2243.

Chitte RR, Nalawade VK, Dey S (1999) Keratinolytic activity from the broth of a feather-degrading thermophilic *Streptomyces thermoviolaceus* strain SD8. Lett Appl Microbiol 28:131-136.

El-Naghy MA, El-Ktatny MS, Fadl-Allah EM, Nazeer WW (1998) Degradation of chicken feathers by *Chrysosporium georgiae*. Mycopathologia. 143:77-84

Ghosh A, Chakrabarti K, Chattopadhyay D (2008) Degradation of raw feather by a novel high molecular weight extracellular protease from newly isolated *Bacillus cereus DCUW*. J Ind Microbiol Biotech. 35:825-834.

Jain Richa, Prakash Chandra Jain, Shyama Charan Agrawal (2011) Feather degradation by *Streptomyces exfoliatus CFS* 1068. Ann Microbiol. DOI 10.1007/s13213-011-0336. 236

Kim JM, Choi YM, Suh HJ (2005) Preparation of feather digests as fertilizer with *Bacillus pumilis KHS-1*. J Microbiol Biotechnol. 15:472-476.

Mazotto Ana Maria, Ana Cristina N. de Melo, Andrew Macrae, Alexandre Soares Rosado, Raquel Peixoto, Sabrina M. L. Cedrola , Sonia Couri, Russolina B. Zingali , Ana Lu´cia V. Villa, Leon Rabinovitch , Jeane Q. Chaves , Alane B. Vermelho (2011) Biodegradation of feather waste by extracellular keratinases and gelatinases from *Bacillus spp*. World J Microbiol Biotechnol.27:1355–1365

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