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Isolation and Screening of Keratinase Producing Bacteria From Chicken Feather Dumping site

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Abstract : The aim of this study was to isolate and screen keratinase producing bacteria from chicken feather dumping sites. Six different feather dumping soil samples, were collected from Aurangabad, Maharashtra area for this study. These six different soil samples were serially diluted and plated for screening of efficient protease producing microorganism. The zone of protease activity of the isolates determined on Skim Milk Agar medium (Himedia M-763) varied .Among them, one strain was produced higher level of zone of clearance by its protease production. Based on morphology and biochemical analysis the isolate were identified as Bacillus sp. Fermentation using feather as a substrate was carried out on minimal salt media for 4 days which resulted in almost complete degradation of feather. The optimum conditions for keratinase production were temperature 37°C, pH 7.4 and initial substrate concentration 1%.
Keywords : Chicken feather, Keratin, Keratinase, Bacillus.

Introduction:-

Keratin forms a major component of the epidermis and its appendages viz. hair, feathers , nails, horns, hoofs, scales and wool. On the basis of secondary structural confirmation , keratins have been classified into α (α -helix of hair and wool) and β (β-helix of feather) [1]. The consumption of chicken leaves behind tons of feathers as a waste. Worldwide, around 18,500 lakh tons of poultry feather is generated annually, of which India's contribution alone is 3500 tons.[2] In its native state, the feather keratin is insoluble and not degradable by commonly known proteolytic enzymes such as trypsin, pepsin and papain not only because of their tight secondary structure but also because the peptide chains are held together by disulfide linkages (Williams et al., 1990; Kim et al., 2001; Schrooyen et al., 2001).[3-5] Nonetheless, feathers do not accumulate in nature and can be degraded efficiently by myriad of microorganisms due to the action of keratinolytic proteases – keratinases (Onifade et al., 1998) .[6]Keratinase is an extracellular enzyme used for the biodegradation of keratin. Keratinase is produced only in the presence of keratin substrate. Keratinase attacks the disulfide bond of keratin to degrade it. Some microbes have been reported to produce keratinase in the presence of keratin substrate. Keratinase attacks the disulfide bond of keratin to degrade it. Some microbes have been reported to produce keratinase in the presence of keratin substrate. Keratinase producing microorganisms have ability to degrade chicken feathers, hairs, nails, wool etc. (Gradisar et al 2005, Cai et al 2008)[7-8] Keratinases (E.C. 3.4.21/24/99) are a particular class of proteolytic enzymes that display the capability of degrading insoluble keratin substrates[9].

Keratinases which are produced by these keratinolytic organisms could be used to degrade feather waste and further the digested product could be excellent material for producing animal feed, fertilizers or natural gas (Tamilmani et al., 2008)[10]. Till date most of purified keratinases known cannot completely solubilize native keratin, their exact nature and uniqueness for keratinolysis is still not clear [11]. Biodegradation of poultry waste by keratinases is an environment friendly biotechnological process, which converts this abundant waste into low cost, nutrient rich animal feed.[12-13] keratinolytic enzymes have applications in the detergent, medical, cosmetic and leather industries; they can be used in prion degradation and as pesticides.[14-16]

Materials and Methods:-

Sample Collection :-

The soil samples were collected from the various feather dumping sites of Aurangabad , Maharashtra , India. Soil samples were collected from 3 to 4 cm depth and transferred in sterile plastic bags by sterile spatula. The samples were brought to Microbiology research laboratory for further processing. See table-1:

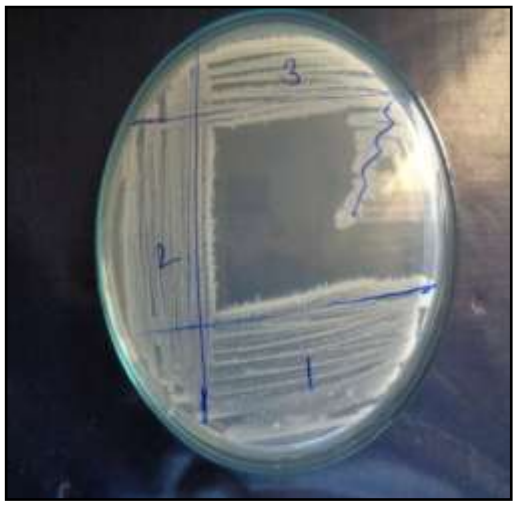
Table-1 :Tabulation for Samples Description

Sr.no.	**Sampling Sites(Feather Dumping Sites)**	**Sampling Sites GPS Locations.**
1	Apna chicken shop, Durgah Aurangabad.	N19°51.601’ E075°19.861’
2	Harsul T point, Aurangabad.	N19°55.174’ E075°21.15’
3	Near chitegaon village paithan road Aurangabad.	N19°45.940’ E075°17.356’
4	Near jakat plaza paithan road Aurangabad.	N19°48.296’ E075°17.328’
5	Near Waluj nagar road, Aurangabad.	N19°47.998’ E075°13.694’
6	Chiken shop near railway Station Aurangabad.	N19°51.742’ E075°18.786’

Tabulation for sample description.

Isolation of Bacteria:-

Isolation of bacteria was performed by serial dilution and plating method on Nutrient agar medium. One gram of soil sample was transferred in 10 ml of sterilized distilled water and mixed properly. Serial dilution was made upto 10^{-6} . 0.1 ml of the diluted sample was inoculated in the nutrient agar medium plates from each dilution. The petriplates were rotated clockwise and anticlockwise to spread the sample uniformly. Plates were incubated at 37°C for 24-48 hours. The bacterial isolates were further subcultured on nutrient agar medium to obtain pure culture . Pure culture of isolates were maintained in nutrient agar media slants at 4°C for further studies. See figure 1 for bacteria pure culture.



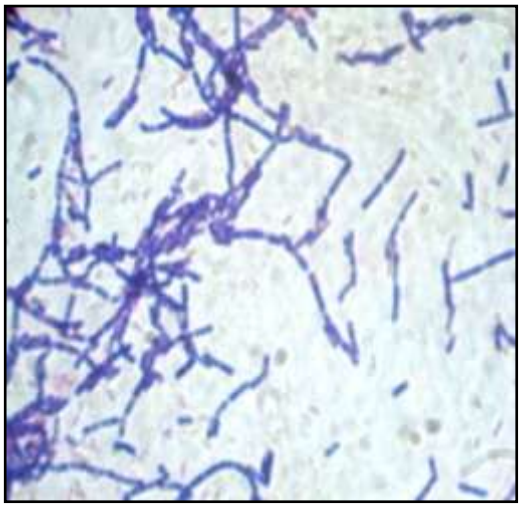


Fig. 1 Pure culture of isolate **Fig. 2 Gram's staining**

Primary Screening of Keratinolytic Bacteria:-

Skim Milk Agar medium (Himedia M 763) was sterilized at 121°C for 15 min. at 15 lbs pressure. The isolates were streaked on the medium. The zone formed around the colonies due to production of protease enzyme was considered as positive result. The organisms screened with skim milk agar medium were subcultured by growing the bacterium in nutrient broth medium at 37°C for 24 hours. See figure 3.

Surface Sterilization of Feathers:-

The feathers were cut with scissors into small pieces of 3-4 cm long & washed several times with tap water. Defatting of feather pieces was done by soaking them in a mixture of chloroform : Methanol (1:1) for 2 days. Followed by Chloroform : acetone: methanol (4:1:3) for 2 days. The solvent was replaced every day. The feathers were finally washed several times with tap water, to eliminate solvent residual.

Secondary Screening of Keratinolytic Bacteria :-

The selected protease producing bacteria were subsequently grown in feather consisted of (g/L^{-1}), NaCl- 0.5 ; $MgCl_2.6H_2O$-0.1 ; $CaCl_2$-0.06 ; KH_2PO_4 -0.7 ; K_2HPO_4 -1.4 ; and feather- 1 g ; pH of the medium adjusted to 7.5 (using 1 N NaOH) in which feather were the only sources of carbon and nitrogen (Lin et al;1995). Cultures were grown at 37°C at 120 rpm for 96 hrs. Keratinolytic strain that completely broke down feathers in the medium selected for further study. SeeFig. 4

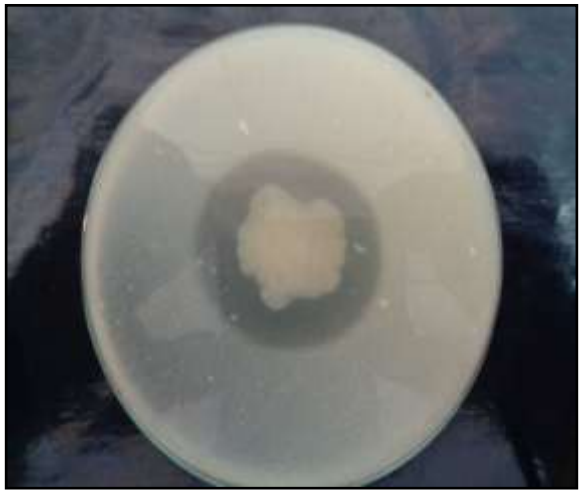


Fig. 3 Production of zone of clearence in Skim milk agar media by Proteolytic Bacteria

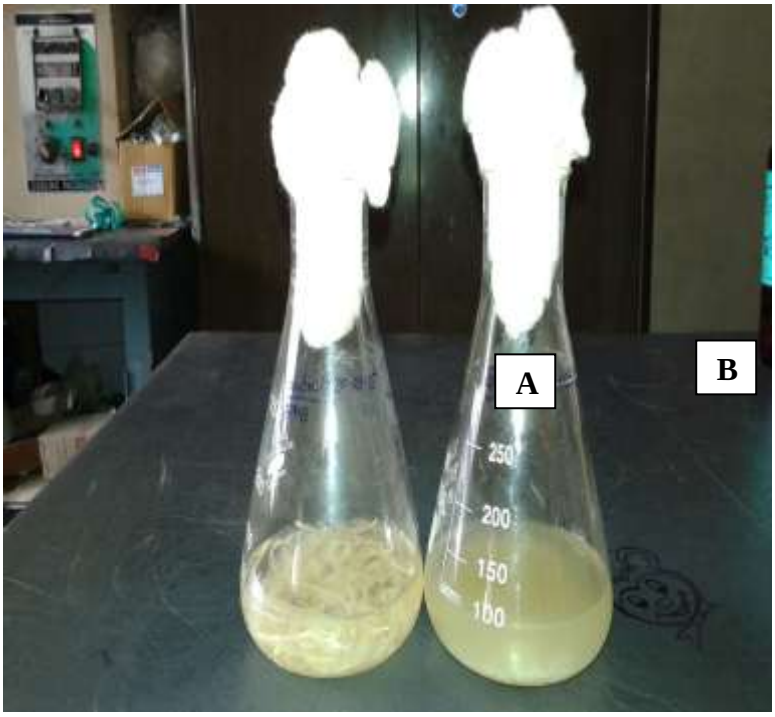


Fig. 4 Degradation of chicken feathers by the bacterial strain isolated from soils of Aurangabad feather dumping sites , in submerged cultivation at 37°C . A) feather control without the bacterial strain. B) Feather after 96 hrs of incubation with the bacterial strain showed complete degradation.

Characterisation and Identification of Keratinolytic Bacteria:-

Culture Characterization :-

The isolates were observed under the microscope , the colony morphology was noted with respect to colour, shape, size, nature of colony and pigmentation[17]. See table-2: tabulation for results of morphological and cultural characteristics of isolate on Nutrient agar media**.**

Table-2 : Tabulation for results of morphological and cultural characteristics of isolate on Nutrient agar media :-

Colony Characters	Result
Shape	Round
Size	2mm
Colour	Creamy
Margin	Entire
Opacity	Opaque
Elevation	Convex
Consistency	Butyrous
Gram Character	Gram positive rod
Growth	Rapid
Motility	Motile
Endospore stain	Spores present

Microscopic Observation:-

Bacterial isolates were Gram stained and observed under a high power magnifying lens in light microscope. Endospore staining and motility tests were performed to observe the morphology and motility of the cells. See fig. 2 for Gram's stain results.

Biochemical Characterization:-

The bacterial isolates were characterized biochemically by Indole test, Methyl red test, Vogus proskaur's test , Citrate utilization test and Carbohydrates test . See table 3 : tabulation for results of various biochemical tests of bacteria.

Table 3 – Tabulation for results of various biochemical tests of bacteria

Biochemical Test	**Results**
Indole	Negative
Methyl Red	Negative
Vogus Proskaur's	Negative
Citrate utilization	Negative
Glucose	Positive
Adonitol	Negative
Arabinose	Negative
Lactose	Positive
Sorbitol	Positive
Mannitol	Positive
Rhamnose	Negative
Sucrose	Positive
Oxidase	Positive
Nitrate reduction	Negative
Casein hydrolysis test	Positive
Starch hydrolysis test	Positive

Result and Discussion:-

Soil samples were collected from six different feather dumping sites (Table:1) . Bacteria were isolated by serial dilution method. About 37 bacterial colonies were isolated , which were streaked on Skim Milk Agar plates for confirmation of protease activity. Among this 17 bacterial strains showed protease activity by zone of clearence. Single bacterial strain , which was showing maximum zone of clearance was selected for further study of Keratinase activity. Bacterial identification was conducted on morphological, physiological and biochemical tests. Results were compared with Bergey's Manual of Determinative Bacteriology, 8th edition (Buchanan and Gibbons , 1974).These results suggested that the strain belongs to Bacillus sp.

Conclusion:-

In this study we isolated the *Bacillus* sp. capable of producing keratinase from habitats of feather dumping sites. The search for promising strain of Keratinase producers is a continuous process . The isolate which shows the higher Keratinase activity was selected for biochemical characterization and identification. The organism was identified as *Bacillus* sp. On the basis of data obtained in present work it can be concluded that *Bacillus* sp. can be employed in the production of Keratinase. The degradation of feathers with Keratinolytic bacteria is the best ecofriendly approach in the poultry feather waste management.

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References:-

1. Akhtar, W. and Edwards H.G.M., Fourier-transform Raman spectroscopy of mammalian and avian keratotic biopolymers.SpectrochimActa .,1997, 53: 81-90.
2. Onifade AA, A1-Sane NA, Al-Musallam AA, Al-Zarban S. Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Biores Technol., 1998, 66(1):1–11.
3. Williams CM, Richter CS, Mackenzie JM, Shih JCH , Isolation, identification, and characterization of a feather-degrading bacterium. Appl. Environ. Microbiol.1990, 56:1509-1515.
4. Kim JM, Lim WJ, Suh HJ . Feather-degrading *Bacillus* species from poultry wastes. Process Biochem., 2001., 37:287-291.
5. Schrooyen PMM, Dijkstra PJ, Oberthur RC, Bantjes A, Feijen J. Partially carboxymethylated feather keratins. 2. Thermal and mechanical properties of films. J. Agric. Food Chem. 2001.,49:221-230.
6. Onifade AA, Al-Sane NA, Al-Musallam AA, Al-Zarban S . A review: potentials for biotechnological application of keratin-degra-ding microorganisms and their enzymes for nutritional improvement of feather and other keratins as livestock feed resources. Bioresour. Technol.,1998, 66:1-11.
7. Gradisar, J. Friedrich, I. Krizaj and R. Jerala, *Applied Environmental Microbiology. 2005.*,71. 3420 - 3426.
8. Cai, B. Lou and X Zheng, J. Zhejiang . *University Science* B 2008.,9, 60.
9. Langeveld JPM, Wang JJ, Van De Wiel, Shih DFM, Garssen C, Bosser A, Shih JCH, 'Enzymatic degradation of prion protein in brain stem from infected cattle and sheep', J.Infect. Dis., 2003,188, (11), 1782-1789.
10. Tamilmani P, Umamaheswari A, Vinayagam A and Prakash B., Production of an extracellular feather degrading enzyme by *Bacillus licheniformis* isolated from poultry farm soil in Namakkal district (Tamilnadu), International journal of poultry Science,2008, 7 (2) :184-188.
11. Ramnani P, Singh R and Gupta R, Keratinolytic potential of Bacillus licheniformis RG1: structural and biochemical mechanism of feather degradation. Can J Microbiol, 2005, 51:191-196.
12. Ramnani P, Singh R and Gupta R, Keratinolytic potential of Bacillus licheniformis RG1: structural and biochemical mechanism of feather degradation. Can J Microbiol 2005, 51:191-196.
13. A. A. Khardenavis, A. Kapley, and H. J. Purohit, "Processing of poultry feathers by alkaline keratin hydrolyzing enzyme from *Serratia* sp. HPC 1383," *Waste Management*, 2009,vol. 29, no. 4, pp. 1409–1415.
14. A. M. Mazotto, A. C. N. de Melo, A. Macrae et al., "Biodegradation of feather waste by extracellular keratinases and gelatinases from *Bacillus* spp," *World Journal of Microbiology and Biotechnology*,2010, vol. 27, no. 6, pp. 1355–1365.
15. S. M¨uller-Hellwig, M. H. Groschup, R. Pichner et al.,"Biochemical evidence for the proteolytic degradation of infectious prion protein PrPsc in hamster brain homogenates by food borne bacteria," *Systematic and Applied Microbiology*, 2006,vol. 29, no. 2, pp. 165–171.
16. X. Y. Yue, B. Zhang, D. D. Jiang, Y. J. Liu, and T. G. Niu, "Separation and purification of a keratinase as pesticide against root-knot nematodes," *World Journal of Microbiology and Biotechnology*. In press.
17. J. G. Cappuccino and N. Sherman, Microbiology: A Laboratory Manual (Benjamin-cummings Publishing Company, 1996,New York.

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