ISOLATION, IDENTIFICATION AND CHARACTERISATION OF KERATIN DEGRADING MICROORGANISMS FROM POULTRY FARM SOIL AND THEIR FEATHER DEGRADATION POTENTIAL

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INTRODUCTION

The day by day increase in consumption of meat received from chicken is causing harsh effect to environment, as the waste from the chicken birds, more particularly the feather are not properly treated. While in nature, the deterioration of feather is slow, generating sulfurous compounds, causing environmental problem. Feathers, which are almost pure keratin proteins, are produced in large amounts and constitute a waste by product at poultry processing plant. Feathers are made up primarily of keratin which is resistance to common proteolytic enzyme such as pepsin, trypsin and papain. World-wide poultry processing plants produce millions of tons of feathers as a waste product annually, which consists of approximately 90% keratin; the keratin is largely responsible for their high degree of recalcitrance if remain untreated. Keratinase are a particular class of proteolytic enzymes that display the capability of degrading insoluble keratin substrates (Brandelli, 2008)[5].

Keratinophilic fungi are generally considered as soil saprophytes. Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophilic fungi. Keratin decomposition in soil leads to an increase in carbon, and nitrogen ratio in soil. They are therefore fast growing nonpathogenic keratinophilic fungi which can be utilized for the recycling of keratin in soil and may be exploited for their biotechnological potential in industry.

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 producing microorganisms have the ability to degrade chicken feather, hair, nails, wool etc. Keratinolytic enzymes are widespread in nature and are produced by several microorganisms including bacteria such as *Bacillus sp*., *Fervidobacterium islandicum* , *Elizabethkingia meningoseptica* KB042 , *Pseudomonas aeruginosaKS1* and Actinomycetes such as *Streptomyces sp*. and fungi such as *Chrysosporium tropicum* , *Trichoderma atroviridae* , *Doratomycesmicrosporus* ; *Paecilomyces arquandii* ; *Scopulariopsis brevicaulis* ; *Alternaria, Paecilomyces, Penicillium, Curvularia* and several *Aspergillus sp.*

Keratinase which are produced by these keratinolytic organisms could be used to degrade feather waste and further the digested products could be an excellent material for producing animal feed, fertilizers or natural gas.

AIM AND OBJECTIVE

Considering the serious issue of releasing sulfurous compounds from poultry waste in to environment, the study was in to environment, the study was conducted with the following objectives. To isolate, identify and characterize the keratin degrading micro organism from poultry farm soil To study their degradation potential.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from regular feather dumping site of poultry farm soil from puramannur, malappuram district. The samples were taken from 30 cm depth from the surface of the soil. The samples were collected in sterile polythene covers and stored in refrigerator until analysis was carried out.

Processing of chicken feathers and Preparation of feather meal broth
Chicken feathers were washed thoroughly with tap water and dried. The dried feathers were defatted by soaking in diethyl ether for 24 hrs, and washed thoroughly with tap water and distilled water, air dried and cut into small pieces. The processed feathers referred as Feather meal. The medium used for keratinase production contained the following constituents;

Isolation and screening of keratinolytic bacteria
Soil suspensions were made with 0.9% saline and inoculated in feather meal broth and incubated on rotary shaker at room temperature. After visible turbidity was observed, serial dilutions of the culture suspensions was conducted.
The isolated bacterial colonies showing zone of clearance one skim milk agar were selected for further studies. The isolates were characterized for colony characteristics, morphological characteristics and biochemical characteristics and identified with Bergey's manual of determinative bacteriology.

MICROSCOPIC EXAMINATION
To differentiate the Gram positive and gram negative nature of the bacterial isolate and also for observing the microscopic morphology the isolate was subjected for Gram staining. Hanging drop method was used to observe the motility character of the pure isolates.

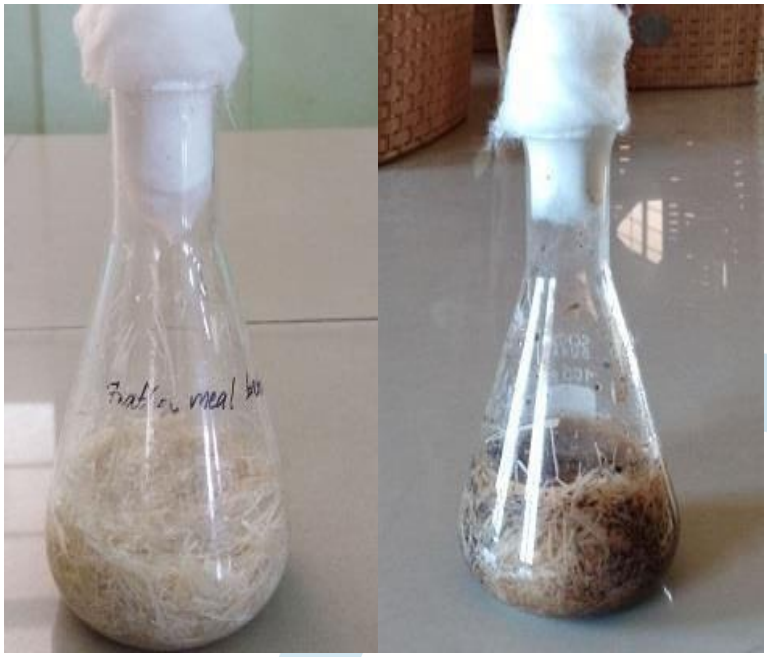
Biochemical And Physiological Examination
The bacterial isolates that exhibited maximum level of resistance against the drugs tested were subjected for identification, using the following routine biochemical and physiological tests.
The tests include Indole, Methyl Red, voges- Proskauer and Citrate (IMViC)tests, Carbohydrate fermentation test, Oxidation tests, Urease tests and Catalase test.

Isolation of keratinolytic fungi
The keratinophilic fungi were isolated using 'hair baiting techniques'. In this technique sterile Petri plates were half filled with soil and short strand of sterilized chicken feathers were spread over the surface of soil. About 10-12 ml sterile water was added to Petri plates for the facilitation of fungal spores to germinate. Petri plates were incubated at 30˚C for 3-4 weeks. After 3-4 weeks the colonies were observed on surface of feathers, were picked up and grown on Potato Dextrose Agar, for purification and identification.

RESULTS

Soil samples were inoculated in feather meal broth to obtain bacterial isolates which are feather degrading and were capable of producing extracellular keratinase, using feather (keratin) as a sole carbon source. After 5-7 days incubation the flasks showed turbidity.
The same culture suspension was serially diluted up to 10^{-6} with distilled water and plated on skim milk agar for selection of keratinolytic bacteria. After enrichment of poultry soil sample, it was found that 3 isolates among 15 exhibited feather degradation activity within 5 days.
From this one colony showing largest zone of clearance are further selected and characterized. The colony and morphological characteristics of the bacterial isolate are shown in Table.

BEFORE INCUBATION AFTER INCUBATION



Colony and morphological characteristics

Color	White
Zone diameter in mm	12 mm
Size and shape	Large, circular
Opacity	Opaque
Elevation	Convex, umbonate
Margin	Irregular
Consistency	Dry
Gram staining	Gram positive long rods
Endospore staining	Spore forming
Motility	Non motile

BIBILIOGRAPHY

[1] Gupta R. And Ramnani P. "Microbial keratinases and their prospective applications an overview, ApplMicrobiol Biotechnol", 2006, vol 70,pp 21-33.

[2]Govinden G, Puchooa D. Isolation and characterization of feather degrading bacteria from mauritian soil. Afri J Biotech. 2012;11(71):13591-13600.

[3]. Xu B, Zhong , Tang X, Yang Y, Huang Z. Isolation and characterization of a new keratinolytic bacterium that exhibits significant feather-degrading capability. Afri J of Biotech. 2009;8(18):4590-4596.

[4]Prescott, L. M., Harley, J. P. and Klein, D. A. (1999). Microbiology. 4th edition, McGraw-Hill companies New Delhi, pp 151-310.

[5]Brandelli, A. (2008). Bacterial keratinases: useful enzymes for bioprocessing agroindustrial wastes and beyond. Food Bioprocess Technol. 1: 105-116.

[6]Karthikeyan, R., Balaji, S. and Sehgal, P. K. (2007). Industrial application of keratins – A review. J. Sci. Ind. Research. 66: 710-715.

[7]Kaluzewska, M., Wawrzkiewics, K., and Labarzewsk, J. (1999).Microscopic of keratin substrates subjected to the action of the enzyme of *Streptomyces fradiae.* Int. J.Biodeterior., 31: 101-107.

[8]Akhtar, W. and Edwards H.G.M. (1997).Fourier-transform Raman spectroscopy of mammalian and avian keratotic biopolymers.SpectrochimActa 53: 81-90.

[9]Hornik, C., Krishan, K., Yusuf, F., Scaal, M., and Biand-Saberi, A. (2005). Dermo1misexpression induces dense dermis, feathers and scales. Developmental Bio. 277(1): 42-50.

[10]. Kreplak, L., Doucet, J., Dumas, P. and Briki, F., (2004). New aspects of the á- helix to â-sheet transition in stretched hard á-keratin fibers. Biophys J.87:640–647.

[11]. Schrooyen, P.M.M., Dijkstra, P.J., Oberthu¨r, R.C., Bantjes, A.andFeijen, J. (2001) Partiallycarboxymethylated feather keratins. 2. Thermal and mechanical properties of films. J Agric Food Chem 49, 221–230.

[12]. Ramnani P, R Singh and R Gupta. 2005. Keratinolytic potential of *Bacillus licheniformis*RG1 : structural and biochemical mechanism of feather degradation. Can. J. Microbiol.51:191- 196.

[13]. Farag, A.M. and Hassan, M.A., (2004).Purification, characterization and Aimmobilization of a keratinase from

Aspergillusoryzae. Enzyme Microb Technol.34: 85–93.
[14]. Gupta, R. and Ramnani, P., (2006). Microbial keratinase and their prospective application: an overview. *Appl.* MicrobiolBiotechnol.70(1): 21-33.
[15]. Grazziotin, A., Pimentel, F. A., de Jong, E.V. and Brandelli, A., (2006). Nutritional improvement of feather protein by treatment with microbial keratinase.Anim Feed SciTechnol.126(1-2): 135-144.
[16]. Macedo, A.J., da Silva, W.O.B., Gava, R., Driemeier, D., Henriques, J.A.P. and Termignoni, C., (2005). Novel keratinase from *Bacillus subtilis*S14 exhibiting remarkable dehairing capabilities.Appl. Environ Microbiol. 71(1): 594-596.
[17] Latkowski JM, Freedberg IM. "Epidermal cell kinetics, epidermal differentiation, and keratinization". In: Freedberg, I.M., Eisen, A.Z., Wolff, K. (eds). (1999). Fitzpatrick's Dermatology in General Medicine. McGraw- Hill, New York, pp.133-144
[18] Korkmaz H. Hur H. ,Dincer S. " Characterization of alkaline keratinase of Bacillus licheniformis strain HK-1 frompoultry waste" Ann. Microbiol.2004, vol 54,pp 201 – 211
[19] Ionata E. ,Canganella F. ,Bianconi G. ,Benno Y. ,Sakamoto M. ,Capasso A. , Rossi M. , La Cara F. " A novelkeratinase from Clostridium sporogenesbv. peenavoransbv. nov., a thermotolerant organism isolated from solfataric muds" Microbiol. Res.2008,vol 163, pp 105- 112.
[20] Rai S.K, Konwarh R., Mukherjee A.K., "Purification, Characterization and biotechnological application of an alkaline β-keratinase produced by Bacillus subtilis RM-01 in solid-state fermentation using chickenfeather as substrate" Biochem. Eng. J. 2009, vol 45,pp 218-225
[21] Mazotto A.M. , Cristina A.N. , De Melo N. , Macrae A. ,Rosado A. , Peixoto R. , Cedrola S.M.L, Couri S., ZingaliR.B. ,Villa A.L.V. , Rabinovitch L. ,Chaves J.Q. ,Vermelho A.B., "Biodegradation of feather waste by extracellular keratinases and gelatinases from Bacillus Spp" World. J. Microbiol. Biotechnol. 2010, vol 27, pp 1355-1365
[22] Shrinivas D. And Naik G. R. "characterization of alkaline thermostable keratinolytic protease from thermo alkalophilic *Bacillus halodurans*jb 99 exhibiting dehairing activity", Int. Biodeterioation& Biodegradation, 2011,
vol 65(1), pp 29-35
[23] Ramnani P., Singh R. And Gupta R. " keratinolytic potential of bacillus licheniformis rg1: structural and biochemical mechanism of feather degradation", can J. Microbiol, 2005,vol 51, pp 191-196
[24] Tamilmani P. Umamaheswari A, Vinayagam A and Prakash B. "Production of extracellular feather degrading enzyms by Bacillus licheniformis isolated from poultry farm soil in Namakal Districk (Tamilnadu)",International Journal of Poultry Science,2008,vol 7 (2), p184-188.
[25] Vigneshwaran C., Shanmuga S. and Satish kumar T. "screening and characterization of keratinase from Bacillus licheniformis isolated from Namakkal poultry farm".2010,vol 2(4), pp 89-96.
[26] VeslavaMatikeviciene, Danute Masiliuniene and SauliusGrigiskis." Degradation of keratin containing waste by Bacteria with keratinolytic activity", Environmental Technology Resources Proceeding of the 7th International Scientific and Practical Conference. 2009, Volume 1
[27] Suntornsuk, W. and L. Suntornsuk "Feather degradation by Bacillus sp FK 46 in submerged Cultivation". Bioresour. Technol.2003,vol 86,pp 239-243.
[28] Sastry T.P., Sehgal P.K, Gupta B and Mahendra Kumar " Solublised keratins as Novel filler in the retaining of upper leather", Leather Science.1986,vol 33,pp 345-359
[29] Alexandre, J.M., O.B. Walter, G. Renata, D. David, P.H. Joao Antonio and T. Carlos "Novel keratinase from Bacillus subtilis S14 exhibiting remarkable dehairing capabilities". Appl. Environ. Microbiol., 2005,vol71,pp 594-596.
[30]. Akhtar, W. and Edwards H.G.M., Fourier-transform Raman spectroscopy of mammalian and avian keratotic biopolymers.SpectrochimActa .,1997, 53: 81-90.
[31]. 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.
[32]. Gradisar, J. Friedrich, I. Krizaj and R. Jerala, *Applied Environmental Microbiology. 2005*.,71. 3420 - 3426.
[33]. Cai, B. Lou and X Zheng, J. Zhejiang . *University Science* B 2008.,9, 60.
[34].Shih JCH, Wang JJ, Rojanatavorn K. Increased production of Bacillus keratinase by chromosomal
integration of multiple copies of the kerA gene.Biotechnol Bioeng. 2006; 87:459-464.
[35].Manczinger L, Rozs M, VagvolgyiCS,Kevei F. *World Journal of Microbiology & Biotechnology.*2003, 19, 35–39.
[36]. Zerdani I, M Faid and AMalki. 2004. Feather wastes digestion by new isolated strains *Bacillus* sp. in Morocco. African J. of Bitechnol. 3(1):67-70